STUDIES ON THE TOXICITY OF TBT AND EFFICACY OF FEW THERAPEUTIC TREATMENTS IN SOME VITAL TISSUES OF DEVELOPING CHICK

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By

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CERTIFICATE

I have pleasure forwarding this thesis of **Ms. Madhavi Thakar** entitled, "*Studies on the Toxicity of TBT and Efficacy of Few Therapeutic Treatments in Some Vital Tissues of Developing Chick*", for acceptance of the Degree of Ph.D. in *Zoology*, in Faculty of *Science*, of Saurashtra University, Rajkot, India.

This thesis contains interpretation of original experimental findings observed by the candidate in the field of Animal Physiology and Toxicology of the broad subject Zoology.

It is further certified that Ms. Madhavi Thakar has put in more than seven terms of research work in my laboratory.

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(MADHAVI THAKAR)

CONTENTS

INTRODUCTION

The environment is complex and diverse. It includes several distinct ecosystem types. The physical and chemical properties of ecosystems can have profound effects on the biological activity and impact of chemicals and other xenobiotics. Our life has now been simplified and as a result made it more leisure. Our improved way of living has introduced various chemicals, in which certain chemicals are non-selective in their mode of activity whereas some chemicals are adversely affected and incorporated in to countless consumers products for example pharmaceuticals, personal care products, food additives, plasticizers, municipal, industrial agricultural, and much more. Though these chemicals are of advantage and therefore important to us for the continuity of healthy living they have side effects on us too. All chemical retardants are released in to the environment as waste by-products. These chemical retardants produce pollution.

In above context each and every corner of the world is murmuring about the pollution. Solving our existing problems of environmental contamination and mitigating the effects of contaminants on living organisms are difficult because of the incredible variety of sources and forms of pollution. Even an abbreviated list of pollutants would include thousands of industrial by-products, pesticide residues from chemicals that have been banned from use, a variety of toxic metals and chemicals in mining waste, many compounds produced by burning fossil fuels, chemicals used in electrical generation and transport machinery and fuel additives. Each pollutant has the potential to disrupt ecosystems. Some have minimal effects, others have contaminated soils so that plants or animals from these areas cannot be eaten.

Anthropogenic effects, processes, objects, or materials are those that are derived from human activities, as opposed to those occurring in natural environments without human influences. The term is often used in the context of environmental externalities in the form of chemical or biological wastes that are produced as by-products or otherwise purposeful human activities. Many different chemicals are regarded as pollutants, ranging from simple inorganic ions to complex organic molecules. Every class of pollutants has its own specific ways of entering the environment and its own

specific dangers. Many organic compounds are basic fabrics of living organisms. Molecules built of carbon and of carbon and hydrogen are non-polar and have little to no water solubility. They have little to no electrical charge. The behavior of organic compounds depends upon their molecular structure, size and shape and the presence of functional groups that are important determinants of toxicity. It is important to know the structure of organic compounds, in order to predict their fate in living organisms and the environment. These organic compounds have adverse effect on human as well as on wild life which are termed as persistent organic pollutants (POPs). Many POPs were widely used during the boom in industrial production after World War II, when thousands of synthetic chemicals were introduced into commercial use. POPs include a range of substances as following: (a) intentionally produced chemicals currently or once used in agriculture, disease control, manufacturing, or industrial processes. Examples include PCBs, which have been useful in a variety of industrial applications (e.g., in electrical transformers and large capacitors, as hydraulic and heat exchange fluids, and as additives to paints and lubricants) and DDT, which is still used to control mosquitoes that carry malaria in some parts of the world. (b) Unintentionally produced chemicals, such as dioxins, that result from some industrial processes and from combustion (for example, municipal and medical waste incineration and backyard burning of trash).

A short list of twelve identified POPs, known as the 'dirty dozen' are Aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, hexachlorobenzene, mirex, polychlorinated biphenyls, polychlorinated dibenzo-p dioxins, polychlorinated dibenzofurans, and toxaphene. Since then, this list has generally been accepted to include such substances as polycyclic aromatic hydrocarbons (PAHs) and certain brominated flame retardants, as well as some organometallic compounds such as tributyltin (TBT). Persistent organic pollutants (POPs) are organic compounds that are resistant to environmental degradation through chemical, biological, and photolytic processes. Because of this, they have been observed to persist in the environment, to be capable of long-range transport, bioaccumulate in human and animal tissue, biomagnify in food chains, (Ritter *et al.*, 2007). The groups of compounds that make up POPs are also classed as PBTs (Persistent Bioaccumulative and Toxic) or even TOMPs (Toxic Organic Micro Pollutants). Chemical characteristics of POPs include low water solubility, high lipid solubility, semi-volatility, and high molecular masses.

POPs with molecular weights lower than 236 g/mol are less toxic, less persistent in the environment, and have more reversible effects than those with higher molecular masses. The semi-volatility of chemicals allows them to travel long distances through the atmosphere before being deposited, and if these compounds are unable to transport directly, indirect routes include attachment to particulate matter, and through the food chain. Thus POPs can be found all over the world, including in areas where they have never been used and remote regions such as the middle of oceans. The chemicals semi-volatility also means that they tend to volatilize in hot regions and accumulate in cold regions, where they tend to condense and stay. The ability of POPs to travel great distances is part of the explanation for why countries that banned the use of specific POPs. Exposure to POPs can take place through diet, environmental exposure, or accidents. One important factor of their chemical properties such as lipid solubility results in the ability to pass through biological phospholipid membranes and bioaccumulate in the fatty tissues of living organisms. Studies have linked POPs exposures to declines, diseases, behavioral abnormalities and birth defects in a number of wildlife species, including certain kinds of fish, birds, and mammals. In people, reproductive, developmental, behavioral, neurologic, endocrine, and immunologic adverse health effects have been linked to POPs. People are mainly exposed to POPs through contaminated foods. Less common exposure routes include drinking contaminated water and direct contact with the chemicals. A number of populations are at particular risk of POPs exposure, including people whose diets include large amounts of fish, shellfish, or wild foods that are high in fat and locally obtained. POPs work their way through the food chain by accumulating in the body fat of living organisms and becoming more concentrated as they move from one creature to another. When contaminants found in small amounts at the bottom of the food chain biomagnify, they can pose a significant hazard to predators that feed at the top of the food chain. This means that even small releases of POPs can have significant impacts. Therefore, even if production of all POPs ceased today, they would continue to pollute the environment for many years to come.

Organotins, or butyltins (BTs), are a group of organometallic compounds that were first synthesized in the 1930s, but did not gain wide commercial use until the 1960s and beyond (Tanabe, 1999). The environmental impact of organotin as a group of compounds has been the subject of a large amount of research in the past 10 years.

Triorganotin compounds are more toxic than mono, di, or tetra organotin forms; and tributyltin compounds are the most toxic triorganotin compounds tested. Tributyltin (TBT) compounds are organic derivatives of tetravalent tin (Sn^{4+}) and have the general formula $(CH_3\text{-}CH\text{-}CH)_3$ Sn - R where R is a covalently linked anion or group. The nature of the covalently-linked anion or group influences the physical and chemical properties of the resulting TBT derivative, in particular its solubility in water and vapor pressure (IPCS, 1990). Tributyltin (TBT) was widely introduced into the marine environment in the 1980s as the bioactive component of antifouling paints, which were used to prevent the attachment of barnacles, algae, and other organisms to boat hulls. The commercially important tributyltin derivatives include TBT oxide, TBT benzoate, TBT methacrylate, TBT chloride, TBT hydroxide and TBT fluoride. These compounds were developed to be used as antifouling paints for a wide range of maritime activities. Tributyltin (TBT) compounds are metabolized to dibutyltin (DBT) and at last monobutyltin (MBT). The world annual production of organotins has been estimated at 50,000 tons (Fent, 1996). Perhaps only 25 organotin compounds are presently produced and used to any great extent (Laughlin and Linden, 1985). Worldwide synthesis of tributyltin compounds is about 900 metric tons annually for all applications (Laughlin *et al*., 1986 a).

Triorganotin are used as general biocides against microbial and invertebrate pests and in marine antifouling paints (Laughlin and Linden, 1985). The first antifouling paints incorporating an organotin compound as a biocide were developed in 1961. Because of their effectiveness and availability in a variety of colors (Stebbing, 1985), the use of TBT in antifouling paints on ships, boats, nets, docks probably contributes most to direct release of organotin into the aquatic environment (Clark *et al.*, 1988; Hall and Pinkney, 1985). It is also act as active ingredient of many bactericides (Diez *et al*., 2002), fungicides, insecticides, acaricides, wood preservatives, in water cooling towers, as slime control in paper mills (Rajendran *et al*., 2001). Tributyltin as TBTO was first used in antifouling paints in Europe between 1959 and 1961 as a replacement for, or in addition to, copper, mercury and lead-based paints. By 1985**,** an estimated 20-30% of vessels worldwide utilized tributyltin-containing antifouling paint systems**.** TBT-containing copolymer paints are effective in controlling biofouling due to its durability, high efficiency, and reasonable cost. These improved

antifoulant systems had an important impact on the international maritime economy. In 1970s TBT was designated as a marine environmental pollutant.

Several studies address the fate of tributyltin in an estuarine environment (Lee *et al*., 1989; Seligman *et al*., 1989; Stang and Seligman, 1987; Donard and Weber, 1985). When TBT is introduced into natural waters, partitioning occurs and TBT leaves the aqueous phase and preferentially adsorbs onto suspended particles. The reported amount of TBT that is adsorbed onto suspended particles ranges from 10 to 95% and varies with the conditions present (IPCS, 1990). Data suggest that in salinities approaching that of seawater, TBT is more strongly adsorbed, while in less saline water, TBT adsorption is reduced. Once adsorbed, degradation of TBT occurs by biological action. Lee *et al*., (1989) reported that TBT degradation in unaltered estuarine water occurred faster in sunlight than in darkness, with half-lives ranging from 3-13 days. This is consistent with another report of half-lives in the marine environment ranging from 4-14 days (Seligman *et al*., 1989). Lee *et al*., (1989) noted that TBT degradation did not occur in the water lacking biotic components. Many studies address the fate of TBT in marine and estuarine sediments (Dowson *et al*., 1993 a; Dowson *et al*., 1993 b; Stang *et al*., 1992; Kram *et al*., 1989; Krone *et al*., 1989; Seligman *et al*., 1989; Unger *et al*., 1988; Maguire *et al*., 1985; Maguire and Tkacz, 1985; Maguire, 1984; and Maguire *et al*., 1983). TBT degradation is slower in sediment than in water, with half-lives in months instead of days.

Stang and Goldberg (1989) reported the average rate of degradation of TBT in fresh water is about the same as that in marine water; however, Stallard *et al*., (1987) reported that TBT degradation may occur at a slower rate in fresh water than in seawater. One group has investigated the concentration and fate of TBT during sewage sludge treatment in Zurich, Switzerland (Fent *et al*., 1991). The authors found that municipal wastewater and sewage were contaminated by organotin compounds; raw sewage sludge contained TBT, DBT, and MBT residues ranging from 0.28-0.83 mg/kg. Like the situation in sediment, TBT in wastewater and sewage sludge is primarily adsorbed onto particulate matter. They monitored the fate of the organotin compounds at various conditions (aerobic, anaerobic, mesophilic, thermophilic) and found the degradation rate of TBT during sludge treatment to be low, regardless of the conditions.

Since the late 197Os, when the ecological and economic effects of TBT on an important commercial stock of oysters in Arcachon Bay, France became evident, several groups have studied the environmental persistence and fate of TBT in aquatic ecosystems. It soon became evident that this long-acting, effective antifouling biocide had a detrimental effect on non-target organisms. The threshold concentration for biological effects was determined to be low, at about 20 ng TBT per liter of seawater. Based on this information, in 1982 France became the first country to take regulatory act by banning the use of TBT paints on all pleasure craft of less than 25 m in length, in an attempt to protect French oyster culture farms. In 1988, the federal government enacted the Organotin Antifouling Paint Control Act, which prohibited the use of butyltin paints on boats except for aluminum boats. Additional regulations were enacted in 1990 that limited the leaching of butyltins from bottom paint to no more than 4 mg/cm^2/day for boats longer than 25 feet and required that certification be required to perform the application of butyltin paints. Control measures have now been implemented in most industrial countries.

TBT was widely used as an antifouling agent in marine paints until it was observed to accumulate in aquatic animals and cause severe damage to the aquatic ecosystem (Harino *et al.*, 2000). A tri substituted organotin, tributyltin (TBT), causes the main risk for humans (RPA, 2005). Humans are exposed to TBT mainly via seafood in the diet (RPA, 2005). Another report also shows that TBT accumulation in foodstuff is serious threat for the human health (Chien *et al.*, 2002). Human exposure to organotin compounds arises from drinking water that has been contaminated with industrial effluents and through leaching of the compounds from polyvinyl chloride water pipes (Snoeij *et al.*, 1987). TBTO is a potent skin irritant and an extreme eye irritant (IPCS, 1990). Results of other studies pointed to induction of apoptosis by TBT (Aw *et al*., 1990; Raffray and Cohen, 1991; Raffray and Cohen, 1993; Grundler *et al*., 2001). Gennari and colleagues reported that low doses of tributyltin chloride inhibited immature thymocyte proliferation, whereas high doses induced apoptotic cell death (Gennari *et al*., 1997). The high lipid solubility of TBT allows for rapid membrane permeability and affects the intracellular environment, inducing cytotoxicity (Gadd, 2000). Various organs are vulnerable by TBT toxicity such as neurons (O'Callaghan

et al., 1988), hepatocytes (Jurkiewicz *et al*., 2004) muscle (Harino *et al*., 1998) and sex organs (Heidrich *et al*., 2001).

In India, TBT compounds had been used as antifouling agents in marine paints earlier, however, there is a ban on the usage of these paints is in force now. There are few studies on the distribution of butyltin residues in water and sediment samples collected from the east coast of India (Rajendran, *et al.,* 2001). Not much is known about the organotin concentrations in marine waters of the south Asian region in general and in Indian waters in particular (Bhosle *et al.,* 2004). TBT and DBT were detected in sea water, biofilm, and animal samples collected from the Dona Paula Bay, west coast of India.

REVIEW OF LITERATURE

Tributyltin is one of the controversial POP and also one of the most toxic substitutes of organotin compounds, globally introduced into the environment by anthropogenic activities (Goldberg, 1986). Tributyltin has been called the most toxic substance ever intentionally introduced into the marine environment (Mee and Fowler, 1991; IPCS, 1990). TBT is one hundred to one thousand times more toxic to laboratory animals than the zinc and copper compounds it replaced (Lenihan *et al*., 1990; Stallard *et al*., 1987).

The toxicity of Tributyltin compounds has become unique focusing point for research because of the extensive uses of TBT includes biocide (fungicide, bactericide, insecticide) in paints and coatings used for marine antifouling applications, preservative for wood, textiles, paper, leather (White *et al.*, 1999). The environmental and economic impact of TBT did not become evident until the deformative and reproductive failures of *Crassostrea gigas* (an important commercial stock of oyster) were noted in Arcachon Bay, France during the late 1970s (Alzieu, 1991; Mee and Fowler, 1991). About the evidence toxicity of TBT to nontarget species led to restricted use of TBT or uses under government regulations among many industrialized countries (van Wezel *et al.*, 2004). Following the partial bans on the use of organotin- based anti-fouling paints, water concentrations of tributyltin (TBT) have dropped dramatically, albeit with hotspots remaining in areas of intense shipping activity (Waite *et al*., 1991, 1996; Stewart, 1996). However, there is increasing evidence to show that organotin species are persistent in marine and freshwater sediments, that act as both reservoirs of the element and sources for the secondary introduction of organotins to the environment (Valkirs and Seligman, 1986; Langston *et al*., 1987; Waldock *et al*., 1990; Langston and Burt, 1991; Steur Lauridsen and Dahl, 1994; Watanabe *et al*., 1995, 1997; Harris *et al*., 1996). Despite such restrictions, TBT persists in many areas at levels considered to be chronically toxic to the most susceptible organisms (Stab *et al*., 1995; Cardwell *et al.*, 1999), because of its degradable products dibutyltin and monobutyltin remain in marine and wetland sediments and soil for a long time (Sarradin *et al.*, 1995). Examples of observed TBT levels in global marine water samples include: 200 ng/L, Mediteranean Sea, Corsica

(Michel *et al*., 2001); 242 ng/L, Mondavi estuary, India (Bhosle *et al*., 2004); 610 ng/L, Yam O, Japan (Cheung *et al*., 2003); 3.20 μg/L, Singapore (Basheer *et al*., 2002); and 14.7 μg/L, Bahrain (Alzieu, 1998). Global sediment levels are much higher; some examples include: 670 ng/g, Sao Paulo, Brazil (Godoi *et al*., 2003); 560 ng/g, Yam O, Japan (Cheung *et al*., 2003); 5.0 μg/g, Barcelona, Spain (Diez *et al*., 2002); and 340 μg/g, Great Barrier Reef, Australia (Haynes & Loong, 2002).

Tributyltin compounds have been found in water, sediment, and biota in areas close to pleasure boating activity, especially in or near marinas, boat yards, and dry docks; in fish nets and cages treated with antifouling paints; and in areas near cooling systems (IPCS, 1990). As reported in IPCS (1990), tributyltin levels have been found to reach 1.58 g/litre in seawater and estuaries; 7.1 g/litre in fresh water; 26.3 mg/kg in coastal sediments; 3.7 mg/kg in freshwater sediments; 6.39 mg/kg in bivalves; 1.92 mg/ kg in gastropods; and 11 mg/kg in fish. The deposition of TBT contaminated sediment on land might lead to a leaching of TBT into the ground. The disposal of sewage sludge is another major pathway of TBT into soil. Thus, the ecotoxicological impact of TBT on microbial activity and terrestrial organisms is of concern. Hall and Pinkney (1984), Rexrode (1987), and Bryan and Gibbs (1991) summarized bioassay studies that demonstrated the toxicity and sublethal effects of TBT in estuarine biota.

Once in the marine environment, TBT can be taken up by marine organisms through exposure to TBT contaminated water and sediments, or ingestion of TBT contaminated food sources. The mechanisms of the storage and the elimination of TBT by the organism depend upon the ability of organism to metabolize the compound (Lee, 1991). TBT is poisonous to a range of organisms from plankton to higher-level organisms (Tanabe *et al*., 1998) including humans (Heidrich *et al*., 2001; Nielsen and Rasmussen, 2004). The toxic effects of TBT compounds has been reported in different test species, organ and cell types and its mode of action was explained in multiple ways (Boyer, 1989; Fent, 1996; EFSA, 2004; Inadera, 2006). It seems to be capable to interrupt cellular components and physiological processes.

A range of cellular effects of TBT compounds has been reported, including disruption of the cytoskeleton, perturbation of plasma membranes and membrane bound transporters, interruption of ion fluxes, a rise in the intracellular Ca^{2+} concentration,

mitochondrial damage, disturbance of energy metabolism, production of reactive oxygen species, and inhibition of DNA, RNA, and protein synthesis (Boyer, 1989; EFSA, 2004). There are few studies on the distribution of butyltin residues in marine fishes (Kannan *et al.,* 1995a), marine mammals (Tanabe *et al.,* 1998), green mussels (Kan-Atirekalp *et al.,* 1990), and water and sediment samples from the east coast of India (Rajendran *et al.,* 2001).

Organotins can alter enzyme activity levels in many organs and tissues including brain, liver, and kidney (WHO, 1980; Davies and Smith, 1982; Maguire *et al*., 1982; Arakawa and Wada, 1984; Dwivedi *et al*., 1985 b; Blunden and Chapman, 1986). The toxicity of triorganotin compounds is probably due to their ability to bind to proteins and to inhibit mitochondrial oxidative phosphorylation (Davies and Smith, 1982; Blunden and Chapman, 1986). Triorganotins also interfere with phagocytosis and exocytosis and other pathways where sulfhydryl groups play a pivotal role (Elferink *et al*., 1986).

Bioconcentration and accumulation of tributyltin in the food chain is well documented; bioconcentration factors of up to 500,000 have been reported in some species (Laughlin, 1996) and up to 7000 have been reported in laboratory investigations with molluscs and fish, and higher values have been reported in field studies (IPCS, 1990). Bioaccumulation in bivalves is especially high because of the low capacity for metabolism. In molluscs, uptake from food is more important than uptake directly from water. Higher BCFs in microorganisms (between 100 and 30 000) may reflect adsorption rather than uptake into cells (IPCS, 1990). A recent publication reported a range of BCFs in the Pacific oyster (*Crassostrea gigas*) of 2400-7800. Another publication reported a range of biomagnification factors in marine mammals of 0.6-6.0 (Madhusree *et al*., 1997). Although it has been suggested that tributyltin accumulates in organisms because of its solubility in fat (IPCS, 1990). Although tributyltin residues in blubber of marine mammals where levels were considerably higher in other tissues, notably liver (Iwata *et al*., 1997; Kannan *et al*., 1998; Kim *et al*., 1996 a,b; Tanabe, 1998; Tanabe *et al*., 1998). A group of researchers had determined organotin compounds in the food web of a shallow freshwater lake; in birds in the food web, the highest concentrations of organotin compounds were also in liver and kidney, not in subcutaneous fat.

There are a number of reports on the occurrence of tributyltin residues in marine organisms. Levels of total butyltin residues (the sum of detected tributyltin, dibutyltin, and monobutyltin) of 5-230 ng/g in muscle of fish (Kannan *et al*., 1997), 300 ng/g in liver and kidney of marine birds (Guruge *et al*., 1997), and 13-395 ng/g in muscle of marine mammals have been reported (Iwata *et al*., 1997; Kannan *et al*., 1997). In marine mammals, much higher total butyltin residues were reported for blubber (48-744 ng/g), kidney (25-3210 ng/g), and liver (40-11 340 ng/g) (Iwata *et al*., 1997; Kannan *et al*., 1996, 1997, 1998; Kim *et al*., 1996 a,b,c; Madhusree *et al*., 1997; Tanabe, 1998; Tanabe *et al*., 1998). TBTO can be transferred across the blood brain barrier and from the placenta to the fetus. Following 14 days of oral administration, steady-state levels in tissue are reached after 3-4 weeks. Absorbed material is rapidly and widely distributed among tissues (principally the liver and kidney). Metabolism in mammals is rapid; metabolites are detectable in the blood within 3 h of TBTO administration. The principal metabolite appears to be the hydroxybutyl compound, which is unstable and rapidly splits to form the dibutyl derivative and butanol. In *in vitro* studies, it has been shown that TBTO is a substrate for mixed-function oxidases, but these enzymes are inhibited by very high concentrations of TBTO. The rate of TBTO loss differs with different tissues. TBTO and its metabolites are eliminated principally via the bile. The calculated half-time for elimination of TBTO residues in mice is 29 days (Brown *et al*., 1977). Tributyltin metabolism also occurs in lower organisms, but it is slower, particularly in molluscs, than in mammals. The capacity for bioaccumulation is, therefore, much greater in lower organisms than in mammals.

The exposure of terrestrial organisms to tributyltin results primarily from its use as a wood preservative, tributyltin compounds are toxic to insects exposed topically or via feeding on treated wood (IPCS, 1990). The LD 50 values for tributyltin compounds applied topically to the thorax of newly emerged insects range from 0.48% to 0.72% (dilutions with acetone) for the house fly (*Musca domestica*), from 0.29% to 0.69% for the mosquito (*Anophelese stephensi*), and from 0.52% to 0.87% for the cotton stainer (*Dysdercus cingulatus*). TBTO is toxic to honey bees (*Apis mellifera*) housed in hives made from TBTO treated wood (1.9 kg/m^3). TBTO is toxic to bats (*Pipistrellus pipistrellus*) housed in roosting cages treated with TBTO, but this result was not statistically significant, owing to high mortality in controls. The acute toxicity

of TBTO to wild mice (deer mice, *Peromyscus maniculatus* and house mice *Mus musculus*) is moderate.

Humans may come into contact with tributyltin compounds during the production of the active ingredients as well as during the formulation and use of end products such as antifouling paints. Further contact may arise during the removal of old paint coatings. The first documented case of organotin poisoning of humans was in 1880 when workers complained of headaches, general weakness, nausea, and diarrhea after exposure to triethyltin acetate vapors (Reiter and Ruppert, 1984). In addition, Exposure of mammals to organotin compounds can induce epilepsy, amnesia, and memory defects (Feldman *et al.*, 1993). There are some recent preliminary data (Takahashi *et al*., 1998) on the occurrence of total butyltin residues in human liver. The average concentration in four samples was 84 ng/g wet weights (range 59-96 ng/g). Accidental exposures of human**s** to organotin compounds have been documented (Saary and House, 2002). It was reported that exposure to organotins affects mammalian reproduction. Trans-placental transfer of organotin was documented in the rat (Noland *et al*., 1983). In utero exposure of rats to tributyltin chloride reduced maternal weight gain and fetal weights in a dose and phase-specific pattern (Ema *et al*., 1995); dose-dependent pre- or post-implantation loss (Harazono *et al*., 1998) and fetal toxicity (Itami *et al*., 1990) were observed.

Metabolism of butyltin compounds by cytochrome P450 enzymes has been suggested to play an important role in the induction of biological effects. Tributyltin was found to undergo hydroxylation followed by dealkylation to produce dibutyltin, monobutyltin, and inorganic compounds in the presence of microsomes and nicotinamide adenine dinucleotide phosphate (NADPH) *in vitro* (Casida *et al*., 1971; Fish 1984; Fish *et al*., 1976; Kimmel *et al*., 1977). Moreover, several studies have shown a variety of metabolites in rat (Matsuda *et al*., 1993) and mouse liver (Ueno *et al*., 1997) formed during the metabolism of TBTC *in vivo*. Food chain accumulation of tributyltin (TBT) has been shown in meat and fish products (Iwata *et al*., 1997; Kannan *et al*., 1998; Hoch, 2001). The deposition level of TBT compounds were considerable in liver tissue reported by researchers. (Iwata *et al*., 1997; Kannan *et al*., 1998; Kim *et al*., 1996 a,b; Madhusree *et al*., 1997; Tanabe, 1998; Tanabe *et al*., 1998). The various authors cited suggest protein binding in liver to be the major

mechanism of bioaccumulation. Liver impairment, as judged by increased serum levels of transaminases, was described in two cases of acute oral intoxication with triphenyltin (Lin *et al*., 1998; Wu *et al*., 1990). Hepatitis was also reported in three subjects who ingested between 20 and 50 grams of a preparation containing 45% triphenyltin acetate (Lin and Hsueh, 1993). It has been reported that a single dose of dibutyltin dichloride of 50 mg/kg produced inflammation of the common bile duct of Wistar rats (Barnes and Magee, 1958). Autopsy of a chemical worker who died following exposure to a combination of methyltin salts revealed massive fatty degeneration of liver cells and necrosis (Rey *et al.,* 1984). Fatty degeneration was observed at necropsy in animals killed after a 95 day exposure period to 4- 6 mg/m^3 (0.30–0.45 ppm) tributyltin chloride (Gohlke *et al.,* 1969).

Histopathology, consisting of atrophy and slight necrosis of the liver, was seen in rats exposed to 2 mg tin/m^3 (0.41 ppm) as a mixture of tributyltin bromide (0.39 ppm), dibutyltin dibromide (0.02 ppm), and hydrocarbon impurities for up to 80 days as part of a study of reproductive function (Iwamoto, 1960). Acute intestinal pancreatitis was observed by Merkord and Hennighausen (1989). Ueno *et al.,* (1994) has reported that TBTC and DBTC could cause hepatotoxicity, as evaluated by serological criteria, after oral administration to mice, whereas MBTC did not induce liver injury. Furthermore, the same researcher groups has reported in year of 2003 histopathological changes like necrosis and capillary hemorrhage in the livers of mice treated with TBTC or DBTC (Ueno *et al.,* 2003). Moreover, swelling and collapse of mitochondria were also observed in mice livers. In experimental animals, butyltin compounds have been shown to induce inflammation of the bile duct associated with hepatic lesions (Barnes and stoner, 1958; Krajnc *et al*., 1984) and to cause hepatotoxicity, detected by serological criterion, after oral administration to mice. Boyer (1989) has reported that tri and dibutyltin compounds induced lesions in the liver, bile duct and pancrease of mice or rats and found that the mitochondria function impairment being the main toxic effects of TBT in haepatocytes (Jurkiewicz *et al*., 2004). Effects of TBTO (purity 96%) on haematology and serum chemistry were assessed in groups of three and four adult male cynomolgus monkeys that ingested doses of 0 or 0.160 mg/kg body weight per day, respectively, 6 days/week for 22 weeks (0 and 0.14 mg/kg body weight per day, actual intake) (Karrer *et al*., 1992).) In addition, Pancreatic and hepatic toxicities was evident by Merkord *et al*., (2001).

TBT is known to have neurotoxic effect in organisms (Fent, 1996). Triethyltin and trimethyltin compounds have been shown to cause severe neurotoxicity. Triethyltin causes interstitial edema throughout the white matter in the spinal cord and various regions of the brain; less marked damage occurs in the peripheral nervous system. Trimethyltin also causes severe and permanent damage to the central nervous system. In this case, however, the effect is neuronal necrosis, rather than oedema. In a 4 week study, rats fed a dietary concentration of 320 mg/kg (equivalent to 30 mg/kg body weight per day) exhibited apoptosis or enophthalmia and slight ataxia (Krajnc *et al*., 1984). Crofton *et al*., (1989) measured brain weight and motor activity in developmental studies. There was some suggestion of neurotoxicity (based on decreased brain weight in pups) at exposures in excess of 10 mg/kg body weight per day, but no reported effects at 5 mg/kg body weight per day. Organotin compounds, including tributyltin, have been shown to induce apoptosis in immortalized neuronal cell lines (Thompson *et al*., 1996) and in pheochromocytoma PC12 cells (Viviani *et al*., 1995). Although TBTO induces apoptosis in neural cells *in vitro*, it does not cause neurotoxicity in whole animals. Neurotoxic effects of organotin compounds have been found in accidentally exposed humans also (Ross *et al.,* 1981). The signs and symptoms were severe headache, vomiting, vertigo, photophobia, anorexia, increased tendency to sleep, memory loss, and psychiatric disturbances. Striking interstitial oedema of the cerebral white matter was found in the victims, and reproduced as a specific effect of organotin compounds in experimental animals. The detailed mechanisms that cause the shift of the fluid into the central nervous system in organotin intoxications remain uncertain. A group of researchers showed that an oral dose of TBTO induced a transient increase in the permeability of the blood-brain barrier of the true capillaries in the hypothalamus. The tight junctions temporarily opened very early after treatment with TBTO and resealed rapidly. Accumulated TBTO at the tight junctions caused the temporary replacement of calcium ion by tin, which induces a transient increase in paracellular ion permeability throughout the blood-brain barrier (Hara *et al*., 1994). The experimental exposure of rodents to organotin compounds produced behavioral and neurological symptoms (Brown *et al*., 1979). Organotin has a high specificity for the hippocampus, and was found to elevate reactive oxygen species (ROS) in the hippocampus of treated rats (Lebel *et al*., 1990).

However, studies on occurrence of TBT in brain of fish were limited (Martin *et al*., 1989; Rouleau *et al*., 1998; Harino *et al*., 2000). Several studies report the occurrence of TBT in the brains of rainbow trout (Martin *et al*., 1989), Japanese sea perch (*Lateolabrax japonicus*), white croaker (*Pennehia argentatus*) and yellow tail (Harino *et al*., 2000). Rouleau *et al*. (1998) also indicated uptake of ^{113}Sn in the brains of rainbow trout fed [^{113}Sn] TBT. Fent and Meier (1992) noted that TBT toxicity in minnows caused abnormal swimming behavior, related to the alteration in muscle or nerve tissues. Similar studies were also carried out by Wang and Huang, (1998). They concluded that chronic TBT exposure caused a decrease in swimming activity in thorn fish due to its consequence effects on muscle and nerve tissues. In addition, a few studies pointed out the behavioral effects of TBT on fish (Triebskorn *et al*., 1994; Nakayama *et al*., 2004 a, b). The causal mechanism of these behavioral effects of TBT is complicated and currently obscure, but the ability of TBT to permeate neural tissue may be one of the important toxic factors. Some studies have pointed out the behavioral effects of TBT on fish. Triebskorn *et al*., (1994) reported that TBTO treated fish exhibited abnormal swimming pattern. Recently, Nakayama *et al*., (2004 a, b) revealed that TBT affected the general and sexual behavior of male medaka. The accumulation of TBT in the blood of fish may result from binding of TBT to a TBT binding protein, which has been identified in the blood of Japanese flounder (Shimasaki *et al*., 2002; Oba *et al*., 2007). Lipid bilayer structure and dynamics play a pivotal role for membrane proper functioning, as a selective barrier and a matrix for enzymes (Bloom *et al*., 1991). Thus, the cytotoxic effects of a variety of drugs and pollutants are suggested to result from their incorporation into the lipid bilayer and a consequence of the ability to affect and modulate lipid membrane physical properties (Sikkema *et al*., 1995). It has previously been demonstrated that organotins induce cell damage. In particular, trisubstituted organotin compounds act as potent cell membrane toxicants leading to perturbations of plasma membranes and membrane bound enzymes. However, although several studies described the toxicity of organotin compounds, only few data are reported on their effect on the structural organization and on the physico-chemical properties of model membranes. It is well known that phospholipids (PL) play many important roles in biological membranes. Their first role is the formation of a bi-dimensional barrier through which controlled fluxes of molecules and information connect the external and the cellular environments. Moreover, the structural and the physico-chemical properties of

the lipid bilayer in which proteins and other membrane components are dispersed, affect membrane functional activities. The important roles of lipids are also suggested by their large variety and by the specificity of lipid composition for different membrane types. Both di and trialkyltin compounds are inhibitors of oxygen uptake in tissues and mitochondria (Fent, 1996).

Butyltins possess both lipophilic and ionic properties that promote bioaccumulation in lipids and binding to macromolecules upon exposure. Due to characteristic of high lipophilicity of TBT (logKow between 2.3 and 4.4, depending on physico-chemical conditions) (Rudel, 2003), biological membranes have been considered supposed targets for its mode of action (White *et al.,* 1999; Gadd, 2000). As TBT compounds have affinity towards lipid, these compounds binds several membrane bound proteins such as anion channels (Powers and Beavis, 1991) and alter their normal activities and perturbation of membrane enzymes was also mediated (Celis *et al.,* 1998). The effect of organotin compounds on membrane permeability has been studied using model membranes by Cullen *et al.,* (1997) and on membrane structure by Heywood and Waterfield (1989). The wide range of organisms, bacteria included, affected by the toxic effects of TBT (White *et al*., 1999; Alzieu, 2000; Petersen and Gustavson, 2000; Qun-Fang *et al*., 2002; Smith *et al*., 2003; Jensen *et al*., 2004; White and Tobin, 2004) suggests that molecular cell components common to all living systems, namely biomembranes, may constitute the main target of this lipophilic xenobiotic.

The effects of triorganotin on mitochondria have been studied for over 50 years, little is known about how they react with proteins. Approximately as far as 60 years ago, the pioneering work was done by Aldridge (1976) who earlier described that TBT-Cl is powerful inhibitor of ATP synthesis from different organisms. He also showed that there was an inhibitory effect of TBT *in vitro* on the osmoregulatory enzyme Na^+ K^+ ATPase. Subsequent *in vivo* experiments conducted to evaluate their potential effect on osmoregulation have focused on organotins in aqueous suspension, rather than sedimentary sources. In the latter studies, no changes in blood osmolalities were found in freshwater adapted rainbow trout (*Oncorhynchus mykiss*) exposed to acutely toxic concentrations of tributyltin oxide (Chliamovitch and Kuhn, 1977). A similar observation was made by Pinkney *et al*., (1989) for juvenile striped bass (*Morone saxatilis*) adapted to 50% seawater and exposed to sublethal concentrations of

tributyltin oxide; these authors also found a significantly enhanced Na^+ K^+ ATPase activity. TBT inhibits Na^+ K^+ ATPase and ionophores controlling exchange of Cl^-, Br^-, F^- and other ions across cell membranes (Selwyn, 1976).

A study conducted in yeast suggests that the target for TBTO action is the mitochondrial ATPase (Veiga *et al*., 1997). Reports of TBT toxicity to organisms belonging to mitochondrial function impairment (Jurkiewicz *et al*., 2004) being the main toxic effects. Stridh *et al.,* (1999) reported that low concentrations of TBTC triggered an immediate depletion of intercellular ATP followed by necrotic death in Jurket cells and showed that the mode of cell death was typically apoptotic when ATP levels were maintained by the addition of glucose. The tissue dependence of enzyme inhibition by alkyltins was ascribed to the different membrane fatty acid composition (Trigari *et al.,* 2001) which may deeply affect the access to or the interaction of the toxicant with membrane bound enzyme complexes (Pagliarani *et al.,* 2006). TBT compounds are also reported to inhibit the ATPase activities and ATPase synthesis (Gruber and Marshansky, 2008; Pelletier *et al.,* 2006). In ATPase enzyme system, particularly Mg^{++} ATPase is susceptive to TBT and strongly inhibited by the TBT as observed in mammals (Nishikimi *et al.,* 2001). Several lines of evidence suggest that TBT causes an increase in intracellular calcium in various cells, including thymocytes (Chow *et al.,* 1992), hepatocytes (Kawanishi *et al.,* 2001), and PC12 cells (Viviani *et al.*, 1995). TBT is potent inhibitors of ATP synthesis and oxidative phosphorylation in mitochondria was documented by Fent (1996). Moreover, it was suggested that a major mitochondrial site of action is the F_0 segment of F_0F_1-ATPase complex, but the specific site of action has not been clearly established (Fent, 1996). The mitochondrial ATPase-ATPsynthase or F_0F_1 complex, the membrane-bound complex involved in the final reaction that links carbon substrate utilization to ATP synthesis, is long recognized to be inhibited by alkyltins in mammals and yeasts (Stockdale *et al*., 1970; Cain and Griffiths, 1977; Emanuel *et al*., 1984; Nishikimi *et al*., 2001) and reported as one of the few mitochondrial protein complexes known to react with triorganotins (Powers and Beavis, 1991). The differential inhibition by triorganotins of ATP synthesis and hydrolysis pointed out in bovine heart submitochondrial particles suggested possible toxicant binding to different functional groups (Emanuel *et al*., 1984).

The impairment of mitochondrial functions at low micromolar concentrations is long known as one of the main biochemical effects of TBT toxicity (Sone and Hagihara, 1964; Cain and Griffiths, 1977; Saxena, 1987) and TBTCl is now currently defined a mitochondrial toxin (Bragadin *et al*., 2003; Tiano *et al*., 2003; Jurkiewicz *et al*., 2004). Photophosphorylation and ATPase activity inhibition by TBT were also reported in phytoplankton (Pelletier *et al*., 2006).

To further characterize the effect of organotins on trout erythrocyte components, structural (Zolese *et al*., 1999) and functional (Santroni *et al*., 1997) studies on trout Hbs were performed. In fact, proteins can be molecular targets for trialkyltins, because these compounds can coordinate with certain amino acids. It is known that TBT can form monodentate ligands (Fent, 1996), with amino acids containing amino or -SH groups. However, the observation that organotins can interact only with a limited number of proteins suggests the requirement for a specific tridimensional structure rather than for a single chemical group (Santroni *et al*., 1997).

Interference of TBT with mitochondrial function and energy production was detected at the highest dose level tested and has also been reported previously (Baken *et al*., 2006). Inhibition of oxidative phosphorylation, loss of ATP synthase activity, and reduction of cellular ATP levels were for instance reported to be a direct effect of TBTO by others, and these findings are now substantiated by down-regulation of related genes (Snoeij *et al*., 1986 b; Boyer, 1989; von Ballmoos *et al*., 2004). Mitochondrial dysfunction may also be related to apoptosis, and both processes produce reactive oxygen species which may explain the increased glutathione synthesis.

TBT is the most potent of the trialkyltins in inhibiting the ATPase activity by an oligomycin-like effect (Stockdale *et al*., 1970), though it binds to different site(s) from that of oligomycin (Dawson and Selwyn, 1975). The ATPase inhibition by triphenyltin in beef-heart mitochondria (Byington, 1971) was hypothetically ascribed to toxicant binding to hydroxyl residues of F_0 moiety (Papa *et al*., 1982). Recently, bacterial ATP synthase was defined as the biochemical target of TBT, which at micromolar concentrations blocks the proton channel probably through noncovalent interactions with the a subunit (von Ballmoos *et al*., 2004) and 96% reduces the rate

of ATP-driven F_0F_1 rotation of the ATPase turbine (Ueno *et al*., 2005). In intact mitochondria TBT enters as butyl $3Sn^+$ aquo-cation through the lipidic bilayer (Bragadin *et al*., 2003). However, apart from this and other mechanisms involved (Snoeij *et al*., 1987), the direct interaction of TBT with the F_0F_1complex is widely recognized (Stockdale *et al*., 1970; Cain and Griffiths, 1977; Dawson and Selwyn, 1975; Powers and Beavis, 1991; Nishikimi *et al*., 2001; von Ballmoos *et al*., 2004; Ueno *et al*., 2005), but the underlying mechanism is far from being understood, both in prokaryotes and eukaryotes. in the gills and in the mantle of the mussel *M. galloprovincialis* the mitochondrial Mg-ATPase is susceptive to TBT and strongly inhibited by the toxicant, as proven in mammals (Stockdale *et al*., 1970; Emanuel *et al*., 1984; Nishikimi *et al*., 2001) and yeasts (Cain and Griffiths, 1977). Consistently, the functionality of bacterial H^+-synthase, which displays structural and functional similarity to the mitochondrial enzyme complex of prokaryotes, was found to be blocked by TBT (von Ballmoos *et al*., 2004; Ueno *et al*., 2005). The literature concerning the binding of these toxicants to biological molecules is scarce (Buck-Koehntop *et al*., 2006). Only recently attention has been focused on the possible molecular mechanism of TBT toxicity. In TBTCl, tin and carbon atoms are covalently bound while the bond connecting tin to chlorine is ionic (Smyth, 1941), thus two oppositely charged ions TBT^+ and Cl^- occur in aqueous solutions. However, according to Hoch (2001), the alkaline pH employed in the *in vitro* assays shift most of TBTCl to a neutral form and partially converts it to tributyltin oxide: the uncharged molecules can easily penetrate membrane bilayer and also combine with H^+ ions to form positively charged tributyltin ions (Aldridge and Rose, 1969). Under the experimental conditions adopted, probably both ionic and non-ionic toxicant forms occurred and interacted with mitochondrial membranes.

Susa *et al*., (1995) has noted the protective effect of 2,3- Dimercapto-1-Propanol on Bis (Tributyltin) oxide-induced cell injury was confirmed to be accompanied by a decrease in cellular tin content. It was also noted from the study that the 2,3-Dimercapto-1-Propanol was able to prevent TBTO induced stimulation of lipid peroxidation and decrease in levels of non-enzymatic and enzymatic antioxidants in isolated rat hepatocytes.

AIMS AND OBJECTIVES

Aims of the study

The aims of the present study were to evaluate the (a) *in vivo* TBT toxicity at the tissue level after its intoxication at sub lethal doses, (b) effects of continuous sub-acute exposure durations on the enzymes in the selected tissues and (c) their possible recovery after natural washing of the toxicant upon withdrawal and protective effects of few therapeutic treatments in developing male white Leghorn chick. In a nutshell, in the present study, an attempt was made to understand the toxicity of TBT on membrane transport system in particular and osmoregulatory mechanism in general in few tissues of male chick.

Objectives of the study

As per the aims mentioned above, following objectives were set forth:

1. To study the effects of sub lethal dose and duration dependent TBT toxicity on few enzyme systems at the tissue level in developing male white Leghorn chick.

2. To study the effects of natural washing upon withdrawal of toxic source and some therapeutic treatments on few enzyme systems of the TBT intoxicated chick at the tissue level.

HYPOTHESES TESTED

The basic questions which led to the commencement of this piece of research were revolving around the toxicity of selected TBT on the vital cellular physiological processes of the test organism. Therefore, few hypotheses were tested in this proposed work which was made in Null form as follows:

Sr. No.	Hypotheses Proposed
1	Dose dependent TBT toxicity may not be causing significant alterations on few key enzymes in selected organs of the chick.
2	Exposure duration dependent TBT toxicity may not be causing significant alterations on few key enzymes in selected organ systems of the chick.
3	Dose dependent TBT toxicity may not influence the membrane integrity and transmembrane transport of ions and metabolites in the selected tissues.
4	Exposure duration dependent TBT toxicity may not influence the membrane integrity and transmembrane transport of ions and metabolites in the selected tissues.
5	The therapeutic treatments given will not be effective against the toxicity of TBT.

MATERIALS AND METHODS

TOXICANT & CHEMICALS

In the present investigation, TBT (Tributyltin) trade and other names also include Alumacoat, Bioclean, Flotin, Fungitrol was used as a source of TBT. (Bis-tributyltin) oxide (TBTO), ($C_{24}H_{54}OSn_2$), structural formula $(CH_3CH_2CH_2CH_2)_3Sn\text{-}O\text{-}Sn(CH_3CH_2CH_2CH_2)_3$ with purity 96% was procured from Sigma Aldrich Pvt. Ltd. According to physical property datasheet of Tributyltin oxide, is a slightly yellow combustible liquid with highly irritating odour having molecular weight 596.08 and lipophilic characteristics. All other chemicals used in this study were of analytical grade. Double distilled water was used for all reagent preparation whenever it concern.

ANIMAL MODEL & ETHICAL ISSUES

Male chick (White leghorn strain, "Broiler"), *Gallus gallus* was selected as experimental animal model. As the studies were conducted on the growing animals, experiments were commenced with one-day-old animals. The animals with the body weight of 30 ± 5 g were considered for experimental use. They were obtained from a poultry farm situated in the Rajkot city and maintained in the departmental animal house facilities in iron cage (36"×24"×24") and in highly hygienic condition with due permission from the Animal Experiment Control and Monitoring Authority, Govt. of India. The experiment was conducted according to the animal ethics committee guidelines vide CPCSEA registration No. 757/03/a/CPCSEA (letter: CP6EA/CH/RF/ACK-2003, 29-7-2003). Growing animals were fed with a poultry starter mash (ingredients-cereal, soybean meal, wheat, grain, corn, pulses) manufactured by Hindustan lever Ltd., and tap water was always made available *ad libitum*. As growing chicks need heat, filamentous light bulbs (a total output of 400 W) were arranged around the iron cage up to one week of their age. The infected and moribund animals were not included in the experiment.

EXPERIMENTAL DESIGN :

Sr. no	Animal groups	TBT intoxication duration in days	Dose of TBT in $mgkg^{-1}$ $bwday^{-1}$	Dose of therapeutic agents in $mgkg^{-1}$ bw day^{-1}	Duration of therapeutic treatment in days	TBT + Therapeutic treatment duration in days	Scheduled day of sacrification
1	Control $_1$	1^{st} - 6^{th}	Only Corn oil	-	-	0+0	7^{th}
		1^{st} -12^{th}					13^{th}
2	Toxicated $_1$	1^{st}- 6^{th}	0.06	-	-	6+0	7^{th}
		1^{st} - 12^{th}				12+0	13^{th}
3	Toxicated $_2$	1^{st}- 6^{th}	0.6	-	-	6+0	7^{th}
		1^{st}-12^{th}				12+0	13^{th}
4	Control $_2$	1^{st}-6^{th}	Only Corn oil	-	-	0+0	9^{th} 11^{th} 13^{th}
		1^{st} -12^{th}					15^{th} 17^{th} 19^{th}
5	Withdrawal $_1$	1^{st} - 6^{th}	0.06	-	7^{th} -8^{th} 7^{th} -10^{th} 7^{th} -12^{th}	6+2 6+4 6+6	9^{th} 11^{th} 13^{th}
		1^{st} -12^{th}			13^{th} -14^{th} 13^{th} -16^{th} 13^{th} -18^{th}	12+2 12+4 12+6	15^{th} 17^{th} 19^{th}
6	Withdrawal $_2$	1^{st} -6^{th}	0.6	-	7^{th} -8^{th} 7^{th} -10^{th} 7^{th} -12^{th}	6+2 6+4 6+6	9^{th} 11^{th} 13^{th}
		1^{st} -12^{th}			13^{th} -14^{th} 13^{th} -16^{th} 13^{th} -18^{th}	12+2 12+4 12+6	15^{th} 17^{th} 19^{th}
7	Vitamin B $_1$	1^{st} -6^{th}	0.06	20	7^{th} -8^{th} 7^{th} -10^{th} 7^{th} -12^{th}	6+2 6+4 6+6	9^{th} 11^{th} 13^{th}
		1^{st} -12^{th}			13^{th} -14^{th} 13^{th} -16^{th} 13^{th} -18^{th}	12+2 12+4 12+6	15^{th} 17^{th} 19^{th}
8	Vitamin B $_2$	1^{st} -6^{th}	0.6	20	7^{th} -8^{th} 7^{th} -10^{th} 7^{th} -12^{th}	6+2 6+4 6+6	9^{th} 11^{th} 13^{th}
		1^{st} -12^{th}			13^{th} -14^{th} 13^{th} -16^{th} 13^{th} -18^{th}	12+2 12+4 12+6	15^{th} 17^{th} 19^{th}
9	Vitamin C $_1$	1^{st}-6^{th}	0.06	50	7^{th} -8^{th} 7^{th} -10^{th} 7^{th} -12^{th}	6+2 6+4 6+6	9^{th} 11^{th} 13^{th}
		1^{st} -12^{th}			13^{th} -14^{th} 13^{th} -16^{th} 13^{th} -18^{th}	12+2 12+4 12+6	15^{th} 17^{th} 19^{th}
10	Vitamin C $_2$	1^{st} -6^{th}	0.6	50	7^{th} -8^{th} 7^{th} -10^{th} 7^{th} -12^{th}	6+2 6+4 6+6	9^{th} 11^{th} 13^{th}
		1^{st} -12^{th}			13^{th} -14^{th} 13^{th} -16^{th} 13^{th} -18^{th}	12+2 12+4 12+6	15^{th} 17^{th} 19^{th}

DOSING AND TREATMENT

Animals were exposed to different sub lethal doses of TBT selected as $1/10^{th}$ of LD_{50} value, i.e. 0.6 mg kg^{-1} body weight day^{-1} and $1/100^{th}$ of LD_{50} value, i.e., 0.06 mg kg^{-1} body weight day^{-1} for 2 different exposure durations 6 and 12 days. Due to lipophilic characteristics of TBT selected doses were prepared by dissolving it in corn oil.

Vitamin B complex and Vitamin C (ascorbic acid) being constituents of animal physiology, were selected as therapeutic agents. Medically available Vitamin C (ascorbic acid) is manufactured by Hindustan pharmaceuticals and Vitamin B complex (commercial name Neurobion Forte, a combination of Thamine hydrochloride-100 mg, Riboflavin sodium phosphate-5 mg, Pyridoxine hydrochloride-100mg, Cyanocobalamin-1000 mcg, Nicotinamide 100 mg, D-panthenol 50 mg) is manufactured by Merck limited were utilized by making their proper dose. 50 mg kg^{-1} vitamin C and 20 mg kg^{-1} Vitamin B complex were prepared by diluting ampule contain in double distilled water and store in a cool place. During experimental days, treatment was given in morning hours between 9:00 a.m. to 10:00 a.m. and intramuscular route of exposure was preferred for treatment. Animal group wise description is given below.

Control $_1$ group: Animals of this group were given only corn oil as per their body weight because corn oil served as vehicle for toxicant. From this group half of the animals were sacrificed on 7^{th} day and remaining were sacrificed on 13^{th} day of experiment.

Toxicated $_1$ group: A number of animals were received 0.06 $mg^{-1}kg^{-1}bw\ d^{-1}$ dose of TBT intramuscularly from the starting of the experiment up to 6 days and a number of animals were received 0.06 $mg^{-1}kg^{-1}bw\ d^{-1}$ dose of TBT intramuscularly from the starting of the experiment up to 12 days and sequentially sacrificed on 7^{th} and 13^{th} day of experiment.

Toxicated $_2$ group: In this group all animals were received 0.6 $mg^{-1}kg^{-1}bw\ d^{-1}$ dose of TBT intramuscularly from the starting of the experiment. From this, half of the

animals were given dose up to 6 days and half of the animals were given dose up to 12 days and sacrificed on 7^{th} and 13^{th} days respectively.

Control $_2$ group: Animals of this group were kept without any treatment and a number of animals were sacrificed on 9^{th}, 11^{th}, 13^{th}, 15^{th}, 17^{th} and 19^{th} day respectively.

Withdrawal $_1$ group: Animals preintoxicated by 0.06 mg kg^{-1}bw d^{-1} dose of TBT for 6 as well as 12 days, were kept without any treatment for natural washing of the toxicant for next 2, 4 and 6 days and sacrificed consecutively on 9^{th}, 11^{th}, 13^{th}, 15^{th}, 17^{th} and 19^{th} day of experiment.

Withdrawal $_2$ group: Animals preintoxicated by 0.6 mg kg^{-1}bw d^{-1} dose of TBT for 6 as well as 12 days, were kept without any treatment for natural washing of the toxicant for next 2, 4 and 6 days and sacrificed consecutively on 9^{th}, 11^{th}, 13^{th}, 15^{th}, 17^{th} and 19^{th} day of experiment.

Vitamin B_1 complex group: Animals who were preintoxicated by 0.06 mg kg^{-1}bw d^{-1} dose of TBT for 6 as well as 12 days, were given vitamin B complex for next 2, 4 and 6 days, and sacrificed serially on 9^{th}, 11^{th}, 13^{th}, 15^{th}, 17^{th}, 19^{th} day of experiment.

Vitamin B_2 complex group: Animals who were preintoxicated by 0.6 mg kg^{-1}bw d^{-1} dose of TBT for 6 as well as 12 days, were given vitamin B complex for next 2, 4 and 6 days, and sacrificed serially on 9^{th}, 11^{th}, 13^{th}, 15^{th}, 17^{th}, 19^{th} day of experiment.

Vitamin C_1 group: Animals preintoxicated by 0.06 mg kg^{-1}bw d^{-1} dose of TBT for 6 days and 12 days were given vitamin C further 2, 4 and 6 days and sacrificed on 9^{th}, 11^{th}, 13^{th}, 15^{th}, 17^{th}, 19^{th} day of experiment respectively.

Vitamin C_2 group: Animals preintoxicated by 0.6 mg kg^{-1}bw d^{-1} dose of TBT for 6 days and 12 days were given vitamin C further 2, 4 and 6 days and sacrificed on 9^{th}, 11^{th}, 13^{th}, 15^{th}, 17^{th}, 19^{th} day of experiment respectively.

Abbereviation used in Figures and elsewhere

- **6IT-** 6 days TBT intoxication period.
- **6IT+2DT**- 6 days TBT preintoxicated animals received therapeutic treatment for next 2 days.
- **6IT+4DT**- 6 days TBT preintoxicated animals received therapeutic treatment for next 4 days.
- **6IT+6DT**- 6 days TBT preintoxicated animals received therapeutic treatment for next 6 days.
- **12IT**- 12 days TBT intoxication period.
- **12IT+2DT**- 12 days TBT preintoxicated animals received therapeutic treatment for next 2 days.
- **12IT+4DT**- 12 days TBT preintoxicated animals received therapeutic treatment for next 4 days.
- **12IT+6DT**- 12 days TBT preintoxicated animals received therapeutic treatment for next 6 days.

ENZYME PREPARATION

On the scheduled day in early morning, the animals were anesthetized and liver, kidney, brain and muscle tissues were quickly harvested. To avoid loss of enzymatic activity and cell autolysis, tissues were placed in bottle filled with chilled SEI buffer (pH 7) containing Sucrose (0.30M) EDTA (0.02M) and Imidazole (0.10M). To get enzyme fraction, tissue was weighed and homogenized in a chilled SEI buffer in Elvenhjem-potter homogenizer. For proper homogenization sterile sand was used. Tissue homogenate was centrifuged at 7000 RPM for 7 minute in cooling centrifuge at 4º C. Thus formed supernatant was collected to obtain G-6-pase enzyme fraction. Remaining pellet was suspended in SEID buffer (pH 7) containing sucrose (0.30M) EDTA (0.02M) Imidazole (0.10M) Deoxycolate disodium salt (0.1%) and further centrifuged at 7000 RPM for 7 minute. After second centrifugation supernatant was collected and utilized for ATPase enzyme estimation. Collected supernatant were freeze at 0-4º C until assay.

ENZYME ASSAYS

Activities of Total, Na^+ K^+, Ca^{++}, Mg^{++}, Ca^{++} HCO_3^- and Mg^{++} HCO_3^-ATPases were estimated as per the method of Zaugg (1982) with appropriate modifications by Lakshmi *et al*., (1991) using KH_2PO_4 as standard and Glucose-6-phosphatase was estimated as per the method of Shimeno *et al*., (1982). The activity of above mentioned enzymes were measured as per the rate of inorganic phosphate (p*i*) released from the substrate. So after the reaction was stopped, Fiske and Subbarow (1925) method was conducted to estimate released inorganic phosphate from the substrate. The specific activity of enzymes was expressed as µM inorganic phosphate released mg $protein^{-1}$ hr^{-1}. To calculate the specific activities of the enzymes studied, protein content of each sample was estimated as per the method of Lowry *et al*., (1951) using bovine serum albumin as a standard. The detailed procedures are as follows:

(a) Total ATPase

The activity of total ATPase was estimated as per the method of Zaugg (1982). The reaction mixture contained $MgCl_2$ (0.02 M), NaCl (0.10 M), KCl (0.07 M), Imidazole (0.01 M) with final pH of 7.0. Aliquots of tissue homogenate were added to this solution and mixed thoroughly using REMI cooling centrifuge. The reaction was initiated by adding 5 mM ATP disodium salt and incubated for 20 minutes in a water bath at 37 ^{0}C. The reaction was stopped by adding chilled Perchloric acid (0.95%). A normal reaction mixture incubated without tissue homogenate served as blank.

(b) Na^+-K^+ ATPase

The activity of this enzyme was estimated as per Zaugg (1982). The incubation medium containing $MgCl_2$ (0.02 M), NaCl (0.1 M), KCl (0.07 M), imidazole (0.1 M) and Ouabain ($5X10^{-4}$ M) adjusted to pH 7.0 with 0.01 M NaOH. The assay procedure was exactly similar to that of total ATPase. The activity of Na^+-K^+ ATPase was calculated by subtracting the activity obtained using this medium from the total ATPase activity. A normal reaction mixture incubated without tissue homogenate served as blank.

(c) Ca^{++} ATPase

The reaction mixture for Ca^{++} ATPase was prepared according to Zaugg (1982) method with appropriate modifications. Aliquots of tissue extracts were incubated in a reaction mixture containing $CaCl_2$ (3 mM) in 20 mM Tris-HCl buffer at pH 8.0. The reaction was initiated by adding 5 mM ATP solution and incubated at 20 ^{0}C for 20 minutes. A normal reaction mixture incubated as above without tissue homogenate served as blank. The reaction was terminated by adding 5% Chilled TCA (trichloroacetic acid).

(d) Mg^{++} ATPase

The activity of Mg^{++} ATPase was estimated as per the method of Zaugg (1982) with slight modifications. The reaction mixture was $MgCl_2$ (3 mM) in tris-HCl buffer (20 mM) at pH 8.0. Aliquots of tissue homogenates were incubated in the presence of 5 mM ATP solution for 20 minutes at 20 °C. The reaction was brought to a stop by adding 5% chilled TCA (Trichloroacetic acid). Normal reaction mixture incubated without tissue homogenate served as blank.

(e) $Ca^{++}HCO_3^-$ ATPase

Enzyme aliquot was drawn in to $CaHCO_3$ Contains 5mM $CaCl_2$, 20mM $NaHCO_3$, 1mM Ouabain, 5mM ATP Na Salt, 50mM Tris HCl, pH-8). All the tubes shaken thoroughly using cyclo mixer and kept in a room temperature for 10 minute incubation. Then 5mM ATP (pH-7) was added as substrate to all the tubes. All the tubes were kept at room temperature for 20 minutes in water bath. 15% TCA was added in all the tubes to stop the reaction and all the tubes were kept on ice- bath.

(f) $Mg^{++}HCO_3^-$ ATPase

Enzyme aliquot drawn in to $MgHCO_3$ Contains 5mm $MgCl_2$, 20mM $NaHCO_3$, 1mM Ouabain, 5mM ATP Na Salt, 50mM Tris HCl, pH-8. All the tubes shaken thoroughly using cyclo mixer and kept in a room temperature for 10 minute incubation. Then

5mM ATP (pH-7) was added as substrate to all the tubes. All the tubes were kept at room temperature for 20 minutes. 15% TCA was added in all the tubes to stop the reaction. Next, all the tubes were kept on ice- bath.

(g) Glucose-6-phosphatase

The activity of Glucose-6-phosphatase (G6Pase) was analyzed according to Shimeno (1982) with slight modifications. In enzyme extract, 40 mM glucose-6-phosphate was added and incubated for 30 min at 37 °C. The reaction was brought to stop by adding 5% chilled TCA (Trichloroacetic acid). Normal reaction mixture incubated without tissue homogenate served as blank.

Inorganic Phosphate

After terminating the reaction, the activities of all the enzymes were measured as the rate of released inorganic phosphate (Pi) from the substrate. This inorganic phosphate was then estimated by the method of Fiske and Subbarow (1925). The samples were treated with H_2SO_4 (5 N) followed by ammonium molybdate solution (2.5%). Suitable aliquots of reducing reagent containing 1-amino-2-napthol-4-sulphonic acid (0.08 M), sodium bisulphite (1.05 M) and sodium sulphite (1.05 M), was added to the above mentioned samples. The absorbance of the resulting blue color was measured at 660 nm. The values were calculated from the calibration curve and are expressed as µ mol inorganic phosphate (Pi) liberated per hour (µ mol pi/h).

Protein

To calculate the specific activities of the enzymes studied, protein content of each sample was estimated as per the method of Lowry *et al*., (1951). Aliquots of tissue homogenate as described above were added to the reaction mixture containing NaOH (0.10 M), Na_2CO_3 (0.20 M), sodium potassium tartarate (1 %) and $CuSO_4$ (0.50 %). To develop the blue color, Folin-phenol reagent dilute with distilled water was added, mixed thoroughly and incubated for 30 minutes at room temperature. The resulting color was read at 660 nM on a Spectrophotometer. Protein content of tissue samples was then calculated from the calibration curve and expressed as mg g^{-1} fresh weight of tissue. From the protein content the specific activity of the entire enzyme studied were

calculated, and expressed as u mol. Inorganic phosphate liberated per mg protein per hour (µ mol. Pi mg Protein^{-1} h^{-1}).

DATA ANALYSIS

The collected data were subjected to appropriate statistical analysis for their validity, reliability and cumulative acceptability. The collected data was then subjected to various statistical analyses for their cumulative acceptability. Specialized analyses like two-factor ANOVA, single factor ANOVA and Student's t-test were employed wherever necessary for their cumulative acceptability. All statistical procedures were computed as per Sokal and Rohlf (1969).

RESULTS

The results of the present study showed notable changes in the ATPase enzyme activity of different tissues of chick exposed to sublethal doses of TBT for different exposure durations. However, different therapeutic treatment against TBT toxicity revealed mixed trends of recovery of the enzymatic activity in respective exposure durations.

Liver

Total ATPase

In liver tissue, the activity of Total ATPase was inhibited due to 0.06 mg/kg dose of TBT after 6 and 12 days of exposure durations (Fig. 1 a & b). In therapeutic groups, 6 days pre-intoxicated animals were kept without any treatment for natural washing of the toxicant and another animals were exposed for different applications of medically available therapeutic agents like vitamin B complex and Vitamin C for next 2, 4 and 6 days. As a result, after 8 days of experiment, the activity of Total ATPase was highly recovered by both Vitamin C as well as withdrawal group (Natural washing of the toxicant). As compared to above mentioned groups, slight less enzymatic recovery was observed by application of vitamin B complex. After 10 days, the activity of Total ATPase was recovered by vitamin C and after that group slight less recovery was noted by vitamin B complex and withdrawal group respectively. After 12 days, measured Total ATPase activity was recovered by vitamin C and withdrawal group. Moreover, minimal recovery was noted by vitamin B complex group (Fig. 1 a). However as a result of therapeutic treatment, highest recovery in case of enzymatic activity was noted in animals treated with vitamin C agent after 14 days of duration. On the other hand vitamin B complex showed moderate recovery and withdrawal group showed less recovery. After 16 days of experiment, animals treated with vitamin B complex for 4 days showed highest effective result to restore Total ATPase activity. In addition, this trend was also followed by withdrawal and vitamin C treated group respectively. By graphical representation it is very clear that after 18 days, withdrawal group showed recovery near about to control level. Vitamin B complex and vitamin C group also showed notable recovery to restore Total ATPase activity (Fig. 1 b).

In liver tissue, the activity of Total ATPase was stimulated by intoxication of 0.6 mg/kg sublethal dose of TBT after 6 days and inhibited after 12 days of exposure duration (Fig. 1 c & d). After intoxication by TBT, detoxification was done by different therapeutic agents over period of 2, 4 and 6 days. After 8 days, animals kept without any treatment showed highest notable recovery in Total ATPase activity. Next to withdrawal group, vitamin B complex treatment noted moderately effective on the Total ATPase activity and less effect was observed by vitamin C on Total ATPase activity. After 10 days, the activity of Total ATPase was reached up to the control level in animals treated with vitamin B complex. Next to it, vitamin C and withdrawal group showed enzymatic recovery in this duration. After 12 days, liver Total ATPase activity was subsequent restored by vitamin C, vitamin B complex and at last by withdrawal group (Fig. 1 c). In therapeutic studies after 14 days, animals received vitamin C agent demonstrated notable recovery in the Total ATPase level in liver tissue. Similar result was also observed in case of Vitamin B complex treated animals. Whereas, TBT preintoxicated animals were kept for natural washing of the toxicant showed negligible changes in the activity of Total ATPase. A very interesting result was found after 16 days, where the activity of Total ATPase stands equally in withdrawal group as well as vitamin B complex treated group. Both group leads to the highest recovery after 16 days of duration. On the other hand slight less recovery was observed by vitamin C. After 18 days, the activity of Total ATPase was gratefully restored by both withdrawal group and vitamin B complex treated group. On the other hand, slight less enzyme restoration was observed by vitamin C treated animals after 18 days of treatment (Fig. 1d).

Na^+ K^+ ATPase

The activity of Na^+ K^+ ATPase of liver tissue was slightly stimulated in animals treated with 0.06 mg/kg dose of TBT for 6 days followed by 12 days of exposure duration as compared to their respective control (Fig. 2 a & b). TBT pre-intoxicated animals were further receiving therapeutic treatments for next 2, 4 and 6 days of duration. After 8 days, vitamin B complex as well as animals of withdrawal group proved to be quite effective to recover the Na^+ K^+ ATPase level of liver. As compared to other exhibited groups vitamin C showed less recoverable effect on Na^+ K^+ ATPase. After 12 days, the activity of Na^+ K^+ ATPase was very poorly restored by

vitamin C and withdrawal group. In addition vitamin B complex in this duration showed totally null effect to restore the Na^+ K^+ ATPase activity (Fig. 2 a). After 14 days of experimental period, the activity of Na^+ K^+ ATPase was stepwise recovered by withdrawal, vitamin B complex and vitamin C treated group respectively. Thus in this duration vitamin C agent showed maximum recovery of Na^+ K^+ ATPase of liver tissue. The results obtained after 16 days showed maximum Na^+ K^+ ATPase restoration by vitamin B complex group. Then after slight less change was observed in animals kept for natural washing of the toxicant and minimum recovery was observed in case of treatment of vitamin C agent. The results obtained after 18 days showed highest Na^+ K^+ ATPase restoration in animals treated with vitamin C agent. Next to it, withdrawal and vitamin B complex showed almost similar effect on Na^+ K^+ ATPase (Fig. 2 b).

The activity of Na^+ K^+ ATPase was increased by given 0.6 mg/kg dose for 6 days exposure duration and drastically decreased after 12 days of exposure duration (Fig. 2 c & d). After 8 days, animals kept without any treatment showed maximum recovery of Na^+ K^+ ATPase of liver tissue. On the other hand, vitamin C treatment showed near about similar position towards the control level in restoration of enzymatic activity. After 10 days, the activity of Na^+ K^+ ATPase was highest recovered by vitamin C application. Moderate recovery was profound by vitamin B complex treated group and at last by withdrawal group. After 12 days by all therapeutic agents, the Na^+ K^+ ATPase was not much recovered. Similar range of recovery in enzymatic activity was noted in vitamin B complex and vitamin C treated group after 12 days. Totally negligible recovery was observed in case of withdrawal group in this duration (Fig. 2 c). After 14 days, the activity of Na^+ K^+ ATPase was recovered by application of vitamin B complex and vitamin C group. When 12 days TBT pre-intoxicated animals were kept for natural washing of the toxicant showed quite negligible recovery of enzyme activity. After 16 days, the activity was high regained by only withdrawal group. Between both vitamin groups only vitamin B complex agent showed moderate recovery and less recovery was noted by vitamin C group. After 18 days, all applied therapeutic treatments were observed quite effective to recover activity of Na^+ K^+ ATPase of liver. By graphical representation it is obvious that vitamin B complex gives maximum protection against TBT toxicity. With above stated controversy,

withdrawal group showed less recovery as compared to vitamin B complex and vitamin C showed lesser recovery (Fig. 2 d).

Ca^{++} ATPase

The activity of Ca^{++} ATPase of liver tissue was inhibited exposed to 0.06 mg/kg dose of TBT for 6 days and stimulated after 12 days of exposure duration (Fig. 3 a & b). After 8 days, vitamin C treated animals showed highest Ca^{++} ATPase recovery in liver tissue. This phenomenon of recovery was slightly less expressed in animals served as withdrawal group. At last vitamin B complex was found to be less effective among all given therapies. After 10 days, highly recovery in enzymatic activity was observed by given medically available agent named vitamin C. Equally to vitamin C, withdrawal group also showed remarkable influence on enzyme restoration. Furthermore vitamin B complex was not able to prove its efficacy in this duration. After 12 days, an interesting result was observed as the activity of Ca^{++} ATPase was stepwise recovered by subsequent group of withdrawal, vitamin B complex and vitamin C (Fig. 3 a). Estimated Ca^{++} ATPase was gratefully recovered by withdrawal group as compared to another vitamin C and Vitamin B complex treated groups in 14 days of duration. The similar result was also observed in case of 16 days of exposure duration. When 12 days TBT preintoxicated animals were subjected to medically available therapeutic agents like vitamin B complex and vitamin C showed maximum effect to restore the enzymatic activity as compared to withdrawal group after 18 days of experiment (Fig. 3 b).

After intoxication by higher sublethal dose of TBT the activity of liver Ca^{++} ATPase was increased during 6 days of exposure duration. However quite opposite trend was observed after 12 days where the activity was decreased (Fig. 3 c & d). After 8 days of experiment, the activity of Ca^{++} ATPase was recuperated by vitamin C agent. Next to it, withdrawal group and at last vitamin B complex had been found effective on Ca^{++} ATPase. It has been indicated by graphical representation, that pre-intoxicated animals kept for natural washing of the toxicant showed highest efficacy to restore the Ca^{++} ATPase activity. Enzymatic activity of withdrawal group had achieved complete control level after 10 days of duration. Next to this group, vitamin C and vitamin B complex had proven its efficacy to recover the Ca^{++} ATPase activity in liver tissue. After 12 days gradual increased recovery of Ca^{++} ATPase was observed by vitamin C,

vitamin B complex and withdrawal group respectively (Fig. 3 c). After 14 days, challenge of natural washing of toxicant as a source of withdrawal, was determined as highest successive group to recover enzyme amongst all other therapeutic groups. Ca^{++} ATPase of withdrawal group has tried to reach to the control level. Furthermore, vitamin C showed moderate effect and at last vitamin B complex group had been seen with minimum effect on enzymatic activity. As a result after 16 days of experiment, vitamin C was determined more efficient to recover the activity of enzyme. In this duration withdrawal group was noted as moderate effective source to restore the enzyme and much less recovery was noted in case of vitamin B complex group. However after 18 days, progressively improvement in Ca^{++} ATPase activity was observed in withdrawal group, vitamin C and vitamin B complex treated groups respectively (Fig. 3 d).

Mg^{++} ATPase

As a result of intoxication by 0.06 mg/kg dose of TBT, the activity of Mg^{++} ATPase was decreased as compared to control level after 6 days and increased after 12 days of exposure period (Fig. 4 a & b). After 8 days of experiment, Mg^{++} ATPase of vitamin C treated group and withdrawal group indicated paramount achievement to restore the enzyme at control level. As compared to these therapies vitamin B complex indicated less recovery. It is clear from the figure that the activity of Mg^{++} ATPase was efficiently recovered only in withdrawal group after 10 days of treatment. The activity of Mg^{++} ATPase was reached up to almost control level in withdrawal group. Treatment by vitamin B complex had given slight lesser effect than withdrawal group. Amongst all given therapeutic treatments vitamin C treated group showed negligible effect on Mg^{++} ATPase activity after 10 days of exposure period. Results after 12 days denoted gradually recovery in Mg^{++} ATPase activity by vitamin C, vitamin B complex treatment and at last by withdrawal group respectively (Fig. 4 a). Activity of Mg^{++} ATPase was reached near about to control level in both the groups of withdrawal as well as vitamin C treated group after 14 days of experimental period. In this controversy application of vitamin B complex did not show much effect on enzyme activity. Quite amazing result was obtained after 16 days of experiment, where given all therapeutic treatments had given almost equal contribution to restore Mg^{++} ATPase activity. Application of vitamin B complex to TBT pre-intoxicated animals showed greatness to improve the usual Mg^{++} ATPase activity after 18 days of

experiment. Next to vitamin B complex, less influence was detected by both withdrawal and vitamin C treated groups (Fig. 4 b).

When animals were intoxicated by higher sublethal dose of TBT for continuous 6 days, stimulatory behavior was observed in case of liver Mg^{++} ATPase (Fig. 4 c) and Inhibitory effect was observed after 12 days exposure duration (Fig. 4 d). 6 days TBT pre-intoxicated animals received Vitamin B complex and vitamin C showed sequentially gradual enzymatic recovery as compared to withdrawal after 8 days of experiment. On the other hand 6 days pre-intoxicated animals were kept as source of withdrawal served maximum recovery of Mg^{++} ATPase as compared to both therapeutic agents like vitamin B complex and vitamin C in 10 and 12 days of experimental durations (Fig. 4 c). After intoxication, different therapeutic treatments were given to the animals amongst them, vitamin B complex treated group and withdrawal group was found to be most successful to recover the enzyme activity after 14 days of experiment. On the other hand effect of vitamin C was not that much helpful. After 16 days of treatment animals of withdrawal and vitamin C treated groups demonstrated their maximum effect on Mg^{++} ATPase recovery as compared to vitamin B complex treated animals. With contradiction of above statement after 18 days, vitamin B complex was recorded as highly effective therapeutic agent, next to it withdrawal group and at last less recovery was observed in vitamin C treated group (Fig. 4 d).

$Ca^{++}HCO_3^-$ ATPase

By given lower sublethal dose of TBT the activity of $Ca^{++}HCO_3^-$ ATPase was decreased in case of 6 days and increased in case of 12 days of exposure duration (Fig. 5 a & b). After 8 days of total treatment period, animals received vitamin C showed effective recovery. Whereas, vitamin B complex showed moderate recovery and as compared to vitamin B complex and vitamin C withdrawal group showed quite less recovery. After 10 days of experiment, enzymatic recovery was followed the similar trend earlier observed in 8 days of duration. After 12 days, vitamin C treated animals showed highest recovery of $Ca^{++}HCO_3^-$ ATPase activity. Next to it, animals of withdrawal group showed less effect and vitamin B complex in this duration was not at all effective to recover the enzymatic activity (Fig. 5 a). All over 14 days of treatment, the activity of $Ca^{++}HCO_3^-$ ATPase was highly influenced by withdrawal

treatment. In this duration treatment of natural washing of entered TBT molecules showed highest recovery of enzyme. Next to it, vitamin B complex showed not that much recovery. In this duration vitamin C treated group was not able to recover the $Ca^{++}HCO_3^-$ ATPase activity. $Ca^{++}HCO_3^-$ ATPase activity was almost repaired after 16 days of duration as evident from the graphical representation. It is quite surprising that all three therapeutic group viz., withdrawal, vitamin C and vitamin B complex treated groups showed almost equal performance to recover the enzymatic activity. $Ca^{++}HCO_3^-$ ATPase stood almost near to control level in all three treatment groups. After 18 days, treatment of vitamin B complex showed highest recovery amongst all other treatment groups. Near about similar effect on enzymatic activity was also observed by vitamin C treated group and withdrawal group (Fig. 5 b).

In case of $Ca^{++}HCO_3^-$ ATPase, the activity of this enzyme was stimulated only in animals received 0.6 mg/kg dose of TBT for 6 days and an inhibitory expression was noted over 12 days of intoxication period (Fig. 5 c & d). The results indicated that the activity of $Ca^{++}HCO_3^-$ ATPase was highly improved by withdrawal group in all three durations viz., 8, 10 and 12 days. Withdrawal group had proved highest ability on $Ca^{++}HCO_3^-$ ATPase to get its usual activity. Except withdrawal group none of the therapeutic treatment showed its ability to recover the enzymatic activity after 8 days of duration. Furthermore after 10 days activity of $Ca^{++}HCO_3^-$ ATPase of vitamin B complex and vitamin C treated group also showed recovery but not as strong as expressed in withdrawal group. After 12 days vitamin B complex registered its efficacy but not that much high, expressed in withdrawal group (Fig. 5 c). $Ca^{++}HCO_3^-$ ATPase was highly recovered only in vitamin C treated animals in all three durations Viz., 14, 16 and 18 days. None of other therapy was seem to be effective than vitamin C (Fig. 5 d).

$Mg^{++}HCO_3^-$ ATPase

The activity of liver $Mg^{++}HCO_3^-$ ATPase was stimulated in 0.06 mg/kg intoxicated animals for 6 days followed by 12 days exposure duration (Fig. 6 a & b). It was find out through data that $Mg^{++}HCO_3^-$ ATPase got its usual activity only in animals treated with vitamin B complex both in 8 and 10 days of durations. Here vitamin C also showed its therapeutic effect on enzyme but not that much great observed in vitamin B complex. At last 12 days of period, the activity of $Mg^{++}HCO_3^-$ ATPase was

progressively repaired and got its normal activity in vitamin C, vitamin B complex and withdrawal treatment respectively (Fig. 6 a). After treatment with different therapeutic agents, the activity of $Mg^{++}HCO_3^-$ ATPase was found to be more usual only vitamin C treated animals after both 14 and 16 days of experiment. Next to vitamin C group, vitamin B complex and withdrawal group also found to be effective on enzyme but not much impressive recovery was noted. Among all durations, maximum recovery was recorded after 18 days of experiment in which activity of $Mg^{++}HCO_3^-$ ATPase reached near about control level in vitamin B complex treated animals. Moreover, withdrawal group also noted effective on enzyme but vitamin C was not much helpful than other applied therapies in this duration (Fig. 6 b).

The activity of $Mg^{++}HCO_3^-$ ATPase of liver was gratefully increased in lower exhibited dose of TBT over 6 days of duration and highly inhibited after 12 days of duration (Fig. 6 c & d). After 8 days of treatment period, the activity of $Mg^{++}HCO_3^-$ ATPase got its normal mode only by vitamin B complex and withdrawal treatment. Among all treatment groups vitamin C treated group indicated highest recovery after 10 days of experimental duration. After 12 days application of vitamin C and B complex denoted as maximum recoverable group. Withdrawal did not show any recovery in this duration (Fig. 6 c). When 12 days TBT intoxicated animals were subjected to vitamin C agent received highest recovery of this enzyme after 14 days of experiment. Withdrawal group had been seen with its moderate recovery on $Mg^{++}HCO_3^-$ ATPase. While minimum recovery was noted in vitamin C treated group in this duration. After 16 days of experiment, vitamin B complex had proven its efficacy to recover the enzyme activity. Animal group of natural washing of TBT had also found moderate recovery of the enzyme. In this duration vitamin C had given minute protective effect on enzyme activity. Highest improvement in $Mg^{++}HCO_3^-$ ATPase was denoted only in vitamin B treated group after 18 days of experiment. Group of vitamin C had also achieved level of improvement in $Mg^{++}HCO_3^-$ ATPase activity. As compared to both medically available vitamin B complex and C, withdrawal group had noted its less efficiency to recover the enzyme (Fig. 6 d).

G-6-Pase

Activity of G-6-Pase was highly inhibited by lower exhibited dose of TBT for 6 and 12 days of duration (Fig. 7 a & b). The activity of G-6-Pase was tried to get its usual

activity only in vitamin C and withdrawal group. On the other hand vitamin B complex had not seen as effective agent on G-6-Pase activity in 8 days duration. By treatment of vitamin C, vitamin B complex and withdrawal group the activity of G-6-Pase was gradually increased and reached more than half of the control level after 10 days of experimental period. Animals treated as source of withdrawal group had proved its maximum effect against damaged by TBT after 12 days of exposure. Next to it, vitamin B complex also tried to recover the enzyme. Furthermore vitamin C was not found to be efficient to recover the enzyme (Fig. 7 a). Activity of G-6-Pase was highly recovered by withdrawal group after 14 days of experiment. After 16 days application of vitamin C demonstrated its efficiency to recover the enzyme amongst all other therapies. After 18 days highest recovery was noted in case of vitamin B complex treated animals. Activity of this group found to reach near above half of the control level. Withdrawal and vitamin C treated group had not proved efficiency in this duration (Fig. 7 b).

Liver G-6-Pase activity was inhibited by exhibited dose of 0.6 mg/kg over 6 and 12 days of exposure duration (Fig. 7 c & d). When 6 days TBT preintoxicated animals reared without any treatment showed maximum G-6-Pase recovery level where the activity reached near to control level. Medically available therapeutic agent Viz., vitamin B complex and vitamin C showed almost similar recovery level of enzyme where they achieved half of the control level after 8 days of experimental period. After 10 days among all therapeutic treatments, group of vitamin C denoted as highest efficient group in enzyme recovery point of view. On the other hand vitamin B complex and withdrawal group possess moderate recovery of G-6-Pase enzyme. After 12 days G-6-Pase enzyme activity got its normal condition only in vitamin B complex group. Next to it, vitamin C treated group and withdrawal group had noted minimum recovery (Fig. 7 c). After 14 days vitamin B complex treated animals and second withdrawal animals showed restoration of G-6-Pase. After 18 days highest enzyme activity repaired by vitamin C agent. All other therapies viz., vitamin B complex and withdrawal demonstrated its poor effect on enzyme activity. After 18 days G-6-Pase had achieved its normal activity level due to application of vitamin B complex. Whilst, all other therapeutic treatments were not much effective as compared to vitamin B complex group (Fig. 7 d).

KIDNEY

Total ATPase

By intoxication with lower sublethal dose of TBT for continuous 6 days, the activity of Total ATPase of kidney was stimulated (Fig. 8 a). When lower sublethal dose of TBT was subjected to animals for 12 days the activity of Total ATPase was inhibited respective to their control (Fig. 8 b). After treatment with different therapeutic agents, the activity of Total ATPase got its regular condition because of natural washing of toxicant. As compared to withdrawal group, vitamin B complex and vitamin C could not admit its efficiency to recover the enzyme after 8 days of experimental period. After 10 days, almost all therapeutic treatment admitted its efficacy to recover the enzyme. Among all therapeutic treatment withdrawal group was noted as highest effective group in which the total ATPase activity of kidney stood totally to control level. In group of vitamin B complex and vitamin C slight less recovery of enzyme was observed. After 12 days, Total ATPase recovery was highest in vitamin B complex treated group, than vitamin C showed slight less recovery and at last withdrawal group showed minimum recovery as compared to all other therapeutic treatments (Fig. 8 a). After 14 days of period, the activity of Total ATPase was stepwise recovered in subsequent group of vitamin C, vitamin B complex and withdrawal group. However, after 16 days vitamin B complex approved highest recovery of Total ATPase enzyme. In this duration vitamin C treated animals showed moderate recovery and minimum recovery in enzymatic activity was noted by withdrawal group. After 18 days among all therapeutic groups vitamin C showed highest recovery and withdrawal showed lowest recovery level of enzyme activity (Fig. 8 b).

The activity of Total ATPase of kidney was stimulated intoxicated with higher sublethal dose of TBT for 6 days (Fig. 8 c). The activity of Total ATPase was inhibited by 0.6 mg/kg dose of TBT after 12 days of exposure (Fig. 8 d). An interesting result was observed in case of Total ATPase activity after its detoxication period, where the Activity of Total ATPase was stepwise showed recovery by serially withdrawal, vitamin B complex and vitamin C treated groups after only 8 and 12 days of treatment period. After 10 days, the activity of Total ATPase was recovered in both the group vitamin B complex and vitamin C. As compared to vitamin treatment

withdrawal group was not that much showed recovery in this duration (Fig. 8 c). After 14 days of treatment withdrawal group denoted its maximum effect to recover the enzymatic activity, where activity of Total ATPase was reached to the control level. Medically available vitamin B complex and vitamin C showed not that much recovery after 14 days of duration. Moreover after 16 days the activity of Total ATPase was recovered by withdrawal group. Then after serially less recovery was noted by vitamin B complex and vitamin C treated group. After 18 days, Total ATPase was highly repaired by medically available agent named vitamin B complex. Next to it, vitamin C and withdrawal group also showed moderate effect on enzyme activity (Fig. 8 d).

Na^+ K^+ ATPase

The activity of Na^+ K^+ ATPase showed variation in their activity. By given lower sublethal dose of TBT the Na^+ K^+ ATPase of kidney was stimulated after 6 days and activity was inhibited after 12 days of exposure period (Fig. 9 a & b). When 6 days TBT preintoxicated animals were further kept without any treatment showed highest Na^+ K^+ ATPase recovery. Vitamin B complex treated animals also showed recovery but not greater than withdrawal group. As evident from the figures that vitamin C was totally failed to restore the Na^+ K^+ ATPase after 8 days of duration. After 10 days, Na^+ K^+ ATPase was massively changed and tried to reach the usual activity only in withdrawal group. As compared to withdrawal group, vitamin treatment to animals showed less recovery. Among 8, 10 and 12 days of duration the activity of Na^+ K^+ ATPase was poorly recovered only after 12 days of duration. Moreover treatment with vitamin had noted remarkable recovery. On the other hand in this duration the activity of Na^+ K^+ ATPase was negligibly recovered by withdrawal group (Fig. 9 a). The activity of Na^+ K^+ ATPase was remarkably recovered after 14, 16 and 18 days of treatment duration. After 14 days, recovery in Na^+ K^+ ATPase was observed in ascending order by vitamin C, vitamin B complex and withdrawal group. After 16 days application of vitamins to individual groups showing massive restoration of Na^+ K^+ ATPase as compared to group of withdrawal. After 18 days of treatment, activity of Na^+ K^+ ATPase was regained its usual condition due to application of vitamin C. Vitamin B complex also demonstrate remarkable recovery. However withdrawal group was marked as very less efficient group in enzymatic recovery point of view only in this duration (Fig. 9 b).

The activity of Na^+ K^+ ATPase was slightly stimulated by given higher sublethal dose of TBT after 6 days of duration while activity was inhibited after 12 days as compared to their control group (Fig. 9 c & d). After 8 days of experiment, Na^+ K^+ ATPase showed its higher recovery in vitamin C treated group. Then after recovery level was decreased in vitamin B complex group and minimum recovery was recorded in withdrawal group. After 10 days, animals treated with vitamins showed maximum effect on restoration of enzyme. After 12 days, vitamin C treated group stood first on recovery point of view. In this duration withdrawal did not show recovery of Na^+ K^+ ATPase activity (Fig. 9 c). After 14 days of treatment with vitamin B complex and withdrawal group, the activity of Na^+ K^+ ATPase achieved near about normal activity as compared to treatment of vitamin C in kidney tissue. Na^+ K^+ ATPase was reached up to half of the control level by natural washing of TBT molecules after 16 days. Moreover, near about withdrawal level was achieved by vitamin B complex group but vitamin C did not show that much efficient recovery of Na^+ K^+ ATPase of kidney. After 18 days, animals treated with vitamin B complex had proven its ability to recover the enzyme. The activity of this group possess near to control level. Whilst withdrawal and vitamin C treated group denoted as less recoverable group after 18 days of experimental period (Fig. 9 d).

Ca^{++} ATPase

Documented Ca^{++} ATPase in kidney tissue was highly inhibited only in animals administered with 0.06 mg/kg dose of TBT for exposure duration of 6 and 12 days (Fig. 10 a & b). In therapeutic studies, the activity of Ca^{++} ATPase was recovered by vitamin C treatment among all other therapeutic treatments after 8 days of period. As a result after 10 days, first vitamin B complex and then vitamin C treated group showed recovery of Ca^{++} ATPase of kidney tissue. With this contradiction after 12 days, highest recovery was noted by vitamin C treated group and less recovery was noted by vitamin B complex treated group as compared to withdrawal group (Fig. 10 a). When 12 days TBT preintoxicated animals received vitamin B complex for next 2 days induced Ca^{++} ATPase recovery, where the activity of Ca^{++} ATPase reached to the control level. Whereas, withdrawal and vitamin C treated group had admitted its moderate recovery after 14 days of experiment. When 12 days TBT preintoxicated kept as source of withdrawal leads to the maximum restoration of Ca^{++} ATPase after

14 days of duration. After 18 days, highest attention was paid by vitamin B complex treated group because its ability to restore the Ca^{++} ATPase activity. In this duration both the groups of vitamin treatment showed its Ca^{++} ATPase activity more than half of the control level (Fig. 10 b).

Ca^{++} ATPase of kidney was highly inhibited in both the exposure durations viz., 6 and 12 days by exhibited higher sublethal dose of TBT (Fig. 10 c & d). After 8 days, natural washing of toxicant group had given massive recovery of enzymatic activity as compared to treatment of vitamins. Similar enzymatic recovery trend was also noted after 10 days of duration. After 12 days of treatment animals treated with different vitamins showed highest proficiency to recover the enzyme (Fig. 10 c). When 12 days TBT preintoxicated animals kept without any treatment of therapeutic agent showed their great affinity towards recovery of Ca^{++} ATPase. After 14 days, vitamin C had also proven its moderate effect to recover the enzyme. In case of 16 days of duration therapeutic treatment of vitamin C induced Ca^{++} ATPase recovery. Observed recovery level of Ca^{++} ATPase was not that much higher after 18 days, in which highest Ca^{++} ATPase recovery was noted by vitamin B complex and withdrawal group (Fig. 10 d).

Mg^{++} ATPase

The activity of Mg^{++} ATPase was considerably suppressed by 0.06 mg/kg dose of TBT for continuous 6 and 12 days of exposure durations (Fig. 11 a & b). When 6 days TBT preintoxicated animals lived without any therapeutic treatment served as withdrawal group had proved its supreme efficiency to recover the Mg^{++} ATPase of kidney tissue. Application of vitamins did not show recovery as compared to withdrawal group after 8 days of duration. As earlier noted in 8 days, the equivalent result in enzymatic recovery was also noted after 10 days of duration. After 12 days highest attention was paid by vitamin C treatment in enzyme recovery. Next to it, vitamin B complex and at last withdrawal showed minimum recovery of Mg^{++} ATPase (Fig. 11 a). After 14 days of experiment withdrawal group had promoted first dominant group to recover the Mg^{++} ATPase activity. The activity of this group had tried to reach near to the control level. Vitamin B complex also found to be effective to restore the enzyme. Vitamin C did not show that much potentiality to recover Mg^{++} ATPase in this duration (Fig. 11 b).

The activity of Mg^{++} ATPase was inhibited by higher sublethal dose of TBT after 6 days of duration and stimulated after 12 days of duration (Fig. 11 c & d). When 6 days TBT preintoxicated animals were further challenged to vitamin B complex showed highest ability to recover Mg^{++} ATPase activity. Withdrawal group had proven moderate recovery of Mg^{++} ATPase of kidney and less recovery was profound in case of vitamin C treated groups after 8 days of experiment. After 10 days of experimental period, the activity of Mg^{++} ATPase was highly repaired by only withdrawal group. Vitamin C and vitamin B complex categorized under less recoverable group in this duration only. The activity of Mg^{++} ATPase got its usual activity by all given therapeutic agents after 12 days of experimental period. In this duration animals received vitamin treatment showed massive recovery in Mg^{++} ATPase. Withdrawal group also showed recovery but less than vitamin treated groups (Fig. 11 c). After 14 days of duration, the activity of Mg^{++} ATPase was repaired by withdrawal group but vitamin B complex and vitamin C did not show much recovery in this duration. After 16 days of experiment, vitamin B complex treated group showed highest recovery. In enzymatic recovery point of view withdrawal seem to average as compared to all other treatment groups. Furthermore, Mg^{++} ATPase was minimum recovered by vitamin C group. The activity of Mg^{++} ATPase was regained by vitamin treatment after 16 days of experimental duration. Whereas, withdrawal group did not show Mg^{++} ATPase recovery as compared to vitamin treated groups in this duration. After 18 days of duration, the activity of Mg^{++} ATPase was highly repaired by vitamin C treated group. On the other hand, vitamin B complex showed moderate enzymatic recovery and negligible recovery was noted by withdrawal group after 18 days of duration (Fig. 11 d).

$Ca^{++}HCO_3^-$ ATPase

In case of $Ca^{++}HCO_3^-$ ATPase of kidney, more or less inhibition was expressed due to given lower sublethal dose of TBT after 6 and 12 days of intoxication period (Fig. 12 a & b). In therapeutic studies, it was noted that enzyme recovery was serially increased in consecutive groups of vitamin C, vitamin B complex and withdrawal after 8 days of duration. After 10 days, vitamin B complex had proven its efficacy to recover the $Ca^{++}HCO_3^-$ ATPase activity. Moderate recovery was noted by vitamin C and lesser recovery was observed by withdrawal group after 10 days of duration. The

activity of $Mg^{++}HCO_3^-$ ATPase was recovered by vitamin C followed by vitamin B complex after 12 days of duration (Fig. 12 a). After 14 days of treatment period, the activity of $Ca^{++}HCO_3^-$ ATPase tried to get its natural condition in vitamin C treated group. However, vitamin B complex and withdrawal did not show much protective effect on enzymatic activity. From decreasing to increasing manner, $Ca^{++}HCO_3^-$ ATPase was sequential recovered by withdrawal, vitamin C, vitamin B complex treated group after 16 days of duration. As earlier noted in 16 days of duration the similar enzyme recovery pattern was also followed by 18 days of duration (Fig. 12 b).

Activity of $Ca^{++}HCO_3^-$ ATPase of kidney was stimulated by given higher sublethal dose of TBT in both 6 and 12 days of exposure durations (Fig. 12 c & d). After 8 days, vitamin B complex and withdrawal group designated highest efficient group to recover the $Ca^{++}HCO_3^-$ ATPase activity. Among all therapeutic groups vitamin C treated group did not show recovery in enzyme. With the contradiction of above statement after 10 days maximum attention in term of enzymatic recovery was paid by vitamin B complex treated group. As compared to vitamin B complex none of any therapy seems to be effective to restore the enzymatic activity after 12 days of duration. $Ca^{++}HCO_3^-$ ATPase was followed the similar trend of recovery as earlier noted in 8 days of duration (Fig. 12 c). After 14 days of treatment period, animals received vitamin C agent noted as highest recoverable group in enzymatic activity. Vitamin B complex also showed its efficiency to recover the enzyme in this duration. Withdrawal group did not show much recovery in $Ca^{++}HCO_3^-$ ATPase enzyme. After 16 days vitamin treatment had again proven its efficacy to restore the enzyme. Among all therapeutic treatment none of them showed enzymatic recovery after 18 days of treatment (Fig. 12 d).

$Mg^{++}HCO_3^-$ ATPase

The activity of $Mg^{++}HCO_3^-$ ATPase of kidney tissue was stimulated after 6 days of duration and inhibited after 12 days of duration by administration of 0.06 mg/kg dose of TBT (Fig. 13 a & b). After 8 days of therapeutic treatments, animals did not received any therapeutic agents indicated highest recovery of $Mg^{++}HCO_3^-$ ATPase activity as compared to both vitamin B complex and vitamin C treated groups. In addition, vitamin B complex also followed similar pattern of recovery after 10 days.

Treatment of vitamin C against TBT not at all affects to recover the $Mg^{++}HCO_3^-$ ATPase after 10 days of duration. As a result after 12 days treatment, highest recovery was also profound in vitamin B complex treated group. The similar trend was also followed by vitamin C treated group. As compared to both vitamin treated groups withdrawal showed less effect on enzyme restoration (Fig. 13 a). Activity of $Mg^{++}HCO_3^-$ ATPase was progressively recovered by withdrawal, vitamin B complex, vitamin C treated groups after 14 days of duration. After 16 days withdrawal group was observed as highest potential group to recover the enzyme, where the activity of $Mg^{++}HCO_3^-$ ATPase reached very near to the control level. After 18 days of treatment, vitamin B complex treatment noted as potential to recover $Mg^{++}HCO_3^-$ ATPase activity. Groups of withdrawal and vitamin C treatment showed analogous level of enzymatic recovery (Fig. 13 b).

The activity of $Mg^{++}HCO_3^-$ ATPase was more or less stimulated in higher dose groups after 6 and 12 days of exposure durations (Fig. 13 c & d). Therapeutic studies revealed that the activity of $Mg^{++}HCO_3^-$ ATPase was recovered by withdrawal group and vitamin treatment did not effective on enzymatic recovery after 8 days of treatment. After 10 days, the activity of $Mg^{++}HCO_3^-$ ATPase was highly recovered by vitamin C group where, the activity of this group reached to the control level. On the other hand, remaining therapeutic groups did not show recovery in this duration. After 12 days remarkable recovery was observed by withdrawal group followed by vitamin B complex group (Fig. 13 c). After 14 days of study, both withdrawal as well as vitamin B complex group was noted as highest recoverable group. However vitamin C treatment could not able to recover the enzymatic activity in this duration. After 16 days only withdrawal group was marked as highest efficient group to recover the enzymatic activity. Vitamin treatments did not show recovery in this duration. After 18 days of treatment, exhibited all therapeutic groups were able to recover the $Mg^{++}HCO_3^-$ ATPase activity (Fig. 13 d).

G-6-Pase

By sublethal exposure of 0.06 mg/kg TBT, the activity of G-6-Pase was highly reduced as compared to their respective control group after 6 and 12 days of duration (Fig. 14 a & b). After 8 days of experiment, vitamin C proved its ability to recover the

usual G-6-Pase activity. As compared to vitamin C treatment, withdrawal and vitamin B complex did not show much recovery in this duration. With contradiction of above statement, the activity of G-6-Pase was repaired by vitamin B complex as well as withdrawal group after 10 days of duration. Vitamin C treated group showed minimum recovery in this duration. Whilst application of vitamin B complex indicated as highest efficient group in term of G-6-Pase recovery after 12 days of duration. In this duration vitamin C was noted as moderate recoverable group (Fig. 14 a). After 14 days of duration, protective effect of vitamin C had noted On G-6-Pase enzyme recovery. In this group the activity of G-6-Pase had achieved the control level. Vitamin B complex observed as moderate potential agent upon recover the G-6-Pase activity. Among all therapeutic groups withdrawal had shown minimum effect to recover the enzyme. After 16 days vitamin C had proven its ability to recover the enzyme as compared to other therapeutic groups. After 18 days withdrawal group showed highest recovery of G-6-Pase whereas application of vitamin treatment did not show recovery (Fig. 14 b).

Massive inhibition was induced in G-6-Pase activity by higher sublethal dose of TBT after 6 and 12 days of duration (Fig. 14 c & d). After 8 days, activity of G-6-Pase was highly protected by withdrawal group where occurrence of natural washing had shown efficiency to recover the enzyme. Usual G-6-Pase activity was gained moderately in vitamin B complex treated group. Very less effect in restoration of G-6-Pase activity was observed in vitamin C treated group. A quite surprising result was observed after 10 days of duration where the activity of G-6-Pase was highly repaired by all given therapeutic treatments. G-6-Pase activity of vitamin C treated group showed complete recovery of enzyme activity and achieved control level. After that withdrawal and vitamin B complex had equal effect on recovery of G-6-Pase activity. After 12 days of duration vitamin B complex treated group showed maximum recovery. Besides withdrawal and vitamin C treatment could not recover the enzyme (Fig. 14 c). After 14 days, highest enzyme recovery occurred in withdrawal group. As compared to withdrawal group vitamin B complex showed less recovery. On the other hand, vitamin C treatment did not show recovery of G-6-pase enzyme. Similar trend was also observed in 16 and 18 days of exposure durations (Fig. 14 d).

BRAIN

Total ATPase

Estimated activity of brain Total ATPase was highly stimulated by exposure of sublethal dose of TBT after 6 days. This trend was also observed after 12 days exposure in brain tissue (Fig. 15 a & b). The activity of Total ATPase was highly recovered by vitamin treatment as compared to withdrawal group after 8 days of duration. After 10 days the activity of G-6-Pase was somewhat recovered by vitamin C, vitamin B complex and withdrawal group. The similar recovery pattern was also observed in 12, 14 and 16 days of duration (Fig. 15 a). After 18 days vitamin C treatment seen to be highly efficient to recover the G-6-Pase activity where the activity achieved to the control level. Withdrawal showed average recovery whilst, vitamin B complex showed minimum recovery of enzyme (Fig. 15 b).

The activity of Total ATPase was highly stimulated by given higher sublethal dose of TBT after 6 as well 12 days of exposure duration (Fig. 15 c & d). After 8 days of experiment, the activity of Total ATPase was considerably recovered by vitamin B complex. Whereas, vitamin C treated group and withdrawal group revealed less effect on the enzyme. After 10 days, vitamin treated groups showed high level of recovery in the Total ATPase activity in brain tissue. As compared to vitamin treatment, withdrawal showed minimum effect on the restoration of enzyme. The similarity in result was observed after 12 days of duration (Fig. 15 c). After 14 days, vitamin B complex treated group was denoted as highest appreciable group in enzymatic recovery point of view. Whereas, comparable average recovery was noted in vitamin C treated group. Withdrawal did not show potentiality to restore the Total ATPase enzyme activity. Amongst 14, 16 and 18 days of experimental period, the highest recovery in Total ATPase was designated by vitamin B complex treatment after 16 days of duration. Where, the activity of Total ATPase achieved near about control level. However withdrawal group also showed its effect on enzyme further vitamin C treated group showed not that much recovery after 16 days. On the other hand after 18 days, vitamin C proved its efficiency to recover the enzyme while, withdrawal and vitamin B complex showed equal proportion of Total ATPase recovery (Fig. 15 d).

Na^+ K^+ ATPase

A highly stimulatory trend was followed by Na^+ K^+ ATPase due to intoxication of 0.06 mg/kg of dose after 6 and 12 days of duration (Fig. 16 a & b). The activity of Total ATPase was highest recovered by vitamin B treated group. Slight less recovery was observed by withdrawal as well as vitamin C treated group only after 8 days of duration. By graphical representation it was obvious that brain Na^+ K^+ ATPase was highly repaired in 10 days of duration. Vitamin B complex treated group showed highest ability to recover the enzyme where activity of Na^+ K^+ ATPase achieved control level. Furthermore slight less recovery was noted by vitamin C and withdrawal group as compared to vitamin B complex group after 10 days of duration. After 12 days gradually increasing recovery was observed by withdrawal, vitamin C and vitamin B complex treated group (Fig. 16 a). The activity of brain Na^+ K^+ ATPase was recovered in ascending order by vitamin C, vitamin B complex and withdrawal group after 14 days of experimental period. The highest recovery in Na^+ K^+ ATPase was observed in withdrawal group following by vitamin B complex group only in 16 days of duration. Vitamin C treated group showed minimum recovery. After 18 days, equal degree of recovery was noted in both withdrawal and vitamin C treated group. Apart from this, Vitamin B complex had also proven recovery of enzyme but not much greater noted in other therapeutic groups (Fig. 16 b).

The activity of Na^+ K^+ ATPase of brain was stimulated due to exhibited higher sublethal dose of TBT for 6 days and inhibited after 12 days of duration (Fig. 16 c & d). In the therapeutic studies, vitamin C showed its highest protective effect against TBT toxicity on enzyme recovery after 8 days of experiment. Comparable less recovery was observed by withdrawal group followed by vitamin B complex treated group. In both the durations of 10 and 12 days higher recovery was observed in vitamin B complex treated group as compared to other therapeutic groups (Fig. 16 c). After 14 and 16 days of experimental period activity of Na^+ K^+ ATPase of brain gained its usual activity only after treatment with therapeutic agent of vitamin B complex. On the other hand after 18 days vitamin C treatment encouraged maximum recovery of Na^+ K^+ ATPase. In this duration average recovery was observed by withdrawal group (Fig. 16 d).

Ca^{++} ATPase

The activity of Ca^{++} ATPase of brain was more or less inhibited in lower sublethal TBT intoxicated groups during both the durations 6 and 12 days (Fig. 17 a & b). The activity of Ca^{++} ATPase of brain was remarkably repaired by vitamin B complex treatment. Slight less recovery was observed in vitamin C treated group. Whereas, withdrawal group did not show any enzymatic recovery after 8 days of duration. After 10 days also vitamin B complex treated group showed remarkable enzymatic recovery. Next to it, average recovery was noted in withdrawal and vitamin C treated group. After 12 days of duration, all exhibited groups showed its efficiency to recover the Ca^{++} ATPase of brain (Fig. 17 a). After 14 days of duration, potential recovery was triggered by vitamin B complex. Then after withdrawal group and vitamin C treatment showed less recovery of Ca^{++} ATPase enzyme. After 16 days of treatment, vitamin application promoted high recovery as compared to withdrawal group. Quite reversible result was observed after 18 days of duration where the activity of Ca^{++}ATPase was regained by process of natural washing of the toxicant and achieved to the control level. Apart from the withdrawal group, vitamin treatment showed less enzymatic recovery in mentioned duration (Fig. 17 b).

Remarkable inhibitory pattern was noted after 6 and 12 days of experiment in higher toxicated groups (Fig. 17 c & d). The activity of Ca^{++} ATPase was regained its usual activity in withdrawal group only after 8 days of duration. Whereas, moderate level of restoration of enzyme was noted in vitamin B complex treated group. But in this duration vitamin C treatment did not show protective effect over enzymatic activity. After 10 days, vitamin treatment was documented extremely active to restore the Ca^{++} ATPase enzyme as compared to withdrawal group. After 12 days, exhibited all therapeutic groups showed enzymatic recovery in which withdrawal group was denoted as leading group to recover the enzyme (Fig. 17 c). After 14 days of experiment, highest possible recovery was noted in withdrawal group. Vitamin application did not show much efficiency to recover the enzyme in this duration. After 16 days, peak recovery was noted by vitamin B complex group following by withdrawal group. After 18 days of experiment, TBT intoxicated animals kept without any treatment showed maximum recovery in Ca^{++} ATPase in brain in which the

activity of withdrawal tried to reach to the control level. Moderate recovery was noted by vitamin B complex treated group and vitamin C did not show that much effect on enzyme (Fig. 17 d).

Mg^{++} ATPase

The activity of Mg^{++} ATPase was more or less inhibited in lower toxicated group as compared to control group after 6 and 12 days of exposure (Fig. 18 a & b). The activity of Mg^{++} ATPase was recovered in vitamin B complex treated group and negligible recovery was noted in withdrawal as well as vitamin C treated group after 8 days of experiment. With the contradiction of above statement, vitamin C and withdrawal group was denoted as highest potential group to recover the Mg^{++} ATPase in brain after 10 days of duration. After 12 days, highest recovery was observed in vitamin B complex treated group (Fig. 18 a). Restoration of Mg^{++} ATPase was highly noted in vitamin B complex treated group in both the 14 and 16 days of duration. The activity of vitamin B complex group reached to the control level. After 18 days of treatment, enzymatic recovery was subsequently increased in withdrawal, vitamin B complex and vitamin C treated group (Fig. 18 b).

Mg^{++} ATPase was stimulated by higher sublethal dose of TBT after 6 days of duration although inhibited after 12 days of exposure duration (Fig. 18 c & d). The activity of Mg^{++} ATPase totally repaired by vitamin B complex treatment. The activity of this group had achieved complete control level. Moderate recovery was triggered by withdrawal group after 8 days of duration. It was noted in this duration, that vitamin C treatment did not show recovery of enzymatic activity. On the other hand after 10 days, withdrawal group revealed highest recovery as compared to both vitamin treatments. After 12 days, all three therapeutic groups showed immense recovery of enzymatic activity. In this duration vitamin application demonstrated highest Mg^{++} ATPase recovery. Withdrawal group had also proven its efficiency to recover the enzyme in this duration (Fig. 18 c). After 14 days of treatment, TBT preintoxicated animals kept without any treatment and served as withdrawal group showed maximum recovery of Mg^{++} ATPase. Activity of Mg^{++} ATPase was moderately gained by vitamin B complex and vitamin C treatment. On the other hand vitamin C treated group showed maximum Mg^{++} ATPase activity after 16 days of duration.

Except vitamin C group other therapeutic groups failed to recover the enzyme. After 18 days, vitamin B complex showed maximum recovery followed by withdrawal group and slight less recovery was noted in vitamin C treated group (Fig. 18 d).

$Ca^{++}HCO_3^-$ ATPase

The activity of $Ca^{++}HCO_3^-$ ATPase of brain was highly suppressed by lower sublethal dose of TBT after 6 and 12 days of experiment (Fig. 19 a & b). The activity of $Ca^{++}HCO_3^-$ ATPase was highly recovered by vitamin B complex treated group after 8 days of duration. Next to it, withdrawal group also demonstrate its moderate effect to recover the enzyme. In this duration vitamin C showed negligible recovery of $Ca^{++}HCO_3^-$ ATPase. After 10 days, animals kept for natural washing recorded maximum recovery as compared to application of vitamin. As earlier noted in 10 days, similar trend in enzymatic recovery was also observed after 12 days duration (Fig. 19 a). the activity of $Ca^{++}HCO_3^-$ ATPase was progressively repaired by subsequent group of withdrawal, vitamin B complex and vitamin C treated group after 14 days of duration. As compared to 14 days, quite opposite trend in enzymatic recovery was observed after 16 days. After 18 days of experimental period, vitamin C treated group showed amazing recovery to restore the $Ca^{++}HCO_3^-$ ATPase in brain tissue. On the other hand withdrawal and vitamin B complex did not show that much recovery (Fig. 19 b).

By given 0.6 mg/kg dose of TBT, the activity of $Ca^{++}HCO_3^-$ ATPase was stimulated after 6 days of duration and inhibited after 12 days of duration (Fig. 19 c & d). The activity of $Ca^{++}HCO_3^-$ ATPase was repaired by natural washing of the toxicant after 8 days of duration, where the activity of this group achieved near about control level. Next to it, slight moderate enzymatic recovery was noted by vitamin B complex following by vitamin C treatment. After 10 days, vitamin B complex had proven its efficiency to recover the $Ca^{++}HCO_3^-$ ATPase. Moreover except vitamin B complex treated group all other therapeutic groups did not show recovery of enzyme. After 12 days of duration, vitamin C treatment to TBT preintoxicated animals showed maximum recovery of $Ca^{++}HCO_3^-$ ATPase. Moreover, equal level of recovery was found in withdrawal and vitamin B complex treated group (Fig. 19 c). After 14 days, $Ca^{++}HCO_3^-$ ATPase was regained by application of vitamin B complex. Withdrawal

and vitamin C revealed less ability to recover the enzyme. As a result after 16 days, highest protective effect was profound in vitamin C treated group. Next to it, vitamin B complex treated group showed average recovery and in this duration minimum recovery was noted in withdrawal group. After 18 days of experiment, vitamin C, vitamin B complex and withdrawal group showed lowest to highest recovery of $Ca^{++}HCO_3^-$ ATPase activity (Fig. 19 d).

$Mg^{++}HCO_3^-$ ATPase

By exposure of lower sublethal dose of TBT, the activity of $Mg^{++}HCO_3^-$ ATPase was stimulated after 6 days of duration on the controversy inhibited after 12 days of exposure duration (Fig. 20 a & b). After 8 days, withdrawal and vitamin B complex groups indicated its greatest ability to recover $Mg^{++}HCO_3^-$ ATPase in brain tissue. However, vitamin C treatment did not show recovery in enzymatic activity. As it was observed after 8 days the similar result was also observed after 10 days of duration. A quite surprising result was observed after 12 days of duration. In this duration all therapeutic groups showed notable recovery of $Mg^{++}HCO_3^-$ ATPase. Activity of $Mg^{++}HCO_3^-$ ATPase in all exhibited therapeutic groups had achieved near about control level (Fig. 20 a). The activity of $Mg^{++}HCO_3^-$ ATPase was highly repaired by vitamin B complex treated group and Moderate recovery was noted in withdrawal group after 14 days of duration. As compared to withdrawal and vitamin B complex treated group, vitamin C treated group could not induce Total ATPase recovery. After 16 days of experimental period, highly $Mg^{++}HCO_3^-$ ATPase recovery was triggered by treatment with vitamin C agent. In this duration moderate enzymatic recovery was demonstrated by withdrawal group. Vitamin B complex treatment showed minor effect to restore the $Mg^{++}HCO_3^-$ ATPase activity. After 16 days of duration, activity of $Mg^{++}HCO_3^-$ ATPase was successfully recovered by withdrawal group. Vitamin B complex also showed enzymatic recovery in this duration. Among all therapeutic treatment application of vitamin C treatment did not show $Mg^{++}HCO_3^-$ ATPase recovery after 18 days of treatment (Fig. 20 b).

More or less stimulation was noted in $Mg^{++}HCO_3^-$ ATPase in animals treated with higher sublethal dose of TBT after 6 and 12 days of intoxication period (Fig. 20 c & d). In term of enzyme recovery, vitamin C revealed its noticeable capacity after 8 days

of duration. The activity of $Mg^{++}HCO_3^-$ ATPase of vitamin C group reached to the total control level. Except vitamin C treatment, other therapeutic groups viz., withdrawal and vitamin B complex was not much capable to restore the $Mg^{++}HCO_3^-$ ATPase after 8 days of duration. After 10 days of experiment remarkable protective effect on $Mg^{++}HCO_3^-$ ATPase was noted in withdrawal as well as in vitamin C treated group. On the other hand application of vitamin B complex could not able to recover the enzymatic activity. To recovery point of view, vitamin C treated group was recognized as highest efficient group after 12 days of duration. Next to it, withdrawal showed average recovery following by vitamin B complex group only after 12 days of duration (Fig. 20 c). Among all therapeutic groups vitamin B complex proved its ability to restore $Mg^{++}HCO_3^-$ ATPase activity after 14 days of exposure duration. In above mentioned duration vitamin C treatment and withdrawal group was not able to recover the enzymatic activity. Among 14, 16 and 18 days of duration, the activity of $Mg^{++}HCO_3^-$ ATPase was highly recovered only in 16 days by treatment with vitamin C agent. The enzymatic activity of vitamin C achieved to the control level. Subsequent effect was noted in vitamin B complex and by withdrawal group. After 18 days of duration, the activity of $Mg^{++}HCO_3^-$ ATPase was highly regained by withdrawal and vitamin B complex groups. Furthermore vitamin C treated group showed slight less recovery as compared to other therapeutic groups (Fig. 20 d).

G-6-Pase

The activity of G-6-Pase of brain was highly decreased in 0.06 mg/kg dose of TBT treated group after 6 and 12 days of duration (Fig. 21 a & b). Among 8, 10 and 12 days of duration the G-6-Pase activity was tremendously regained after 8 days of duration in withdrawal group. In this case occurrence of natural washing of TBT could helpful to recover the G-6-Pase activity. The activity of G-6-Pase was also recovered by vitamin treatment but not much greater than withdrawal group. From increasing to decreasing manner of enzymatic recovery was observed in consequent groups of vitamin B complex, withdrawal and vitamin C group after 10 days of duration. After 12 days of experiment greater recovery was noted in vitamin B complex group. Then after, slight less recovery was noted in vitamin C treated group. As compared to vitamin application withdrawal group was failed to restore the G-6-Pase activity (Fig. 21 a). After 14 days of duration, both vitamin treated group viz.,

vitamin B complex and vitamin C registered its ability to improve the G-6-Pase activity as compared to withdrawal group. On the other hand after 16 days, vitamin B complex treated group alone noted highest efficient group in term of enzymatic recovery. Whereas, remaining therapeutic groups like withdrawal and vitamin C showed negligible effect on G-6-Pase restoration. As a result after 18 days, serially withdrawal, vitamin B complex and vitamin C group showed increasing to decreasing manner of recovery (Fig. 21 b).

Due to exhibited higher sublethal dose of TBT, the activity of G-6-Pase of brain was more or less decreased as compared to their respective control after 6 and 12 days of exposure duration (Fig. 21 c & d). As a result after 8 days of exposure duration, the activity of G-6-Pase was absolutely recovered by natural washing of the toxicant and the activity of this group had achieved the level of control. Whereas, vitamin C treated group showed moderate protective effect against TBT toxicity. After 8 days, very minute recovery was triggered by vitamin B complex group. After 10 days, vitamin C group leads to the highest recovery of brain G-6-Pase. Total control level was achieved by G-6-Pase of vitamin C group. Vitamin B complex also induced recovery but not as great as observed in vitamin C treated group. Withdrawal group did not show enzymatic recovery after 10 days of duration. After 12 days, between given vitamin treatment vitamin B complex was documented much effective on enzymatic activity as compared to vitamin C treatment. In this duration withdrawal was failed to recover the G-6-Pase of brain (Fig. 21 c). After 14 days of treatment among all therapeutic groups the activity of G-6-Pase was highly attempted by treatment of vitamin C. whereas, vitamin B complex and withdrawal showed less ability to recover the enzyme. After 16 days, ascending trend of enzymatic recovery was followed by withdrawal, vitamin B complex and vitamin C treated group. After 18 days, vitamin C was denoted as leading group in enzymatic recovery as compared to other therapeutic treatments (Fig. 21 d).

MUSCLE

Total ATPase

The activity of Total ATPase of muscle was stimulated by given lower sublethal dose of TBT after 6 days and inhibited after 12 days of duration as compared to their respective control group (Fig. 22 a & b). In therapeutic studies, activity of Total ATPase was recovered by subsequent group of vitamin C, vitamin B complex and withdrawal group from lower to higher range after 8 days. As observed after 8 days of duration, quite opposite trend was noted in Total ATPase recovery after 12 days. Total ATPase gained its usual activity by vitamin application in 10 days of duration as compared to withdrawal group. However, withdrawal also showed moderate recovery of enzyme (Fig. 22 a). TBT preintoxicated animals kept without any treatment showed highest enzymatic recovery in 14 and 18 days of duration. As compared to withdrawal group, treatment of vitamin showed minimal recovery of enzyme in both the durations viz.,14 and 18 days. As a result after 16 days vitamin C treated group documented highest effective group. Next to it, withdrawal showed average enzyme recovery following by vitamin B complex (Fig. 22 b).

By given higher sublethal dose of TBT the activity of Total ATPase of muscle was remarkably stimulated after 6 days and inhibited after 12 days of exposure durations (Fig. 22 c & d). After 8 days of treatment, the activity of Total ATPase was repaired from lower to higher level by withdrawal, vitamin B complex and vitamin C treated group respectively. As a result after 10 days, vitamin B complex had proven its highest efficacy to recover the Total ATPase enzyme where the activity of this group reached to the control level. Moreover remaining both group withdrawal and vitamin C showed less recovery. After 12 days, application of vitamin B complex provides highest restoration of Total ATPase of muscle. Withdrawal group was come under moderate effective group followed by vitamin C group (Fig. 22 c). After 14 days of duration maximum protective effect against TBT was noted in vitamin C group. Withdrawal causes less Total ATPase recovery whereas, in this duration vitamin B complex was failed to recover the enzyme. After 16 days of experiment maximum recovery was documented in withdrawal group. In this duration vitamin treatment

against TBT showed recovery in equal ratio. After 18 days animals kept for withdrawal showed effective recovery as compared to both vitamins (Fig. 22 d).

Na^+ K^+ ATPase

The activity of Na^+ K^+ ATPase of muscle was more or less stimulated in 0.06 mg/kg intoxicated animals after 6 and 12 days of duration (Fig. 23 a & b). When TBT preintoxicated animals kept without any treatment for natural washing of the toxicant showed notable recovery of Na^+ K^+ ATPase activity after 8 days. Whilst, vitamin treatment showed less recovery as compared to withdrawal group. After 10 and 12 days of experiment recovery trend of Na^+ K^+ ATPase followed the similar trend. In this duration, enzymatic recovery was increased by subsequent group of withdrawal, vitamin B complex and vitamin C treated group (Fig. 23 a). As a result after 14 days, withdrawal group was established as highest efficient group to protect the enzymatic activity against TBT toxicity as compared vitamin treatment. After 16 days of experimental period vitamin C treatment was denoted quite efficient to recover the Na^+ K^+ ATPase activity in muscle tissue. Then after withdrawal group showed average enzymatic recovery and vitamin B complex treatment had given minimum recovery of Na^+ K^+ ATPase. After 18 days of treatment, Na^+ K^+ ATPase activity was regained by only natural washing of the TBT molecules. The enzymatic activity of withdrawal group was tried to reach to the control level in respective duration. As compared to withdrawal group vitamin application against TBT toxicity revealed less effect to restore the usual activity of Na^+ K^+ ATPase of muscle (Fig. 23 b).

Due to given higher sublethal dose of TBT the activity of Na^+ K^+ ATPase was increased after 6 days of duration on the other hand decreased after 12 days of experimental period (Fig. 23 c 7 d). After 8 days of duration, remarkable enzymatic recovery was profound by vitamin C treated group. As compare to vitamin C less recovery was observed in vitamin B complex group and totally negligible recovery was documented in withdrawal group. As earlier recorded in 8 days of duration, similar result was followed by 10 days duration. After 12 days, the activity of Na^+ K^+ ATPase was repaired by treatment of vitamin B complex. On the other hand as compared to vitamin B complex vitamin C and withdrawal group did not show much recovery of enzyme (Fig. 23 c). When TBT preintoxicated animals treated with

vitamin did not show recovery of Na^+ K^+ ATPase in muscle after 14 days of experimental duration. On the other hand in this duration withdrawal group was established as highest group to recover the enzymatic activity. Also in 16 and 18 days of durations withdrawal noted as highest effective group to recover the Na^+ K^+ ATPase as compared to application of vitamins (Fig. 23 d).

Ca^{++} ATPase

By given 0.06 mg/kg dose of TBT the activity of Ca^{++} ATPase in muscle was increased after 6 days period and decreased after 12 days of period (Fig. 24 a & b). Among existed all therapeutic groups none of the group was able to recover the Ca^{++} ATPase in muscle after 8 days of duration. Furthermore after 10 days, all three therapeutic treatments were able to restore the enzyme activity, in which animals treated with vitamin B complex showed maximum recovery of enzymatic activity. Withdrawal and vitamin C groups were achieved moderate recovery after 10 days of period. The enzymatic recovery point of view similar result was found in 12 days of duration (Fig. 24 a). A quite interesting trend in Ca^{++} ATPase recovery was found in 14, 16 and 18 days of duration. As compared to withdrawal and vitamin C treatment, vitamin B complex registered maximum ability to regain usual Ca^{++} ATPase activity in all three durations (Fig. 24 b).

Remarkable stimulatory behavior of Ca^{++} ATPase was noted in 0.6 mg/kg intoxicated group after 6 days following by 12 days of exposure duration (Fig. 24 c & d). After 8 days of duration, none of the therapeutic group seems to be effective to recover the Ca^{++} ATPase of muscle. After 10 days of experiment, withdrawal and vitamin B complex group proved their ability to restore normal enzymatic activity as compared to vitamin C treatment. After 12 days, withdrawal denoted as highest efficient group to recover the enzyme. However, moderate recovery was noted in vitamin C treated group (Fig. 24 c). By graphical representation, it was obvious that TBT preintoxicated animals kept for natural washing of toxicant registered maximum enzymatic recovery as compared to vitamin administration in both 14 and 16 days of duration. Among all therapeutic groups vitamin B complex group was highest effective to recover the enzyme after 18 days of duration (Fig. 24 d).

Mg^{++} ATPase

The activity of Mg^{++} ATPase in muscle was increased due to applied lower sublethal dose of TBT after 6 and 12 days of duration (Fig. 25 a & b). In existed all three exposure durations the activity of Mg^{++} ATPase was regained only in withdrawal group as compared to vitamin group. In addition, vitamin treatment showed quite less recovery of Mg^{++} ATPase (Fig. 25 a). After 14 days of duration the activity of Mg^{++} ATPase was potentially recovered by vitamin C application as compared to withdrawal and vitamin B complex group. Similar pattern in enzymatic recovery was profound in 16 as well as 18 days of duration where, withdrawal group showed highest protective effect against TBT toxicity as compared to application of vitamin agents (Fig. 25 b).

In 0.6 mg/kg intoxicated animal group, activity of Mg^{++} ATPase of muscle was stimulated during 6 and 12 days of exposure (Fig. 25 c & d). The activity of Mg^{++} ATPase was repaired by vitamin B complex group in 8 days of duration. On the other hand, withdrawal and vitamin C treatment showed equal ratio of recovery. After 10 days, withdrawal group had proved its ability upon enzyme restoration. As compared to withdrawal group vitamin treatment in this duration was failed to recover the enzyme. After 12 days decreasing to increasing pattern in recovery was found by subsequent group of vitamin B complex, withdrawal and vitamin C group (Fig. 25 c). After 14 days of treatment, the activity of Mg^{++} ATPase was regained its usual activity in withdrawal group. On the other hand vitamin treatment showed less protective nature on enzymatic activity. After 16 days, serially increasing recovery trend was followed by vitamin B complex, withdrawal and vitamin C group. After 18 days withdrawal as well as vitamin C treated group noted potential to recover the enzyme as compared to vitamin B complex treatment (Fig. 25 d).

$Ca^{++}HCO_3^-$ ATPase

The activity of $Ca^{++}HCO_3^-$ ATPase was highly stimulated because of 0.06 mg/kg TBT intoxication after 6 days and inhibited after 12 days of duration (Fig. 26 a & b). After 8 days of duration, the activity of $Ca^{++}HCO_3^-$ ATPase of muscle was highly recovered

in withdrawal group as compared to vitamin treatment. The enzymatic recovery was maximum achieved in 10 days of duration among all exhibited durations. In this duration withdrawal group showed maximum recovery of enzyme where the activity of $Ca^{++}HCO_3^-$ ATPase had achieved the total control level. The similar trend in recovery was also followed by vitamin B complex. Vitamin C could not able to restore the enzyme activity after 10 days of duration. After 12 days both the groups viz., withdrawal and vitamin B complex showed maximum recovery as compared to vitamin C treatment (Fig. 26 a). Very surprising result was observed in enzyme recovery in 14 and 16 days of duration. Therapeutic groups showed similar trend of recovery in both above mentioned duration, where vitamin C agent showed greatest protective effect on $Ca^{++}HCO_3^-$ ATPase activity whilst withdrawal and vitamin B complex were not proved that much effective on enzyme activity. After 18 days of experiment, potential recovery was noted in vitamin B complex group, moderate recovery in vitamin C treated group and less recovery was noted in withdrawal group (Fig. 26 b).

Highly stimulatory behavior was noted in $Ca^{++}HCO_3^-$ ATPase due to exhibited dose of 0.6 mg/kg after 6 days following by 12 days of duration (Fig. 26 c & d). TBT preintoxicated animals received vitamin B complex as therapeutic agent had given best result to recover the enzyme activity. In this duration withdrawal also noted as effective group and less recovery was noted in vitamin C treated group. After 10 days, the enzyme activity was recovered by increasing manner in subsequent group of vitamin C, vitamin B and withdrawal group. As a result after 12 days of experiment all existed therapeutic groups were able to regained the $Ca^{++}HCO_3^-$ ATPase activity. However among all group withdrawal had maximum effect, vitamin B complex showed moderate and vitamin C showed least recovery of $Ca^{++}HCO_3^-$ ATPase (Fig. 26 c). The activity of $Ca^{++}HCO_3^-$ ATPase was regained in vitamin C, vitamin B complex and withdrawal group serially after 14 days of experiment. Whilst after 16 days remarkable enzymatic recovery was done by natural washing of toxicant in withdrawal group. The activity of withdrawal group reached to the activity of control group. On the other hand vitamin treatment against TBT toxicity gives moderate result of enzymatic recovery. After 18 days of duration, both the group withdrawal and vitamin B complex had equal recovery effect whilst vitamin C in this duration showed negligible recovery (Fig. 26 d).

$Mg^{++}HCO_3^-$ ATPase

The activity of $Mg^{++}HCO_3^-$ ATPase was stimulated due to 0.06 mg/kg dose of TBT after 6 days and inhibited after 12 days of period (Fig. 27 a & b). In all 8, 10 and 12 days of duration, the activity of $Mg^{++}HCO_3^-$ ATPase was highest recovered in withdrawal group. After 8 days vitamin application were failed to restore the enzymatic activity whilst withdrawal had proven its maximum ability to restore the enzyme. After 10 and 12 days, withdrawal showed maximum recovery and tried to reach to the control level. In above mentioned durations vitamin treatment also showed moderate recovery (Fig. 27 a). After 14 and 16 days of experiment, withdrawal was noted as helpful to restore the enzymatic activity as compared to vitamin treatments. On the other hand after 18 days, vitamin B complex was denoted highest capable group to recover the enzyme as compared to withdrawal and vitamin C treatment (Fig. 27 b).

Remarkable stimulation was noted in muscle $Mg^{++}HCO_3^-$ ATPase in 0.6 mg/kg intoxicated animals after 6 days of duration following by 12 days (Fig. 27 c & d). To recovery point of view similar result was documented in 8 as well as 10 days of duration, where withdrawal and vitamin C were helpful to recover the $Mg^{++}HCO_3^-$ ATPase. However, vitamin B complex treatment against TBT toxicity did not helpful to recover the enzyme. On the contradiction with above statement, the highest enzymatic recovery was followed in vitamin B complex group after 12 days of duration. Similar trend was also followed by withdrawal group. However, vitamin C treatment was not helpful to recover the enzyme in this duration (Fig. 27 c). After 14 days of experiment, the activity of $Mg^{++}HCO_3^-$ ATPase was regained in increasing manner by subsequent group of vitamin C, vitamin B complex and withdrawal group. After 16 days of treatment near about similar recovery ratio was noted in all three exhibited therapeutic groups. After 18 days, vitamin C treatment showed highest recovery, withdrawal with moderate recovery and vitamin B complex showed lesser recovery to restore the enzymatic activity (Fig. 27 d).

G-6-Pase

After 6 and 12 days of duration, the activity of G-6-Pase was remarkably decreased by given lower sublethal dose of TBT as compared to their respective control (Fig. 28 a & b). After 6 days of duration process of natural washing of TBT molecules leads to improve enzymatic activity. In this duration application of vitamin treatment were failed to restore the G-6-Pase activity. After 10 days, the activity of G-6-Pase was restore in increasing manner by group of withdrawal, vitamin B complex, vitamin C respectively. Whereas, after 12 days the G-6-Pase activity follow the similar recovery pattern as earlier noted in 8 days of duration (Fig. 28 a). In all three durations viz., 14, 16 and 18 days the activity was highly repaired by process of natural washing of the toxicant in withdrawal group. On the other hand vitamin applications were not helpful to recover the enzyme after 14 days of duration. Moreover moderate enzymatic recovery was also noted in vitamin treated group after 16 days following by 18 days (Fig. 28 b).

Due to intoxication with 0.6 mg/kg dose of TBT, the activity of G-6-Pase was inhibited after 6 days and stimulated after 12 days of duration (Fig. 28 c 7 d). After 8 days as compared to vitamin treatment withdrawal showed maximum recovery. Between two vitamin groups vitamin B complex treated group showed highest recovery. Moderate recovery was noted in withdrawal group after 10 days. It was quite surprising that after 12 days of experiment, not a single therapeutic group seem to be effective to restore the enzymatic activity (Fig. 28 c). After 14 days, withdrawal showed maximum recovery whilst vitamin treatment were failed to restore the G-6-Pase activity. Also after s16 days, withdrawal group denoted quite efficient to recover the G-6-Pase activity. In this duration the activity reached to the total control level. However, moderate recovery was also found in vitamin treated groups. On the other hand after 18 days, remarkable recovery was noted in vitamin C treated group. Moreover moderate recovery was noted in both withdrawal and vitamin B complex treated group (Fig. 28 d).

DISCUSSION

Wide range of TBT compounds have been used as ingredient of antifouling paint on marine vessels, where it directly leached in to the aquatic environment. Its direct release causes drastic effect on aquatic biota through it TBT enters into the food chain. However, how TBT enters into cell through plasma membrane and its toxic mode of action inside the cell remain uncertain. Present investigation was carried out to find out TBT toxicity on special emphasis on ATPase enzyme system at tissue level and its maximum removal process by different therapeutic treatments on avian system. Among variety of TBT compounds, Bis (Tributyltin) Oxide was selected as a source of TBT due to its maximum application in antifouling paint and in other areas. Selected experimental animal, (white Leghorn strain, "Broiler"), male chick is a great source of food and easily available at poultry farms which supplies chicken meat for human consumption. To evaluate *in vivo* toxicity of TBT on enzymatic level, some membrane associated ATPases such as Total ATPase, Na^+ K^+ ATPase, Ca^{++} ATPase, Mg^{++} ATPase, $Ca^{++}HCO_3^-$ ATPase, $Mg^{++}HCO_3^-$ ATPase and Glucose-6-phosphatase were assayed from liver, kidney, brain and muscle tissues of developing male broiler chick after intoxication by sub lethal doses for continuous sub-acute exposure durations and their therapeutic treatments by vitamin B complex and vitamin C and also process of natural washing of TBT was carried out.

LIVER

The liver is a largest gland, chemically reactant pool of cells and act as a well-equipped laboratory where metabolism of practically all nutritional substances viz., carbohydrates, proteins, lipids, vitamins and minerals take place and heat is produced. Besides its role in digestion, the liver also acts as a synthesis cum storage organ for lipids and glycogen. Since the liver tissue contains large quantity of lipids and possess the capability of synthesizing more, it would naturally be the most vulnerable to the effect of TBT compounds which has strong affinity towards lipid fraction.

In the present investigation, two sublethal doses of TBT were given for two exposure durations to understand the TBT toxicity on ATPase enzyme system at tissue level.

To understand the duration dependent effect or dose dependent effect of TBT, two-way ANOVA among the first control and different toxicated groups was carried out. The results of two way ANOVA clearly showed that estimated ATPases did not show significant difference between exhibited sublethal doses of TBT, except Na^+ K^+ ATPase. The activity of Na^+ K^+ ATPase was significantly altered by doses of TBT. However, any other estimated enzymes did not influenced by different employed doses of TBT. The mechanism behind why Na^+ K^+ ATPase showed significant difference remains uncertain at this stage. On the other hand, different ATPases of liver tissue showed significant difference in their enzymatic activity in different exposure durations (Table 1). The results of two-way ANOVA indicated that the activity of ATPases in liver tissue did not have dose dependent toxicity. However, the activity of enzyme could change only by different exposure durations.

It was noted from the observed results that, in case of liver tissue the activity of Na^+ K^+ ATPase was stimulated and negligible changes occurred in $Mg^{++}HCO_3^-$ ATPase possibly due to lower dose of TBT for 6 days exposure period. However, remaining all other ATPases showed inhibitory trend in this exposure duration. Furthermore, for more confirmation of obtained results, t test between $control_1$ and $toxicated_1$ group was carried out. This revealed that only $Ca^{++}HCO_3^-$ and $Mg^{++}HCO_3^-$ ATPases showed significant changes (Table 2). Thus, from this result it can be stated that lower dose of TBT could not alter the ATPase activity except CO_3^- dependent ATPases. In therapeutic studies, different therapies were given to lower dose of TBT to the preintoxicated animals revealed that vitamin C showed recovery in Total ATPase after 2, 4 and 6 days of treatment, in Na^+ K^+ ATPase after 4 and 6 days of duration, Ca^{++} ATPase after 4 days, $Ca^{++}HCO_3^-$ ATPase after 2 and 6 days and G-6-Pase after 2 days of therapeutic treatment. However, natural washing of TBT molecules also played a beneficial role on enzyme system. By natural washing of TBT Na^+ K^+ ATPase after 2 days, Ca^{++} ATPase after 2 and 6 days, Mg^{++} ATPase after all three treatment durations and G-6-Pase after 4 and 6 days of treatment duration showed greatest recovery. In this case, recovery by vitamin B complex was very less. The obtained result was further checked out by t test between $control_2$ and individual therapeutic group in individual durational scale. The 't' test also supports the obtained results, however, in some cases statistical significance was not observed. Total ATPase after 6 days, Na^+ K^+ ATPase after 4 and 6 days and G-6-Pase after 4 days of

treatment period did not show statistical significance as noted by t test between $control_2$ and $withdrawal_1$ and $control_2$ and Vitamin C_1 (Table 8).

When intoxication by lower dose of TBT expands upto 12 days revealed stimulation in all enzymes studied except Total ATPase and G-6-Pase. However, the results of t test between $control_1$ and $toxicated_1$ group showed that among all estimated enzymes only Na^+ K^+ ATPase had statistically significant variations (Table 2). The therapeutic studies revealed that the activity of Total ATPase after 2 days of treatment, Na^+ K^+ ATPase after 2 and 6 days of treatment, Ca^{++} ATPase after 6 days of treatment, Mg^{++}, $Mg^{++}HCO_3^-$ ATPases after 2 as well as 4 days of treatment, $Ca^{++}HCO_3^-$ ATPase and G-6-Pase after 4 days of treatment with Vitamin C showed recovery in enzymatic activity. However, after mid treatment duration Total and Na^+ K^+ ATPases, and after long treatment duration Mg^{++}, $Ca^{++}HCO_3^-$, $Mg^{++}HCO_3^-$ ATPases and also G-6-Pase showed recovery by vitamin B complex. Furthermore, in this case efficacy of withdrawal is less evident. Withdrawal had paid attention to recover the Ca^{++} ATPase after first two exposure durations and $Ca^{++}HCO_3^-$ ATPase and G-6-Pase were also recovered by withdrawal after first short term of therapeutic treatment duration. The obtained results of therapeutic study revealed that from recovery point of view, Ca^{++} ATPase after 4 days by withdrawal and after 6 days by vitamin C, Mg^{++} ATPase after 4 days treatment by vitamin C, $Mg^{++}HCO_3^-$ ATPase after 2 days treatment by vitamin C and G-6-Pase after 4 days treatment by vitamin C did not show statistical approval as resulted from t test between $control_2$ and Vitamin C_1 and $control_2$ and $Withdrawal_1$ group. Except above mentioned enzymes, obtained result was well supported by t test where difference between $control_2$ and individual therapy was negligible (Table 9).

In liver tissue, resulting effect of toxicity by exposed higher sublethal TBT dose for 6 days exposure revealed except G-6-Pase all other enzymes were stimulated while, G-6-Pase showed inhibitory effect. However, statistical analyses showed that only few ATPases viz., Total, Mg^{++}, and $Ca^{++}HCO_3^-$ ATPases showed significant variation in their activity as proven by t test between $control_1$ and $toxicated_2$ group (Table 2). Therapeutic studies to 6 days exposed $toxicated_2$ group revealed some mix kind of nature in enzymatic recovery. From graphical representation it was speculated that natural washing of toxicant process to TBT preintoxicted animals could help to recover Total and Na^+ K^+ ATPases after 2 days, Ca^{++} and Mg^{++} ATPases after

different therapeutic durations, $Ca^{++}HCO_3^-$ ATPase throughout all therapeutic treatment durations and G-6-Pase after initial treatment duration. However, less evidence of enzymatic recovery was also noted by vitamin B complex and vitamin C. The activity of Total ATPase after 4 days of treatment, $Na^+ K^+$ ATPase and G-6-Pase after 6 days of treatment, Mg^{++} and $Mg^{++}HCO_3^-$ ATPases after first therapeutic duration showed recovery by Vitamin B complex. While, vitamin C seemed to be effective on Total ATPase enzyme after 6 days of treatment period, $Na^+ K^+$ ATPase and G-6-Pase after 4 and 6 days of treatment period, Ca^{++} ATPase after initial therapeutic duration and $Mg^{++}HCO_3^-$ ATPase after last two durations. Thus, obtained results were further ratified with statistical analysis. t test between $control_2$ and individual therapeutic group showed some contradictory results as mentioned below. The t test between control and $withdrawal_2$ clearly demonstrated that there was significant difference in Total ATPase after 2 days indicating withdrawal was failed to achieve the recovery only in this case. t test between $control_2$ and vitamin B_2 proved that there was a significant difference in measured $Na^+ K^+$ ATPase activity after 6 days of period and t test between $control_2$ and vitamin C_2 showed significant difference in $Na^+ K^+$ ATPase after 6 days of therapeutic treatment, in $Mg^{++}HCO_3^-$ ATPase and in G-6-Pase after 4 days of treatment duration indicative no therapeutic effect on the enzymatic recovery (Table 10).

In the present study, when, higher sublethal dose of TBT was given for continuous 12 days of exposure duration, induced inhibition in estimated enzymes except $Ca^{++}HCO_3^-$ ATPase. It may be possible that given high sublethal dose of TBT for long exposure duration cause drastic effect on normal functioning of ATPase enzyme system lead to inhibition of ATPases activity. On the other hand, it is quite surprising that the activity of $Ca^{++}HCO_3^-$ ATPase did not respond toward entered TBT molecules in this duration only. The obtained toxicity result was later on ratified by calculating t test. It was marked from t test between $control_1$ and $toxicated_2$ group that amongst all enzymes only Total ATPase and Ca^{++} ATPase showed statistically significant variation (Table 2). The obtained findings of therapeutic studies showed that vitamin B complex revealed a great positive influence on enzyme system. It was observed by graphical representation that withdrawal had induced notable recovery in the activity of Total ATPase, $Na^+ K^+$ and Mg^{++} ATPase after 4 days of treatment and in Ca^{++} ATPase after initial treatment duration. However, vitamin B complex had

given contribution to recover the Total ATPase after 6 days, Na^+ K^+ and Mg^{++} ATPases and also G-6-Pase after first and last therapeutic exposure durations, Ca^{++} ATPase after 6 days of treatment and $Mg^{++}HCO_3^-$ ATPase after 4 and 6 days of therapeutic treatment durations. Furthermore, enzymatic recovery by application of Vitamin C was less evident in this case. t test between $control_2$ and individual therapeutic group was carried out for more confirmation of obtained results. It was apparent from t test between $control_2$ and vitamin B_2 that after 6 days Total and Mg^{++} ATPases showed significant variations. In addition t test between $control_2$ and Vitamin C_2 revealed that after 4 and 6 days $Ca^{++}HCO_3^-$ ATPase and G-6-Pase showed statistically significant variations (Table 11).

Results of one way ANOVA among $control_2$ and therapeutic groups (6 days exposed by lower sublethal dose group received therapy) showed that in liver tissue, Mg^{++}, $Ca^{++}HCO_3^-$, $Mg^{++}HCO_3^-$ ATPases and Glucose-6-Pase showed significant variations in their enzymatic activity after 2 days of therapeutic treatment period. After 4 days of therapeutic treatment, except Total ATPase other estimated ATPases and Glucose-6-Phosphatase showed statistical significant variations in the activity and after 6 days except Ca^{++} ATPase all other estimated enzymes showed statistical significant variation in their activity (Table 3). However, the results of one way ANOVA among $control_2$ and therapeutic groups (12 days exposed by lower sublethal dose group received therapy) after 2 days of therapeutic treatment demonstrated that except Total and Na^+ K^+ ATPases all other enzymes showed statistically significant difference in their activity. While after 4 days of treatment, except Na^+ K^+ and $Ca^{++}HCO_3^-$ ATPases remaining ATPases and G-6-Pase and after 6 days of therapeutic treatment Mg^{++} and $Ca^{++}HCO_3^-$ ATPases and G-6-Pase showed significant variations in their activity (Table 3). One way ANOVA among control 2 and therapeutic groups (toxicated 2 group exposed for 12 days received therapies) was calculated. The results of one way ANOVA suggests that after 2 days treatment by different therapeutic agents except Ca^{++}, Mg^{++} and $Ca^{++}HCO_3^-$ ATPases the activity of all other enzymes were found to be statistically significant. On the other hand after 4 days therapeutic treatment demonstrated that except Total ATPase and after 6 days all estimated enzymes showed statistical significant variation in their enzymatic activity (Table 3). Results of one way ANOVA amongst $control_2$ and therapeutic groups indicated that after 2 days of therapeutic treatment except Ca^{++}, Mg^{++} and $Ca^{++}HCO_3^-$ ATPases all other

enzymes showed significant variation. However it is quite surprising that the activity of all estimated enzymes were achieved a significant level after 4 days of therapeutic treatment. At the end of the therapeutic treatment except Total and $Mg^{++}HCO_3^-$ ATPases showed significant variation (Table 3).

The results of two way ANOVA among $control_2$ and therapeutic groups showed that within durations significant variations in enzymatic activity was noted while, among therapeutic groups except G-6-Pase no significant changes were observed (Table 4). The results of two way ANOVA among $control_2$ and therapeutic groups showed that within durations highly significant variations in enzymatic activity was noted while, among therapeutic groups except Ca^{++} ATPase no significant changes were observed (Table 5). Between the exposure durations, there was a significant difference in the estimated enzymatic activity however, exhibited different therapeutic groups were not at all found to be statistical significant conducted from two way ANOVA among $control_2$ and therapeutic groups (Table 6 & 7). In this case, recovery was done by only different therapeutic groups however, exposed different durations did not play any significant role to recover the enzymatic activity.

The possible mechanisms by which toxic agents can impair important biochemical processes and physiological functions in living organisms and the degree of response will depend on the actual doses that reach the receptors or target tissues in the dynamic phase (Ariens *et al*., 1976; Jernelov *et al*., 1978, Kundu and Pathak, 2011). Since the liver tissue contains large quantity of lipids and possess the capability of synthesizing more, it would naturally be most vulnerable to the attack of heavy metals which have strong affinity to lipids (Addison *et al*., 1977). Liver impairment, as judged by increased serum levels of transaminases, was described in two cases of acute oral intoxication with triphenyltin (Lin *et al*., 1998; Wu *et al*., 1990). Hepatitis also was reported in three subjects who ingested between 20 and 50 grams of a preparation containing 45% triphenyltin acetate (Lin and Hsueh, 1993).

A significant increase in serum levels of ornithine carbamyl transferase (used as index of hepatotoxicity) was observed in albino mice gavaged once with 58 mg tributyltin chloride/kg (Ueno *et al*., 1994). Further studies by the same group of investigators showed that the liver toxicity of tributyltin chloride could be prevented

by pretreatment of the mice with the cytochrome P-450 inhibitor SKF-525 (Ueno *et al*., 1997). Comparative studies with tributyltin and dibutyltin in mice and guinea pigs showed the mice to be much more sensitive to the hepatotoxicity of tri- and dibutyltin dichloride than guinea pigs (Ueno *et al.*, 2003a), and this was correlated with differential inhibition of mitochondrial respiration in the two species. Earlier experiments suggested that the difference in susceptibility between mice and guinea pigs might be due to the high affinity of butyltins, particularly dibutyltin, for hepatic mitochondria in mice containing higher levels of sulfhydril groups relative to guinea pigs. In a three species comparison, the susceptibilities followed the order: mice > rats > guinea pigs (Ueno *et al*., 2003b). No hepatotoxicity was seen in dogs exposed through the diet to up to 0.62 mg triphenyltin hydroxide/kg/day for up to 52 weeks (Sachsse *et al*., 1987).

KIDNEY

Kidney is predominant excretory organ in animal. The mesonephros persists and forms the anterior portion of the permanent kidneys in fishes and amphibians, but in reptiles, birds, and mammals, it atrophies and for the most part disappears rapidly as the permanent kidney (metanephros) begins to development. Acute nephropathy was reported in three subjects who ingested between 20 and 50 grams of a preparation containing 45% triphenyltin acetate (Lin and Hsueh, 1993). No further information was located regarding renal effects in humans after oral exposure to organotin compounds. Treatment of rats with up to 5.7 mg dibutyltin dichloride/kg/day for 90 days (Gaunt *et al*., 1968) or mice with up to 30 mg dibutyltin dichloride/kg/day (Seinen *et al*., 1977a) for 4 weeks did not induce any significant gross or microscopic alterations in the kidneys. Also, no significant renal effects were reported in rats or mice dosed with up to 6.7 or 19.8 mg dibutyltin diacetate/kg/day, respectively, for 78 weeks (NCI, 1978a).

In the present investigation, results of two way ANOVA did not show significant difference between different sublethal doses of TBT. While, in case of exposed durations highly significant variations was observed in all enzymes excluding Total and Na^+ K^+ ATPases. Rather than dose dependency, the results indicative of duration dependent toxic effect on ATPase enzyme system in kidney tissue (Table 12). The

results of toxicity of TBT in kidney tissue demonstrated that due to lower sublethal intoxication of TBT for continuous 6 days produce enzymatic dysfunction. The activity of Total, Na^{+} K^{+} and $Mg^{++}HCO_3^{-}$ ATPases showed stimulatory behavior after TBT intoxication. Apart of this, opposite action was noted by Ca^{++}, Mg^{++} and $Ca^{++}HCO_3^{-}$ ATPases and G-6-pse where the activity of these enzymes were more or less inhibited. As a validation of obtained results t test was carried out between $control_1$ and $toxicated_1$ group. The activity of Total, Na^{+} K^{+} and Ca^{++} ATPases and also G-6-Pase showed high degree of significant changes as evident from t test (Table 13). It was apparent from the therapeutic treatments, amongst all therapeutic groups withdrawal was quite effective to recover the enzymatic activity. It was observed by graphical representation that in kidney tissue, withdrawal had given positive response after 2, 4 and 6 days of treatment in Total ATPase, after 2 and 4 days of treatment in Na^{+} K^{+}, Mg^{++} as well as $Mg^{++}HCO_3^{-}$ ATPases. However, treatment by vitamin B complex showed recovery in Ca^{++} and $Ca^{++}HCO_3^{-}$ ATPases after 4 days of treatment, in $Mg^{++}HCO_3^{-}$ ATPases after 6 days of treatment and in G-6-Pase after 4 and 6 days of treatment. Evidence of enzymatic recovery by vitamin C was less recorded in this case. However, Na^{+} K^{+} and Mg^{++} ATPases after 6 days of treatment, Ca^{++} and $Ca^{++}HCO_3^{-}$ ATPases after 2 and 6 days of therapeutic treatment and Mg^{++} ATPase after lower exposure duration of therapeutic treatment showed recovery by vitamin C. The obtained result was verified by performing the t test. t test between $control_2$ and $withdrawal_1$ revealed that after 6 days of duration Total ATPase, after 2 days Mg^{++} ATPase and after 4 days $Mg^{++}HCO_3^{-}$ ATPase indicated significant variations. Furthermore, t test between $control_2$ and vitamin B_1 group showed significant variation in $Ca^{++}HCO_3^{-}$ ATPase after 4 days of treatment and G-6-Pase after 6 days of treatment. When t test between $control_2$ and vitamin C_1 group was performed, obtained results clearly suggests that after 6 days of treatment Na^{+} K^{+} and $Ca^{++}HCO_3^{-}$ ATPases and after 2 days of treatment Ca^{++} ATPase shows significant variation in their activity (Table 19).

As a result of lower sublethal TBT exposure for continuous 12 days, inhibitory effect in all estimated ATPases was observed. The inhibition in enzyme activity supposed to be continuous longer exposure duration of intoxication period. In this case kidney tissue considered to the most susceptible for TBT toxicity. However, t test between

$control_1$ and $toxicated_1$ supports only Mg^{++} ATPase activity where statistical significant difference was noted (Table 13).

The graphical representation of the present study revealed maximum enzymatic recovery by the process of natural washing. After 2 days Total and Na^+ K^+ ATPases, after 4 days Ca^{++} ATPase, after both 2 and 4 days Mg^{++} and $Mg^{++}HCO_3^-$ ATPases, after 4 and 6 days $Mg^{++}HCO_3^-$ ATPase and after 6 days G-6-Pase showed notable recovery in withdrawal group. Evidence of enzymatic recovery in this case by vitamin B complex and vitamin C were very less. However, vitamin B complex showed recovery on Total and Na^+ K^+ ATPases after 4 days treatment, on Ca^{++} ATPase after 2 days of treatment and on $Ca^{++}HCO_3^-$ ATPase after 6 days of therapeutic treatment. Furthermore, vitamin C showed recovery only in few ATPases of kidney. The statistical analysis also supports above results however in some cases significant point of view contradiction was noted. t test between $control_2$ and individual therapeutic group revealed that after 2 days $Mg^{++}HCO_3^-$ ATPase, after 6 days G-6-Pase and Total, Mg^{++} and $Mg^{++}HCO_3^-$ ATPases showed statistically significant variation in their activity (Table 20). Toxicity results of higher sublethal dose exposed for 6 days duration focused on inhibition of Ca^{++} and Mg^{++} ATPases and also G-6-Pase and stimulation of Total, Na^+ K^+ and $Ca^{++}HCO_3^-$ ATPases in kidney tissue. Somehow, negligible toxic effect was observed by $Mg^{++}HCO_3^-$ ATPase amongst all studied enzymes. The verification of results with the help of statistical analysis was done. It was documented by t test between $control_1$ and $toxicated_2$ group that only Ca^{++} ATPase and G-6-Pase showed significant variations indicating significant effect of higher sublethal dose of TBT only on these two enzymes (Table 13). As a resulting effect of therapeutic treatments, the recovery by withdrawal group was noted in case of Ca^{++} ATPase and G-6-Pase after 2 and 4 days, in case of Mg^{++} ATPase after 4 days and in case of $Mg^{++}HCO_3^-$ ATPase after 2 days of therapeutic treatment period. Furthermore, medically available vitamin B complex and vitamin C were also seem to be effective to recover the enzymes. As a result of vitamin B complex treatment, recovery in Na^+ K^+ ATPase after 4 days of treatment, in Ca^{++} and $Mg^{++}HCO_3^-$ ATPases and also in G-6-Pase after 6 days of treatment, in Mg^{++} and $Ca^{++}HCO_3^-$ ATPases after 2 and 6 days of treatment duration were noted. Less evidence of recovery by vitamin C was noted in this case. The obtained result was further ratified by performing t test between $control_2$ and individual therapeutic group. Except Total

and Mg^{++} ATPases and G-6-Pase after 6 days of treatment and also Na^{+} K^{+} ATPase after 2 and 6 days of durations all other enzymes showed greatest recovery as approved by t test between $control_2$ and vitamin C_2 group and $control_2$ and vitamin B_2 group (Table 21). Higher sublethal dose of TBT exposed for 12 days duration causes inhibition in Total, Na^{+} K^{+} and Ca^{++} ATPases and also G-6-Pase. However, total opposite effect was noted on $Mg^{++}HCO_3^{-}$ and $Ca^{++}HCO_3^{-}$ ATPases where the activity of these enzymes were stimulated. Furthermore, the results of t test between $control_1$ and $toxicated_2$ groups did not support any of estimated enzymes as these enzymes did not show significant variations in their activity (Table 13). As a result of therapeutic studies, enzymatic recovery was done by withdrawal group in Total and Mg^{++} ATPases after 2 and 4 days of treatment, in Na^{+} K^{+} and $Mg^{++}HCO_3^{-}$ ATPases after 4 days of treatment, in $Ca^{++}HCO_3^{-}$ ATPase after 2 and 6 days of treatment duration and in G-6-Pase after all therapeutic treatment durations. While, vitamin B complex prompted recovery on Total, Ca^{++} and $Mg^{++}HCO_3^{-}$ ATPases after 6 days of treatment, on Na^{+} K^{+} ATPase after 2 and 6 days duration, on Mg^{++} and $Ca^{++}HCO_3^{-}$ ATPases after 4 days of treatment duration. However, in this case less evidence of recovery by vitamin C was noted. Verification of obtained results was done by statistical analysis. Total ATPase, Na^{+} K^{+} ATPase and Ca^{++} ATPase after 4 days of treatment, $Ca^{++}HCO_3^{-}$ ATPase after 6 days of duration and G-6-Pase throughout all exposure durations showed significant changes in their activity as noted from calculations of t test between $control_2$ and $withdrawal_2$ group and also $control_2$ and vitamin C_2 group (Table 22).

In the therapeutic studies, along with t test one way as well as two way ANOVA was also calculated. The results of one way ANOVA among $control_2$ and therapeutic groups for each respective duration indicated amongst all enzymes majority of enzymes showed significant changes in their activity (Table 14). In addition, two way ANOVA among $control_2$ and different therapeutic groups revealed that significant difference in enzymatic activity was noted in case of within durations, while among different therapeutic groups significant changes in enzyme activity were not noted (Table 15) although in some enzymes occurrence of significant changes were also recorded. (Table 16, 17 & 18). In therapeutic studies, exhibited different therapeutic treatments play a vital role to recover the enzyme while different therapeutic treatment durations could not reason for recovery.

In the present investigation the activity of ATPases was measured in the kidney tissue of control as well as TBT intoxicated and therapeutic animals. Among the ATPases, the activity of total ATPases and two another ion dependent ATPases (Ca^{2+} and Mg^{2+} ATPases) were assayed. The result of the present investigation shows an identical trend of inhibition of various ion dependent forms of ATPases in the kidney of control as well as intoxicated groups. The results indicate comparatively less effect on the functioning of the Na^{+}-K^{+} pump in the kidney as describe earlier by Nechcly and Saunders (1977). The drastic inhibition of these two important enzymes prevents a complete destruction of general cellular metabolism and transport of different metabolites through the membranes. The badly effected lysosomal lesions could lead to nephritis and shorten the life span of the subject. The ion dependant ATPases are known to regulate the entry and exit of different ions across the membrane in order to maintain the physiological requirements of the cell. The inhibition of Total ATPase probably disturbs ion pumps especially Na^{+}-K^{+} pump, resulting in a controllable entry of potassium into the cell along the concentration gradient and the water molecule follow along the osmotic gradient. This process may cause swelling of the cell and finally membrane ruptures (Jernelov, 1978). Different ions such as Na^{+}, Cl^{-}, K^{+}, $Ca^{2+,}$ $Mg^{2+,}$ $HCO3^{-}$, are generally excreted by the kidney. However the kidney does not serves as the exclusive pathway for any of the above mentioned ions. Kidney is regarded as the filter plan of the body. It filters the blood removing the harmful metabolic byproducts like ammonia, urea, various ions like Na^{+}, Cl^{-}, HCO_3^{-}, SO_4^{-} etc. Among the others it also eliminates bacteria, drugs and other metallic ions.

Kidney tissue is a soft and delicate tissue, responsible for filtration. In chick, the kidney is of mesonephros type. The results clearly indicate severe effects on the membrane permeability, especially in the Na^{+} K^{+} pump. In an intermediate-duration study, treatment of rats with doses of 2.5 mg/kg/day of tributyltin chloride in the diet for 30 days did not cause any gross kidney alterations (Bressa *et al*., 1991). The renal effects of trimethyltin chloride were examined in male Wistar rats (Opacka and Sparrow, 1985). Gavage administration of single doses (3, 6, or 10 mg/kg) of the tin compound significantly increased urine production over an observation period of 3 days; this effect was dose-related. Water consumption was significantly increased in the high-dose group beginning the first 24 hours after dosing.

The nephron reaches to the peak of its specialization in mammals where it efficiently removes extra, non-essential ions, water, metabolic wastes and other toxic substances from the circulation (Wedeen and Qian, 1991). This process is entirely dependent on the epithelial cells of the tubules. ATPase, being a membrane bound system is responsible for the trans-membrane movements of ions. Results of the present investigations suggest that TBT ions in the circulation caused increased membrane permeability in the epithelial cells of the kidney tubules (Kundu *et al.,* 1995). It is also possible that the epithelial cells tried to remove the chromium ions by enhancing the movement of ions across the membrane.

The results of the statistical analysis in the present investigations also throw lights for insight of what happens during in vitro conditions. The results of the one way ANOVA in the kidney tissue showed a significant variation between the applied doses of the TBT (Table-3) in almost all the enzymes except Mg^{++} ATPase. However in case of the muscle tissue all enzymes were exhibited highly significant variations (Table-4). TBT compounds have affinity towards lipids and disulphide bonds in addition to sulphydryl bonds (Kundu and Patel, 2005; Ramoliya *et al*., 2007).

Results of the present investigation showed not much disturbance in the activity of (Ca^{++})-ATPase and (Mg^{++})-ATPase in the kidney tissue. It seems that the transport of all-important Ca^{++} and Mg^{++} ions in the epithelial cells of the tubule was blocked in lower dose (Nechay and Saunders, 1977; William and Hook, 1977). On the other hand these enzymes were seemingly not much affected in the muscle tissue showing irregular trends. The (Ca^{++})-ATPase and (Mg^{++})-ATPase is responsible for transepithelial regulation of Ca^{++} and Mg^{++} ions. In mammals, the excretion of excess Mg^{++} occurs generally through kidney. However, results of the present investigations indicate a blockade of Ca^{++} and Mg^{++} transport in the epithelial cells of the kidney (Kass and Orrenius, 1999; Wedeen and Qian, 1991). In the case of muscle, the contraction and relaxation processes depends upon these ions, which are generally transported by Ca^{++} and Mg^{++} dependent ATPase systems (Suzuki, 1980) and specific cation and anion dependent ATPases are responsible for the active transport of anions like Ca^{++}, Mg^{++}, HCO_3^-, SO_4^- etc. (Kass and Orrenius, 1999) the enhanced or reduced activity of the above enzymes definitely indicates a high or low uptake and transport

of these cations or anions as the case may be (Van Os *et al*., 1977; Nys and De Laage, 1984). However, the mobilization of Ca^{++}, Mg^{++} and HCO_3^- is also greatly reduced resulting in the malfunction of the muscular bands. This may ultimately affects the muscular rhythms (Lakshmi *et al*., 1991 a, b).

BRAIN

In brain tissue, regarding TBT toxicity results indicated that TBT evoked disturbances in the activity of estimated enzymes in various degrees over doses and exposure durations. Although, the results of two way ANOVA among $control_2$ and different therapeutic groups indicated that different TBT doses did not produce any significant toxic effects on enzyme activity, at the same time significant exposure duration dependent effect was produced in enzyme activity in brain tissue (Table 23). Thus, obtained result from two-way ANOVA revealed a clear indication of duration dependent toxic effect of TBT on enzyme system. Toxicity to brain tissue was done by two sublethal doses of TBT for two exposure durations. It was concluded from graphical representation that activity of Total Na^+ K^+ and $Mg^{++}HCO_3^-$ ATPases were stimulated after 6 days intoxication with lower sublethal dose. However, total opposite nature was came out in case of Ca^{++}, Mg^{++}, $Ca^{++}HCO_3^-$ ATPases and G-6-Pase where the activity of these enzymes were more or less inhibited. In addition, statistical point of view only few enzymes viz., Total, Na^+K^+, Ca^{++} ATPases and G-6-Pase shows significant level as proved by t test between $control_1$ and $toxicated_1$ group (Table 24).

Rey *et al*., (1984) described the neurotoxicity of methyltins primarily by inhalation included headache, tinnitus, deafness, impaired memory, disorientation, aggressiveness, psychotic and other severe neuropsychiatric behavior, syncope, and loss of consciousness as symptoms of exposure. Fortemps *et al*., (1978) reported that intermittently exposed to vapors of dimethyltin dichloride and trimethyltin chloride for about 3 months abruptly developed a status of mental confusion with generalized epileptic seizures, headaches, pain in various organs, and psychological disturbances such as memory defects, vigilance loss, insomnia, anorexia, and disorientation Yanofsky *et al*., (1991) and Feldman *et al*., (1993) described delirium, spatial disorientation, perseveration, inappropriate affect, and memory loss.

In therapeutic studies, selected therapeutic treatments were given to TBT preintoxicated animals, amongst different therapies vitamin B complex showed dominant nature to regain the enzymatic activity. due to application of vitamin B complex the activity of Total and $Ca^{++}HCO_3^-$ ATPases after 2 days treatment, Na^+ K^+ ATPase throughout all treatment durations, Ca^{++} ATPase after first two treatment durations, Mg^{++} and $Mg^{++}HCO_3^-$ATPases after first and last treatment durations and G-6-Pase after 4 and 6 days of duration showed enzymatic recovery. Moreover, occurrences of recovery by vitamin C were also noted. Recovery in Total ATPase after 4 and 6 days of treatment, in Ca^{++} ATPase after 6 days of treatment and in Mg^{++} ATPase after 4 days of treatment was accomplished by vitamin C agent. Furthermore, recovery by withdrawal was also noted in few cases like $Ca^{++}HCO_3^-$, $Mg^{++}HCO_3^-$ ATPases and G-6-Pase enzymes. For more confirmation of data t test was carried out between $control_2$ and individual therapeutic group. t test between $control_2$ and vitamin B_1 revealed that Total, Na^+ K^+, Mg^{++} ATPases after 6 days of treatment and G-6-Pase after 4 days of treatment did not recovered by vitamin B complex as all these enzymes showed highly significant variations. Conclusions of t test between $control_2$ and vitamin C_1 showed that Total and Mg^{++} ATPases could not recover by Vitamin C agent as they possess significant variations between groups (Table 30).

Toxicity manifestations of TBT by exposed lower sublethal dose for long sub-acute duration revealed that all ATPases showed inhibitory behavior except Total and Na^+ K^+ ATPases showed stimulatory behavior. However, t test between $control_1$ and $toxicated_1$ only supported Total, Ca^{++}, Mg^{++} and $Ca^{++}HCO_3^-$ ATPases as significant difference was observed only in these enzymes (Table 24). If moving towards therapeutic treatments to lower sublethal TBT exposed animals, some surprising result was obtained. Amongst all therapeutic treatments withdrawal was came out by its maximum recovery on enzyme system. It is evident from graphs that activity of Total ATPase after first two treatment durations, Na^+ K^+ ATPase throughout all treatment durations, Ca^{++}, $Mg^{++}HCO_3^-$ ATPases and G-6-Pase after 6 days of duration and $Ca^{++}HCO_3^-$ ATPase after 4 days of duration were repaired by natural washing of TBT molecules. In addition, positive influence of vitamin C as well as vitamin B complex on enzyme system was also noted. In the same way recovery was noted by vitamin C in case of Total, Mg^{++}, $Ca^{++}HCO_3^-$ and $Mg^{++}HCO_3^-$ ATPases. However,

statistical approval of therapeutic studies was done by calculating t test between $control_2$ and individual therapeutic group. Total, Na^+ K^+ and $Ca^{++}HCO_3^-$ ATPases showed significant variations in their enzymatic activity noted from t test between $control_2$ and $withdrawal_1$. In addition, $Mg^{++}HCO_3^-$ ATPase and G-6-Pase also showed statistical significant variation derived from t test between $control_2$ and vitamin B_1 group and $control_2$ and vitamin C_1 group (Table 31). In case of Total, Mg^{++}, $Ca^{++}HCO_3^-$ and $Mg^{++}HCO_3^-$ ATPases the activity was stimulated as a reaction of TBT exposure. On the other hand, Ca^{++} ATPase and G-6-Pase showed inhibition. Among above mentioned result only Ca^{++} and Mg^{++} ATPases and G-6-Pase were significantly influenced by higher sublethal dose of TBT as recorded from t test between $control_1$ and $toxicated_2$ group (Table 24). The therapeutic studies demonstrated that withdrawal as well as vitamin B complex was efficient to repair the lost enzyme activity. Withdrawal caused recovery in case of Ca^{++} ATPase after 2 and 6 days of treatment, Mg^{++} ATPase after 4 days of treatment, $Ca^{++}HCO_3^-$ ATPase and G-6-Pase after first treatment duration and $Mg^{++}HCO_3^-$ ATPase after first two exposure durations. In addition, recovery by vitamin B complex was also noted in case of Total ATPase after 2 and 4 days of treatment, Na^+ K^+ ATPase after 4 and 6 days of treatment, Mg^{++} ATPase after first duration, $Ca^{++}HCO_3^-$ ATPase after 4 days and G-6-Pase after 6 days of duration. In this case, recovery of few enzymes was also recovered by vitamin C application. By calculating t test it was judged that the activity of enzyme showed significant variations in few cases. Total ATPase after 6 days, Na^+ K^+ ATPase after 2 and 6 days showed significant variation indicating of not recovery done by therapeutic treatment (Table 32).

As a result of toxic response higher sublethal dose of TBT for 12 days exposure duration, the activity of Na^+ k^+, Ca^{++}, $Ca^{++}HCO_3^-$ ATPases and G-6-Pase were inhibited. On the other hand, Total and $Mg^{++}HCO_3^-$ ATPases showed stimulatory behavior. Somehow why Mg^{++} ATPase could not response towards toxic stress of TBT is uncertain. However, obtained result was passed through statistical analysis, in which a single enzyme did not show significant variations as approved by t test between $control_1$ and $toxicated_1$ group (Table 24). Therapeutic manifestations revealed that vitamin B complex showed highest dominant enzymatic recovery amongst all therapeutic treatments. By graphical representation it was obvious that Total and Na^+ K^+ ATPases after 2 and 4 days of treatment, Ca^{++} ATPase after 4 days

of treatment, Mg^{++} ATPase after 6 days of treatment, $Ca^{++}HCO_3^-$ ATPase and G-6-Pase after first treatment duration and $Mg^{++}HCO_3^-$ ATPase after 2 and 6 days of treatment duration showed recovery by vitamn B complex agent. Furthermore recovery by vitamin C was also noted in case of Total, Na^+ K^+, Mg^{++}, $Ca^{++}HCO_3^-$ and $Mg^{++}HCO_3^-$ ATPases after 4 days of treatment duration and G-6-Pase after 4 and 6 days of treatment durations. Enzymatic recovery was also recorded by withdrawal group but only in few cases. Results of t test between $control_2$ and vitamin B_2 and between $control_2$ and vitamin C_2 revealed that Na^+ K^+ ATPase after 2 days treatment by vitamin B and G-6-Pase after all treatment durations showed significant variations in their activity (Table 33). Along with t test for detailed confirmation of therapeutic studies, two way ANOVA was employed among $control_2$ and therapeutic groups. All over results of two way ANOVA suggests that enzymatic activity showed significant variation in within exhibited therapeutic treatment durations. Moreover, enzymatic activity did not show significant variation among exhibited therapeutic groups (Tables 25, 26, 27, 28, 29). These are suggestive of disturbances in the metabolic activities in the brain cell. The toxic effects of TBT in the brain tissue are very rarely reported. Brain, popularly known as “Black box” is a highly specialized tissue of the body and enters into the organization of the nervous system which is defined as structural and functional bases that regulates animal’s responses to internal and external environment. The basic functions of the nervous system include reception that is i.e. gaining of information from the environments through the excitation of proper receptor and to control the production of appropriate responses. Another important function is integration, the process in which parts are put together to form a whole action. The behavioral and physiological changes are the earliest important indicators of the chronic effect on exposure to any neurotoxic agents. The neurotoxic effects of heavy metals are widely recognized and well documented. These effects include severe damage to the ganglia and formation of hemorrhagic lesions.

All TBTs are slowly metabolized compounds. Therefore, toxic symptoms usually occur after long-term exposure and bioaccumulation (ATSDR, 2005). Effects from acute exposure to organic TBTs may include facial edema, ocular discharge, swollen eyelids, conjunctival hyperemia, visual and hearing disturbances, decreases in diastolic and systolic blood pressure, weakness and numbness of the extremities, neurobehavioral and psychomotor impairment, GI upset, diarrhea, hepatitis,

chloracne, and asymptomatic hyperthyroxinemia (ATSDR, 2005). Trimethyltin is characterized by neuronal necrosis, particularly in the hippocampus, whereas triethyltin treatment causes primarily intramyelinic edema. The neurotoxicity of trimethyltin has been examined in numerous acute-duration studies and in few smaller number of intermediate-duration studies. Bouldin *et al*., (1981) reported the morphological effects of trimethyltin hydroxide in adult and neonatal Long-Evans rats. Both groups were intoxicated with 1 mg/kg. The adults were dosed once a day for 14 days, and the neonates once every alternate day for 26 days. The major finding in both the groups was neuronal necrosis in the neocortex, pyriform cortex, hippocampal formation, basal ganglia, brain stem, spinal cord, and dorsal root ganglia. The neurons of the hippocampal formation and pyriform cortex were most vulnerable to the effects of trimethyltin. Trimethyltin also has been shown to induce neuronal damage in sensory neurons of the central and peripheral nervous system (Chang and Dyer, 1983).

Compared to the above, less information was available for other organotins. An acute exposure reported that a daily dose of 2.5 mg tributyltin bromide/kg for 6 days induced slight tremors and weakness in Sprague-Dawley rats; doses of 1.5 mg/kg caused no adverse effects (Yallapragada *et al*., 1991). Administration of 37.5 or 75 mg tributyltin oxide/kg/day for 3 days to rats induced significant reductions in serotonin, dopamine, and noradrenaline in whole brain preparations (Elsabbagh *et al*., 2002). In general, the reductions were dose-related. ATPase activities also were significantly reduced. In general, the severity of the effects was dose-related. In a 2-year bioassay with tributyltin oxide, no histopathologic alterations were observed in the brain and spinal cord from Wistar rats administered dietary doses of up to 2.5 mg/kg/day (Wester *et al*., 1990). Rats treated acutely with 20 mg dibutyltin laureate/kg/day for 3 days showed decreased motor activity and learning, but that dose also caused lethality (Alam *et al*., 1993). In 78-weeks dietary studies with dibutyltin chloride, there was no evidence of adverse gross or microscopic alterations in the brains of Fischer-344 rats and B6C3F1 mice dosed with up 6.7 and 19.8 mg/kg/day, respectively (NCI, 1978a). No neurological effects have been observed in chronic-duration studies with triphenyltin hydroxide in rats and mice (NCI, 1978b), and dogs (Sachsse *et al*., 1987).

MUSCLE

Muscle tissue has ability to relax and contract and so bring about movement and mechanical work in various parts of the body. In the present investigation two way ANOVA was employed among $control_1$ and toxicated groups clearly indicated that exhibited different sublethal doses of TBT did not able to produce toxic effect on enzyme system. However, in muscle tissue toxicity is due to different exposure durations indicating time intervals play a significant role in this case (Table 34). Toxicity results in muscle tissue regarding lower sublethal dose of TBT exposed for 6 days revealed that except G-6-Pase all other estimated ATPases were stimulated. However, inhibition was observed in case of only G-6-Pase. When results of t test between $control_1$ and $toxicated_1$ demonstrated only Mg^{++}, $Ca^{++}HCO_3^-$ and $Mg^{++}HCO_3^-$ ATPases showed significant variation (Table 35).

Therapeutic treatments to $toxicated_1$ group revealed that highest recovery was noted by withdrawal group, second most vitamin C had given contribution. Furthermore, recovery by vitamin B complex was quite rare. Withdrawal showed to be effective on Total and Na^+ K^+ ATPases after 2 days of treatment, Mg^{++} and $Mg^{++}HCO_3^-$ ATPases throughout all therapeutic durations and $Ca^{++}HCO_3^-$ ATPase after first two exposure durations. Occurrences of recovery by application of vitamin C were noted in case of Total, Na^+ K^+ ATPases after 4 and 6 days of durations and G-6-Pase after mid treatment duration only. In case of vitamin B complex, the activity of Ca^{++}ATPase after all treatment durations and $Ca^{++}HCO_3^-$ ATPase after last exposure duration showed recovery. On account of this, when t test was carried out between $control_2$ and $withdrawal_1$ group $Ca^{++}HCO_3^-$ ATPase after 2 days, $Mg^{++}HCO_3^-$ ATPase after 4 days and G-6-Pase after 6 days showed significant variation. The activity of Ca^{++} ATPase after first and last treatment durations and $Ca^{++}HCO_3^-$ ATPase after last treatment duration showed statistically significant variations in their activity as proved by t test between $control_2$ and vitamin B_1 group (Table 41).

When lower sublethal dose exposed for long period causes, inhibition in Total, Ca^{++} and $Ca^{++}HCO_3^-$ ATPases and G-6-Pase. Although, stimulation was also recorded in case of Na^+ K^+ ATPase, Mg^{++} and $Mg^{++}HCO_3^-$ ATPases did not change their activity against TBT exposure. Amongst all estimated enzymes only Ca^{++} and $Ca^{++}HCO_3^-$

ATPases were well supported by t test between $control_1$ and $toxicated_1$ group as the significant variations was observed (Table 35). In therapeutic studies it was find out that among all treatments withdrawal was the greatest therapeutic treatment and induced recovery in all most all enzymes. Furthermore, application of vitamin B complex and vitamin C had equal contribution in this case. From the graphical representation it was noted that Total, Na^+ K^+ ATPases and G-6-Pase after 2 and 6 days of treatment, Mg^{++} ATPase after 4 and 6 days of treatment, $Mg^{++}HCO_3^-$ ATPase after 2 and 4 days of treatment and G-6-Pase after two days of treatment showed recovery by withdrawal treatment. Enzymatic recovery was also noted by vitamin B complex agent in case of Ca^{++}, $Ca^{++}HCO_3^-$ and $Mg^{++}HCO_3^-$ ATPases after 6 days of treatment and G-6-Pase after mid treatment duration. Evidence of recovery by vitamin C was noted only in case of Total and Na^+ K^+ ATPases after 4 days treatment, Mg^{++} ATPase after first treatment duration, $Ca^{++}HCO_3^-$ ATPase after first two treatment durations. However t test between $control_2$ and $withdrawal_1$ and $control_2$ and vitamin B_1 indicated that only Mg^{++}, $Ca^{++}HCO_3^-$ and $Mg^{++}HCO_3^-$ ATPases after 6 days treatment showed statistical significant difference in their activity (Table 42). Toxicity manifestations of higher sublethal dose for short term exposure revealed that except Ca^{++} ATPase and G-6-Pase all other enzymes were stimulated. However, t test between $control_2$ and $toxicated_2$ supports only Ca^{++}, Mg^{++} and $Ca^{++}HCO_3^-$ ATPases (Table 35).

In therapeutic studies, recovery by withdrawal was noted in case of Ca^{++} and $Ca^{++}HCO_3^-$ ATPases after 4 and 6 days of treatment durations, Mg^{++} ATPase after 4 days of treatment and G-6-Pase after first treatment duration. Enzymatic recovery was also done by application of vitamin B complex in activity of Total and G-6-Pase after 4 and 6 days of treatment, Na^+ K^+ and $Mg^{++}HCO_3^-$ ATPases after 6 days of treatment period, Ca^{++}, Mg^{++} and $Ca^{++}HCO_3^-$ ATPases after first treatment duration. Medically available vitamin C agent found to be quite effective from recovery point of view in case of Total and $Mg^{++}HCO_3^-$ ATPases after first duration, Na^+ K^+ ATase after 2 and 4 days of durations and Mg^{++} ATPase after 6 days of duration. Furthermore, for validation of obtained data t test between $control_2$ and $withdrawal_2$, $control_2$ and vitamin B_2 and $control_2$ and vitamin C_2 was carried out. Results of t test revealed that Total, Na^+ K^+ and Ca^{++} ATPases and G-6-Pase after 4 days of therapeutic treatment duration showed statistical significant difference in their activity (Table 43).

Toxicity results of higher sublethal dose exposed for long period revealed that Mg^{++}, $Ca^{++}HCO_3^-$ and $Mg^{++}HCO_3^-$ ATPases showed stimulation whereas, Total and Na^+ K^+ ATPases showed inhibition. Somehow, why Ca^{++} ATPase and G-6-Pase showed negligible changes in their activity was not well understood. Statistical analysis like t test between $control_1$ and $toxicated_2$ group revealed that only Ca^{++} and $Mg^{++}HCO_3^-$ ATPases showed significant variations (Table 35). In therapeutic studies, amongst all therapeutic treatments process of natural washing of TBT was noted quite effective on enzyme system to TBT preintoxicated animals. From graphs it was find out that withdrawal showed recovery in Total ATPase after 4 and 6 days of treatment durations, Na^+ K^+ and $Ca^{++}HCO_3^-$ ATPases throughout all treatment durations, Ca^{++} ATPase and G-6-Pase after first two exposure durations and Mg^{++} and $Mg^{++}HCO_3^-$ ATPases after first exposure duration. t test between $control_2$ and $withdrawal_2$ revealed that Total, Mg^{++} and $Ca^{++}HCO_3^-$ ATPases after 6 days of treatment by withdrawal process showed significant variations in activity indicating not at all significant recovery was found by withdrawal in these enzymes (Table 44). In therapeutic investigation, recovery by both vitamin applications was less recorded. Ca^{++} ATPase after 6 days and $Mg^{++}HCO_3^-$ ATPase after 4 days showed recovery by vitamin B complex treatment. However, t test between $control_2$ and vitamin B_2 did not support Ca^{++} ATPase as the activity of this enzyme showed significant changes (Table 44). Enzymatic recovery by vitamin C was noted in case of Total ATPase after 2 days of therapeutic treatment and G-6-Pase after last therapeutic treatment duration. Recovery by vitamin C on Mg^{++} ATPase after 4 days of duration did not approve by t test between $control_2$ and vitamin C_2 as this enzyme showed significant variation (Table 44). Along with t test two way ANOVA was also employed between $control_2$ and exhibited therapeutic groups. Results of two way ANOVA revealed that estimated enzymes showed significant variations only in case of within groups. On the other hand, activity of different enzymes did not show significant variations in their activity only in case of among therapeutic exposure durations (Tables 36, 37, 38, 39 & 40).

Treatment of rats with up to 16 mg dioctyltin dichloride/kg/day for 6 weeks did not induce histopathological alterations in skeletal muscle (Seinen and Willems, 1976). No treatment-related alterations in skeletal muscles were observed in a 104-week study in rats dosed with up to 6.2 mg triphenyltin hydroxide/kg/day or in mice dosed with up to 9.8 mg triphenyltin hydroxide/kg/day (Tennekes *et al*., 1989a, 1989b). Beagle dogs

dosed with up to 0.62 mg triphenyltin hydroxide/kg/day for up to 52 weeks showed no gross or microscopic alterations in skeletal muscle or in the sternum bone (Sachsse *et al*., 1987). Similar findings were reported in a 106-week study with tributyltin oxide in rats dosed with to 2.5 mg/kg/day of the chemical (Wester *et al*., 1990).

The Overall results of the present investigations revealed a predominantly exposure duration dependent inhibitory effect of the TBT in almost all the enzymes studies in the tissues of the chick. It is apparent from the present studies that TBT has some sort of indirect effect on various ATPases or the membrane bound ATPase system. The Total ATPase was found to be the most affected enzyme. It is obvious that the mode of action of TBT on the enzyme is different in case of *in vivo* toxicity, where a relatively low dose is sufficient to bring about a significant change in the enzyme activity. Whereas, in case of *in vitro* toxicity, the effects observed may be in the form of direct effect of TBTO on enzyme protein (Matsuda *et al*., 1993). Thus, a relatively high dose is required to bring about alterations in the enzymic levels (Thaker *et al*., 1999, Ohhira *et al*., 2003). Ion dependent ATPases are known to regulate different ions across the membrane, in order to maintain the physiological requirements of the cell. The disturbance of (Na^+,K^+)-ATPase probably disturbed Na^+,K^+ pump, resulting in an uncontrollable entry of Na^+ into the cell along the concentration gradient and the water molecule follows along the osmotic gradient (Thaker *et al*., 1996). This process may cause swelling of the cell and finally membrane ruptures (Kundu *et al*., 1992). The stimulation or inhibition of this enzyme by chromium ions thus prevents the buildup of high ion concentrations in the extra cellular spaces resulting in a blockade of the movement of internal harmful extra ions towards the external medium via the leakage junctions (Kundu *et al*., 1995). The results of the present investigations showed that stimulation of this enzyme might have enhanced the transport of vital ions as well as nutrients enormously in the tissues (Brown, 1984). Similar to ethyltin compounds, ingested butyltin compounds and their dealkylation products distribute to soft tissues, including brain, kidney, and liver (Mushak *et al*., 1982, Krajnc *et al*., 1984). Since the toxicity of TBT was not found to be direct and thus, less, the efficacy of the therapeutic agents used in this study was also found to be minimal. The natural washing of the toxic substances observed in the withdrawal group was found to be the most effective amongst the therapeutic groups. However, the toxicity of TBT appears

to be more exposure duration dependent and through more complicated physiological processes, more studies are needed in this line to understand the pathway of the toxicity. More work should be done in this line. Though in most of the countries TBT is now banned, unauthorized uses may be possible apart from the long half-life of this lipid soluble for which detailed physiological studies need be conducted.

Results of the Hypotheses Tested

Keeping in mind the aforementioned discussions, the following results were observed for the hypotheses which were tested during the present study:

Sr. No.	Hypothesis Tested	Result
1	Dose dependent TBT toxicity may not be causing significant alterations on few key enzymes in selected organs of the chick.	**True**
2	Exposure duration dependent TBT toxicity may not be causing significant alterations on few key enzymes in selected organ systems of the chick.	**False**
3	Dose dependent TBT toxicity may not influence the membrane integrity and transmembrane transport of ions and metabolites in the selected tissues.	**True**
4	Exposure duration dependent TBT toxicity may not influence the membrane integrity and transmembrane transport of ions and metabolites in the selected tissues.	**False**
5	The therapeutic treatments given will not be effective against the toxicity of TBT.	**Partially True**

SUMMARY

1. The aims of the present study were to evaluate the **(a)** TBT toxicity at the tissue level after its intoxication at sub lethal doses for different exposure durations **(b)** their possible recovery after natural washing of the toxicant upon withdrawal and (**c**) protective effects of few therapeutic treatments in chick. In a nutshell, in the present study, an attempt was made to understand the toxicity of TBT on membrane transport system (ATPase system) in particular and osmoregulatory mechanism in general in liver, kidney, brain and muscle tissues in male white Leghorn chick.

2. In the present investigation, TBT (Tributyltin, trade and other names also include Alumacoat, Bioclean, Flotin, Fungitrol) was used as a source of TBT. (Bis-tributyltin) oxide (TBTO), ($C_{24}H_{54}OSn_2$), structural formula $(CH_3CH_2CH_2CH_2)_3Sn\text{-}O\text{-}Sn\ (CH_3CH_2CH_2CH_2)_3$ with 96% purity was procured from Sigma Aldrich (India) Pvt. Ltd. Male White leghorn strain ("Broiler"), chick *Gallus gallus* was selected as animal model. Experiments were commenced with one-day-old animals. The animals with the total body weight of 30-40g were considered for experimental use and maintained in the departmental animal house facilities in iron cage (36"×24"×24") in highly hygienic condition with due permission from the Animal Experiment Control and Monitoring Authority, Govt. of India.

3. Animals were exposed to different sublethal doses of TBT selected as $1/10^{th}$ of LD_{50} value, i.e. 0.6 mg kg^{-1} body weight day^{-1} and $1/100^{th}$ of LD_{50} value, i.e., 0.06 mg kg^{-1} body weight day^{-1} for 2 different exposure durations (6 and 12 days). Due to lipophilic characteristics of TBT, selected doses were prepared by dissolving it in corn oil.

4. Vitamin B complex and Vitamin C (ascorbic acid) were selected as therapeutic agents. Medically available Vitamin C (ascorbic acid) is manufactured by Hindustan pharmaceuticals and Vitamin B complex (commercial name Neurobion Forte, a combination of Thamine

hydrochloride-100 mg, Riboflavin sodium phosphate-5 mg, Pyridoxine hydrochloride-100mg, Cyanocobalamin-1000 mcg, Nicotinamide 100 mg, D-panthenol 50 mg) is manufactured by Merck limited were utilized by making their proper dose. 50 mg kg^{-1} vitamin C and 20 mg kg^{-1} Vitamin B complex were prepared by diluting ampule contain in double distilled water and store in a cool place.

5. Experiments were conducted according to the following design**:** (a) **$Control_1$ group**: Animals of this group were given only corn oil. From this group half of the animals were sacrificed on 7^{th} day and remaining were sacrificed on 13^{th} day of experiment. (b) **$Toxicated_1$ group**: Animals were received 0.06 $mg^{-1}kg^{-1}bw\ d^{-1}$ dose of TBT intramuscularly from the starting of the experiment up to 6 and up to 12 days and sequentially sacrificed on 7^{th} and 13^{th} day of experiment. (c) **$Toxicated_2$ group**: animals were received 0.6 $mg^{-1}kg^{-1}bw\ d^{-1}$ dose of TBT intramuscularly from the starting of the experiment. Half of the animals were given dose up to 6 days and half of the animals were given dose up to 12 days and sacrificed on 7^{th} and 13^{th} days respectively. (d) **$Control_2$ group**: Animals of this group were given only corn oil and sacrificed on 9^{th}, 11^{th}, 13^{th}, 15^{th}, 17^{th} and 19^{th} day respectively. (e) **$Withdrawal_1$ group**: Animals pre-intoxicated by 0.06 mg $kg^{-1}bw\ d^{-1}$ dose of TBT for 6 and 12 days and then kept without any further dose (natural washing) for next 2, 4 and 6 days and sacrificed consecutively on 9^{th}, 11^{th}, 13^{th}, 15^{th}, 17^{th} and 19^{th} day of experiment. (f) **$Withdrawal_2$ group**: Animals pre-intoxicated by 0.6 mg $kg^{-1}bw\ d^{-1}$ dose of TBT for 6 and 12 days and then were kept without dosing for next 2, 4 and 6 days and sacrificed consecutively on 9^{th}, 11^{th}, 13^{th}, 15^{th}, 17^{th} and 19^{th} day of experiment. (g) **Vitamin B_1 complex group**: Animals pre-intoxicated by 0.06 mg $kg^{-1}bw\ d^{-1}$ dose of TBT for 6 and 12 days, were given vitamin B complex for the next 2, 4 and 6 days, and sacrificed serially on 9^{th}, 11^{th}, 13^{th}, 15^{th}, 17^{th}, 19^{th} day of experiment. (h) **Vitamin B_2 complex group**: Animals pre-intoxicated by 0.6 mg $kg^{-1}bw\ d^{-1}$ dose of TBT for 6 and 12 days, were given vitamin B complex for the next 2, 4 and 6 days, and sacrificed serially on 9^{th}, 11^{th}, 13^{th}, 15^{th}, 17^{th}, 19^{th} day of experiment. (i) **Vitamin C_1 group**: Animals pre-intoxicated by 0.06 mg $kg^{-1}bw\ d^{-1}$ dose of TBT for 6 days and 12 days were given vitamin C for further 2, 4 and 6 days

and sacrificed on 9^{th}, 11^{th}, 13^{th}, 15^{th}, 17^{th}, 19^{th} day of experiment respectively. (j) **Vitamin C_2 group**: Animals pre-intoxicated by 0.6 mg kg^{-1}bw d^{-1} dose of TBT for 6 days and 12 days and then were given vitamin C for further 2, 4 and 6 days and sacrificed on 9^{th}, 11^{th}, 13^{th}, 15^{th}, 17^{th}, 19^{th} day of experiment respectively.

6. Activities of Total, Na^+ K^+, Ca^{++}, Mg^{++}, Ca^{++} HCO_3^- and Mg^{++} HCO_3^-ATPases and Glucose-6-phosphatase were estimated using KH_2PO_4 as standard. To calculate the specific activities of the enzymes studied, protein content of each sample was estimated. The collected data were subjected to appropriate statistical analysis for their validity, reliability and cumulative acceptability. Specialized analyses like two-factor ANOVA, single factor ANOVA and Student's t-test were employed wherever necessary for their cumulative acceptability.

7. Results of the present study are indicative of predominantly exposure duration dependent effects of TBT in the **Liver** tissue. Most of the ATPases did not show significant difference between exhibited sublethal doses of TBT, except Na^+ K^+ ATPase. The activity of Na^+ K^+ ATPase was significantly altered by doses of TBT. However, any other estimated enzymes were not influenced by different employed doses of TBT. On the other hand, different ATPases of liver tissue showed significant difference in their enzymatic activity in different exposure durations. In therapeutic studies, different therapies were given to the preintoxicated animals which revealed that vitamin C possibly initiated a recovery process in the enzymic activity in the lower doses of TBT. However, natural washing seemed to have played a beneficial role on the enzyme system. It was evident that in the higher doses, the recovery by vitamin B complex and vitamin C was very less.

8. Results of the present study are indicative of a predominantly exposure duration dependent toxic effect on ATPase enzyme system in **Kidney** tissue. The activity of Total, Na^+ K^+ and $Mg^{++}HCO_3^-$ ATPases showed a general stimulatory behavior after low dose of TBT intoxication. In the rest of the enzymes varying degrees of inhibitory trend was observed. The exact mode of

action of the TBT was not fully understood at this point, but it was evident that the enzymes were not directly affected by TBT. The resulting variations in the enzymatic activity in different doses and exposure durations were possibly due to the indirect effects either through physical deformities caused in the membrane structure or by through some other complex physiological process. It was apparent from the study that amongst therapeutic treatments, natural washing by withdrawal of the toxic stress was comparatively effective to recover the enzymatic activity.

9. In **Brain** tissue, different TBT doses did not produce any significant toxic effects on the enzyme activity, but significant exposure duration dependent effect was clearly evident. The activity of most of the enzymes were sometimes inhibited in the lower dose and stimulated in the higher dose or the other way round. This is possibly an indication of duration dependent indirect toxic effect of TBT on enzyme systems studied. Though the exact mode of action of the TBT was not fully understood at this point, but it was evident that the enzymes were not directly affected by TBT possibly due to blood-brain-barrier. The resulting variations in the enzymatic activity in different doses and exposure durations were probably due to the indirect effects either through physical deformities caused in the membrane structure or by through some other complex physiological process. The therapeutic studies demonstrated that withdrawal as well as vitamin B complex was efficient to repair the lost enzyme activity. In few cases, recovery of the enzyme activity was also evident by vitamin C.

10. In case of **Muscle** tissue, the results of control and toxicated groups showed negligible alterations in all the enzyme activities in different doses of TBT. However, between the toxicated groups, results showed variations in the enzyme activities as the duration of the intoxication increases. It is possible that the transmembrane transport system, represented by all ion dependent membrane bound ATPases affected indirectly by the toxic element. In this case also, dose did not have much effect on the enzymes. However, the exposure durations were in fact severe for the activity of these enzymes in both the doses. The results observed are suggestive of disturbances in the

metabolic activities in the muscle cell by indirect way. Therapeutic treatments to toxicated group revealed that most recovery was noted by withdrawal group followed by vitamin C.

11. The overall results of the present studies suggested a predominantly indirect way of toxicity of TBT in the enzyme systems. This is apparent by observing predominantly exposure duration dependent effects rather than employed doses. TBT is lipid soluble and thus, definitely taken different routes to produce the toxicity in the enzyme systems. It may be possible that TBT affected the metabolite transport channels of the hepatocytes cells in liver by inhibiting this particular enzyme through an indirect way. In case of kidney tissue, variations in the activity of almost all enzymes were observed in case of exposure durations only. It is possible that the toxicant entered the kidney tubule cells through blood and acted on the membrane bound enzyme in an indirect way. In lower dose, the defense mechanism of the cell possibly tried to adjust the imposed stress by stimulating the enzyme activity. This mechanism of adjustment was futile against the onslaught of the toxicant, especially in the increasing exposure durations, leading inhibition of the enzymes. In case of brain and muscle, most of the enzymes studied showed fewer variations in the enzymes activity. In this case also, it was observed that the dose of the toxicant did not influence the enzymes activity significantly, whereas, some impact was seen in exposure durations. It is not known that whether TBT could cross the blood-brain barrier. However, the results indicate a possible indirect effect on the enzymes. In case of muscle tissue, variations were observed in case of exposure durations only.

12. It is obvious from the above discussions that the toxic effects of TBT were enhanced by the time factor. The longer it stays in the system, more damages it caused to the tissue. It is also possible that the chronic exposure or continuous exposure causes more heavy damage than a discontinuous high dose exposure. The present study initiated the debate on the possible physiological effects of TBT on the membrane permeability and transmembrane transport mechanisms of the cell types in respect to their

organic specializations. The study was fairly successful in answering the initial questions which were aimed to be answered. However, the exact nature of toxicity of the TBT will be known after further and detailed investigations in this line.

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TABLES AND FIGURES

Table 1- Results of Two Way ANOVA of ATPases estimated in the **liver** tissue of developing chick after TBT intoxication by two sub lethal doses (0.06 & 0.6 mg kg^{-1} bw day^{-1}) for two exposure durations (6 and 12 days). F critical value for between doses is **5.318** and F critical value for within durations is **3.438**. * mark denotes statistical significance at p < 5% level.

	Total ATPase	$Na^+ K^+$ ATPase	Ca^{++} ATPase	Mg^{++} ATPase	$Ca^{++}HCO3^-$ ATPase	$Mg^{++}HCO3^-$ ATPase	G-6-Pase
Between Doses	0.988	5.951*	3.510	0.034	0.942	0.153	0.548
Within Durations	19.616*	20.984*	23.342*	70.299*	52.238*	41.201*	44.670*

Table 2- Results of student's 't' test between $control_1$ and individual toxicated group of ATPase enzymes estimated in the **liver** tissue of developing chick after two exposure durations (6 and 12 days). The given critical value of 't' is **4.303**. * mark denotes statistical significance at p < 5% level.

Exposure Durations	Groups	Enzymes						
		Total ATPase	$Na^+ K^+$ ATPase	Ca^{++} ATPase	Mg^{++} ATPase	$Ca^{++}HCO_3^-$ ATPase	$Mg^{++}HCO_3^-$ ATPase	G-6-Pase
6 days	C_1 Vs T_1	0.974	4.019	2.717	0.762	5.117*	18.946*	8.481
	C_1 Vs T_2	5.090*	0.846	1.557	7.652*	4.648*	0.220	5.789
12 days	C_1 Vs T_1	0.279	7.039*	1.701	2.751	2.754	0.786	1.627
	C_1 Vs T_2	5.588*	3.959	7.396*	1.665	2.154	1.587	2.699

Table 3- Results of One way ANOVA among $control_2$ and therapeutic groups of ATPase enzymes estimated in the **liver** of developing chick after therapeutic treatments to 6 and 12 days TBT preintoxicted animals by subsequent 2, 4 and 6 days of duration. F critical value is **6.591**. An * mark denotes statistical significance at p < 5% level.

Dose	Duration in Days	Enzymes						
		Total ATPase	$Na^+ K^+$ ATPase	Ca^{++} ATPase	Mg^{++} ATPase	$Ca^{++}HCO_3^-$ ATPase	$Mg^{++}HCO_3^-$ ATPase	G-6-Pase
	6+2	1.934	4.822	5.441	11.253*	90.374*	20.145*	18.386*
0.06	**6+4**	4.163	28.212*	9.477*	13.985*	18.535*	37.097*	53.307*
	6+6	27.673*	32.544*	5.315	8.048*	27.758*	29.104*	25.787*
	12+2	6.275	4.448	27.924*	15.371*	97.384*	12.156*	23.669*
0.06	**12+4**	10.121*	4.193	10.688*	30.960*	1.014	54.637*	15.347*
	12+6	2.491	4.054	5.964	7.741*	7.767*	2.610	99.242*
	6+2	13.691*	14.165*	1.381	0.610	1.579	7.948*	26.948*
0.6	**6+4**	5.662	15.173*	10.235*	67.607*	10.053*	98.532*	79.636*
	6+6	25.303*	29.678*	8.537*	8.901*	8.732*	10.534*	24.781*
	12+2	13.424*	15.299*	1.439	1.577	4.922	51.105*	12.077*
0.6	**12+4**	16.447*	7.092*	17.456*	27.338*	13.727*	33.910*	68.348*
	12+6	3.744	9.373*	30.044*	22.848*	34.151*	2.417	10.809*

Table 4 - Results of two way ANOVA among control$_2$ and therapeutic groups of ATPases enzymes estimated in the **liver** of developing chick after therapeutic treatment to 6 days 0.06 mgkg^{-1} bw day^{-1} TBT preintoxicated animals by subsequent 2, 4 and 6 days of duration. The given F critical value for among groups = **4.844** and F critical value for among durations = **2.818**. * mark denotes statistical significance at p < 5% level.

	Total ATPase	**$Na^+ K^+$ ATPase**	**Ca^{++} ATPase**	**Mg^{++} ATPase**	**$Ca^{++}HCO_3^-$ ATPase**	**$Mg^{++}HCO_3^-$ ATPase**	**G-6-Pase**
Within Groups	0.185	0.043	0.636	1.139	0.785	0.114	5.494*
Among Durations	23.74*	24.16*	23.22*	26.64*	25.37*	21.4*	50.06*

Table 5 - Results of two way ANOVA among control$_2$ and therapeutic groups of ATPases enzymes estimated in the **liver** of developing chick after therapeutic treatment to 12 days 0.06 mgkg^{-1} bw day^{-1} TBT preintoxicated animals by subsequent 2, 4 and 6 days of duration. The given F critical value for among groups is **4.844** and F critical value for among durations is **2.818**. * mark denotes statistical significance at p < 5% level.

	Total ATPase	**$Na^+ K^+$ ATPase**	**Ca^{++} ATPase**	**Mg^{++} ATPase**	**$Ca^{++}HCO_3^-$ ATPase**	**$Mg^{++}HCO_3^-$ ATPase**	**G-6-Pase**
Within Groups	0.011	0.039	5.413*	1.079	0.014	1.026	0.625
Among Durations	5.327*	3.684*	44.590*	13.731*	77.979*	48.784*	68.560*

Table 6 - Results of two way ANOVA among control$_2$ and therapeutic groups of ATPases enzymes estimated in the **liver** of developing chick after therapeutic treatment to 6 days 0.6 mgkg^{-1} bw day^{-1} TBT preintoxicated animals by subsequent 2, 4 and 6 days of duration. The given F critical value for among groups is **4.844** and F critical value for among durations is **2.818**. * mark denotes statistical significance at p < 5% level.

	Total ATPase	**$Na^+ K^+$ ATPase**	**Ca^{++} ATPase**	**Mg^{++} ATPase**	**$Ca^{++}HCO_3^-$ ATPase**	**$Mg^{++}HCO_3^-$ ATPase**	**G-6-Pase**
Within Groups	0.710	1.663	0.583	0.849	0.014	0.142	2.797
Among Durations	18.607*	22.201*	11.610*	38.190*	9.896*	35.513*	47.691*

Table 7 - Results of two way ANOVA among control$_2$ and therapeutic groups of ATPases enzymes estimated in the **liver** of developing chick after therapeutic treatment to 12 days 0.6 mgkg^{-1} bw day^{-1} TBT preintoxicated animals by subsequent 2, 4 and 6 days of duration. The given F critical value for among groups is **4.844** and F critical value for among durations is **2.818**. * mark denotes statistical significance at p < 5% level.

	Total ATPase	**$Na^+ K^+$ ATPase**	**Ca^{++} ATPase**	**Mg^{++} ATPase**	**$Ca^{++}HCO_3^-$ ATPase**	**$Mg^{++}HCO_3^-$ ATPase**	**G-6-Pase**
Within Groups	0.002	0.056	2.436	0.756	0.723	0.251	0.116
Among Durations	9.613*	10.239*	12.690*	5.239*	15.652*	28.979*	16.252*

Table 8 - Results of student's 't' test between $control_2$ and individual therapeutic group of ATPase enzymes estimated in the **liver** tissue of developing chick after therapeutic treatment to 6 days 0.06 $mgkg^{-1}$ bw day^{-1} TBT preintoxicated animals by subsequent 2, 4 and 6 days of duration. The given critical value of 't' is **4.303**. * mark denotes statistical significance at p < 5% level.

Enzymes	2 days			4 days			6 days		
	C_2 Vs W_1	C_2 Vs VB_1	C_2 Vs VC_1	C_2 Vs W_1	C_2 Vs VB_1	C_2 Vs VC_1	C_2 Vs W_1	C_2 Vs VB_1	C_2 Vs VC_1
Total ATPase	4.829*	6.897*	4.861*	2.963	2.425	1.838	5.069*	6.114*	4.875*
$Na^+ K^+$ ATPase	2.652	2.632	2.441	10.082*	4.866*	5.554*	5.665*	6.692*	5.312*
Ca^{++} ATPase	1.740	4.087	0.989	0.425	4.136	0.097	1.076	2.200	2.760
Mg^{++} ATPase	0.144	7.305*	3.704	0.117	0.291	6.470*	1.625	2.034	4.235
$Ca^{++}HCO_3^-$ ATPase	12.307*	10.489*	2.998	5.023*	3.656	4.441*	9.850*	3.824	3.330
$Mg^{++}HCO_3^-$ ATPase	6.682*	2.994	13.633*	4.113	3.906	22.993*	3.972	5.756*	6.432*
G-6-Pase	2.613	3.000	3.009	9.619*	23.325*	10.522*	2.281	2.832	2.820

Table 9 - Results of student's 't' test between $control_2$ and individual therapeutic group of ATPase enzymes estimated in the **liver** tissue of developing chick after therapeutic treatment to 12 days 0.06 $mgkg^{-1}$ bw day^{-1} TBT preintoxicated animals by subsequent 2, 4 and 6 days of duration. The given critical value of 't' is **4.303**. A * mark denotes statistical significance at p < 5% level.

Enzymes	2 days			4 days			6 days		
	C_2 Vs W_1	C_2 Vs VB_1	C_2 Vs VC_1	C_2 Vs W_1	C_2 Vs VB_1	C_2 Vs VC_1	C_2 Vs W_1	C_2 Vs VB_1	C_2 Vs VC_1
Total ATPase	3.010	2.811	0.921	2.369	1.909	4.174	0.839	0.766	5.197
$Na^+ K^+$ ATPase	2.853	2.528	1.328	1.295	1.127	2.653	1.883	1.329	1.050
Ca^{++} ATPase	2.040	5.550*	6.373*	3.568	5.094*	3.011	76.548*	40.915*	9.353*
Mg^{++} ATPase	0.355	229.541*	0.720	7.218*	7.099*	6.774*	3.694	1.961	2.905
$Ca^{++}HCO_3^-$ ATPase	1.237	4.970*	10.448*	0.744	2.111	0.328	18.280*	12.702*	18.185*
$Mg^{++}HCO_3^-$ ATPase	16.617*	15.202*	11.591*	3.492	7.448*	1.357	0.333	0.062	1.701
G-6-Pase	3.412	13.312*	4.727*	50.377*	49.489*	62.712*	10.553*	10.091*	12.370*

Table 10 - Results of student's 't' test between control$_2$ and individual therapeutic group of ATPase enzymes estimated in the **liver** tissue of developing chick after therapeutic treatment to 6 days 0.6 mgkg^{-1} bw day^{-1} TBT preintoxicated animals by subsequent 2, 4 and 6 days of duration. The given critical value of 't' is **4.303**. A * mark denotes statistical significance at p < 5% level.

Enzymes	2 days			4 days			6 days		
	C_2 Vs W_2	C_2 Vs VB_2	C_2 Vs VC_2	C_2 Vs W_2	C_2 Vs VB_2	C_2 Vs VC_2	C_2 Vs W_2	C_2 Vs VB_2	C_2 Vs VC_2
Total ATPase	4.490*	6.541*	4.395*	3.373	0.443	1.509	5.947	4.325*	3.755
$Na^+ K^+$ ATPase	0.920	3.790	4.176	4.454*	2.976	1.790	6.476*	4.597*	4.550*
Ca^{++} ATPase	1.582	1.304	0.345	0.040	3.858	2.400	1.308	3.593	4.359*
Mg^{++} ATPase	4.668*	0.294	0.477	0.340	8.015*	6.948*	0.711	9.817*	8.111*
$Ca^{++}HCO_3^-$ ATPase	0.178	0.928	1.548	1.057	3.526	3.435	3.340	4.109	1.357
$Mg^{++}HCO_3^-$ ATPase	5.337*	2.974	2.252	5.510*	18.014*	4.480*	12.880*	4.598*	4.160
G-6-Pase	0.614	4.720*	5.250*	24.377*	58.952*	8.626*	3.928	1.917	1.976

Table 11 - Results of student's 't' test between control$_2$ and individual therapeutic group of ATPase enzymes estimated in the **liver** tissue of developing chick after therapeutic treatment to 12 days 0.6 mgkg^{-1} bw day^{-1} TBT preintoxicated animals by subsequent 2, 4 and 6 days of duration. The given critical value of 't' is **4.303**. A * mark denotes statistical significance at p < 5% level.

Enzymes	2 days			4 days			6 days		
	C_2 Vs W_2	C_2 Vs VB_2	C_2 Vs VC_2	C_2 Vs W_2	C_2 Vs VB_2	C_2 Vs VC_2	C_2 Vs W_2	C_2 Vs VB_2	C_2 Vs VC_2
Total ATPase	5.371*	3.279	3.125	3.378	3.510	5.177*	5.571*	5.077*	2.338
$Na^+ K^+$ ATPase	4.647*	3.140	3.316	1.295	2.237	3.151	3.283	1.705	3.751
Ca^{++} ATPase	0.711	2.004	1.273	4.429*	11.677*	1.334	47.900*	40.948*	5.717*
Mg^{++} ATPase	1.148	0.299	1.650	1.835	8.572*	2.173	5.034*	2.146	5.529*
$Ca^{++}HCO_3^-$ ATPase	5.070*	3.666	0.396	5.961	17.222*	9.296*	19.203*	20.932*	5.277*
$Mg^{++}HCO_3^-$ ATPase	14.018*	11.133*	5.731*	10.090*	3.678	22.739*	2.554	1.128	1.051
G-6-Pase	5.961*	0.606	8.174*	37.741*	38.808*	55.700*	10.862*	1.894	11.521*

Table 12 - Results of Two Way ANOVA of ATPases estimated in the **kidney** tissue of developing chick after TBT intoxication by two sub lethal doses (0.06 & 0.6 mg kg^{-1} bw day^{-1}) for two exposure durations (6 and 12 days). F critical value for between doses is **5.318** and F critical value for within durations is **3.438**. A * mark denotes statistical significance at p < 5% level.

	Total ATPase	**Na^+ K^+ ATPase**	**Ca^{++} ATPase**	**Mg^{++} ATPase**	**$Ca^{++}HCO3^-$ ATPase**	**$Mg^{++}HCO3^-$ ATPase**	**G-6-Pase**
Between Doses	3.012	3.567	0.757	0.027	2.305	0.785	0.040
Within Durations	0.992	2.931	13.279*	21.844*	15.197*	57.081*	23.238*

Table 13 - Results of student's 't' test between $control_1$ and individual toxicated group of ATPase enzymes estimated in the **kidney** tissue of developing chick after two exposure durations (6 and 12 days). The given critical value of 't' is **4.303**. A * mark denotes statistical significance at p < 5% level.

Exposure Durations	**Groups**	**Enzymes**						
		Total ATPase	**Na^+ K^+ ATPase**	**Ca^{++} ATPase**	**Mg^{++} ATPase**	**$Ca^{++}HCO_3^-$ ATPase**	**$Mg^{++}HCO_3^-$ ATPase**	**G-6-Pase**
6 days	**C_1 Vs T_1**	9.590*	6.494*	6.173*	2.093	0.728	1.767	6.675*
	C_1 Vs T_2	0.406	0.959	5.118*	1.034	0.541	0.100	5.729*
12 days	**C_1 Vs T_1**	0.810	0.856	1.043	5.606	1.620	0.888	0.814
	C_1 Vs T_2	0.608	1.016	2.058	1.468	1.521	4.289	3.123

Table 14 – Results of one way ANOVA among $control_2$ and therapeutic groups of ATPase enzymes estimated in the **kidney** of developing chick after therapeutic treatments to 6 and 12 days TBT preintoxicted animals by subsequent 2, 4 and 6 days of duration. F critical is **6.591**. A * mark denotes statistical significance at p < 5% level.

Dose	**Duration in Days**	**Enzymes**						
		Total ATPase	**Na^+ K^+ ATPase**	**Ca^{++} ATPase**	**Mg^{++} ATPase**	**$Ca^{++}HCO_3^-$ ATPase**	**$Mg^{++}HCO_3^-$ ATPase**	**G-6-Pase**
	6+2	61.263*	48.910*	22.249*	1.731	2.384	26.108*	11.262*
0.06	**6+4**	0.613	1.134	8.032*	40.769*	12.741*	45.225*	3.473
	6+6	11.125*	12.645*	4.550	9.770*	10.126*	42.233*	51.850*
	12+2	2.653	3.902	18.280*	17.574*	39.083*	16.496*	4.809
0.06	**12+4**	10.55*	15.240*	11.999*	6.558	17.825*	23.491*	14.184*
	12+6	18.047*	21.071*	45.354*	37.484*	10.114*	37.803*	48.446*
	6+2	33.057*	34.719*	36.096*	7.512*	19.190*	91.433*	35.951*
0.6	**6+4**	1.561	4.754	14.695*	51.544*	10.045*	37.940*	0.258
	6+6	11.679*	57.707*	0.785	0.870	23.427*	22.837*	16.877*
	12+2	5.630	2.486	8.971*	8.569*	12.333*	40.863*	23.012*
0.6	**12+4**	82.024*	71.757*	10.853*	11.334*	5.011	57.530*	12.373*
	12+6	13.829*	16.600*	16.096*	33.825*	46.337*	13.799*	80.421*

Table 15 - Results of two way ANOVA among control$_2$ and therapeutic groups of ATPases enzymes estimated in the **kidney** of developing chick after therapeutic treatment to 6 days 0.06 mgkg^{-1} bw day^{-1} TBT preintoxicated animals by subsequent 2, 4 and 6 days of duration. The given F critical value for among groups is **4.844** and F critical value for among durations is **2.818**. A * mark denotes statistical significance at p < 5% level.

	Total ATPase	**Na^+ K^+ ATPase**	**Ca^{++} ATPase**	**Mg^{++} ATPase**	**$Ca^{++}HCO_3^-$ ATPase**	**$Mg^{++}HCO_3^-$ ATPase**	**G-6-Pase**
Within Groups	0.018	0.056	0.200	0.012	3.759	0.500	0.081
Among Durations	20.348*	32.389*	29.586*	11.431*	22.800*	53.414*	35.074*

Table 16 - Results of two way ANOVA among control$_2$ and therapeutic groups of ATPases enzymes estimated in the **kidney** of developing chick after therapeutic treatment to 12 days 0.06 mgkg^{-1} bw day^{-1} TBT preintoxicated animals by subsequent 2, 4 and 6 days of duration. The given F critical value for among groups is **4.844** and F critical value for among durations is **2.818**. A * mark denotes statistical significance at p < 5% level.

	Total ATPase	**Na^+ K^+ ATPase**	**Ca^{++} ATPase**	**Mg^{++} ATPase**	**$Ca^{++}HCO_3^-$ ATPase**	**$Mg^{++}HCO_3^-$ ATPase**	**G-6-Pase**
Within Groups	4.847*	4.758	0.116	3.147	0.493	4.728	0.029
Among Durations	8.073*	10.826*	28.062*	20.158*	22.894*	35.162*	66.279*

Table 17 - Results of two way ANOVA among control$_2$ and therapeutic groups of ATPases enzymes estimated in the **kidney** of developing chick after therapeutic treatment to 6 days 0.6 mgkg^{-1} bw day^{-1} TBT preintoxicated animals by subsequent 2, 4 and 6 days of duration. The given F critical value for among groups is **4.844** and F critical value for among durations is **2.818**. A * mark denotes statistical significance at p < 5% level.

	Total ATPase	**Na^+ K^+ ATPase**	**Ca^{++} ATPase**	**Mg^{++} ATPase**	**$Ca^{++}HCO_3^-$ ATPase**	**$Mg^{++}HCO_3^-$ ATPase**	**G-6-Pase**
Within Groups	0.753	2.601	6.008*	0.306	1.142	0.377	0.419
Among Durations	22.936*	26.091*	72.922*	28.612*	22.029*	13.816*	47.710*

Table 18 - Results of two way ANOVA among control$_2$ and therapeutic groups of ATPases enzymes estimated in the **kidney** of developing chick after therapeutic treatment to 12 days 0.6 mgkg^{-1} bw day^{-1} TBT preintoxicated animals by subsequent 2, 4 and 6 days of duration. The given F critical value for among groups is **4.844** and F critical value for among durations is **2.818**. A * mark denotes statistical significance at p < 5% level.

	Total ATPase	**Na^+ K^+ ATPase**	**Ca^{++} ATPase**	**Mg^{++} ATPase**	**$Ca^{++}HCO_3^-$ ATPase**	**$Mg^{++}HCO_3^-$ ATPase**	**G-6-Pase**
Within Groups	1.515	5.514*	0.273	4.128	0.513	0.341	0.833
Among Durations	18.946*	14.683*	22.231*	24.143*	11.758*	40.006*	22.272*

Table 19 - Results of student's 't' test between control$_2$ and individual therapeutic group of ATPase enzymes estimated in the **kidney** tissue of developing chick after therapeutic treatment to 6 days 0.06 mgkg^{-1} bw day^{-1} TBT preintoxicated animals by subsequent 2, 4 and 6 days of duration. The given critical value of 't' is **4.303**. A * mark denotes statistical significance at p < 5% level.

Enzymes	2 days			4 days			6 days		
	C_2 Vs W_1	C_2 Vs VB_1	C_2 Vs VC_1	C_2 Vs W_1	C_2 Vs VB_1	C_2 Vs VC_1	C_2 Vs W_1	C_2 Vs VB_1	C_2 Vs VC_1
Total ATPase	0.940	8.542*	7.943*	0.016	0.800	0.646	27.434*	14.920*	10.297*
$Na^+ K^+$ ATPase	1.182	28.218*	59.097*	0.599	1.330	0.966	28.938*	12.096*	11.88*
Ca^{++} ATPase	5.478*	0.107	7.391*	1.783	3.685	3.322	0.401	0.849	2.637
Mg^{++} ATPase	1.563	9.820*	5.910*	3.583	6.238*	7.307*	9.294*	3.545	1.137
$Ca^{++}HCO_3^-$ ATPase	3.291	2.430	1.053	47.545*	30.368*	19.257*	2.869	2.443	4.905*
$Mg^{++}HCO_3^-$ ATPase	2.897	21.273*	22.784*	4.496*	7.302*	7.049*	5.034*	3.487	4.217*
G-6-Pase	1.724	5.879*	1.800	1.216	1.020	2.343	8.474*	6.470*	5.496*

Table 20 - Results of student's 't' test between control$_2$ and individual therapeutic group of ATPase enzymes estimated in the **kidney** tissue of developing chick after therapeutic treatment to 12 days 0.06 mgkg^{-1} bw day^{-1} TBT preintoxicated animals by subsequent 2, 4 and 6 days of duration. The given critical value of 't' is **4.303**. A * mark denotes statistical significance at p < 5% level.

Enzymes	2 days			4 days			6 days		
	C_2 Vs W_1	C_2 Vs VB_1	C_2 Vs VC_1	C_2 Vs W_1	C_2 Vs VB_1	C_2 Vs VC_1	C_2 Vs W_1	C_2 Vs VB_1	C_2 Vs VC_1
Total ATPase	1.375	1.580	2.111	5.521*	3.364	4.431	11.239*	2.635	4.614*
$Na^+ K^+$ ATPase	1.473	2.123	2.387	7.758*	4.643	4.087	14.458*	3.160	3.451
Ca^{++} ATPase	5.044*	1.031	5.353*	0.746	7.825*	4.463*	11.235*	2.907	5.371*
Mg^{++} ATPase	0.514	1.100	7.078*	0.418	2.548	5.729*	8.261*	5.651*	5.537*
$Ca^{++}HCO_3^-$ ATPase	4.719*	8.951*	1.349	0.430	8.623*	3.009	1.831	18.663*	5.944*
$Mg^{++}HCO_3^-$ ATPase	5.484*	6.369*	5.172*	0.451	15.677*	6.526*	6.628*	4.693*	6.744*
G-6-Pase	8.787*	2.597	0.140	10.308*	10.313*	1.463	23.424*	19.016*	52.222*

Table 21 - Results of student's 't' test between control$_2$ and individual therapeutic group of ATPase enzymes estimated in the **kidney** tissue of developing chick after therapeutic treatment to 6 days 0.6 mgkg^{-1} bw day^{-1} TBT preintoxicated animals by subsequent 2, 4 and 6 days of duration. The given critical value of 't' is **4.303**. A * mark denotes statistical significance at p < 5% level.

Enzymes	2 days			4 days			6 days		
	C_2 Vs W_2	C_2 Vs VB_2	C_2 Vs VC_2	C_2 Vs W_2	C_2 Vs VB_2	C_2 Vs VC_2	C_2 Vs W_2	C_2 Vs VB_2	C_2 Vs VC_2
Total ATPase	8.499*	6.732*	3.135	0.978	0.419	0.340	26.924*	24.174*	5.902*
$Na^+ K^+$ ATPase	10.010*	7.979*	9.628*	0.853	0.887	2.336	29.191*	14.965*	4.897*
Ca^{++} ATPase	2.643	27.702*	16.845*	1.821	2.813	3.988	1.254	0.734	0.472
Mg^{++} ATPase	6.265*	0.574	2.121	2.207	7.378*	4.588*	2.326	3.442	0.979
$Ca^{++}HCO_3^-$ ATPase	5.505*	3.544	6.402*	1.929	47.744*	1.790	9.972*	1.989	5.076*
$Mg^{++}HCO_3^-$ ATPase	2.994	8.550*	20.860*	18.563*	28.645*	2.232	6.010*	3.224	2.215
G-6-Pase	2.386	3.053	7.481*	0.034	0.410	0.451	42.952*	5.764*	43.424*

Table 22 - Results of student's 't' test between control$_2$ and individual therapeutic group of ATPase enzymes estimated in the **kidney** tissue of developing chick after therapeutic treatment to 12 days 0.6 mgkg^{-1} bw day^{-1} TBT preintoxicated animals by subsequent 2, 4 and 6 days of duration. The given critical value of 't is **4.303**. A * mark denotes statistical significance at p < 5% level.

Enzymes	2 days			4 days			6 days		
	C_2 Vs W_2	C_2 Vs VB_2	C_2 Vs VC_2	C_2 Vs W_2	C_2 Vs VB_2	C_2 Vs VC_2	C_2 Vs W_2	C_2 Vs VB_2	C_2 Vs VC_2
Total ATPase	0.060	0.553	2.467	5.493*	7.373*	11.998*	2.280	0.498	9.422*
$Na^+ K^+$ ATPase	0.341	0.200	1.731	7.367*	8.461*	15.612*	13.813*	1.293	3.733
Ca^{++} ATPase	2.130	8.099*	1.316	13.86*	7.037*	40.405*	1.729	1.064	4.701*
Mg^{++} ATPase	2.013	2.470	6.789*	7.588*	4.150	13.580*	2.782	3.580	9.439*
$Ca^{++}HCO_3^-$ ATPase	9.220*	2.464	0.805	3.873	1.065	2.375	27.439*	40.212*	21.408*
$Mg^{++}HCO_3^-$ ATPase	4.136	3.859	3.796	1.219	4.038	36.316*	4.670*	1.720	3.183
G-6-Pase	16.421*	9.454*	31.398*	7.285*	25.937*	28.021*	22.724*	34.507*	45.847*

Table 23 - Results of Two Way ANOVA of ATPases estimated in the **brain** tissue of developing chick after TBT intoxication by two sub lethal doses (0.06 & 0.6 mg kg^{-1} bw day^{-1}) for two exposure durations (6 and 12 days). F critical value for between doses is **5.318** and F critical value for within durations is **3.438**. A * mark denotes statistical significance at p < 5% level.

	Total ATPase	**$Na^+ K^+$ ATPase**	**Ca^{++} ATPase**	**Mg^{++} ATPase**	**$Ca^{++}HCO3^-$ ATPase**	**$Mg^{++}HCO3^-$ ATPase**	**G-6-Pase**
Between Doses	0.767	1.086	0.001	0.062	0.007	4.037	0.102
Within Durations	18.509*	22.470*	22.915*	131.676*	18.424*	3.446*	4.484*

Table 24 - Results of student's 't' test between $control_1$ and individual toxicated group of ATPase enzymes estimated in the **brain** tissue of developing chick after two exposure durations (6 and 12 days). The given critical value of 't' is **4.303**. A * mark denotes statistical significance at p < 5% level.

Exposure Durations	**Groups**	**Enzymes**						
		Total ATPase	**$Na^+ K^+$ ATPase**	**Ca^{++} ATPase**	**Mg^{++} ATPase**	**$Ca^{++}HCO_3^-$ ATPase**	**$Mg^{++}HCO_3^-$ ATPase**	**G-6-Pase**
6 days	**C_1 Vs T_1**	8.842*	5.188*	11.267*	2.381	2.383	1.016	34.580*
	C_1 Vs T_2	3.942	0.582	8.302*	11.324*	1.656	1.942	6.624*
12 days	**C_1 Vs T_1**	28.091*	3.430	4.453*	25.727*	7.158*	1.050	2.006
	C_1 Vs T_2	3.105	1.347	2.350	2.338	1.644	0.260	0.171

Table 25 - One way ANOVA among $control_2$ and therapeutic groups of ATPase enzymes estimated in the **brain** of developing chick after therapeutic treatments to 6 and 12 days TBT preintoxicted animals by subsequent 2, 4 and 6 days of duration. F critical is **6.591.** A * mark denotes statistical significance at p < 5% level.

Dose	**Duration**	**Enzymes**						
		Total ATPase	**$Na^+ K^+$ ATPase**	**Ca^{++} ATPase**	**Mg^{++} ATPase**	**$Ca^{++}HCO_3^-$ ATPase**	**$Mg^{++}HCO_3^-$ ATPase**	**G-6-Pase**
	6+2	9.596*	6.371	14.579*	74.519*	33.571*	18.501*	37.890*
0.06	**6+4**	0.664	0.584	1.386	63.360*	11.927*	34.222*	47.936*
	6+6	11.807*	11.748*	11.471*	6.608*	27.392*	87.519*	22.553*
	12+2	37.963*	28.733*	3.630	3.365	13.289*	25.147*	54.724*
0.06	**12+4**	4.506	7.359*	7.163*	14.218*	23.051*	30.558*	89.035*
	12+6	1.387	2.947	1.384	6.938*	32.411*	29.085*	21.615*
	6+2	17.325*	57.730*	91.723*	14.846*	57.270*	13.940*	28.607*
0.6	**6+4**	6.629*	16.186*	11.470*	25.514*	57.583*	45.650*	17.585*
	6+6	14.088*	12.784*	13.965*	7.225*	55.370*	41.809*	28.988*
	12+2	28.588*	42.828*	6.075	1.529	22.094*	16.020*	54.137*
0.6	**12+4**	14.343*	24.684*	2.867	3.687	30.628*	4.781	47.512*
	12+6	9.357*	8.088*	7.237*	3.249	46.292*	0.811	31.271*

Table 26 - Results of two way ANOVA among control$_2$ and therapeutic groups of ATPases enzymes estimated in the **brain** of developing chick after therapeutic treatment to 6 days 0.06 mgkg^{-1} bw day^{-1} TBT preintoxicated animals by subsequent 2, 4 and 6 days of duration. The given F critical value for among groups is **4.844** and F critical value for among durations is **2.818**. A * mark denotes statistical significance at p < 5% level.

	Total ATPase	**$Na^+ K^+$ ATPase**	**Ca^{++} ATPase**	**Mg^{++} ATPase**	**$Ca^{++}HCO_3^-$ ATPase**	**$Mg^{++}HCO_3^-$ ATPase**	**G-6-Pase**
Within Groups	0.535	1.184	0.563	0.121	0.276	4E-06	0.283
Among Durations	13.055*	16.500*	11.914*	59.480*	26.689*	62.337*	29.970*

Table 27 - Results of two way ANOVA among control$_2$ and therapeutic groups of ATPases enzymes estimated in the **brain** of developing chick after therapeutic treatment to 12 days 0.06 mgkg^{-1} bw day^{-1} TBT preintoxicated animals by subsequent 2, 4 and 6 days of duration. The given F critical value for among groups is **4.844** and F critical value for among durations is **2.818**. A * mark denotes statistical significance at p < 5% level.

	Total ATPase	**$Na^+ K^+$ ATPase**	**Ca^{++} ATPase**	**Mg^{++} ATPase**	**$Ca^{++}HCO_3^-$ ATPase**	**$Mg^{++}HCO_3^-$ ATPase**	**G-6-Pase**
Within Groups	0.601	2.103	0.188	0.001	0.011	0.136	0.481
Among Durations	32.809*	44.920*	3.927*	6.587*	45.795*	33.083*	18.983*

Table 28 - Results of two way ANOVA among control$_2$ and therapeutic groups of ATPases enzymes estimated in the **brain** of developing chick after therapeutic treatment to 6 days 0.6 mgkg^{-1} bw day^{-1} TBT preintoxicated animals by subsequent 2, 4 and 6 days of duration. The given F critical value for among groups is **4.844** and F critical value for among durations is **2.818**.

	Total ATPase	**$Na^+ K^+$ ATPase**	**Ca^{++} ATPase**	**Mg^{++} ATPase**	**$Ca^{++}HCO_3^-$ ATPase**	**$Mg^{++}HCO_3^-$ ATPase**	**G-6-Pase**
Within Groups	3.549	0.718	0.008	0.248	0.099	1.210	3.257
Among Durations	28.406*	41.248*	91.841*	26.438*	44.802*	70.736*	27.280*

Table 29 - Results of two way ANOVA among control$_2$ and therapeutic groups of ATPases enzymes estimated in the **brain** of developing chick after therapeutic treatment to 12 days 0.6 mgkg^{-1} bw day^{-1} TBT preintoxicated animals by subsequent 2, 4 and 6 days of duration. The given F critical value for among groups is **4.844** and F critical value for among durations is **2.818**. A * mark denotes statistical significance at p < 5% level.

	Total ATPase	**$Na^+ K^+$ ATPase**	**Ca^{++} ATPase**	**Mg^{++} ATPase**	**$Ca^{++}HCO_3^-$ ATPase**	**$Mg^{++}HCO_3^-$ ATPase**	**G-6-Pase**
Within Duration	0.105	0.272	0.000	0.324	0.092	0.009	0.024
Between groups	22.809*	27.097*	4.660*	6.605*	23.723*	14.909*	15.706*

Table 30 - Results of student's 't' test between control$_2$ and individual therapeutic group of ATPase enzymes estimated in the **brain** tissue of developing chick after therapeutic treatment to 6 days 0.06 mgkg^{-1} bw day^{-1} TBT preintoxicated animals by subsequent 2, 4 and 6 days of duration. The given critical value of 't' is **4.303**. A * mark denotes statistical significance at p < 5% level.

Enzymes	2 days			4 days			6 days		
	C_2 Vs W_1	C_2 Vs VB_1	C_2 Vs VC_1	C_2 Vs W_1	C_2 Vs VB_1	C_2 Vs VC_1	C_2 Vs W_1	C_2 Vs VB_1	C_2 Vs VC_1
Total ATPase	15.649*	9.124*	2.485	1.475	1.144	0.262	17.608*	11.529*	10.341*
$Na^+ K^+$ ATPase	17.381*	10.456*	2.997	0.868	0.030	0.312	28.529*	13.657*	10.431*
Ca^{++} ATPase	23.809*	1.971	5.054*	0.882	0.487	3.662	3.314	2.270	2.239
Mg^{++} ATPase	12.442*	2.106	12.066*	4.064	35.533*	36.742*	8.056*	9.146*	3.152
$Ca^{++}HCO_3^-$ ATPase	5.585*	3.054	4.639*	2.335	5.222*	2.568	1.046	7.275*	13.323*
$Mg^{++}HCO_3^-$ ATPase	3.685	2.715	43.942*	3.960	2.669	7.538*	0.586	12.534*	10.956*
G-6-Pase	1.010	17.550*	18.164*	7.002*	5.864*	10.865*	3.300	1.821	3.164

Table 31 - Results of student's 't' test between control$_2$ and individual therapeutic group of ATPase enzymes estimated in the **brain** tissue of developing chick after therapeutic treatment to 12 days 0.06 mgkg^{-1} bw day^{-1} TBT preintoxicated animals by subsequent 2, 4 and 6 days of duration. The given critical value of 't' is **4.303**. A * mark denotes statistical significance at p < 5% level.

Enzymes	2 days			4 days			6 days		
	C_2 Vs W_1	C_2 Vs VB_1	C_2 Vs VC_1	C_2 Vs W_1	C_2 Vs VB_1	C_2 Vs VC_1	C_2 Vs W_1	C_2 Vs VB_1	C_2 Vs VC_1
Total ATPase	20.50*	33.96*	54.306*	0.617	1.330	2.666	0.844	2.697	0.054
$Na^+ K^+$ ATPase	63.60*	18.89*	36.305*	1.132	2.288	12.115*	1.270	4.586*	9.695*
Ca^{++} ATPase	1.510	0.358	1.413	3.536	0.108	0.135	0.047	0.916	1.054
Mg^{++} ATPase	0.936	0.346	2.798	16.832*	0.756	2.658	1.845	1.788	1.103
$Ca^{++}HCO_3^-$ ATPase	4.486*	7.973*	2.786	6.156*	2.296	11.060*	5.126*	7.089*	0.791
$Mg^{++}HCO_3^-$ ATPase	1.209	0.519	16.302*	6.671*	4.173	7.670*	0.640	1.426	6.086*
G-6-Pase	31.31*	7.121*	14.036*	13.874*	4.679*	13.366*	3.827	5.871*	5.795*

Table 32 - Results of student's 't' test between $control_2$ and individual therapeutic group of ATPase enzymes estimated in the **brain** tissue of developing chick after therapeutic treatment to 6 days 0.6 $mgkg^{-1}$ bw day^{-1} TBT preintoxicated animals by subsequent 2, 4 and 6 days of duration. The given critical value of 't' is **4.303**. A * mark denotes statistical significance at p < 5% level.

Enzymes	2 days			4 days			6 days		
	C_2 Vs W_2	C_2 Vs VB_2	C_2 Vs VC_2	C_2 Vs W_2	C_2 Vs VB_2	C_2 Vs VC_2	C_2 Vs W_2	C_2 Vs VB_2	C_2 Vs VC_2
Total ATPase	12.554*	2.087	10.699*	7.611*	0.472	1.125	18.001*	10.481*	13.560*
$Na^+ K^+$ ATPase	12.740*	6.946*	4.932*	7.800*	0.975	5.849*	33.016*	11.701*	14.182*
Ca^{++} ATPase	3.513	2.143	25.760*	12.484*	2.760	2.946	4.075	4.623*	4.913*
Mg^{++} ATPase	2.149	0.330	20.072*	0.488	12.561*	12.125*	6.738*	3.291	5.202*
$Ca^{++}HCO_3^-$ ATPase	0.966	3.114	7.713*	8.384*	0.171	9.371*	12.491*	7.531*	0.602
Mg++HCO 3-	0.174	12.07*	13.730*	2.331	6.917*	4.739*	24.066*	44.618*	3.020
G-6-Pase	0.706	20.96*	2.727	7.481*	5.955*	0.140	3.737	2.424	3.157

Table 33 - Results of student's 't' test between $control_2$ and individual therapeutic group of ATPase enzymes estimated in the **brain** tissue of developing chick after therapeutic treatment to 12 days 0.6 $mgkg^{-1}$ bw day^{-1} TBT preintoxicated animals by subsequent 2, 4 and 6 days of duration. The given critical value of 't' is **4.303**. A * mark denotes statistical significance at p < 5% level.

Enzymes	2 days			4 days			6 days		
	C_2 Vs W_2	C_2Vs VB_2	C_2 Vs VC_2	C_2 Vs W_2	C_2 Vs VB_2	C_2 Vs VC_2	C_2 Vs W_2	C_2 Vs VB_2	C_2 Vs VC_2
Total ATPase	18.117*	1.640	6.684*	1.610	0.521	3.531	2.883	2.672	0.773
$Na^+ K^+$ ATPase	14.490*	11.8*	6.282*	14.737*	3.680	4.138	5.810*	12.715*	0.931
Ca^{++} ATPase	0.354	1.667	2.114	0.895	0.564	1.611	0.159	0.486	2.560
Mg^{++} ATPase	0.328	1.296	1.317	23.635*	1.972	0.103	1.159	0.458	2.127
$Ca^{++}HCO_3^-$ ATPase	5.469*	2.213	18.914*	9.646*	7.467*	2.558	2.279	7.766*	10.627*
$Mg^{++}HCO_3^-$ ATPase	1.656	0.878	3.408	9.208*	5.762*	0.068	0.239	0.165	1.073
G-6-Pase	26.329*	5.80*	27.887*	11.422*	8.134*	11.183*	5.425*	8.383*	5.910*

Table 34 - Results of Two Way ANOVA of ATPases estimated in the **muscle** tissue of developing chick after TBT intoxication by two sub lethal doses (0.06 & 0.6 mg kg^{-1} bw day^{-1}) for two exposure durations (6 and 12 days). F critical value for between doses is **5.318** and F critical value for within durations is **3.438**. A * mark denotes statistical significance at p < 5% level.

	Total ATPase	**$Na^+ K^+$ ATPase**	**Ca^{++} ATPase**	**Mg^{++} ATPase**	**$Ca^{++}HCO3^-$ ATPase**	**$Mg^{++}HCO3^-$ ATPase**	**G-6-Pase**
Between Doses	1.495	1.894	0.331	0.009	0.941	0.140	4.287
Within Durations	3.522*	6.005*	6.725*	30.894*	74.670*	90.456*	9.939*

Table 35 - Results of student's 't' test between $control_1$ and individual toxicated group of ATPase enzymes estimated in the **muscle** tissue of developing chick after two exposure durations (6 and 12 days). The given critical value of 't' is **4.303**. A * mark denotes statistical significance at p < 5% level.

Exposure Durations	**Groups**	**Enzymes**						
		Total ATPase	**$Na^+ K^+$ ATPase**	**Ca^{++} ATPase**	**Mg^{++} ATPase**	**$Ca^{++}HCO_3^-$ ATPase**	**$Mg^{++}HCO_3^-$ ATPase**	**G-6-Pase**
6 days	**C_1 Vs T_1**	2.280	2.947	3.091	7.208*	10.702*	5.564*	1.158
	C_1 Vs T_2	0.854	0.676	6.727*	10.215*	10.571*	2.990	0.830
12 days	**C_1 Vs T_1**	0.861	0.369	6.791*	0.205	4.342*	0.048	2.736
	C_1 Vs T_2	2.301	2.326	4.321*	2.141	3.596	10.945*	0.325

Table 36 - One way ANOVA among $control_2$ and therapeutic groups of ATPase enzymes estimated in the **muscle** of developing chick after therapeutic treatments to 6 and 12 days TBT preintoxicted animals by subsequent 2, 4 and 6 days of duration. The tabulated F critical is **6.591.** A * mark denotes statistical significance at p < 5% level.

Dose	**Duration**	**Enzymes**						
		Total ATPase	**$Na^+ K^+$ ATPase**	**Ca^{++} ATPase**	**Mg^{++} ATPase**	**$Ca^{++}HCO_3^-$ ATPase**	**$Mg^{++}HCO_3^-$ ATPase**	**G-6-Pase**
	6+2	14.363*	16.522*	30.353*	4.866	21.109*	51.656*	18.109*
0.06	**6+4**	1.814	4.613	3.114	15.708*	16.070*	89.414*	39.634*
	6+6	10.887*	7.142*	42.170*	32.670*	28.270*	50.918*	39.490*
	12+2	11.815*	12.254*	3.627	2.739	36.680*	71.383*	36.435*
0.06	**12+4**	5.736	14.518*	15.835*	30.825*	14.996*	15.292*	14.307*
	12+6	12.308*	24.631*	3.037	16.139*	10.603*	11.882*	42.007*
	6+2	10.432*	22.235*	38.990*	8.001*	21.582*	16.604*	80.115*
0.6	**6+4**	3.341	20.038*	14.61*1	19.883*	43.365*	10.009*	10.508*
	6+6	21.036*	13.293*	3.882	18.926*	2.329	65.659*	50.311*
	12+2	3.711	8.027*	6.048	19.280*	15.135*	9.752*	33.420*
0.6	**12+4**	8.570*	20.726*	13.051*	25.847*	20.147*	9.299*	54.747*
	12+6	12.687*	58.990*	88.464*	28.845*	32.043*	16.879*	10.602*

Table 37 - Results of two way ANOVA among control$_2$ and therapeutic groups of ATPases enzymes estimated in the **muscle** of developing chick after therapeutic treatment to 6 days 0.06 mg kg^{-1} bw day^{-1} TBT preintoxicated animals by subsequent 2, 4 and 6 days of duration. The given F critical value for among groups is **4.844** and F critical value for among durations is **2.818.** A * mark denotes statistical significance at p < 5% level.

	Total ATPase	**Na^+ K^+ ATPase**	**Ca^{++} ATPase**	**Mg^{++} ATPase**	**$Ca^{++}HCO_3^-$ ATPase**	**$Mg^{++}HCO_3^-$ ATPase**	**G-6-Pase**
Among Duration	3.990	0.396	0.820	0.001	0.177	0.120	0.130
Between groups	19.646*	12.546*	30.361*	64.819*	19.474*	50.044*	20.577*

Table 38 - Results of two way ANOVA among control$_2$ and therapeutic groups of ATPases enzymes estimated in the **muscle** of developing chick after therapeutic treatment to 12 days 0.06 mgkg^{-1} bw day^{-1} TBT preintoxicated animals by subsequent 2, 4 and 6 days of duration. The given F critical value for among groups is **4.844** and F critical value for among durations is **2.818.** A * mark denotes statistical significance at p < 5% level.

	Total ATPase	**Na^+ K^+ ATPase**	**Ca^{++} ATPase**	**Mg^{++} ATPase**	**$Ca^{++}HCO_3^-$ ATPase**	**$Mg^{++}HCO_3^-$ ATPase**	**G-6-Pase**
Among Duration	0.096	0.133	1.278	1.804	1.618	0.615	0.247
Between groups	10.034*	23.949*	9.467*	13.311*	27.205*	47.280*	12.501*

Table 39 - Results of two way ANOVA among control$_2$ and therapeutic groups of ATPases enzymes estimated in the **muscle** of developing chick after therapeutic treatment to 6 days 0.6 mgkg^{-1} bw day^{-1} TBT preintoxicated animals by subsequent 2, 4 and 6 days of duration. The given F critical value for among groups is **4.844** and F critical value for among durations is **2.818**. A * mark denotes statistical significance at p < 5% level.

	Total ATPase	**Na^+ K^+ ATPase**	**Ca^{++} ATPase**	**Mg^{++} ATPase**	**$Ca^{++}HCO_3^-$ ATPase**	**$Mg^{++}HCO_3^-$ ATPase**	**G-6-Pase**
Among Duration	4.834	1.172	0.203	0.100	0.061	0.617	0.249
Between groups	26.389*	27.434*	17.520*	11.819*	28.236*	74.205*	56.549*

Table 40 - Results of two way ANOVA among control$_2$ and therapeutic groups of ATPases enzymes estimated in the **muscle** of developing chick after therapeutic treatment to 12 days 0.6 mgkg^{-1} bw day^{-1} TBT preintoxicated animals by subsequent 2, 4 and 6 days of duration. The given F critical value for among groups is **4.844** and F critical value for among durations is **2.818**. A * mark denotes statistical significance at p < 5% level.

	Total ATPase	**Na^+ K^+ ATPase**	**Ca^{++} ATPase**	**Mg^{++} ATPase**	**$Ca^{++}HCO_3^-$ ATPase**	**$Mg^{++}HCO_3^-$ ATPase**	**G-6-Pase**
Among Duration	0.389	0.002	0.966	0.071	0.497	0.000	0.065
Between groups	6.687*	13.104*	7.917*	32.415*	28.978*	13.805*	14.485*

Table 41- Results of student's 't' test between control$_2$ and individual therapeutic group of ATPase enzymes estimated in the **muscle** tissue of developing chick after therapeutic treatment to 6 days 0.06 mgkg^{-1} bw day^{-1} TBT preintoxicated animals by subsequent 2, 4 and 6 days of duration. The given critical value of 't' is **4.303**. A * mark denotes statistical significance at p < 5% level.

Enzymes	2 days			4 days			6 days		
	C_2 Vs W_1	C_2 Vs VB_1	C_2 Vs VC_1	C_2 Vs W_1	C_2 Vs VB_1	C_2 Vs VC_1	C_2 Vs W_1	C_2 Vs VB_1	C_2 Vs VC_1
Total ATPase	2.969	7.780*	4.777*	1.520	0.213	0.244	27.576*	15.957*	1.501
$Na^+ K^+$ ATPase	1.087	3.492	10.586*	3.374	1.348	0.863	8.540*	1.854	1.381
Ca^{++} ATPase	98.109*	34.849*	85.887*	0.872	0.486	4.107	0.119	8.803*	6.840*
Mg^{++} ATPase	1.267	1.330	2.851	2.387	12.820*	20.863*	3.914	7.351*	4.341*
$Ca^{++}HCO_3^-$ ATPase	14.989*	19.655*	2.890	0.025	0.322	8.204*	6.365*	6.173*	6.155*
$Mg^{++}HCO_3^-$ ATPase	3.169	7.162*	11.348*	1.154*	8.117*	8.345*	0.970	13.191*	4.866*
G-6-Pase	3.191	50.670*	41.988*	17.84*	19.800*	3.064	4.914*	69.294*	54.77*

Table 42 - Results of student's 't' test between control$_2$ and individual therapeutic group of ATPase enzymes estimated in the **muscle** tissue of developing chick after therapeutic treatment to 12 days 0.06 mgkg^{-1} bw day^{-1} TBT preintoxicated animals by subsequent 2, 4 and 6 days of duration. The given critical value of 't' is **4.303**. A * mark denotes statistical significance at p < 5% level.

Enzymes	2 days			4 days			6 days		
	C_2 Vs W_1	C_2 Vs VB_1	C_2 Vs VC_1	C_2 Vs W_1	C_2 Vs VB_1	C_2 Vs VC_1	C_2 Vs W_1	C_2 Vs VB_1	C_2 Vs VC_1
Total ATPase	0.904	3.136	5.302*	4.052	8.974*	0.640	1.261	1.929	14.236*
$Na^+ K^+$ ATPase	2.034	5.026*	6.230*	8.011*	13.193*	1.264	0.959	3.704	5.131*
Ca^{++} ATPase	2.205	4.104	1.214	6.212*	0.587	0.785	4.624*	0.114	0.750
Mg^{++} ATPase	1.629	3.664	4.051	2.400	27.116*	9.343*	5.167*	10.489*	6.398*
$Ca^{++}HCO_3^-$ ATPase	4.549*	5.671*	0.447	4.249	3.227	1.737	18.280*	5.168*	5.292*
$Mg^{++}HCO_3^-$ ATPase	2.987	4.738*	5.734*	0.624	4.820*	1.470	12.548*	6.692*	41.612*
G-6-Pase	0.655	6.328*	11.83*	2.065	1.610	5.751*	1.796	35.949*	25.308*

Table 43 - Results of student's 't' test between control$_2$ and individual therapeutic group of ATPase enzymes estimated in the **muscle** tissue of developing chick after therapeutic treatment to 6 days 0.6 mgkg^{-1} bw day^{-1} TBT preintoxicated animals by subsequent 2, 4 and 6 days of duration. The given critical value of 't' is **4.303**. A * mark denotes statistical significance at p < 5% level.

Enzymes	2 days			4 days			6 days		
	C_2 Vs W_2	C_2 Vs VB_2	C_2 Vs VC_2	C_2 Vs W_2	C_2 Vs VB_2	C_2 Vs VC_2	C_2 Vs W_2	C_2 Vs VB_2	C_2 Vs VC_2
Total ATPase	17.298*	9.961*	7.461*	1.200	0.123	1.063	6.228*	1.501	5.072*
$Na^+ K^+$ ATPase	12.367*	12.333*	9.079*	3.106	2.609	1.846	5.943*	2.238	2.226
Ca^{++} ATPase	88.045*	19.664*	52.957*	2.153	0.979	9.051*	1.129	2.299	1.071
Mg^{++} ATPase	3.766	2.036	3.756	2.895	14.891*	18.662*	5.364*	4.917*	2.850
$Ca^{++}HCO_3^-$ ATPase	8.671*	0.998	43.793*	4.801*	9.423*	10.607*	0.878	0.832	1.669
$Mg^{++}HCO_3^-$ ATPase	2.489	3.749	1.897	0.910	13.750*	9.433*	1.158	2.306	12.952*
G-6-Pase	86.254*	51.816*	35.661*	19.425 *	4.235	46.430*	4.224	12.103*	37.104*

Table 44 - Results of student's 't' test between control$_2$ and individual therapeutic group of ATPase enzymes estimated in the **muscle** tissue of developing chick after therapeutic treatment to 12 days 0.6 mgkg^{-1} bw day^{-1} TBT preintoxicated animals by subsequent 2, 4 and 6 days of duration. The given critical value of 't' is **4.303**. A * mark denotes statistical significance at p < 5% level.

Enzymes	2 days			4 days			6 days		
	C_2 Vs W_2	C_2 Vs VB_2	C_2 Vs VC_2	C_2 Vs W_2	C_2 Vs VB_2	C_2 Vs VC_2	C_2 Vs W_2	C_2 Vs VB_2	C_2 Vs VC_2
Total ATPase	0.934	3.963	0.323	1.053	4.258	5.846*	5.707*	23.307*	6.634*
$Na^+ K^+$ ATPase	1.391	3.527	2.841	0.354	8.176*	9.777*	3.802	7.763*	10.189*
Ca^{++} ATPase	0.175	2.416	3.457	0.938	2.949	6.386*	6.257*	6.379*	6.394*
Mg^{++} ATPase	2.383	4.234*	4.340*	7.078*	9.233*	8.509*	11.956*	39.905*	23.477*
$Ca^{++}HCO_3^-$ ATPase	2.322	3.079	6.193*	0.211	4.608*	2.747	4.449*	2.031	8.625*
$Mg^{++}HCO_3^-$ ATPase	2.192	3.317	6.142*	2.297	1.933	2.749	13.811*	25.835*	25.775*
G-6-Pase	3.513	18.192*	2.731	1.133	4.525*	5.743*	1.512	12.886*	2.746

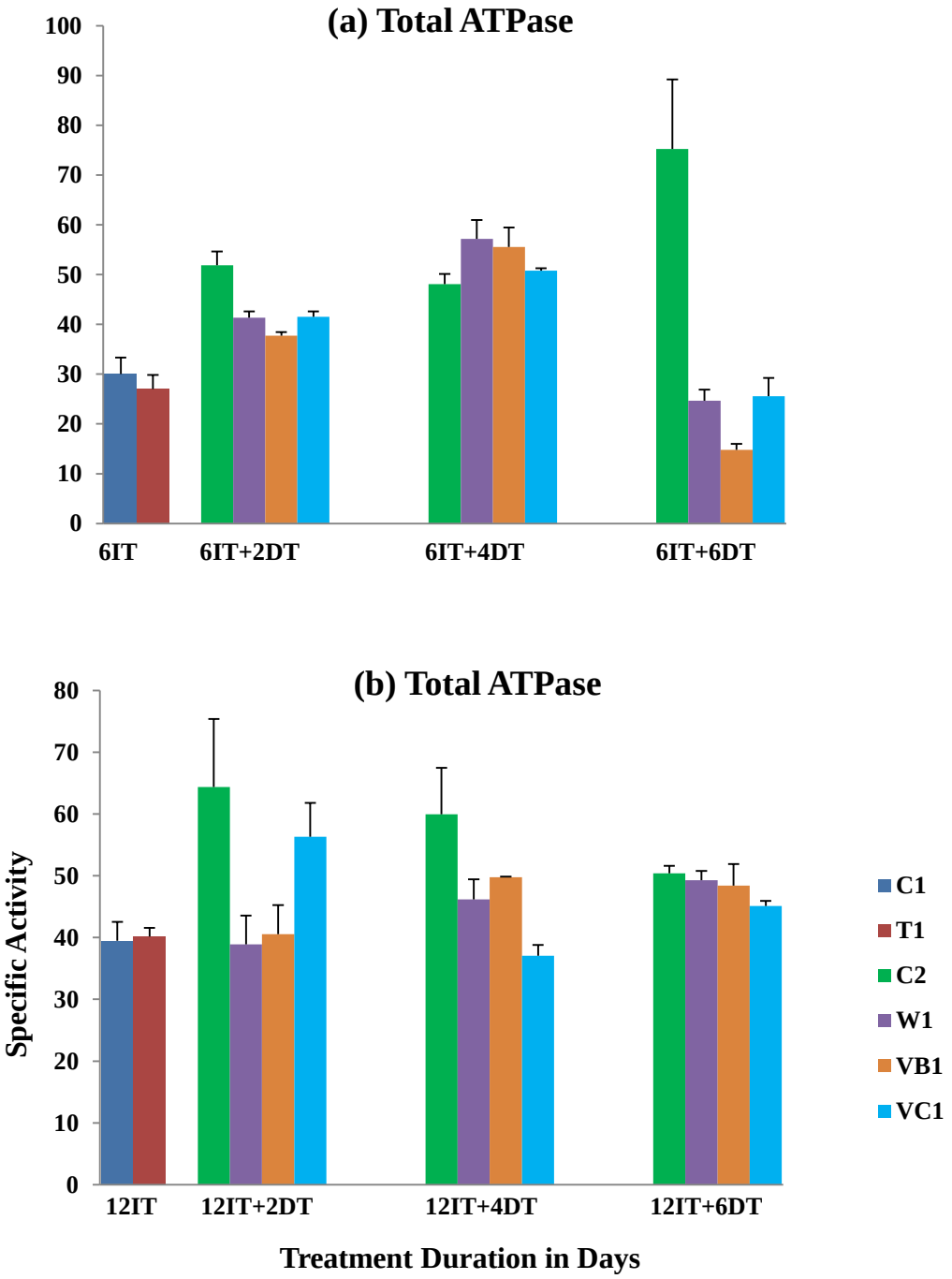


Fig 1 - Changes in the specific activity of **Total ATPase** of chick **live**r. (a) TBT dose 0.06 mg kg^{-1}bw d^{-1} exposed for 6 days (b) TBT dose 0.06 mg kg^{-1}bw d^{-1} exposed for 12 days and its possible recovery by natural washing of toxicant (withdrawal), Vitamin B complex and Vitamin C for next 2, 4, 6 days. Data expressed are mean of specific activity ± SD. Abbreviations used in graphs are mentioned in materials and methods chapter.

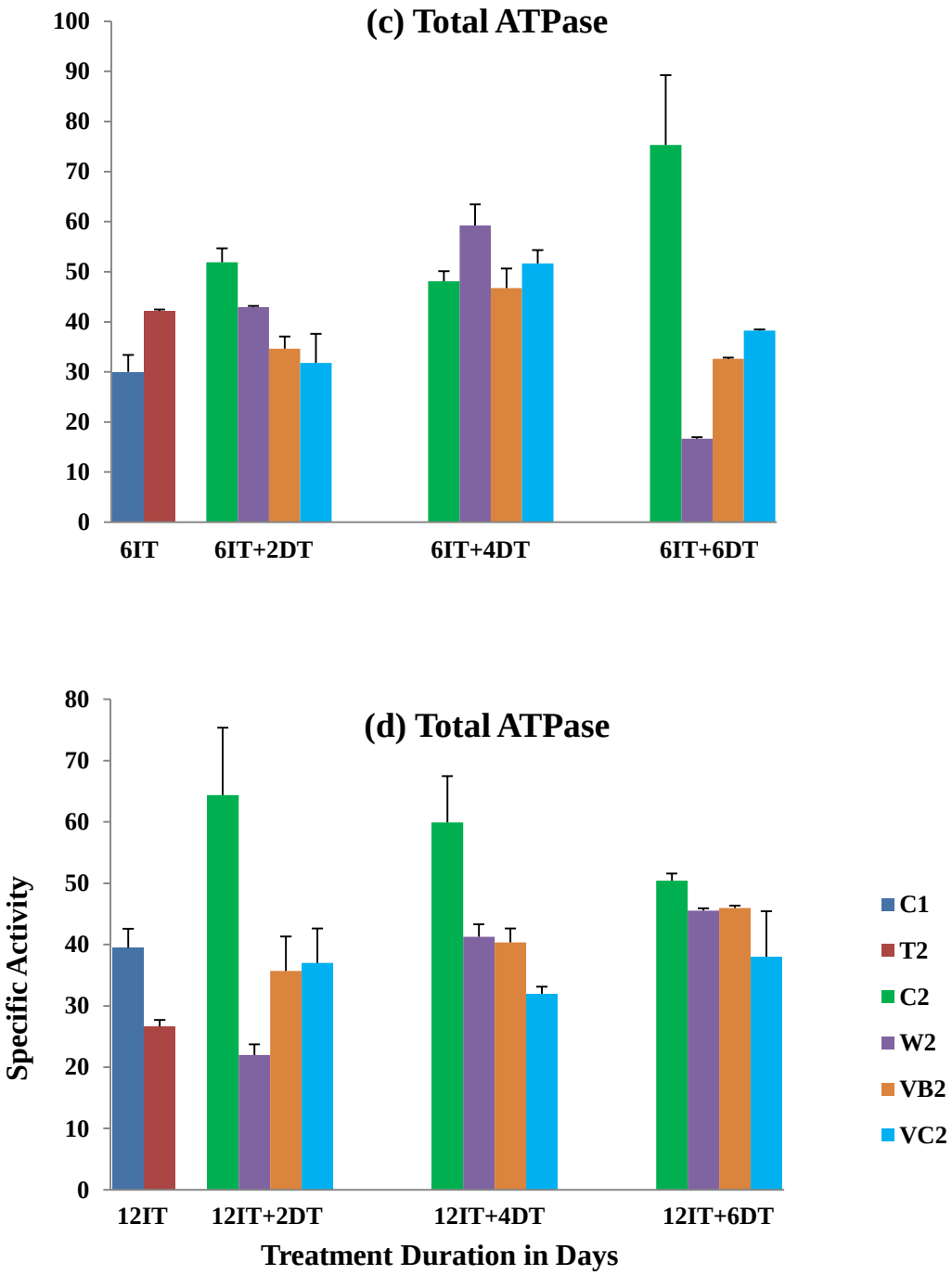


Fig 1 - Changes in the specific activity of **Total ATPase** of chick **live**r. (c) TBT dose 0.6 mg kg^{-1}bw d^{-1} exposed for 6 days (d) TBT dose 0.6 mg kg^{-1}bw d^{-1} exposed for 12 days and its possible recovery by natural washing of toxicant (withdrawal), Vitamin B complex and Vitamin C for next 2, 4, 6 days. Data expressed are mean of specific activity ± SD. Abbreviations used in graphs are mentioned in materials and methods chapter.

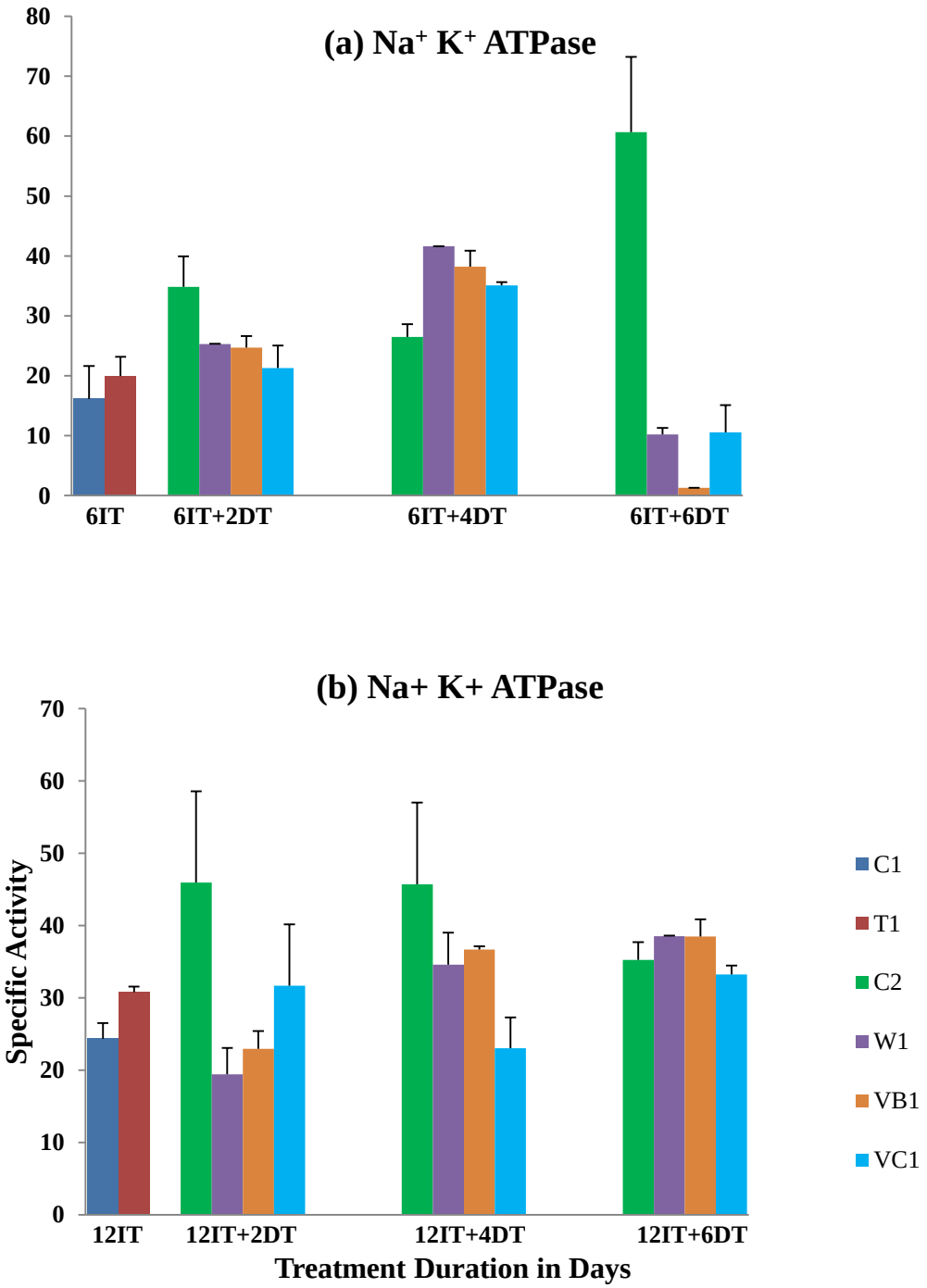


Fig 2 - Changes in the specific activity of **Na^+ K^+ ATPase** of chick **liver**. (a) TBT dose 0.06 mg kg^{-1}bw d^{-1} exposed for 6 days (b) TBT dose 0.06 mg kg^{-1}bw d^{-1} exposed for 12 days and its possible recovery by natural washing of toxicant (withdrawal), Vitamin B complex and Vitamin C for next 2, 4, 6 days. Data expressed are mean of specific activity ± SD. Abbreviations used in graphs are mentioned in materials and methods chapter.

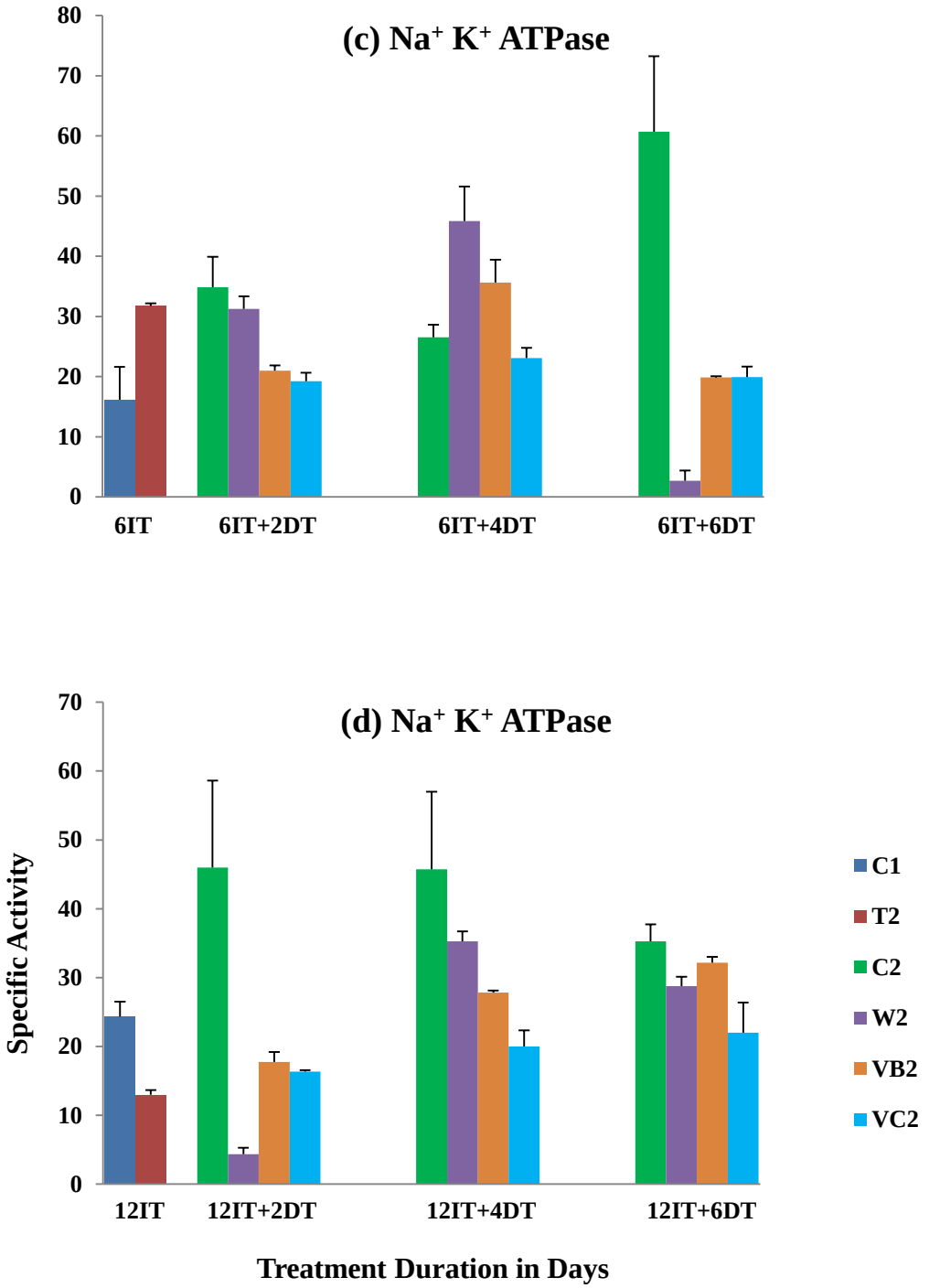


Fig 2 - Changes in the specific activity of **Na^+ K^+ ATPase** of chick **liver**. (c) TBT dose 0.6 mg kg^{-1}bw d^{-1} exposed for 6 days (d) TBT dose 0.6 mg kg^{-1}bw d^{-1} exposed for 12 days and its possible recovery by natural washing of toxicant (withdrawal), Vitamin B complex and Vitamin C for next 2, 4, 6 days. Data expressed are mean of specific activity ± SD. Abbreviations used in graphs are mentioned in materials and methods chapter.

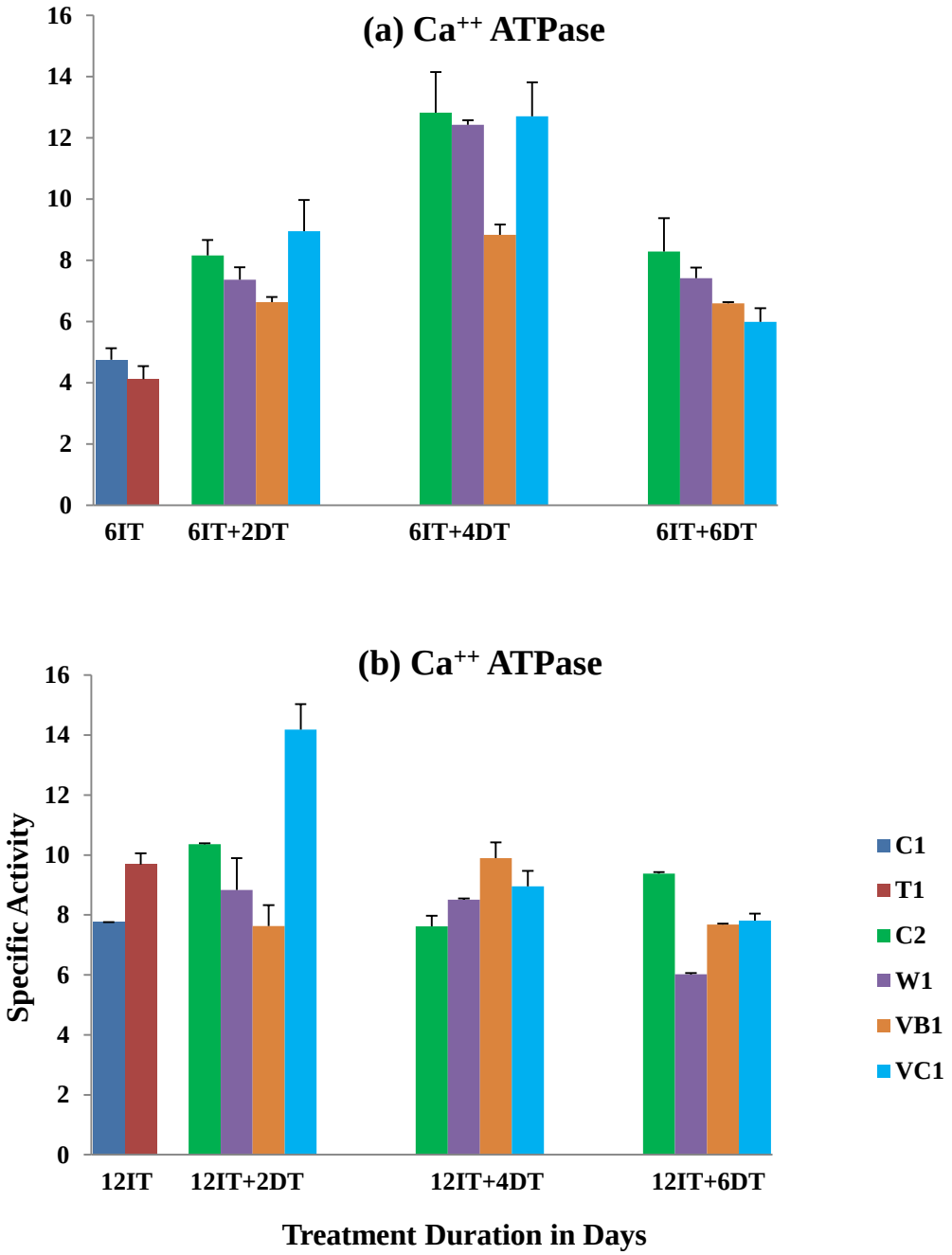


Fig 3 - Changes in the specific activity of **Ca^{++} ATPase** of chick **liver**. (a) TBT dose 0.06 mg kg^{-1}bw d^{-1} exposed for 6 days (b) TBT dose 0.06 mg kg^{-1}bw d^{-1} exposed for 12 days and its possible recovery by natural washing of toxicant (withdrawal), Vitamin B complex and Vitamin C for next 2, 4, 6 days. Data expressed are mean of specific activity ± SD. Abbreviations used in graphs are mentioned in materials and methods chapter.

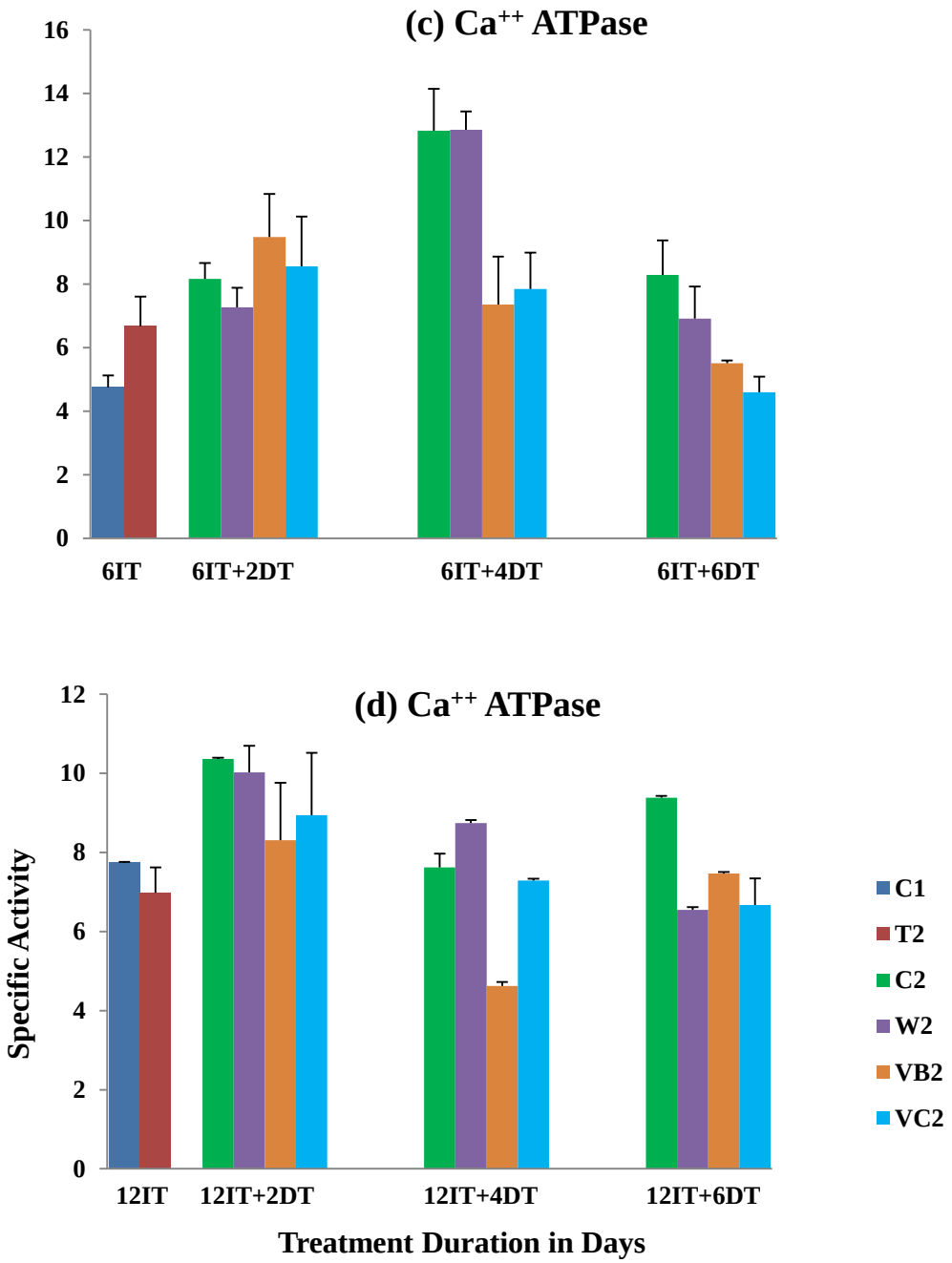


Fig 3 - Changes in the specific activity of **Ca^{++} ATPase** of chick **liver**. (c) TBT dose 0.6 mg kg^{-1}bw d^{-1} exposed for 6 days (d) TBT dose 0.6 mg kg^{-1}bw d^{-1} exposed for 12 days and its possible recovery by natural washing of toxicant (withdrawal), Vitamin B complex and Vitamin C for next 2, 4, 6 days. Data expressed are mean of specific activity ± SD. Abbreviations used in graphs are mentioned in materials and methods chapter.

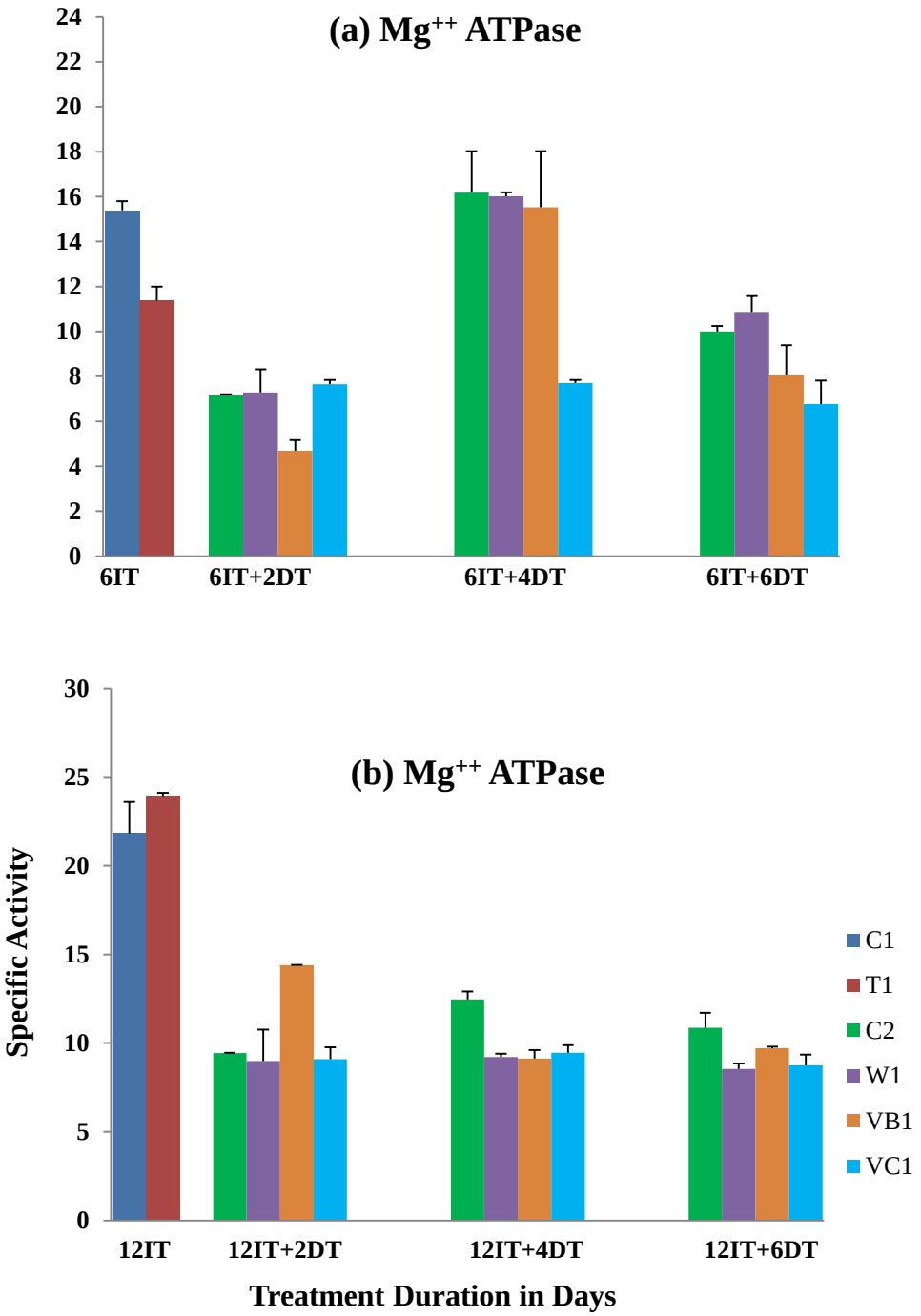


Fig 4 - Changes in the specific activity of **Mg^{++} ATPase** of chick **live**r. (a) TBT dose 0.06 mg kg^{-1}bw d^{-1} exposed for 6 days (b) TBT dose 0.06 mg kg^{-1}bw d^{-1} exposed for 12 days and its possible recovery by natural washing of toxicant (withdrawal), Vitamin B complex and Vitamin C for next 2, 4, 6 days. Data expressed are mean of specific activity ± SD. Abbreviations used in graphs are mentioned in materials and methods chapter.

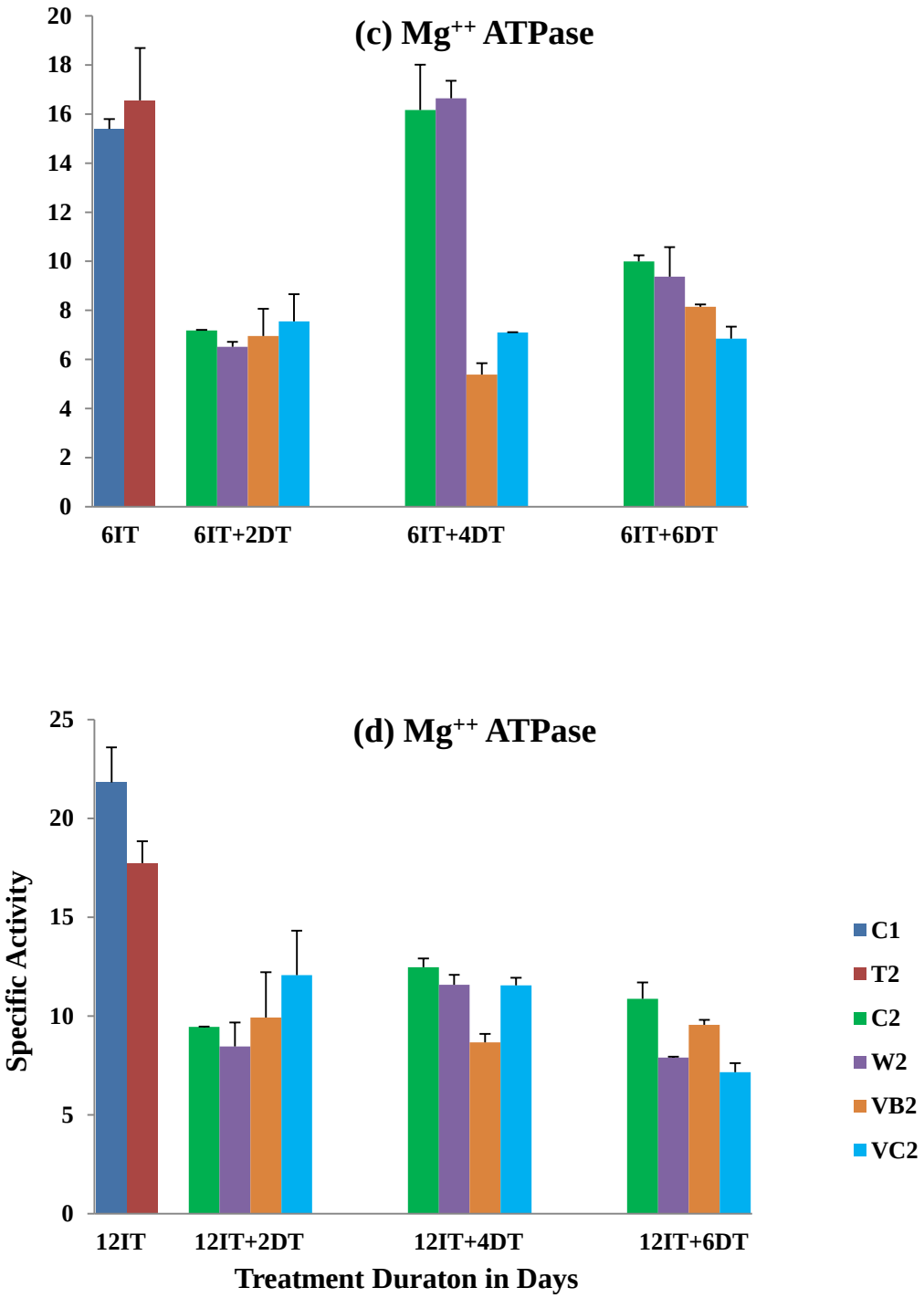


Fig 4 - Changes in the specific activity of **Mg^{++} ATPase** of chick **live**r. (c) TBT dose 0.6 mg kg^{-1}bw d^{-1} exposed for 6 days (d) TBT dose 0.6 mg kg^{-1}bw d^{-1} exposed for 12 days and its possible recovery by natural washing of toxicant (withdrawal), Vitamin B complex and Vitamin C for next 2, 4, 6 days. Data expressed are mean of specific activity ± SD. Abbreviations used in graphs are mentioned in materials and methods chapter.

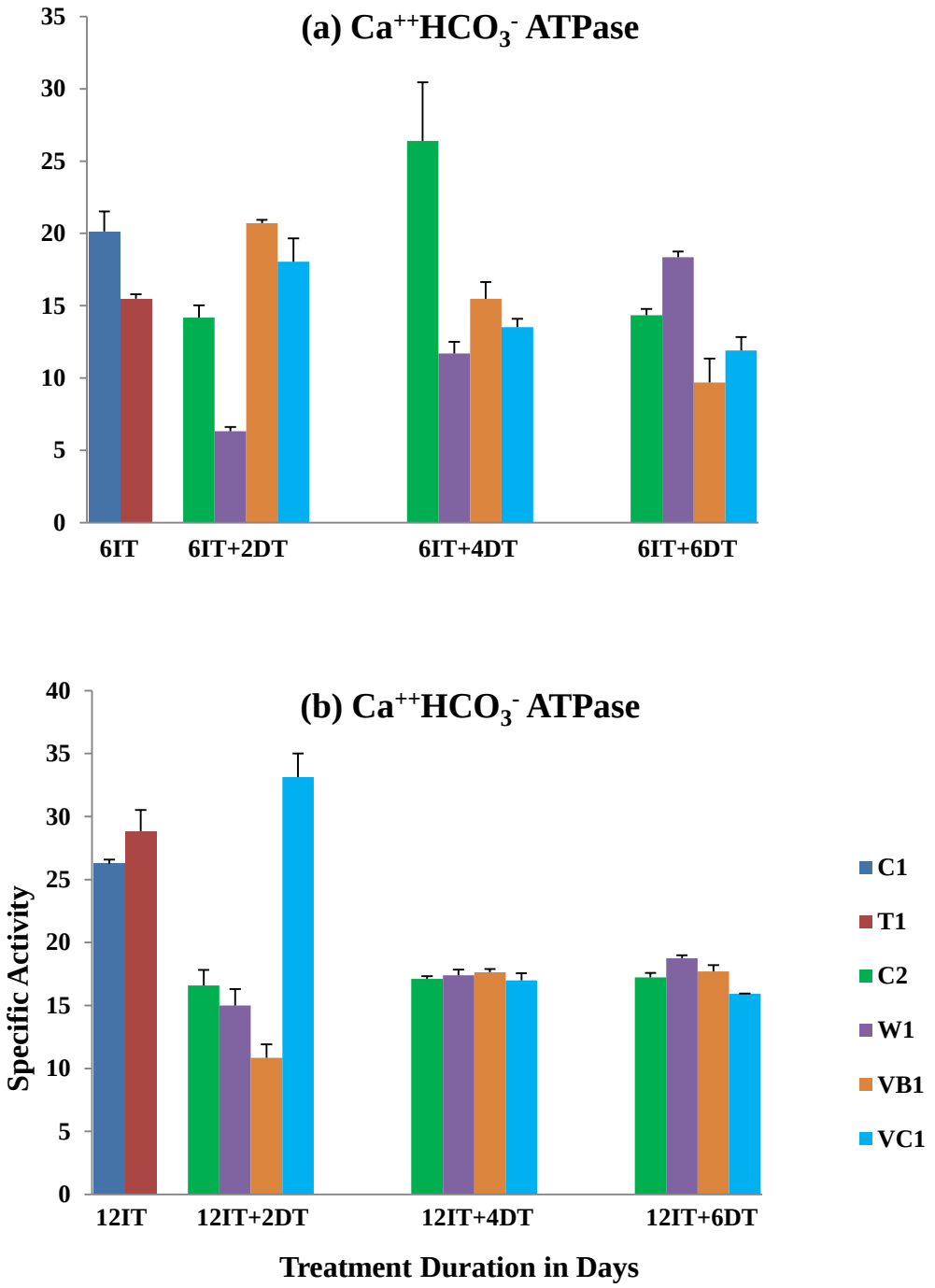


Fig 5 - Changes in the specific activity of **$Ca^{++}HCO_3^-$ ATPase** of chick **liver**. (a) TBT dose 0.06 mg kg^{-1}bw d^{-1} exposed for 6 days (b) TBT dose 0.06 mg kg^{-1}bw d^{-1} exposed for 12 days and its possible recovery by natural washing of toxicant (withdrawal), Vitamin B complex and Vitamin C for next 2, 4, 6 days. Data expressed are mean of specific activity ± SD. Abbreviations used in graphs are mentioned in materials and methods chapter.

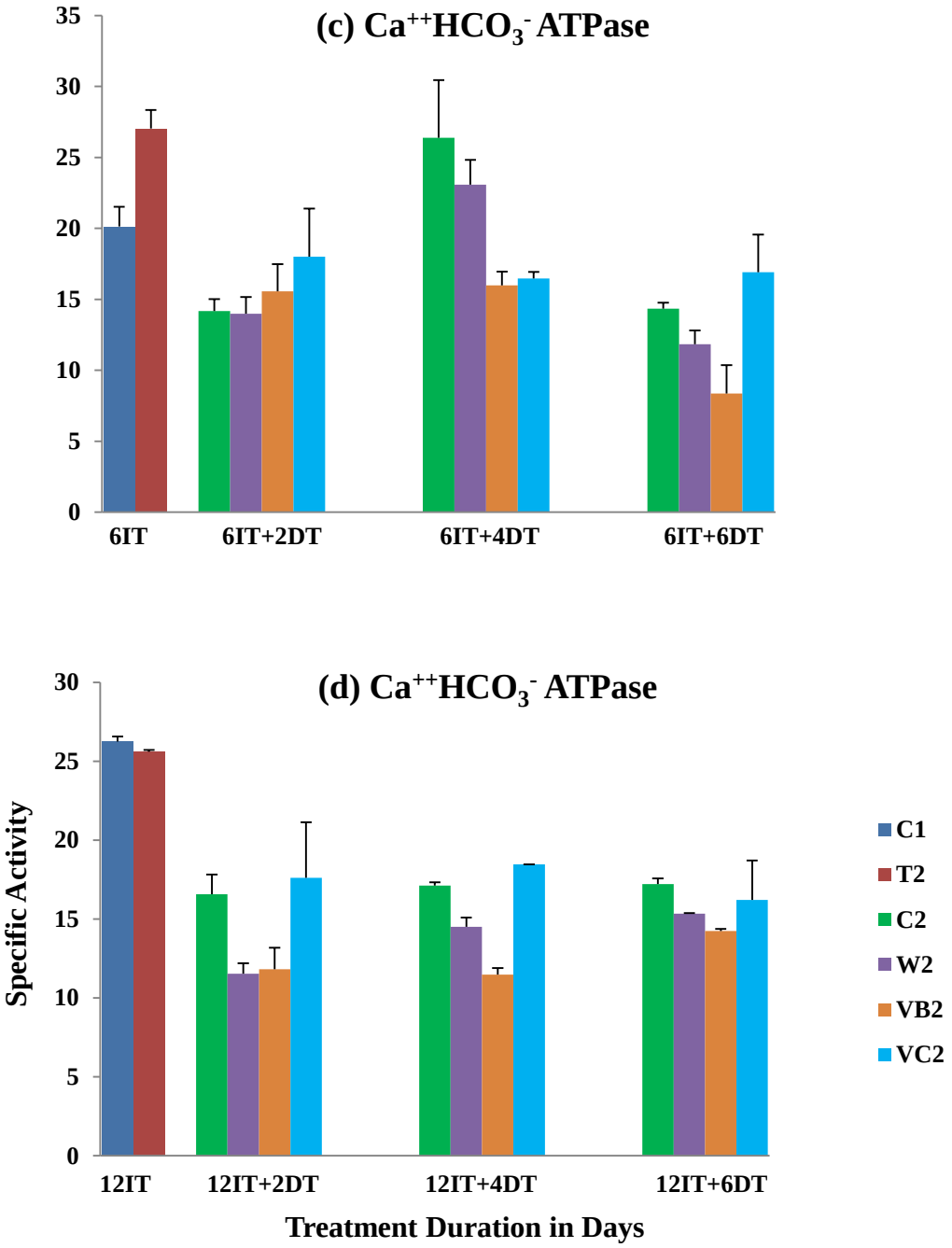


Fig 5 - Changes in the specific activity of **$Ca^{++}HCO_3^-$ ATPase** of chick **live**r (c) TBT dose 0.6 mg kg^{-1}bw d^{-1} exposed for 6 days (d) TBT dose 0.6 mg kg^{-1}bw d^{-1} exposed for 12 days and its possible recovery by natural washing of toxicant (withdrawal), Vitamin B complex and Vitamin C for next 2, 4, 6 days. Data expressed are mean of specific activity ± SD. Abbreviations used in graphs are mentioned in materials and methods chapter.

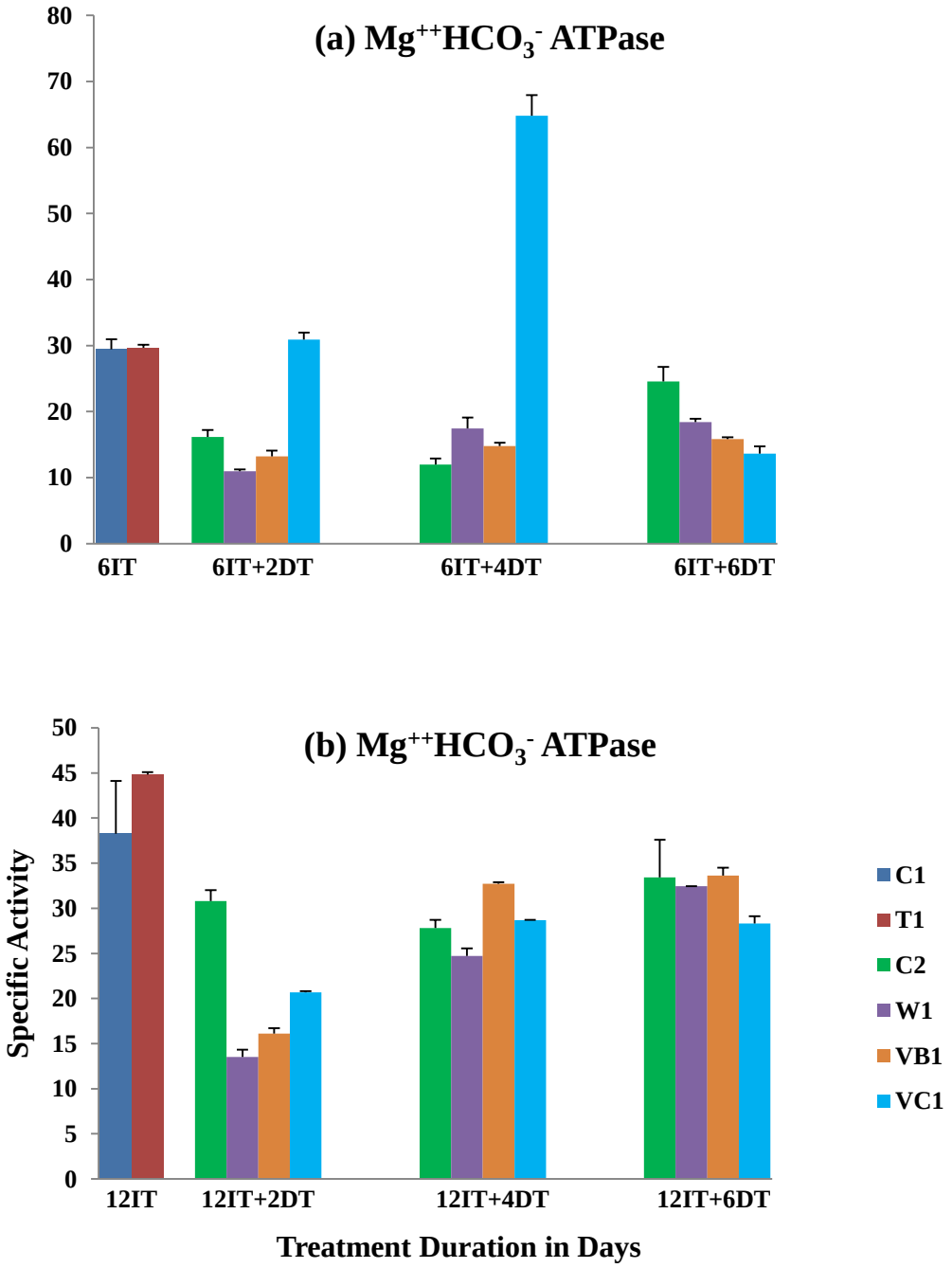


Fig 6 - Changes in the specific activity of **$Mg^{++}HCO_3^-$ ATPase** of chick **live**r. (a) TBT dose 0.06 mg kg^{-1}bw d^{-1} exposed for 6 days (b) TBT dose 0.06 mg kg^{-1}bw d^{-1} exposed for 12 days and its possible recovery by natural washing of toxicant (withdrawal), Vitamin B complex and Vitamin C for next 2, 4, 6 days. Data expressed are mean of specific activity ± SD. Abbreviations used in graphs are mentioned in materials and methods chapter.

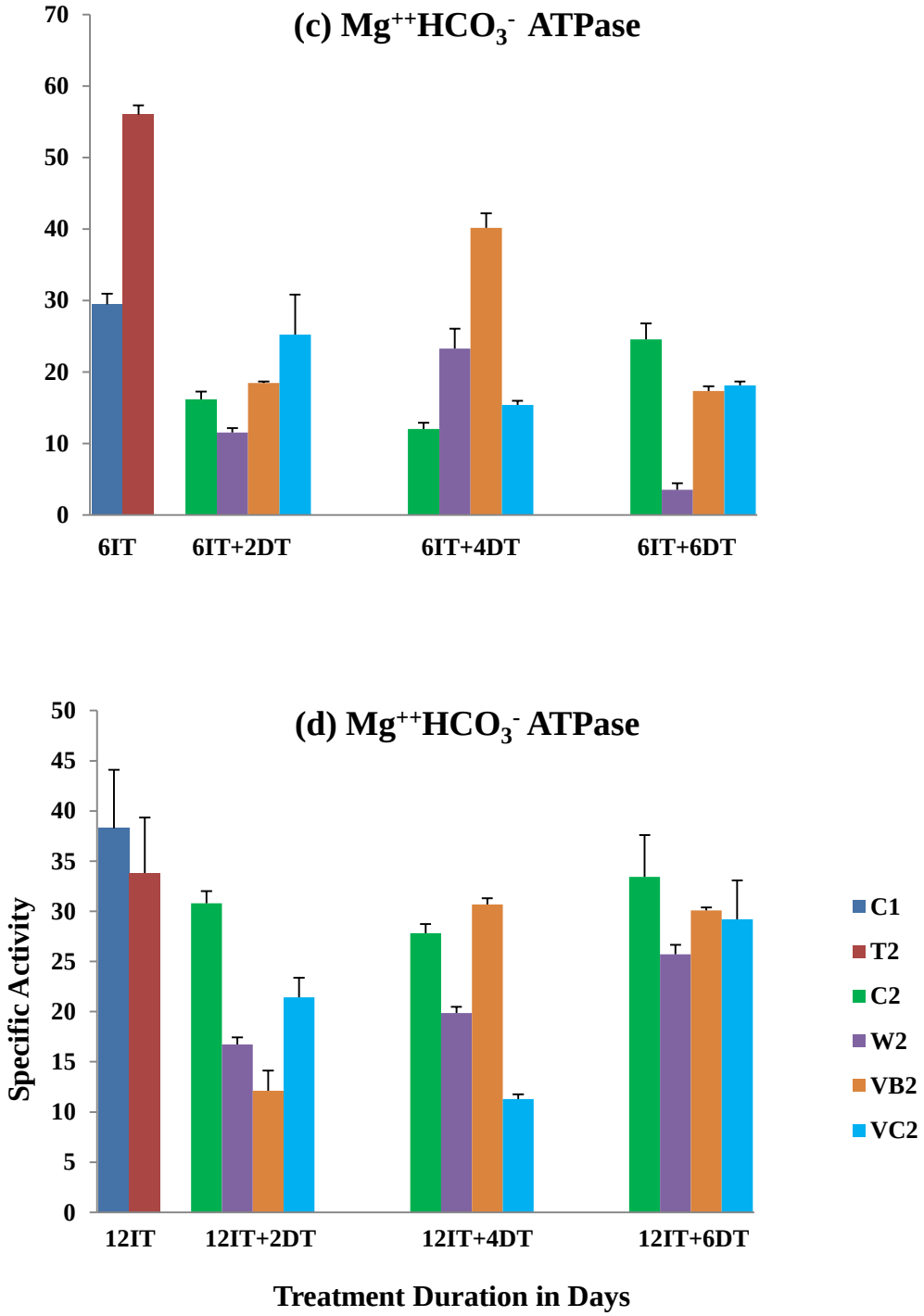


Fig 6 - Changes in the specific activity of **$Mg^{++}HCO_3^-$ ATPase** of chick **live**r. (c) TBT dose 0.6 mg kg^{-1}bw d^{-1} exposed for 6 days (d) TBT dose 0.6 mg kg^{-1}bw d^{-1} exposed for 12 days and its possible recovery by natural washing of toxicant (withdrawal), Vitamin B complex and Vitamin C for next 2, 4, 6 days. Data expressed are mean of specific activity ± SD. Abbreviations used in graphs are mentioned in materials and methods chapter.

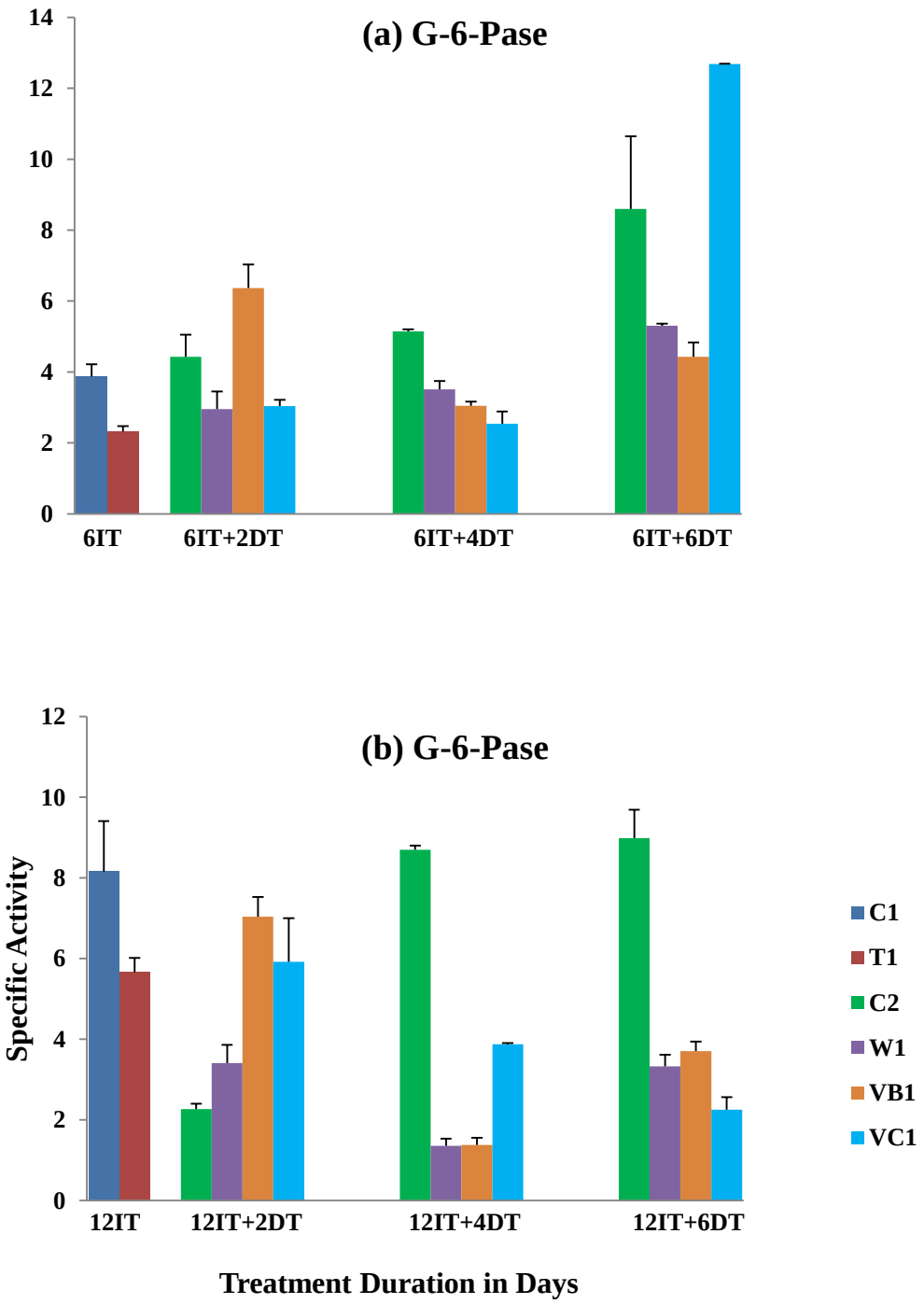


Fig 7 - Changes in the specific activity of **Glucose-6-Phosphatase** of chick **live**r. (a) TBT dose 0.06 mg kg^{-1}bw d^{-1} exposed for 6 days (b) TBT dose 0.06 mg kg^{-1}bw d^{-1} exposed for 12 days and its possible recovery by natural washing of toxicant (withdrawal), Vitamin B complex and Vitamin C for next 2, 4, 6 days. Data expressed are mean of specific activity ± SD. Abbreviations used in graphs are mentioned in materials and methods chapter.

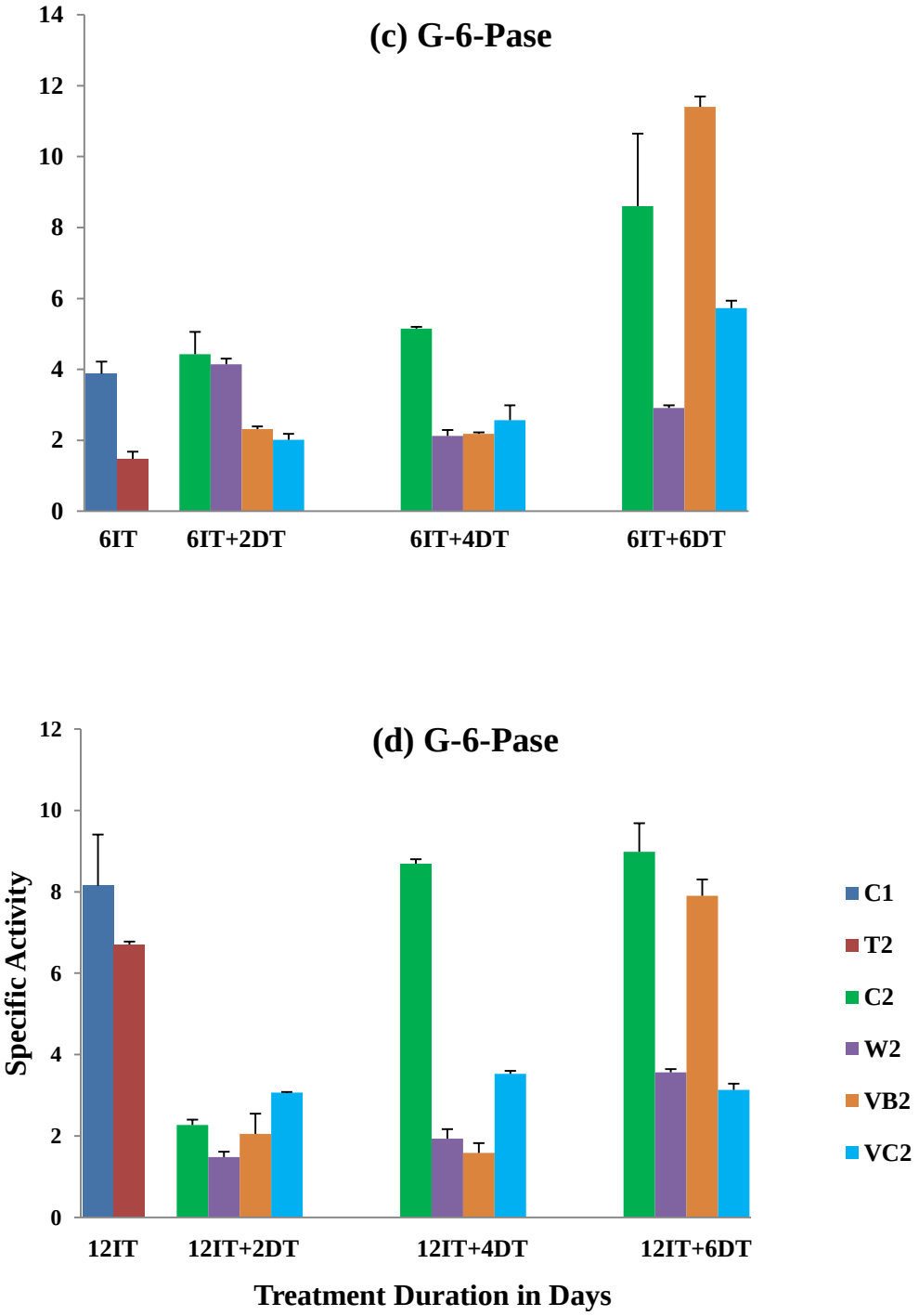


Fig 7 - Changes in the specific activity of **Glucose-6-Phosphatase** of chick **live**r. (c) TBT dose 0.6 mg kg^{-1}bw d^{-1} exposed for 6 days (d) TBT dose 0.6 mg kg^{-1}bw d^{-1} exposed for 12 days and its possible recovery by natural washing of toxicant (withdrawal), Vitamin B complex and Vitamin C for next 2, 4, 6 days. Data expressed are mean of specific activity ± SD. Abbreviations used in graphs are mentioned in materials and methods chapter.

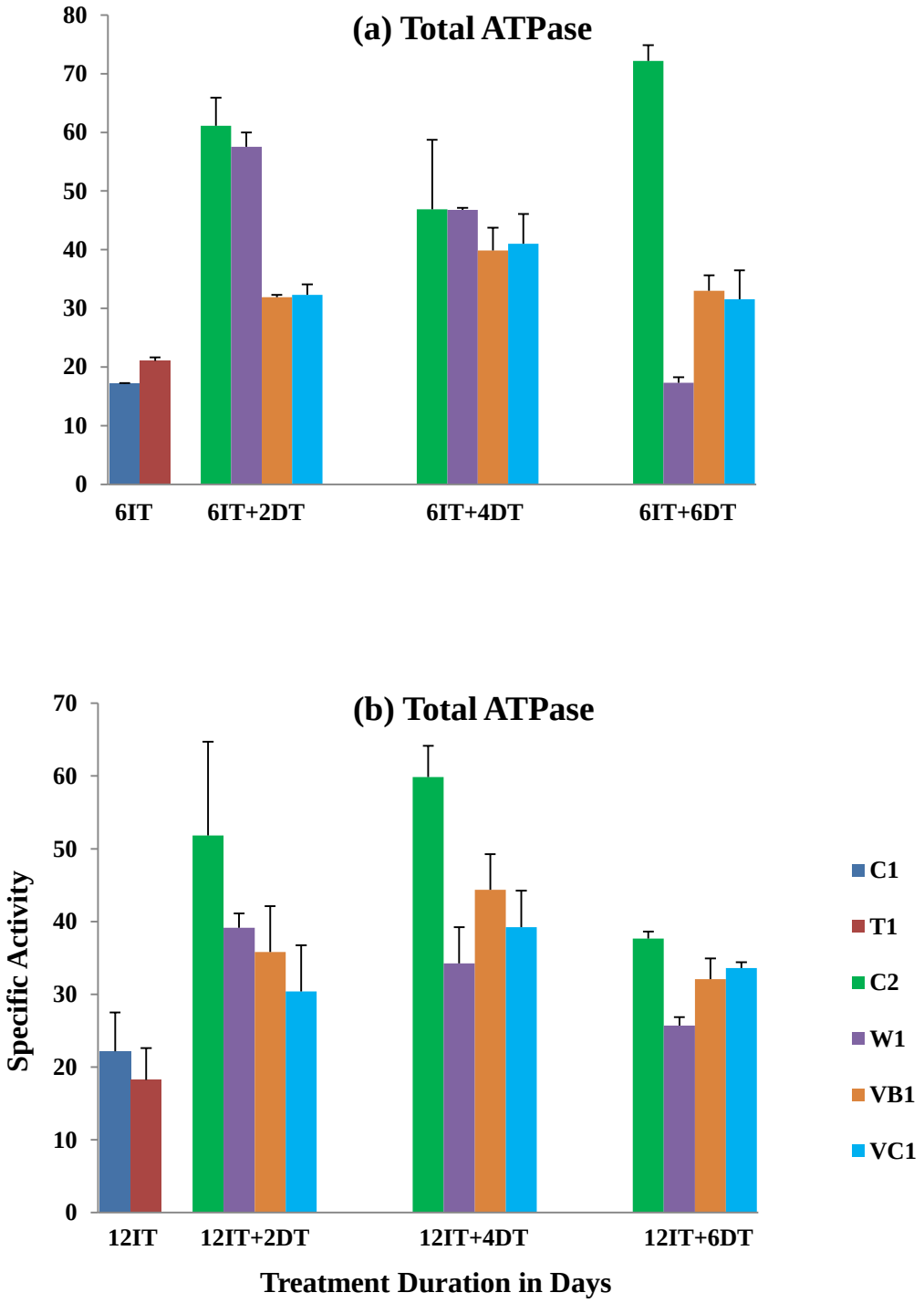
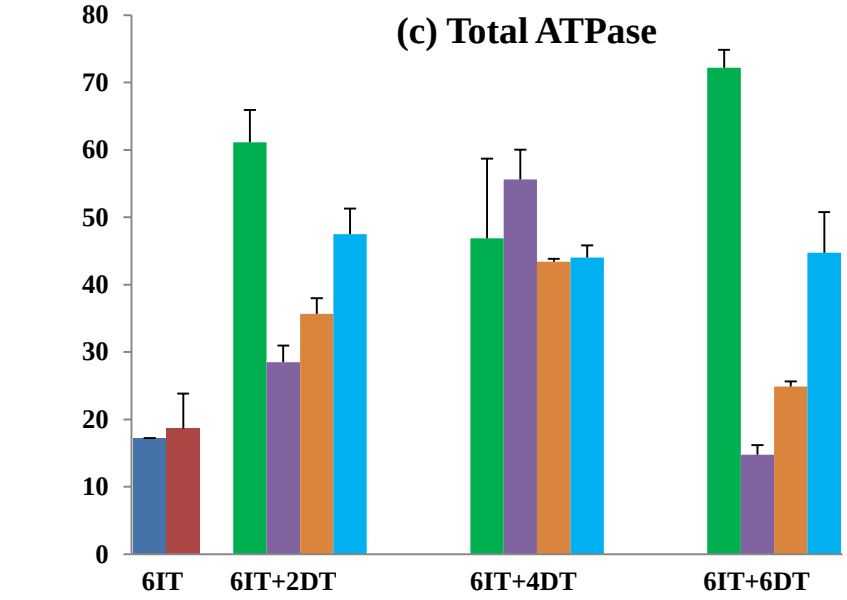


Fig 8 - Changes in the specific activity of **Total ATPase** of chick **kidney**. (a) TBT dose 0.06 mg kg^{-1}bw d^{-1} exposed for 6 days (b) TBT dose 0.06 mg kg^{-1}bw d^{-1} exposed for 12 days and its possible recovery by natural washing of toxicant (withdrawal), Vitamin B complex and Vitamin C for next 2, 4, 6 days. Data expressed are mean of specific activity ± SD. Abbreviations used in graphs are mentioned in materials and methods chapter.



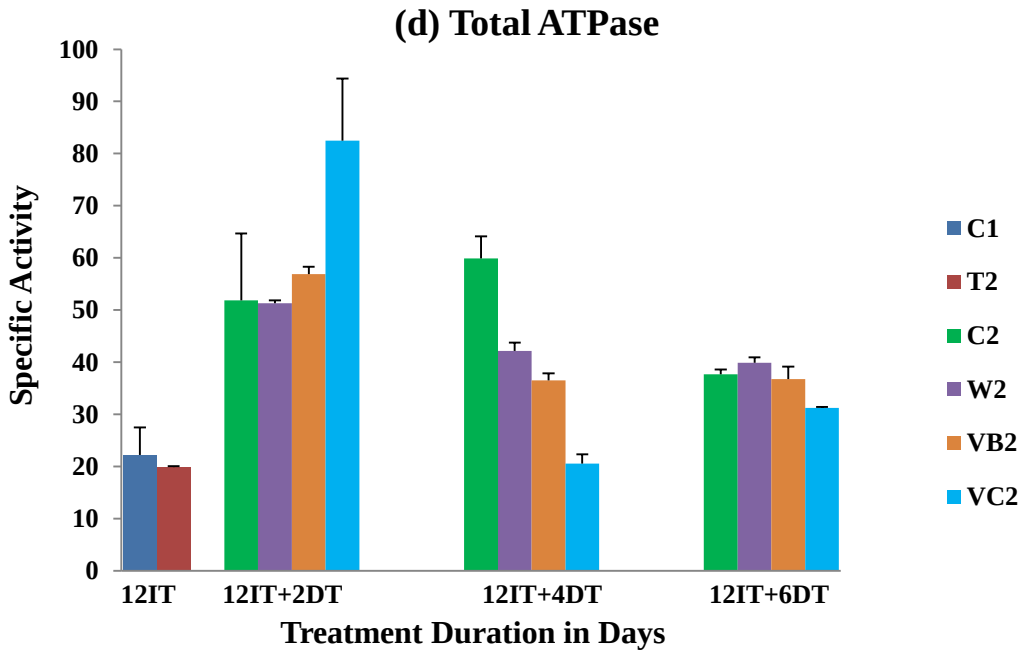


Fig 8 - Changes in the specific activity of **Total ATPase** of chick **kidney**. (c) TBT dose 0.6 mg kg^{-1}bw d^{-1} exposed for 6 days (d) TBT dose 0.6 mg kg^{-1}bw d^{-1} exposed for 12 days and its possible recovery by natural washing of toxicant (withdrawal), Vitamin B complex and Vitamin C for next 2, 4, 6 days. Data expressed are mean of specific activity ± SD. Abbreviations used in graphs are mentioned in materials and methods chapter.

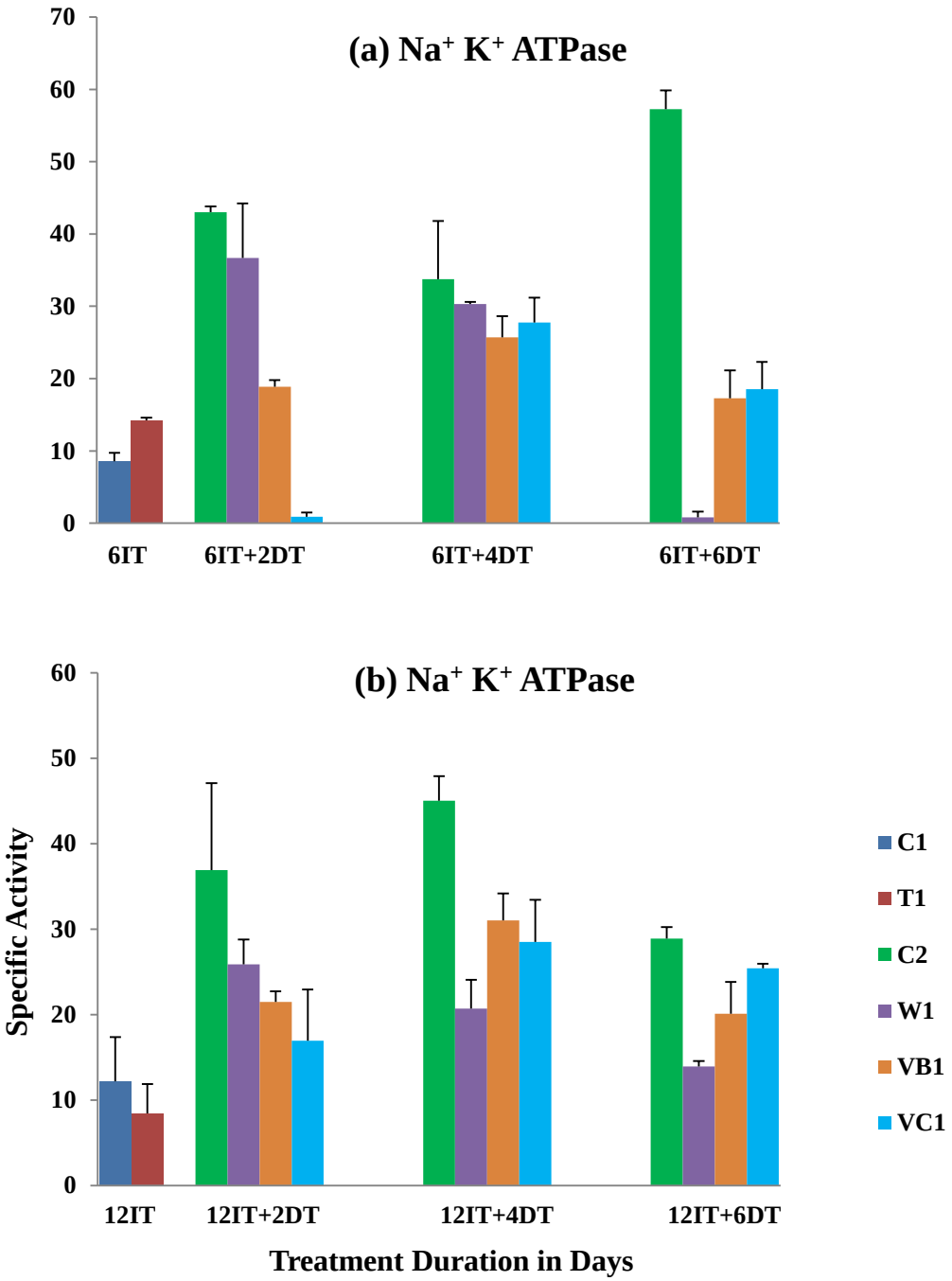


Fig 9 - Changes in the specific activity of **Na^+ K^+ ATPase** of chick **kidney**. (a) TBT dose 0.06 mg kg^{-1}bw d^{-1} exposed for 6 days (b) TBT dose 0.06 mg kg^{-1}bw d^{-1} exposed for 12 days and its possible recovery by natural washing of toxicant (withdrawal), Vitamin B complex and Vitamin C for next 2, 4, 6 days. Data expressed are mean of specific activity ± SD. Abbreviations used in graphs are mentioned in materials and methods chapter.

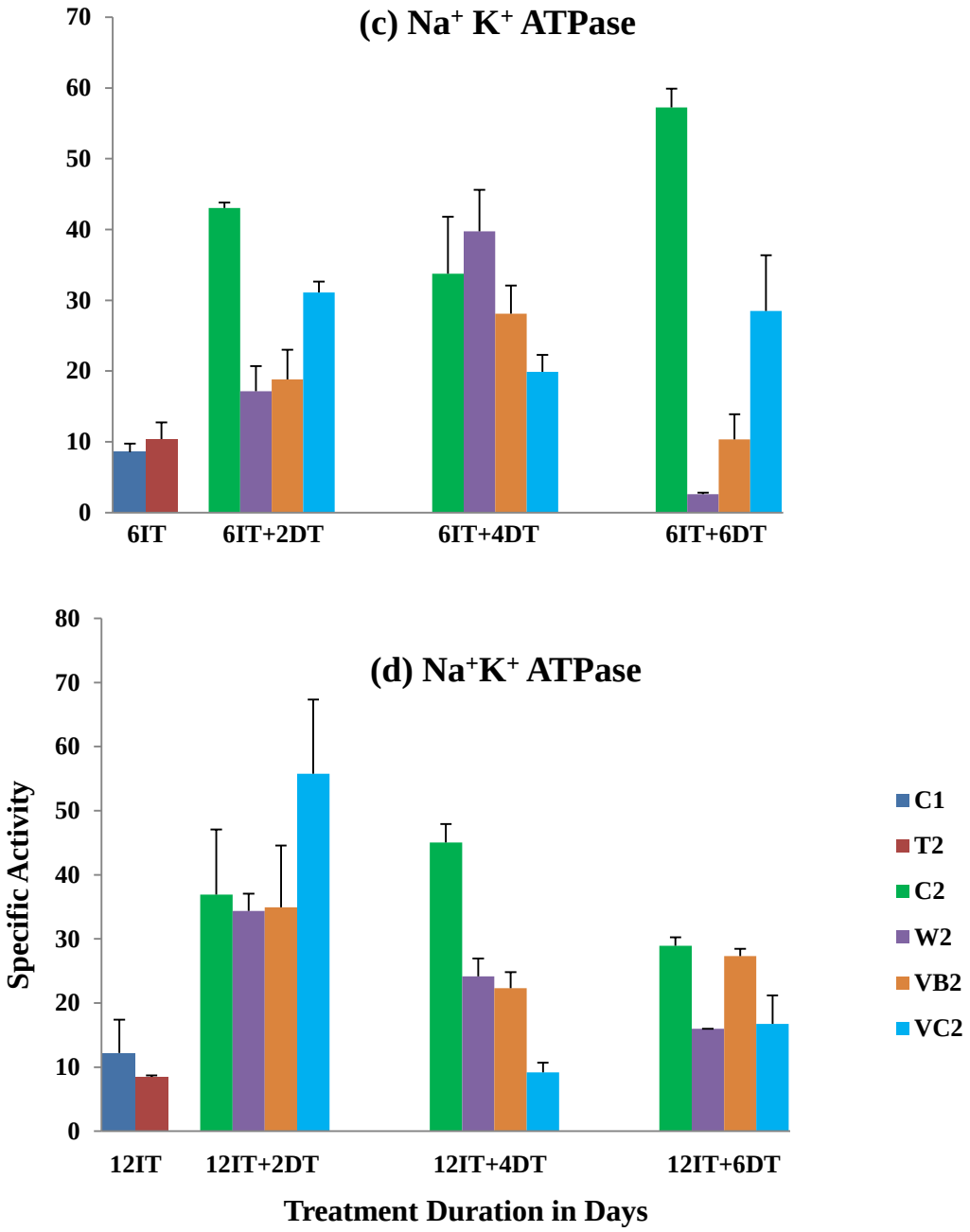


Fig 9 - Changes in the specific activity of **Na^+ K^+ ATPase** of chick **kidney**. (c) TBT dose 0.6 mg kg^{-1}bw d^{-1} exposed for 6 days (d) TBT dose 0.6 mg kg^{-1}bw d^{-1} exposed for 12 days and its possible recovery by natural washing of toxicant (withdrawal), Vitamin B complex and Vitamin C for next 2, 4, 6 days. Data expressed are mean of specific activity ± SD. Abbreviations used in graphs are mentioned in materials and methods chapter.

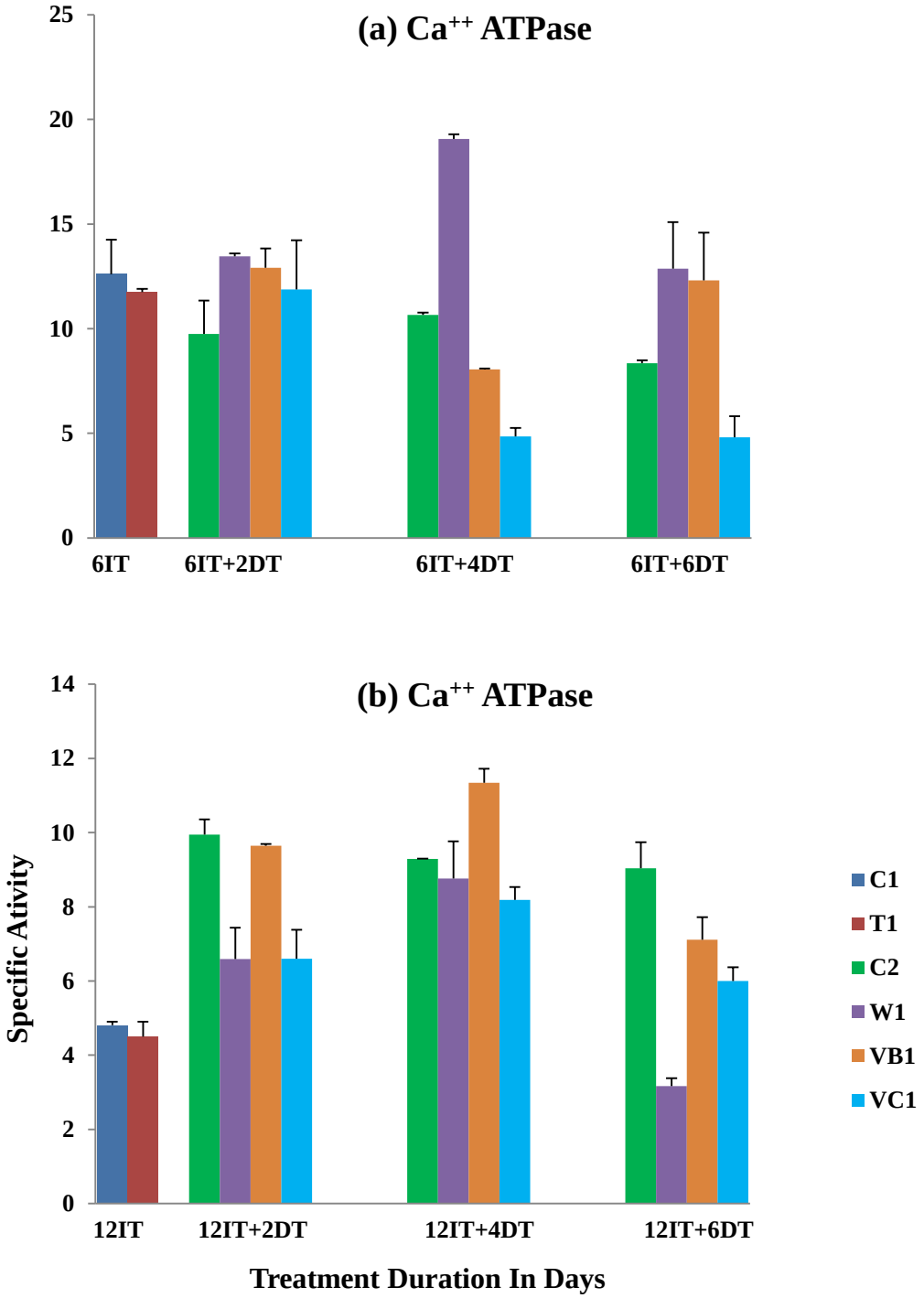
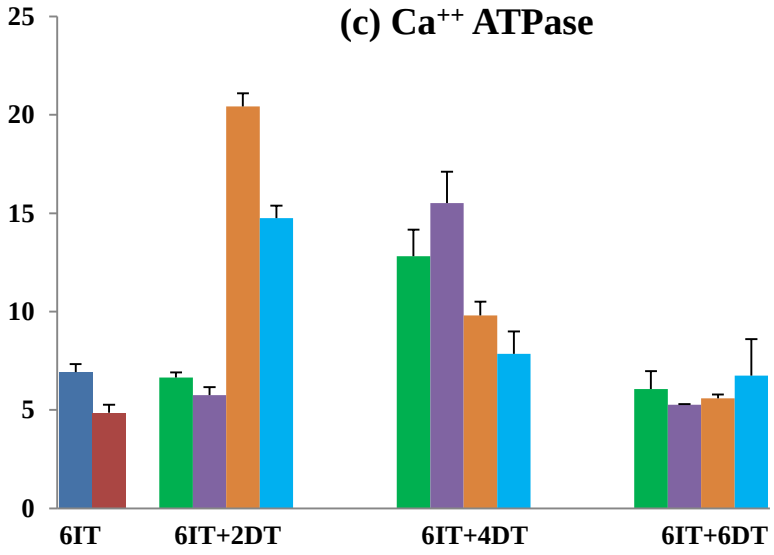


Fig 10 - Changes in the specific activity of **Ca^{++} ATPase** of chick **kidney**. (a) TBT dose 0.06 mg kg^{-1}bw d^{-1} exposed for 6 days (b) TBT dose 0.06 mg kg^{-1}bw d^{-1} exposed for 12 days and its possible recovery by natural washing of toxicant (withdrawal), Vitamin B complex and Vitamin C for next 2, 4, 6 days. Data expressed are mean of specific activity ± SD. Abbreviations used in graphs are mentioned in materials and methods chapter.



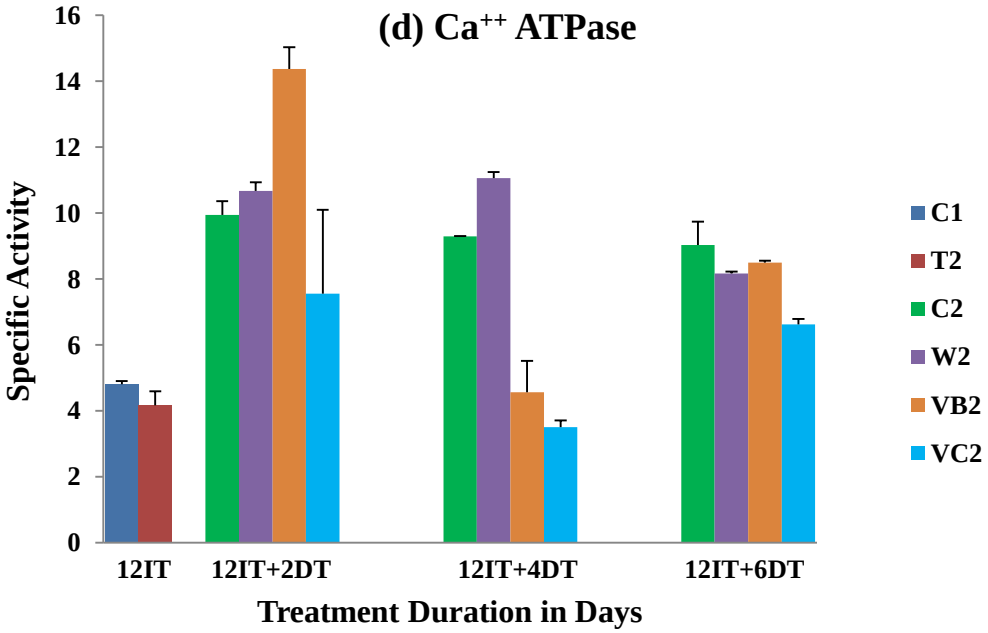


Fig 10 - Changes in the specific activity of **Ca^{++} ATPase** of chick **kidney**. (c) TBT dose 0.6 mg kg^{-1}bw d^{-1} exposed for 6 days (d) TBT dose 0.6 mg kg^{-1}bw d^{-1} exposed for 12 days and its possible recovery by natural washing of toxicant (withdrawal), Vitamin B complex and Vitamin C for next 2, 4, 6 days. Data expressed are mean of specific activity ± SD. Abbreviations used in graphs are mentioned in materials and methods chapter.

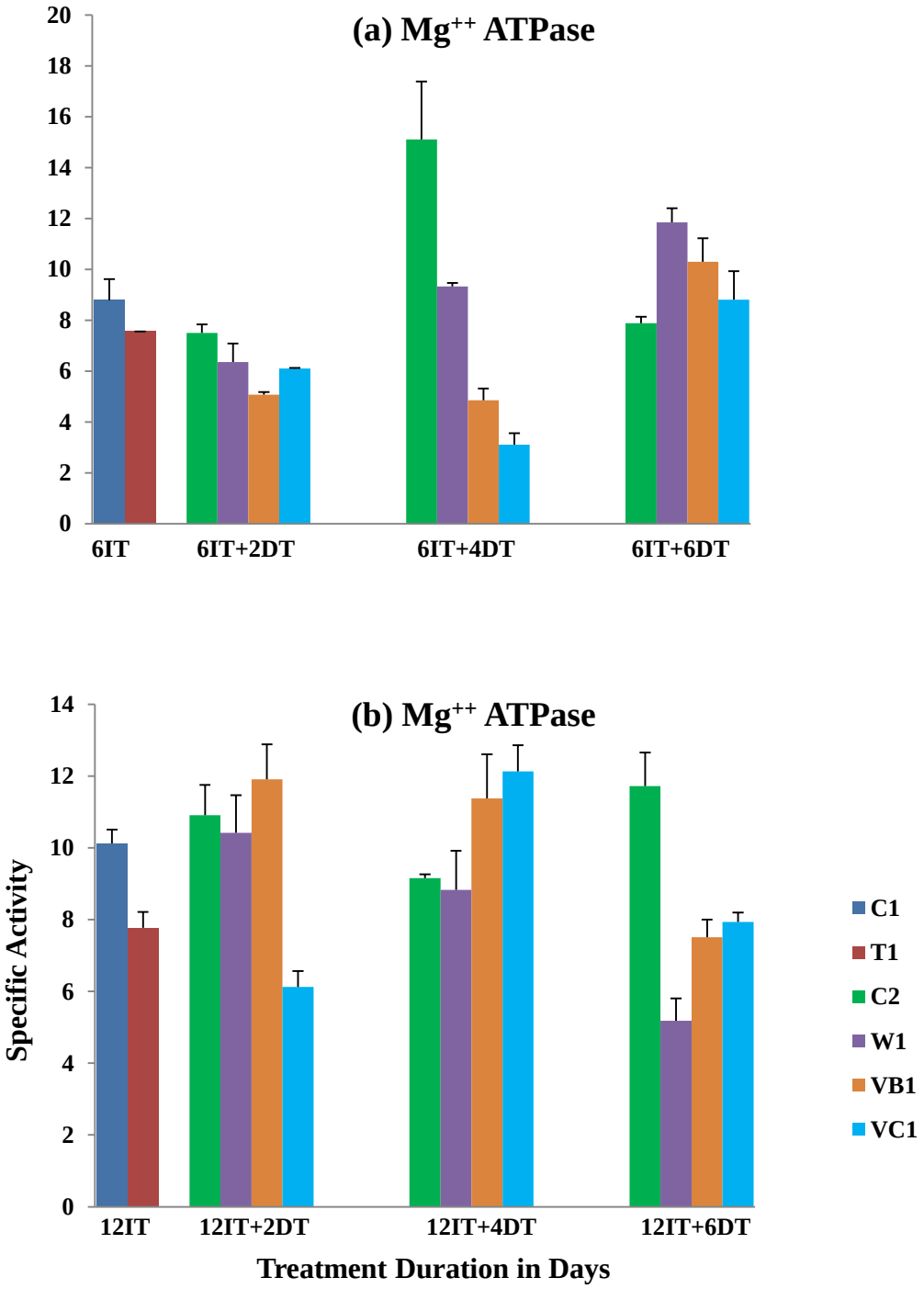
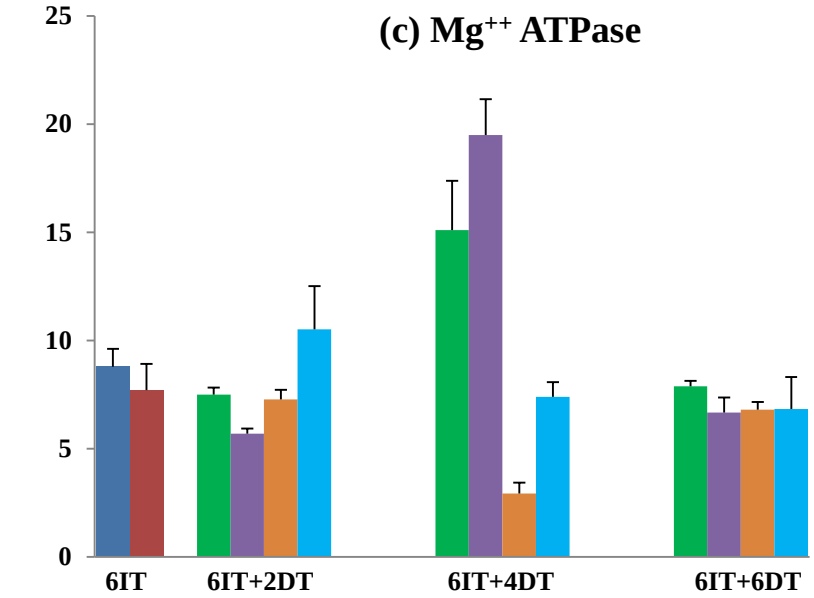


Fig 11 - Changes in the specific activity of **Mg^{++} ATPase** of chick **kidney**. (a) TBT dose 0.06 mg kg^{-1}bw d^{-1} exposed for 6 days (b) TBT dose 0.06 mg kg^{-1}bw d^{-1} exposed for 12 days and its possible recovery by natural washing of toxicant (withdrawal), Vitamin B complex and Vitamin C for next 2, 4, 6 days. Data expressed are mean of specific activity ± SD. Abbreviations used in graphs are mentioned in materials and methods chapter.



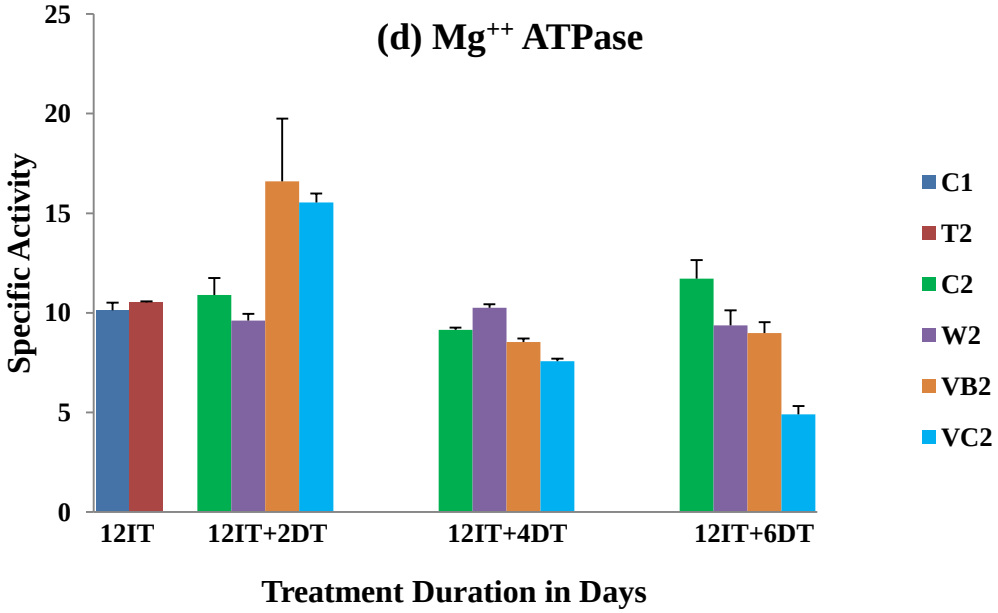


Fig 11 - Changes in the specific activity of **Mg^{++} ATPase** of chick **kidney**. (c) TBT dose 0.6 mg kg^{-1}bw d^{-1} exposed for 6 days (d) TBT dose 0.6 mg kg^{-1}bw d^{-1} exposed for 12 days and its possible recovery by natural washing of toxicant (withdrawal), Vitamin B complex and Vitamin C for next 2, 4, 6 days. Data expressed are mean of specific activity ± SD. Abbreviations used in graphs are mentioned in materials and methods chapter.

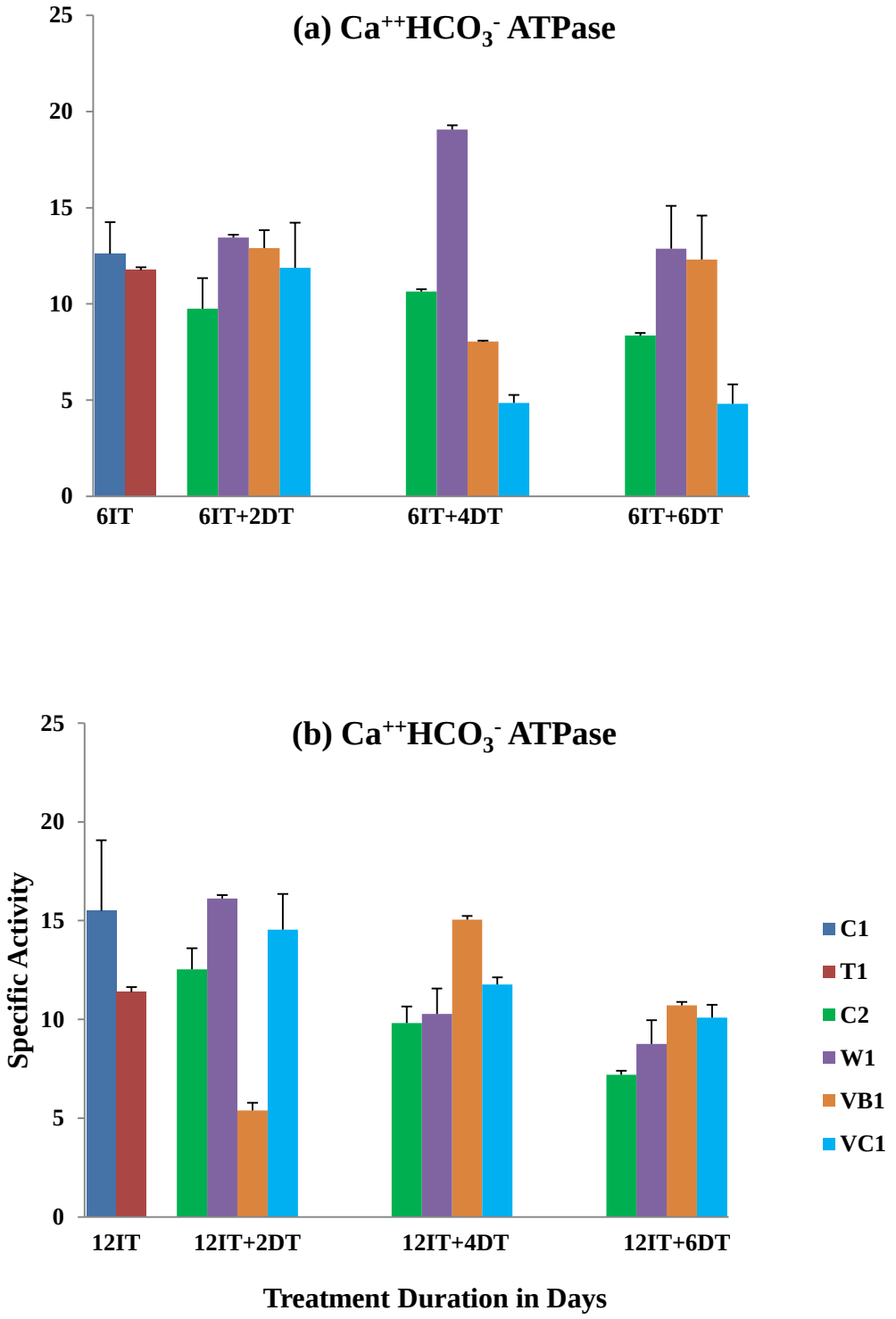


Fig 12 - Changes in the specific activity of **$Ca^{++}HCO_3^-$ ATPase** of chick **kidney**. (a) TBT dose 0.06 mg kg^{-1}bw d^{-1} exposed for 6 days (b) TBT dose 0.06 mg kg^{-1}bw d^{-1} exposed for 12 days and its possible recovery by natural washing of toxicant (withdrawal), Vitamin B complex and Vitamin C for next 2, 4, 6 days. Data expressed are mean of specific activity ± SD. Abbreviations used in graphs are mentioned in materials and methods chapter.

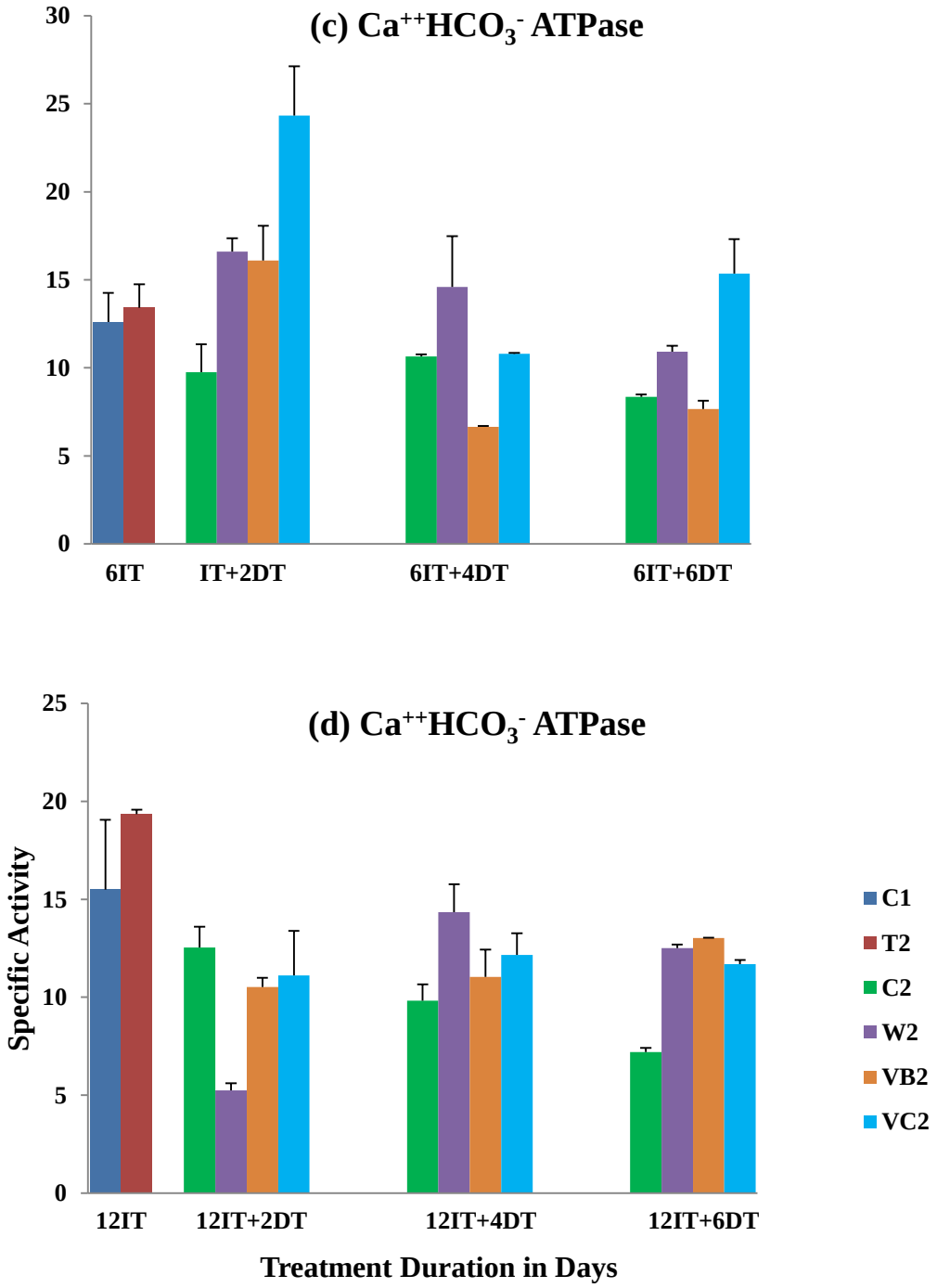


Fig 12 - Changes in the specific activity of **$Ca^{++}HCO_3^-$ ATPase** of chick **kidney**. (c) TBT dose 0.6 mg kg^{-1}bw d^{-1} exposed for 6 days (d) TBT dose 0.6 mg kg^{-1}bw d^{-1} exposed for 12 days and its possible recovery by natural washing of toxicant (withdrawal), Vitamin B complex and Vitamin C for next 2, 4, 6 days. Data expressed are mean of specific activity ± SD. Abbreviations used in graphs are mentioned in materials and methods chapter.

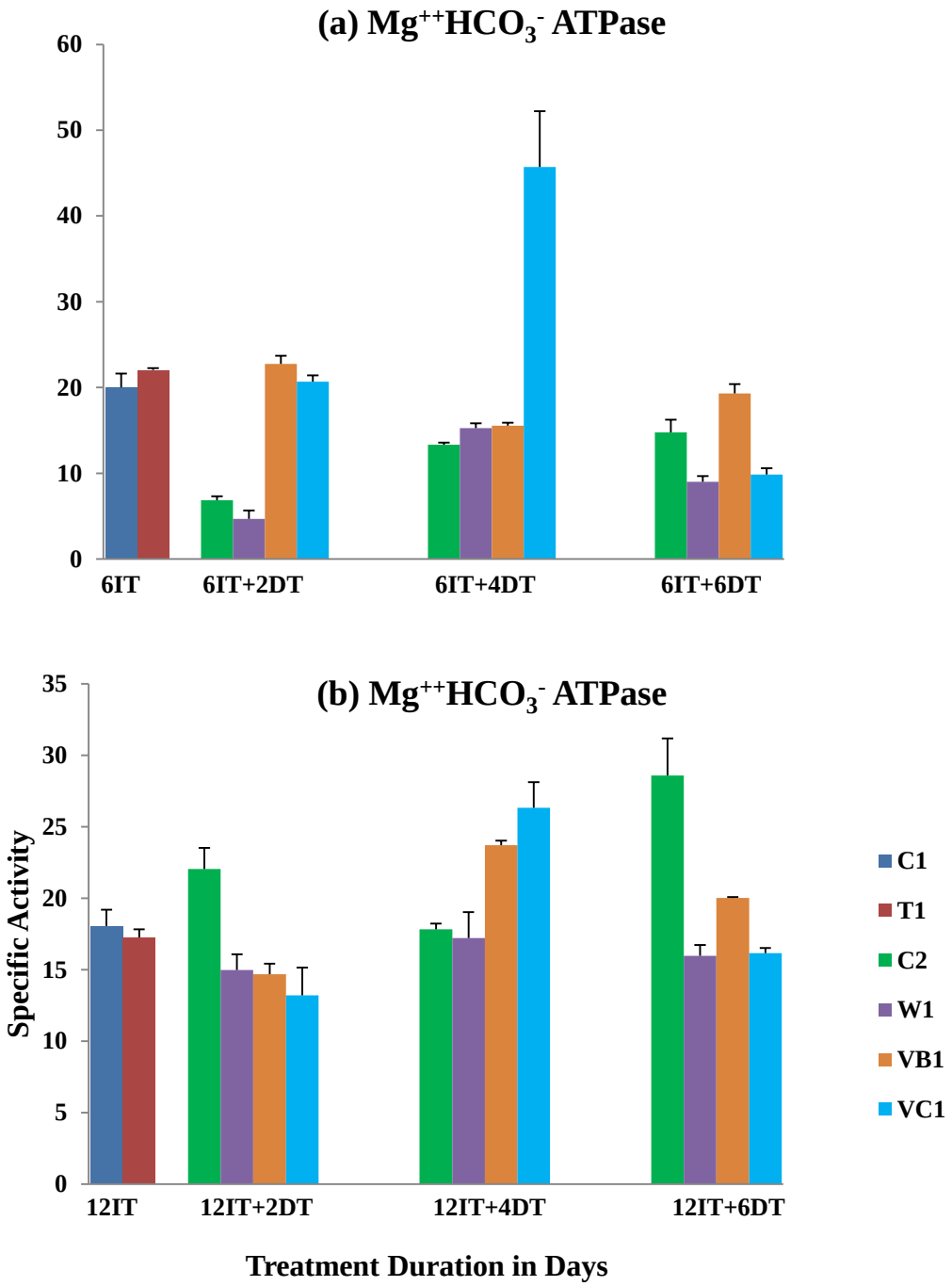


Fig 13 - Changes in the specific activity of **$Mg^{++}HCO_3^-$ ATPase** of chick **kidney.** (a) TBT dose 0.06 mg kg^{-1}bw d^{-1} exposed for 6 days (b) TBT dose 0.06 mg kg^{-1}bw d^{-1} exposed for 12 days and its possible recovery by natural washing of toxicant (withdrawal), Vitamin B complex and Vitamin C for next 2, 4, 6 days. Data expressed are mean of specific activity ± SD. Abbreviations used in graphs are mentioned in materials and methods chapter.

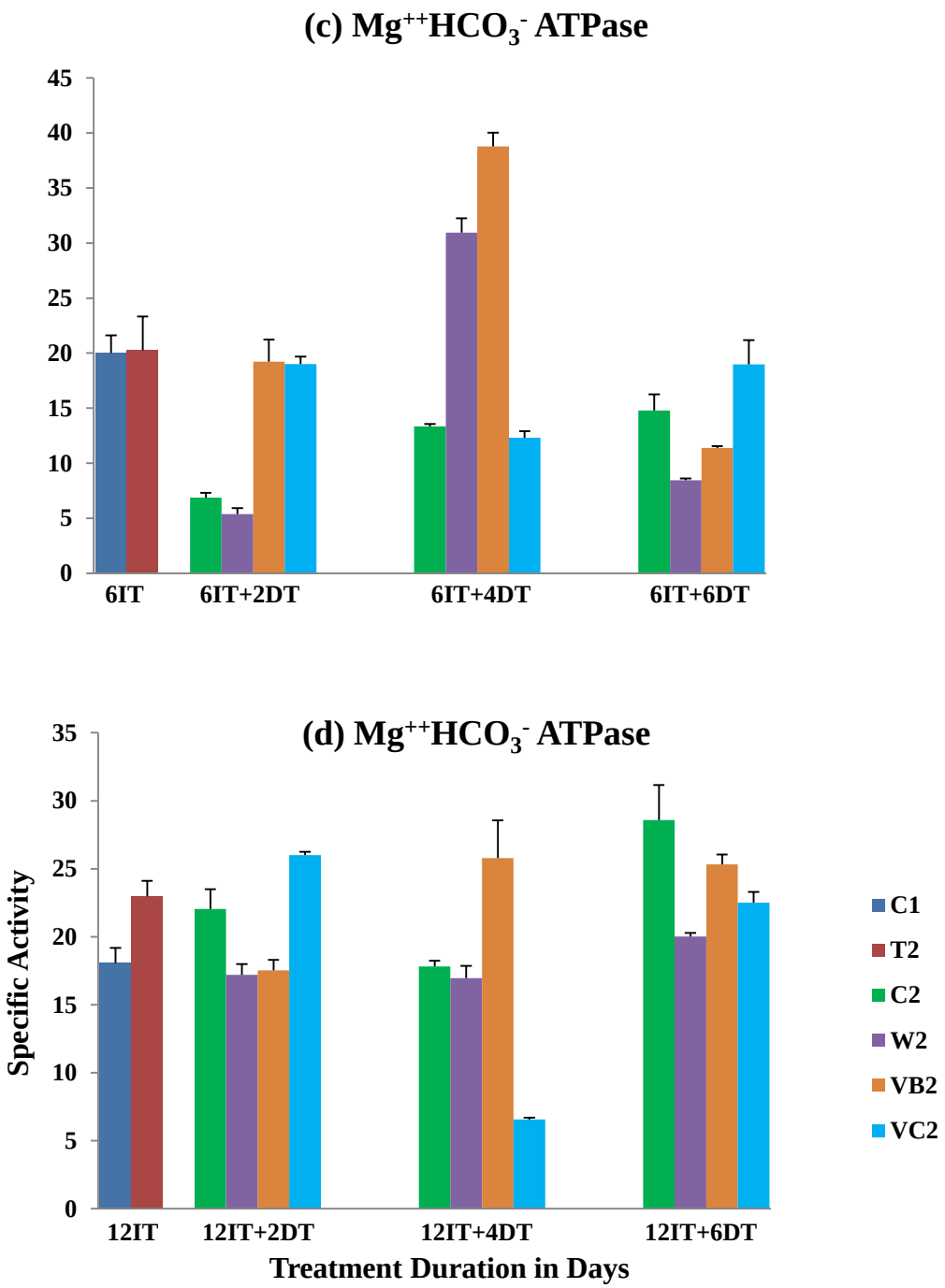


Fig 13 - Changes in the specific activity of **$Mg^{++}HCO_3^-$ ATPase** of chick **kidney.** (c) TBT dose 0.6 mg kg^{-1}bw d^{-1} exposed for 6 days (d) TBT dose 0.6 mg kg^{-1}bw d^{-1} exposed for 12 days and its possible recovery by natural washing of toxicant (withdrawal), Vitamin B complex and Vitamin C for next 2, 4, 6 days. Data expressed are mean of specific activity ± SD. Abbreviations used in graphs are mentioned in materials and methods chapter.

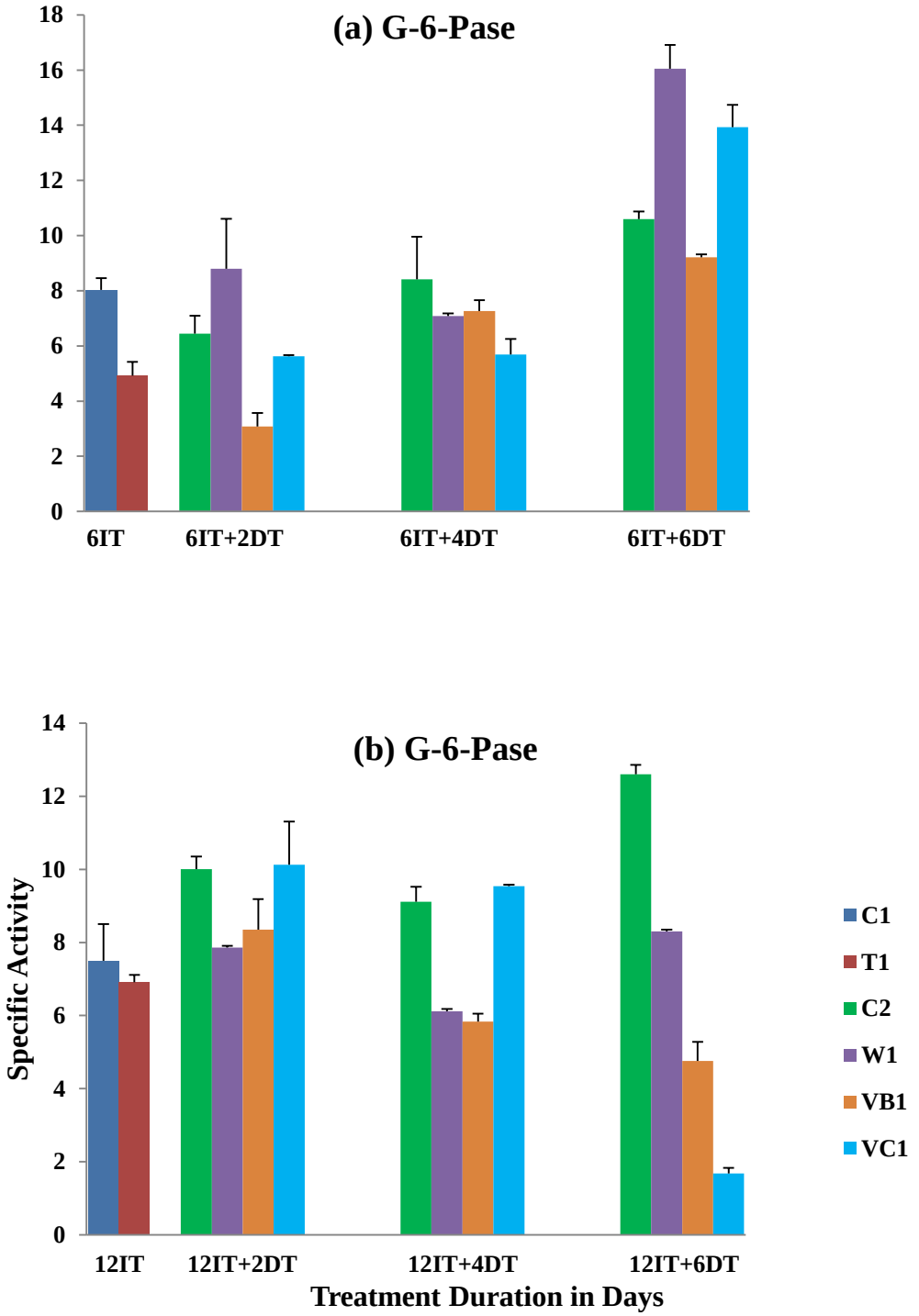


Fig 14 - Changes in the specific activity of **Glucose-6-Phosphatase** of chick **kidney**. (a) TBT dose 0.06 mg kg^{-1}bw d^{-1} exposed for 6 days (b) TBT dose 0.06 mg kg^{-1}bw d^{-1} exposed for 12 days and its possible recovery by natural washing of toxicant (withdrawal), Vitamin B complex and Vitamin C for next 2, 4, 6 days. Data expressed are mean of specific activity ± SD. Abbreviations used in graphs are mentioned in materials and methods chapter.

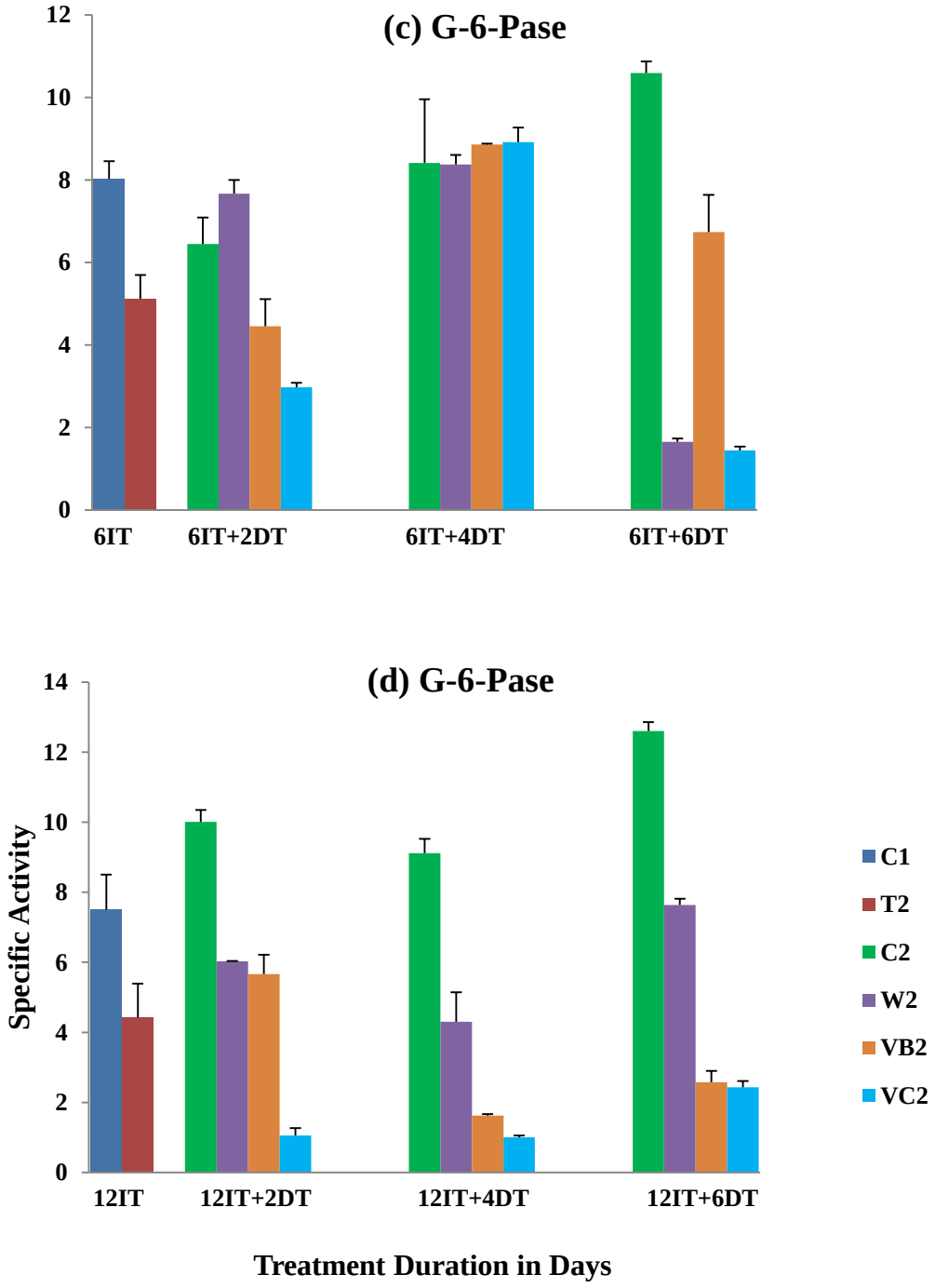


Fig 14 - Changes in the specific activity of **Glucose-6-Phosphatase** of chick **kidney**. (c) TBT dose 0.6 mg kg^{-1}bw d^{-1} exposed for 6 days (d) TBT dose 0.6 mg kg^{-1}bw d^{-1} exposed for 12 days and its possible recovery by natural washing of toxicant (withdrawal), Vitamin B complex and Vitamin C for next 2, 4, 6 days. Data expressed are mean of specific activity ± SD. Abbreviations used in graphs are mentioned in materials and methods chapter.

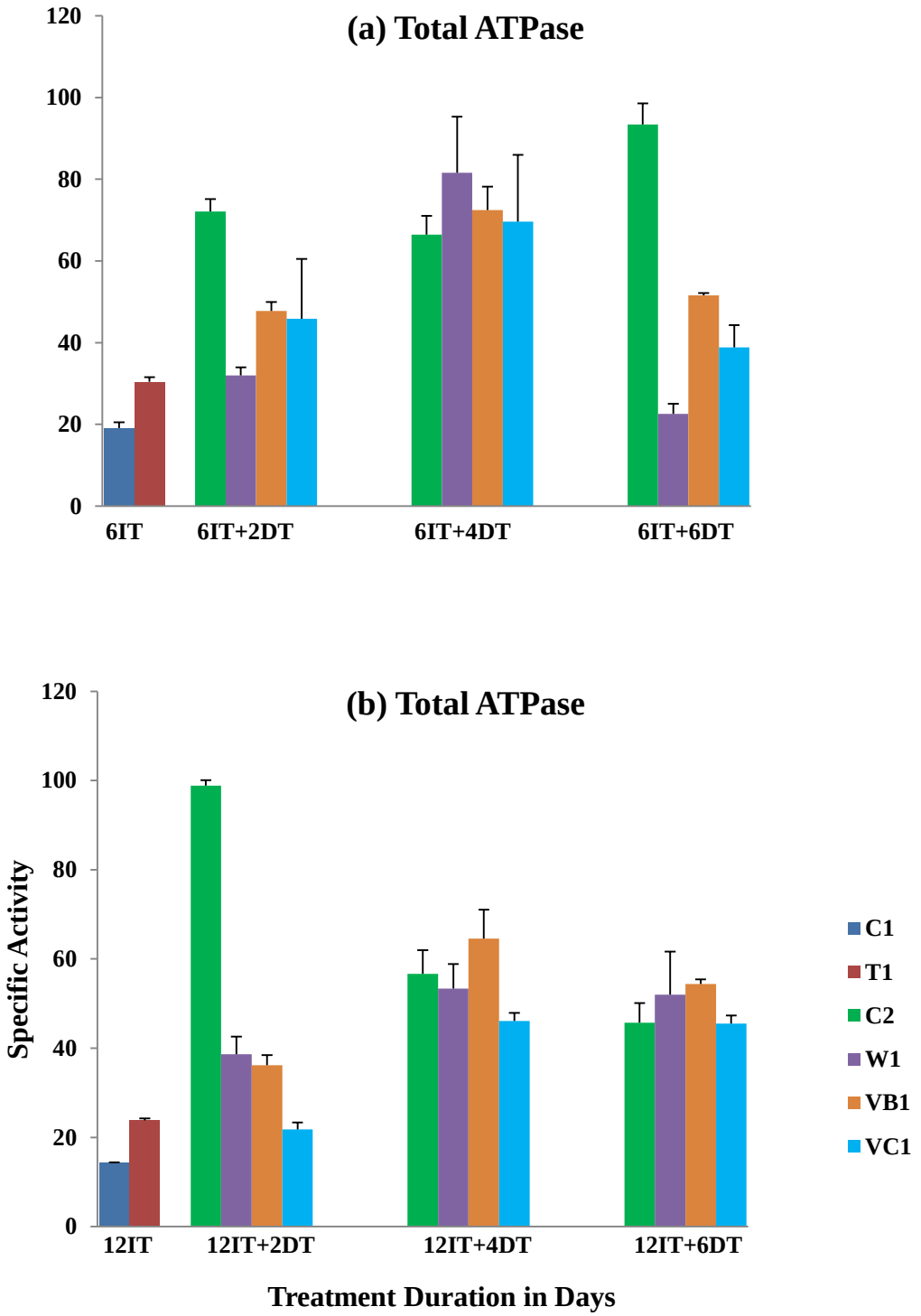


Fig 15 - Changes in the specific activity of **Total ATPase** of chick **brain**. (a) TBT dose 0.06 mg kg^{-1}bw d^{-1} exposed for 6 days (b) TBT dose 0.06 mg kg^{-1}bw d^{-1} exposed for 12 days and its possible recovery by natural washing of toxicant (withdrawal), Vitamin B complex and Vitamin C for next 2, 4, 6 days. Data expressed are mean of specific activity ± SD. Abbreviations used in graphs are mentioned in materials and methods chapter.

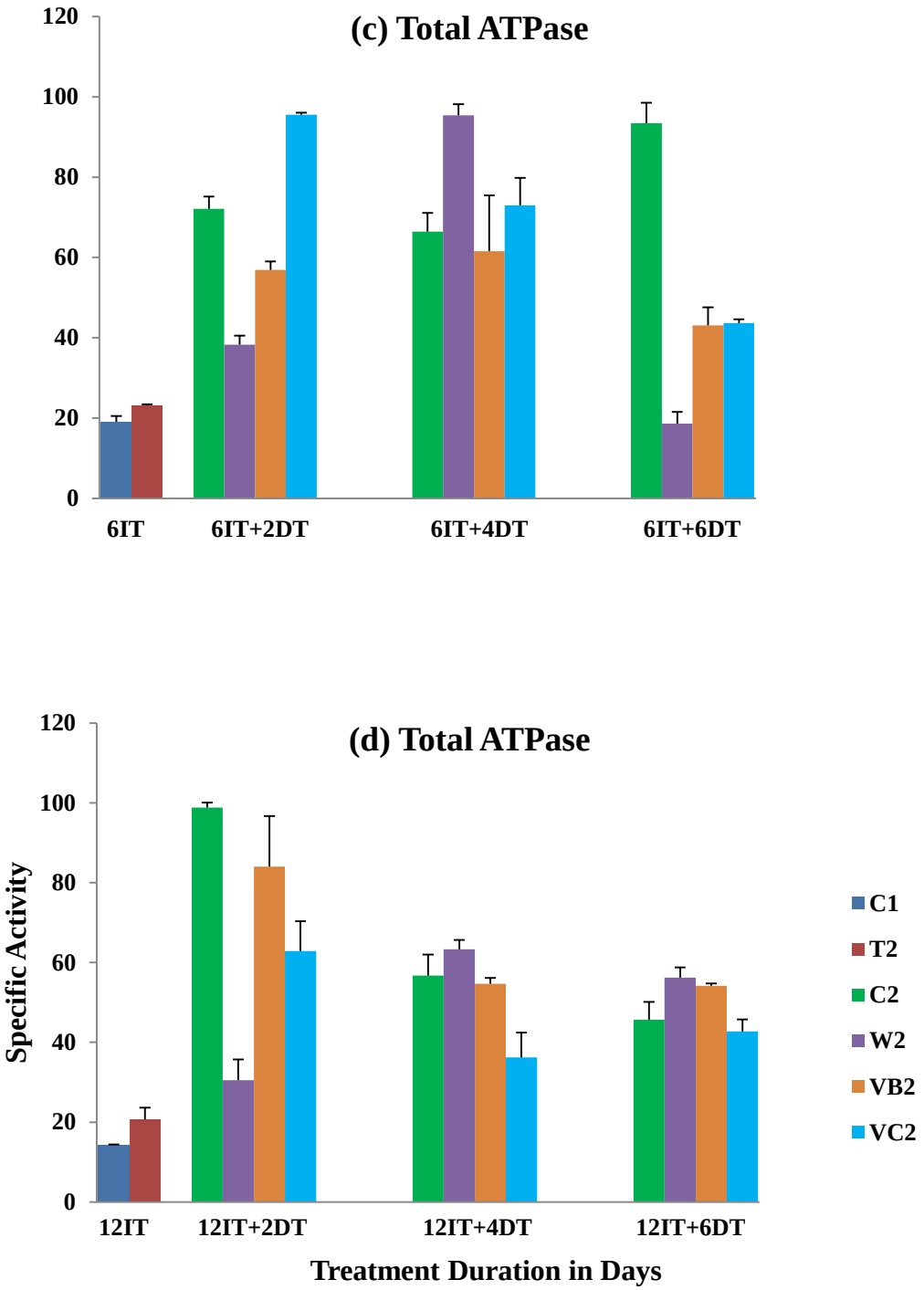


Fig 15 - Changes in the specific activity of **Total ATPase** of chick **brain**. (c) TBT dose 0.6 mg kg^{-1}bw d^{-1} exposed for 6 days (d) TBT dose 0.6 mg kg^{-1}bw d^{-1} exposed for 12 days and its possible recovery by natural washing of toxicant (withdrawal), Vitamin B complex and Vitamin C for next 2, 4, 6 days. Data expressed are mean of specific activity ± SD. Abbreviations used in graphs are mentioned in materials and methods chapter.

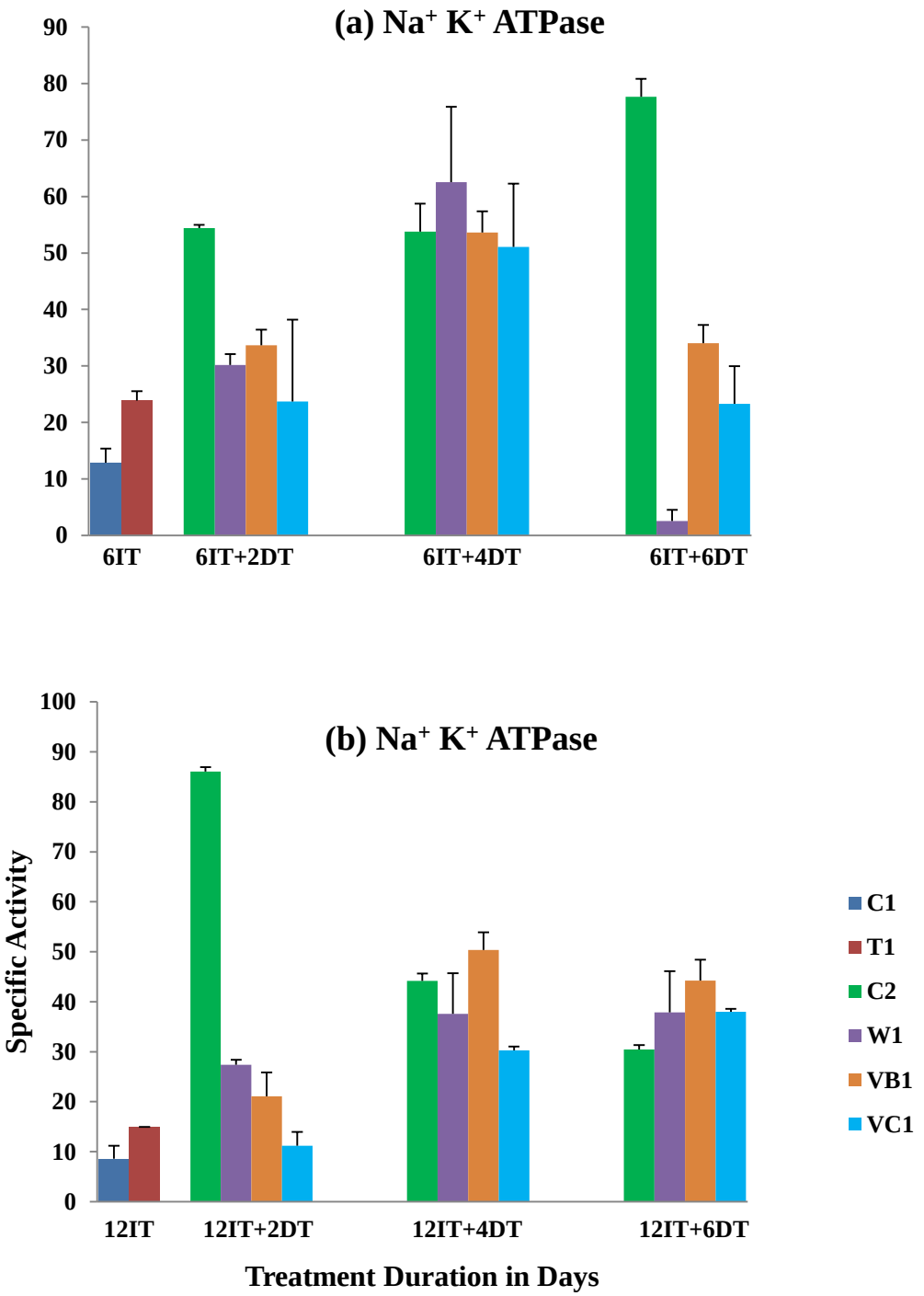


Fig 16 - Changes in the specific activity of **Na^+ K^+ ATPase** of chick **brain**. (a) TBT dose 0.06 mg kg^{-1}bw d^{-1} exposed for 6 days (b) TBT dose 0.06 mg kg^{-1}bw d^{-1} exposed for 12 days and its possible recovery by natural washing of toxicant (withdrawal), Vitamin B complex and Vitamin C for next 2, 4, 6 days. Data expressed are mean of specific activity ± SD. Abbreviations used in graphs are mentioned in materials and methods chapter.

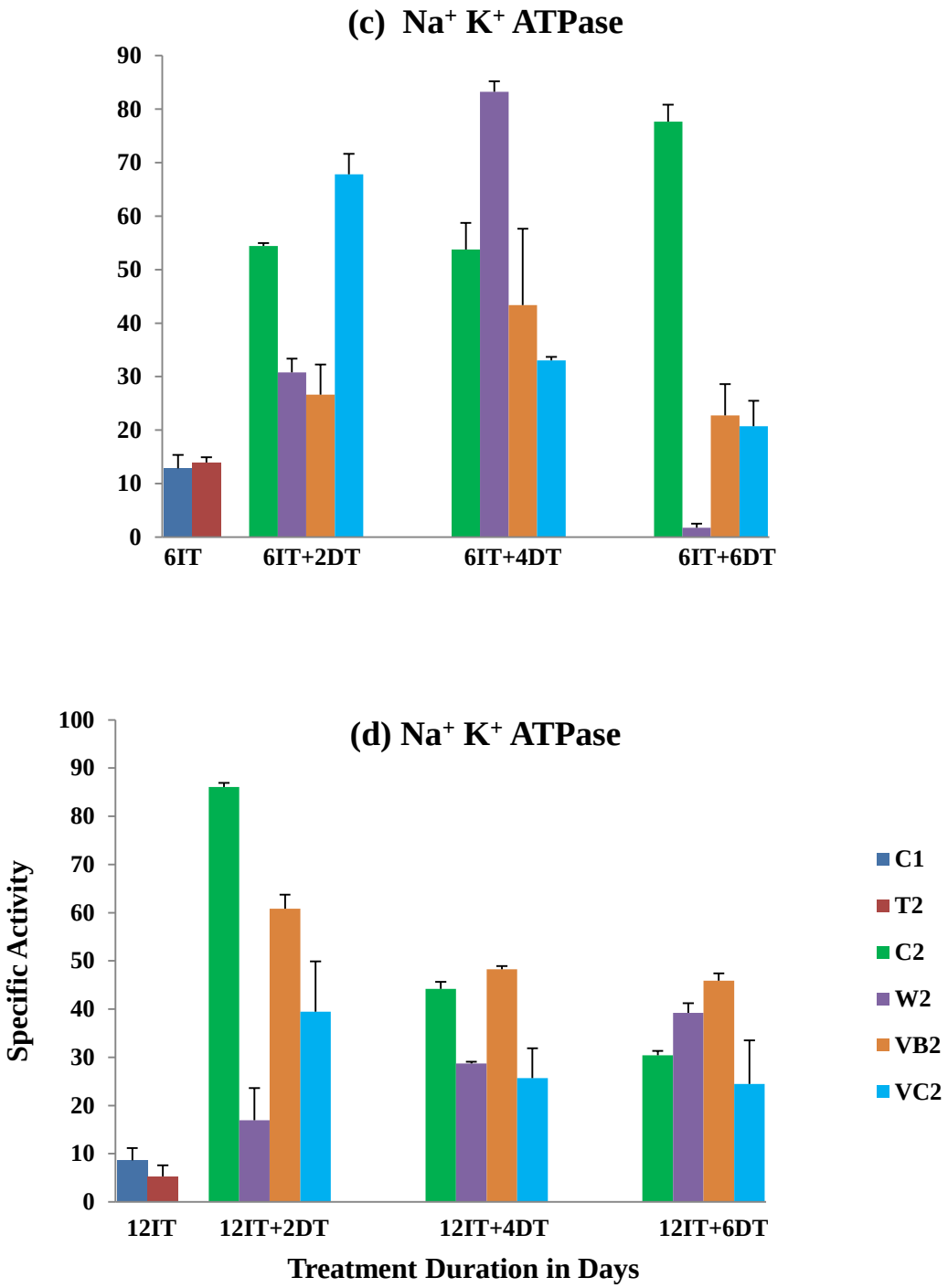


Fig 16 - Changes in the specific activity of **Na^+ K^+ ATPase** of chick **brain**. (c) TBT dose 0.6 mg kg^{-1}bw d^{-1} exposed for 6 days (d) TBT dose 0.6 mg kg^{-1}bw d^{-1} exposed for 12 days and its possible recovery by natural washing of toxicant (withdrawal), Vitamin B complex and Vitamin C for next 2, 4, 6 days. Data expressed are mean of specific activity ± SD. Abbreviations used in graphs are mentioned in materials and methods chapter.

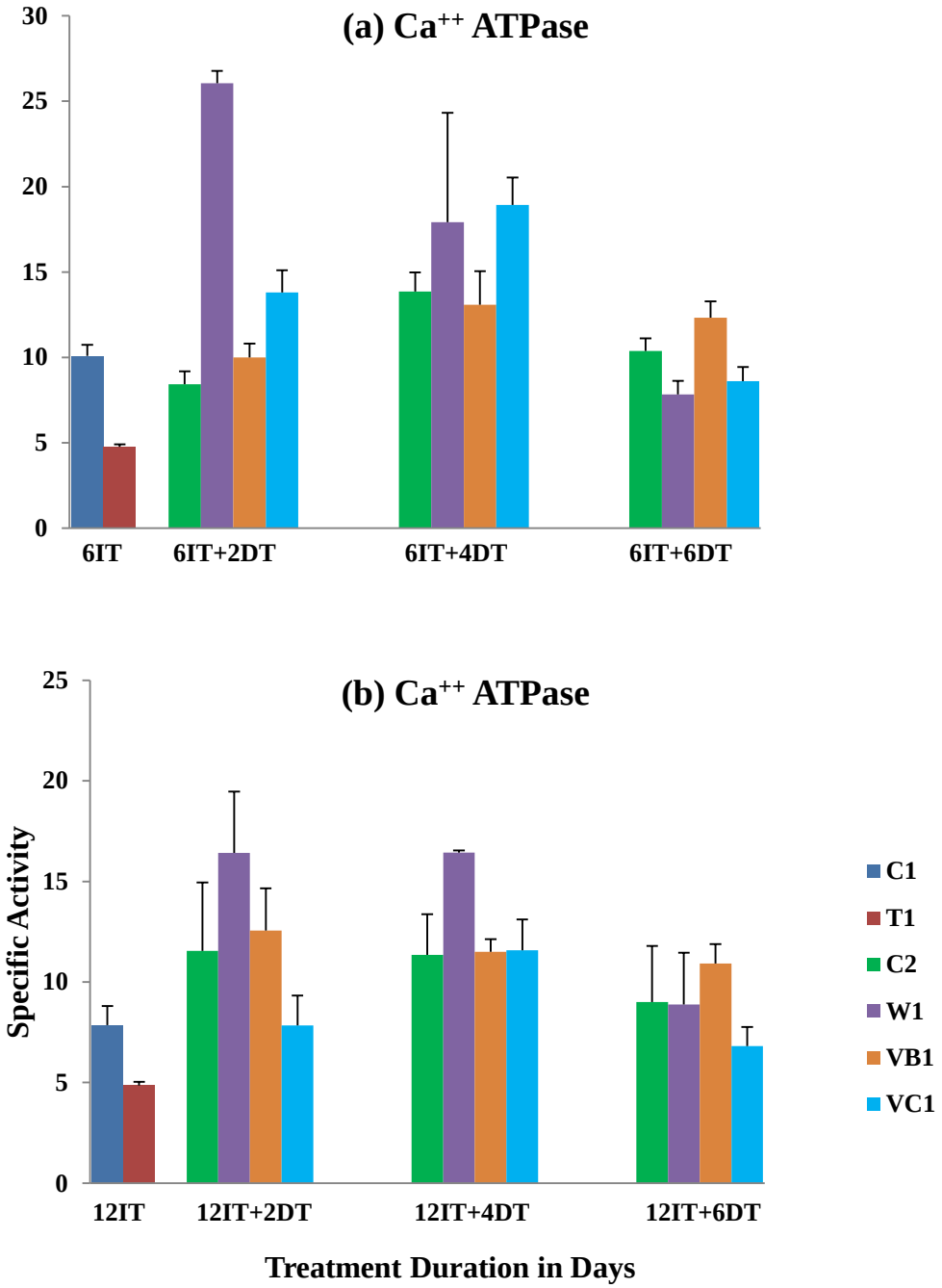


Fig 17 - Changes in the specific activity of **Ca^{++} ATPase** of chick **brain**. (a) TBT dose 0.06 mg kg^{-1}bw d^{-1} exposed for 6 days (b) TBT dose 0.06 mg kg^{-1}bw d^{-1} exposed for 12 days and its possible recovery by natural washing of toxicant (withdrawal), Vitamin B complex and Vitamin C for next 2, 4, 6 days. Data expressed are mean of specific activity ± SD. Abbreviations used in graphs are mentioned in materials and methods chapter

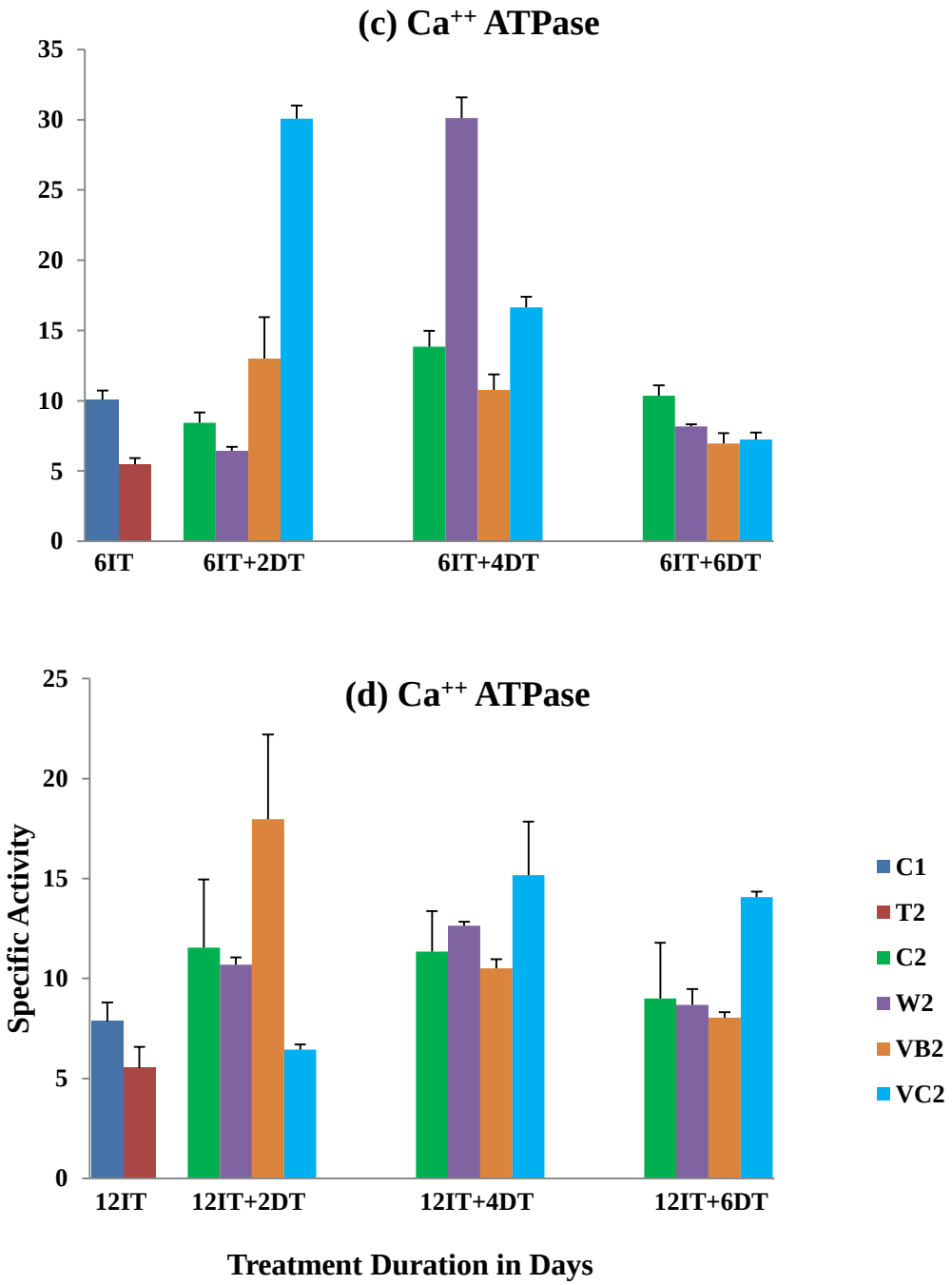


Fig 17 - Changes in the specific activity of **Ca^{++} ATPase** of chick **brain**. (c) TBT dose 0.6 mg kg^{-1}bw d^{-1} exposed for 6 days (d) TBT dose 0.6 mg kg^{-1}bw d^{-1} exposed for 12 days and its possible recovery by natural washing of toxicant (withdrawal), Vitamin B complex and Vitamin C for next 2, 4, 6 days. Data expressed are mean of specific activity ± SD. Abbreviations used in graphs are mentioned in materials and methods chapter.

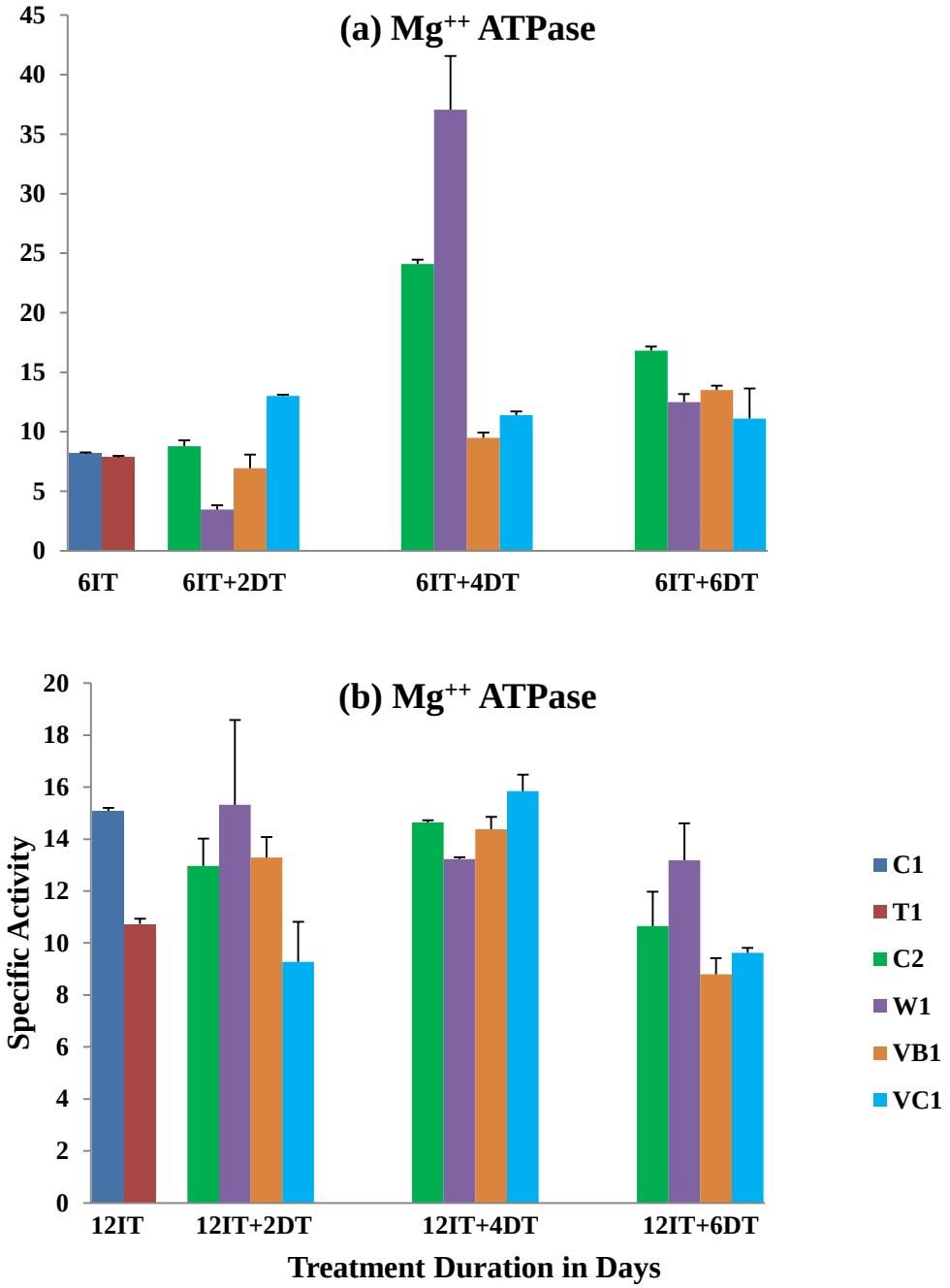


Fig 18 - Changes in the specific activity of **Mg^{++} ATPase** of chick **brain**. (a) TBT dose 0.06 mg kg^{-1}bw d^{-1} exposed for 6 days (b) TBT dose 0.06 mg kg^{-1}bw d^{-1} exposed for 12 days and its possible recovery by natural washing of toxicant (withdrawal), Vitamin B complex and Vitamin C for next 2, 4, 6 days. Data expressed are mean of specific activity ± SD. Abbreviations used in graphs are mentioned in materials and methods chapter.

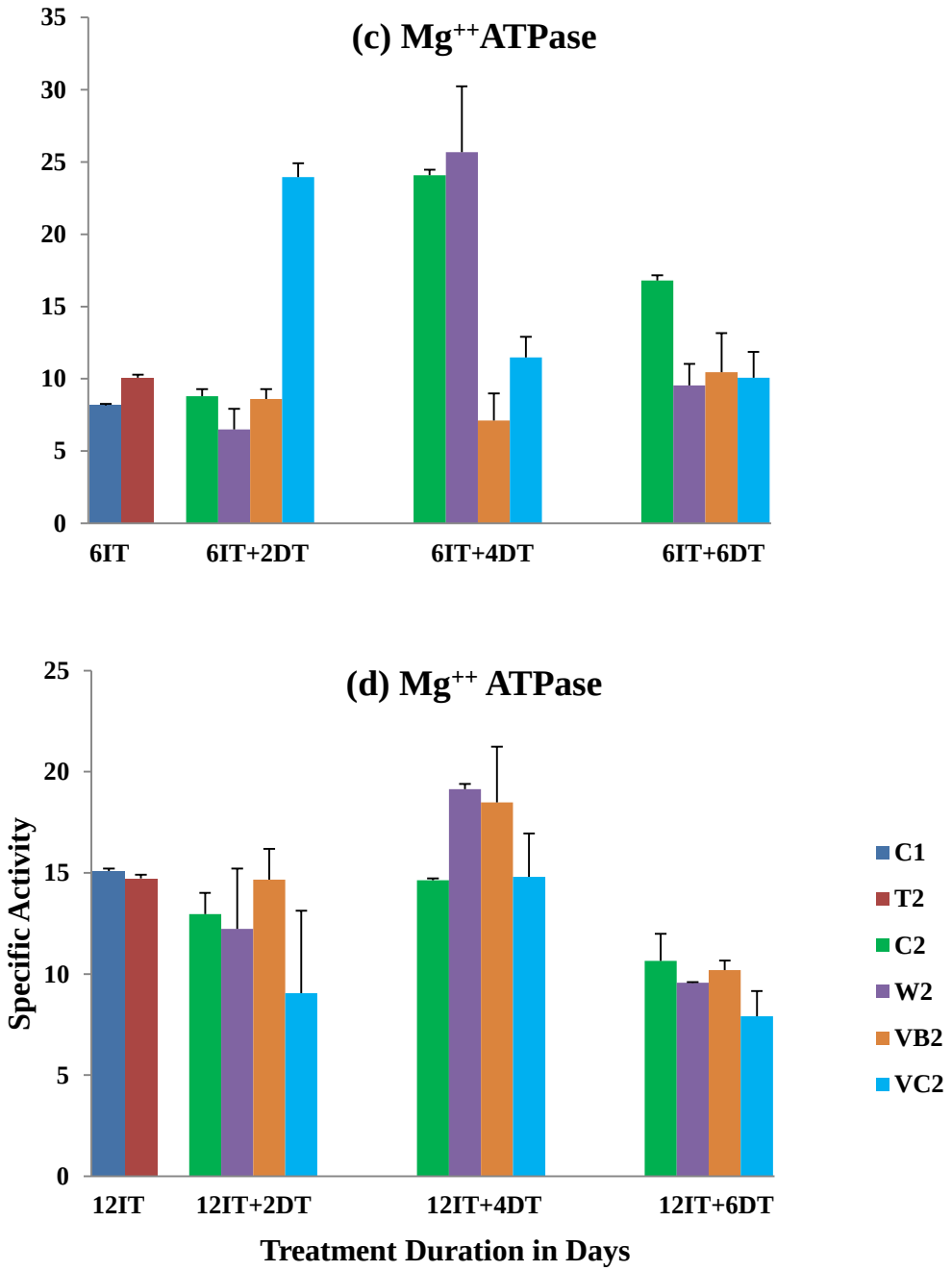


Fig 18 - Changes in the specific activity of **Mg^{++} ATPase** of chick **brain.** (c) TBT dose 0.6 mg kg^{-1}bw d^{-1} exposed for 6 days (d) TBT dose 0.6 mg kg^{-1}bw d^{-1} exposed for 12 days and its possible recovery by natural washing of toxicant (withdrawal), Vitamin B complex and Vitamin C for next 2, 4, 6 days. Data expressed are mean of specific activity ± SD. Abbreviations used in graphs are mentioned in materials and methods chapter.

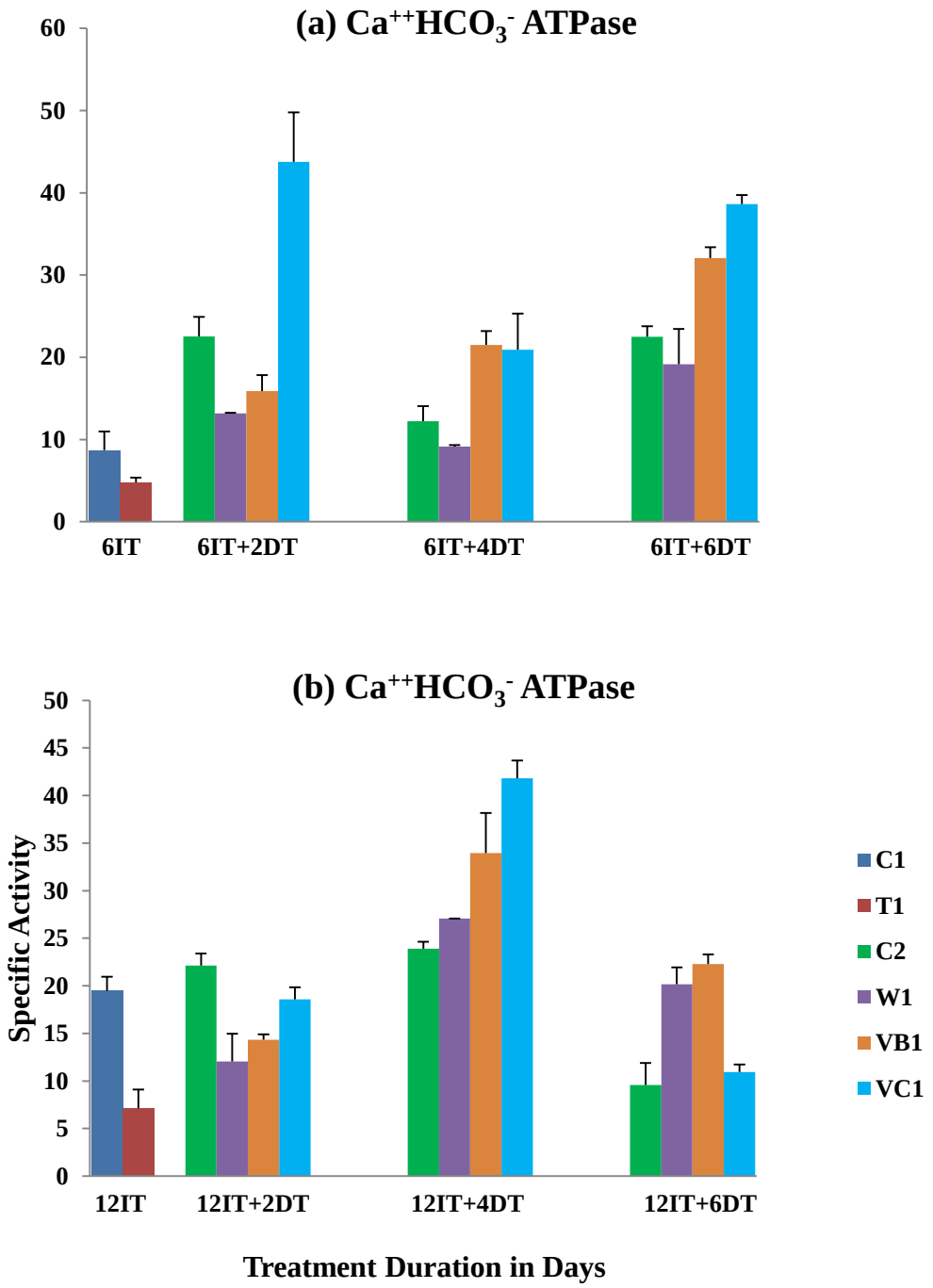


Fig 19 - Changes in the specific activity of **$Ca^{++}HCO_3^-$ ATPase** of chick **brain**. (a) TBT dose 0.06 mg kg^{-1}bw d^{-1} exposed for 6 days (b) TBT dose 0.06 mg kg^{-1}bw d^{-1} exposed for 12 days and its possible recovery by natural washing of toxicant (withdrawal), Vitamin B complex and Vitamin C for next 2, 4, 6 days. Data expressed are mean of specific activity ± SD. Abbreviations used in graphs are mentioned in materials and methods chapter.

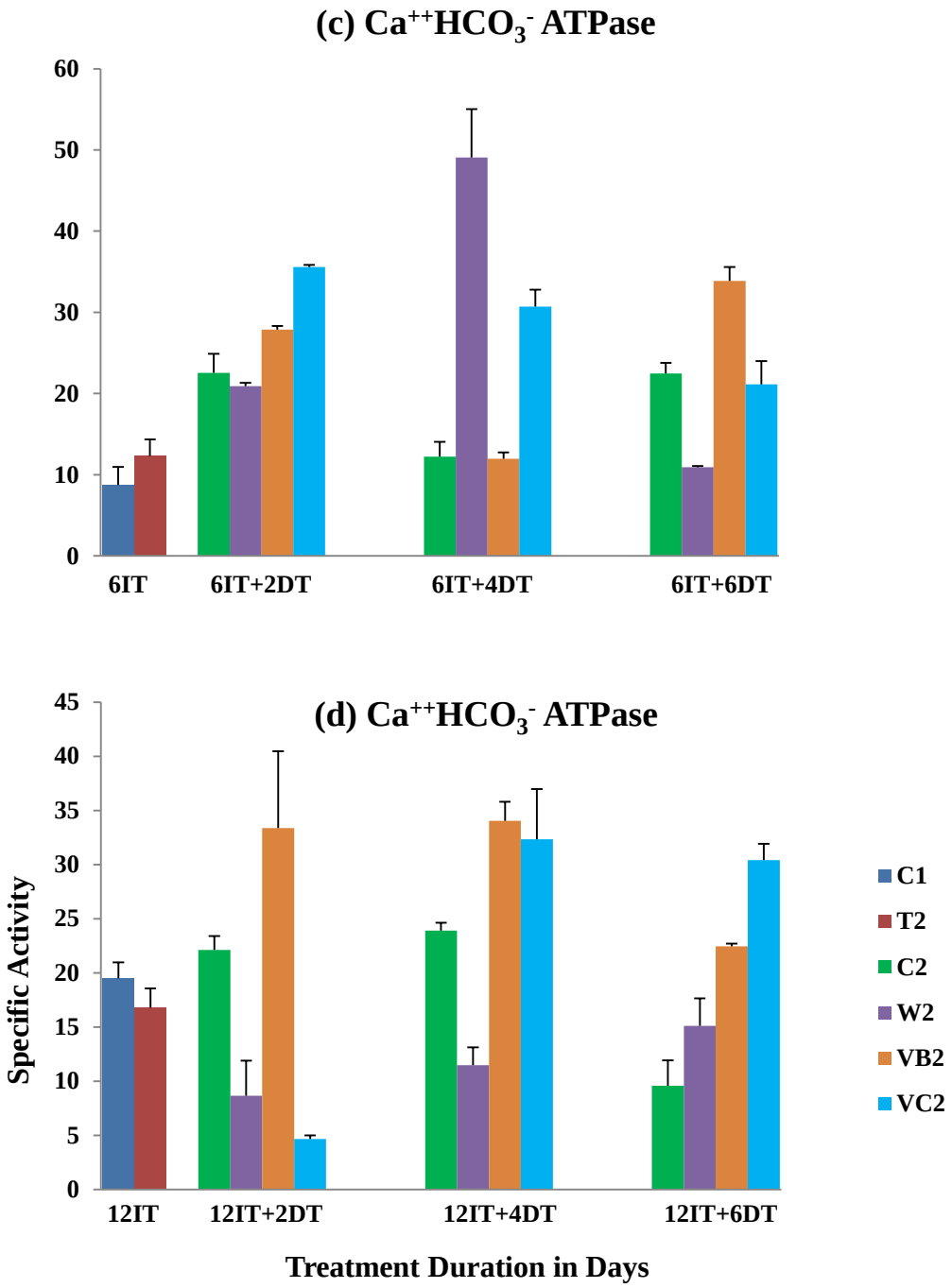


Fig 19 - Changes in the specific activity of **$Ca^{++}HCO_3^-$ ATPase** of chick **brain**. (c) TBT dose 0.6 mg kg^{-1}bw d^{-1} exposed for 6 days (d) TBT dose 0.6 mg kg^{-1}bw d^{-1} exposed for 12 days and its possible recovery by natural washing of toxicant (withdrawal), Vitamin B complex and Vitamin C for next 2, 4, 6 days. Data expressed are mean of specific activity ± SD. Abbreviations used in graphs are mentioned in materials and methods chapter.

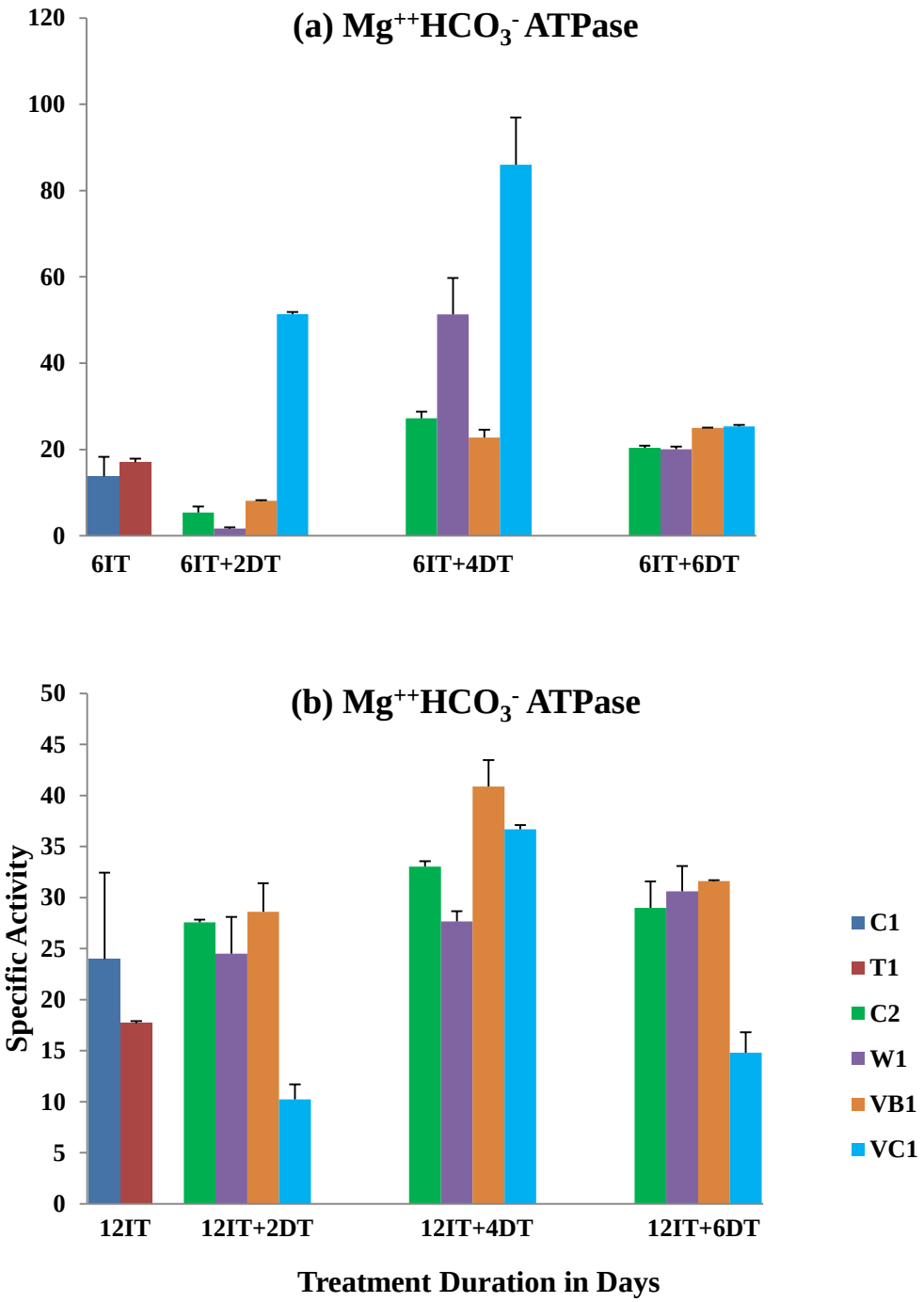


Fig 20 - Changes in the specific activity of **$Mg^{++}HCO_3^-$ ATPase** of chick **brain**. (a) TBT dose 0.06 mg kg^{-1}bw d^{-1} exposed for 6 days (b) TBT dose 0.06 mg kg^{-1}bw d^{-1} exposed for 12 days and its possible recovery by natural washing of toxicant (withdrawal), Vitamin B complex and Vitamin C for next 2, 4, 6 days. Data expressed are mean of specific activity ± SD. Abbreviations used in graphs are mentioned in materials and methods chapter.

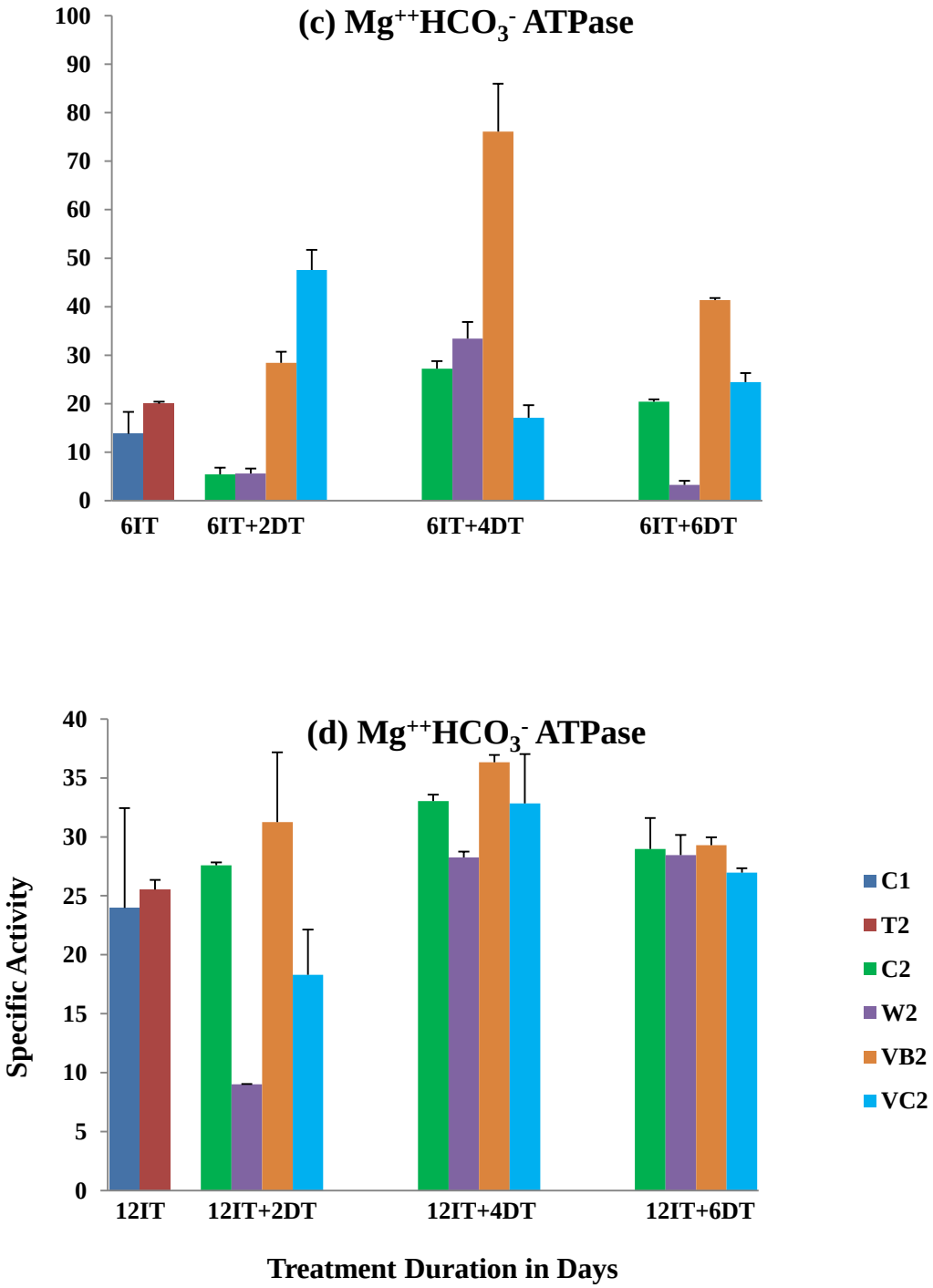


Fig 20 - Changes in the specific activity of **$Mg^{++}HCO_3^-$ ATPase** of chick **brain.** (c) TBT dose 0.6 mg kg^{-1}bw d^{-1} exposed for 6 days (d) TBT dose 0.6 mg kg^{-1}bw d^{-1} exposed for 12 days and its possible recovery by natural washing of toxicant (withdrawal), Vitamin B complex and Vitamin C for next 2, 4, 6 days. Data expressed are mean of specific activity ± SD. Abbreviations used in graphs are mentioned in materials and methods chapter.

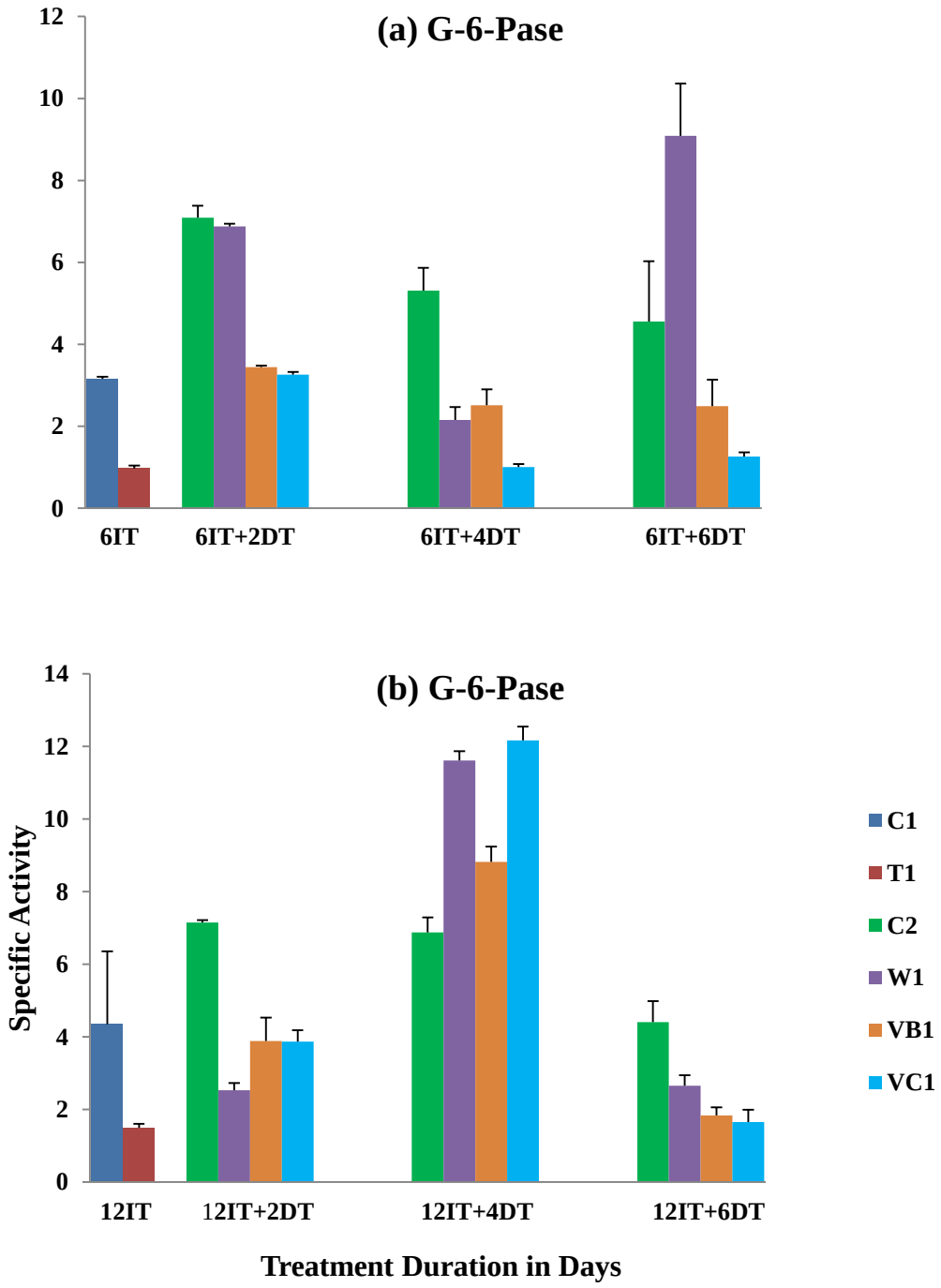


Fig 21 - Changes in the specific activity of **Glucose-6-Phosphatase** of chick **brain**. (a) TBT dose 0.06 mg kg^{-1}bw d^{-1} exposed for 6 days (b) TBT dose 0.06 mg kg^{-1}bw d^{-1} exposed for 12 days and its possible recovery by natural washing of toxicant (withdrawal), Vitamin B complex and Vitamin C for next 2, 4, 6 days. Data expressed are mean of specific activity ± SD. Abbreviations used in graphs are mentioned in materials and methods chapter.

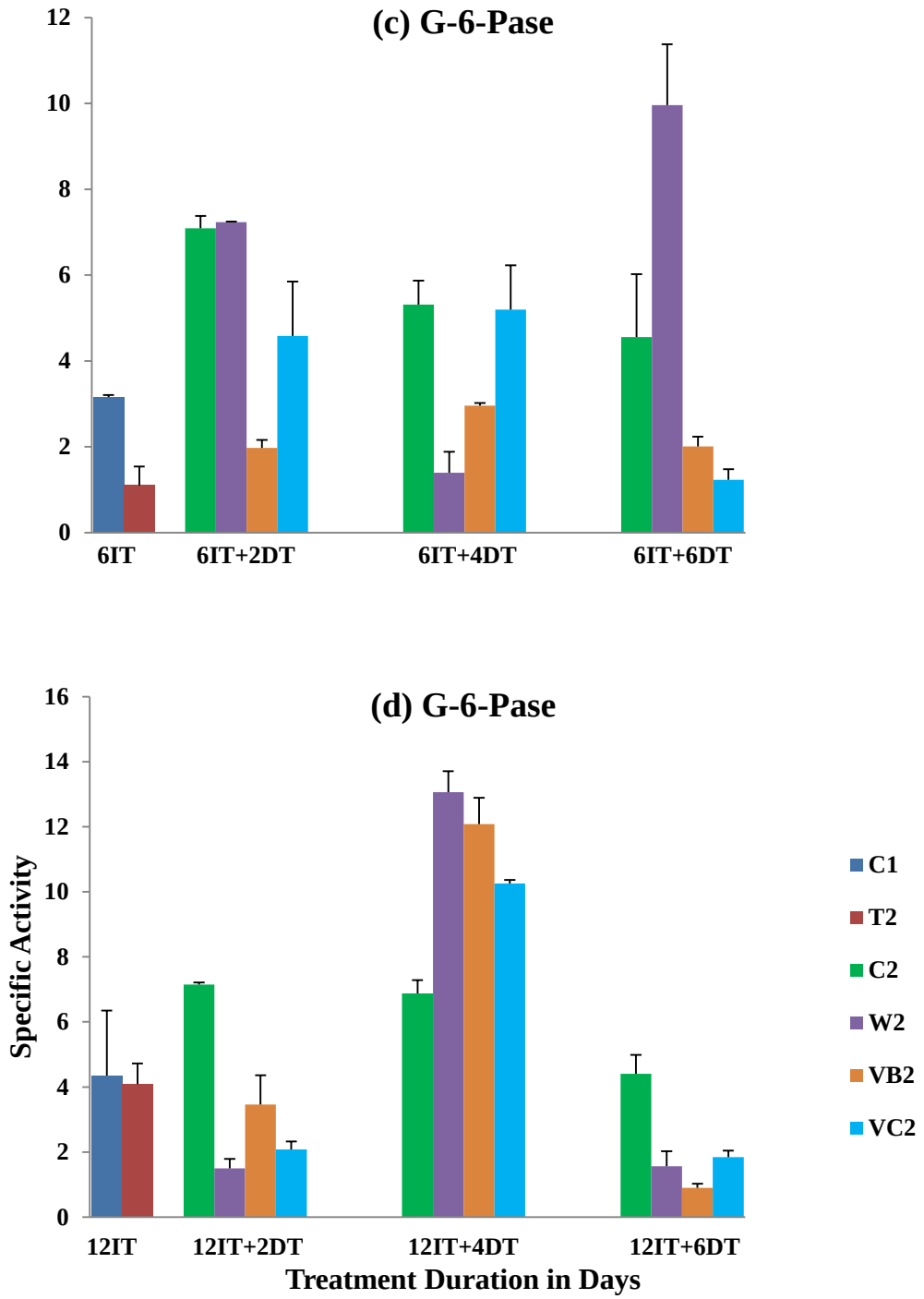


Fig 21 - Changes in the specific activity of **Glucose-6-Phosphatase** of chick **brain**. (c) TBT dose 0.6 mg kg^{-1}bw d^{-1} exposed for 6 days (d) TBT dose 0.6 mg kg^{-1}bw d^{-1} exposed for 12 days and its possible recovery by natural washing of toxicant (withdrawal), Vitamin B complex and Vitamin C for next 2, 4, 6 days. Data expressed are mean of specific activity ± SD. Abbreviations used in graphs are mentioned in materials and methods chapter.

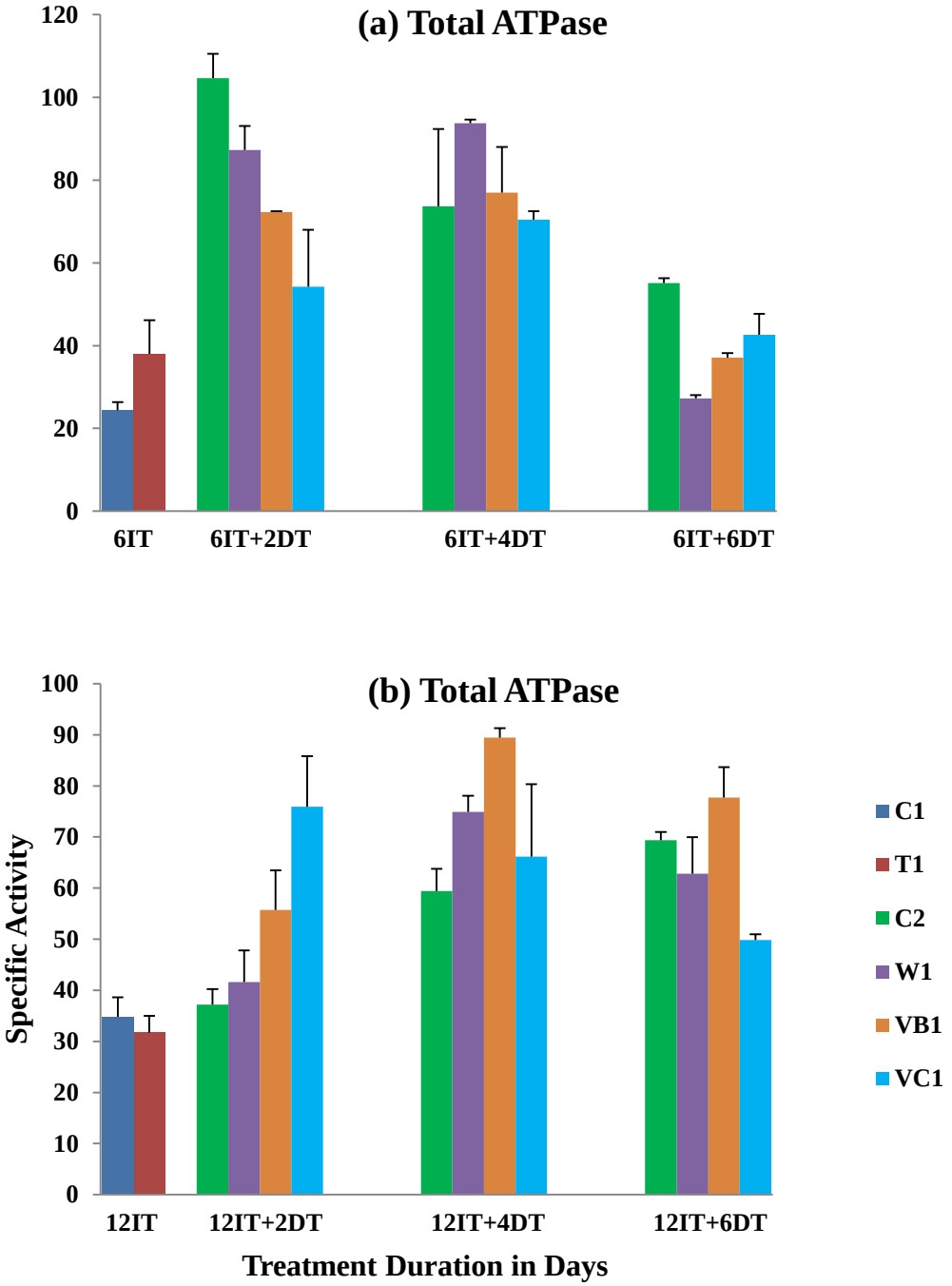


Fig 22 - Changes in the specific activity of **Total ATPase** of chick **muscle**. (a) TBT dose 0.06 mg kg^{-1}bw d^{-1} exposed for 6 days (b) TBT dose 0.06 mg kg^{-1}bw d^{-1} exposed for 12 days and its possible recovery by natural washing of toxicant (withdrawal), Vitamin B complex and Vitamin C for next 2, 4, 6 days. Data expressed are mean of specific activity ± SD. Abbreviations used in graphs are mentioned in materials and methods chapter

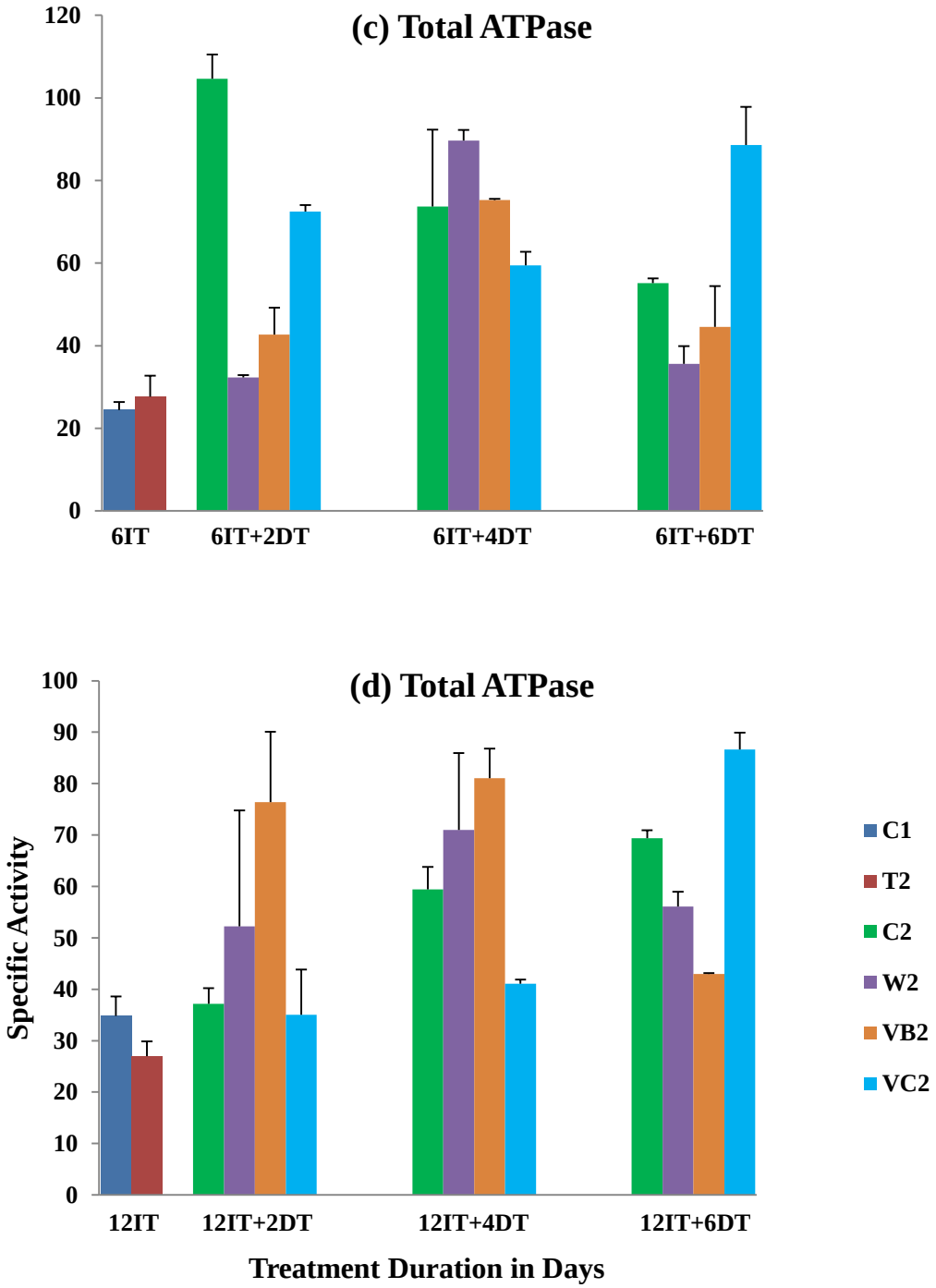


Fig 22 - Changes in the specific activity of **Total ATPase** of chick **muscle**. (c) TBT dose 0.6 mg kg^{-1}bw d^{-1} exposed for 6 days (d) TBT dose 0.6 mg kg^{-1}bw d^{-1} exposed for 12 days and its possible recovery by natural washing of toxicant (withdrawal), Vitamin B complex and Vitamin C for next 2, 4, 6 days. Data expressed are mean of specific activity ± SD. Abbreviations used in graphs are mentioned in materials and methods chapter.

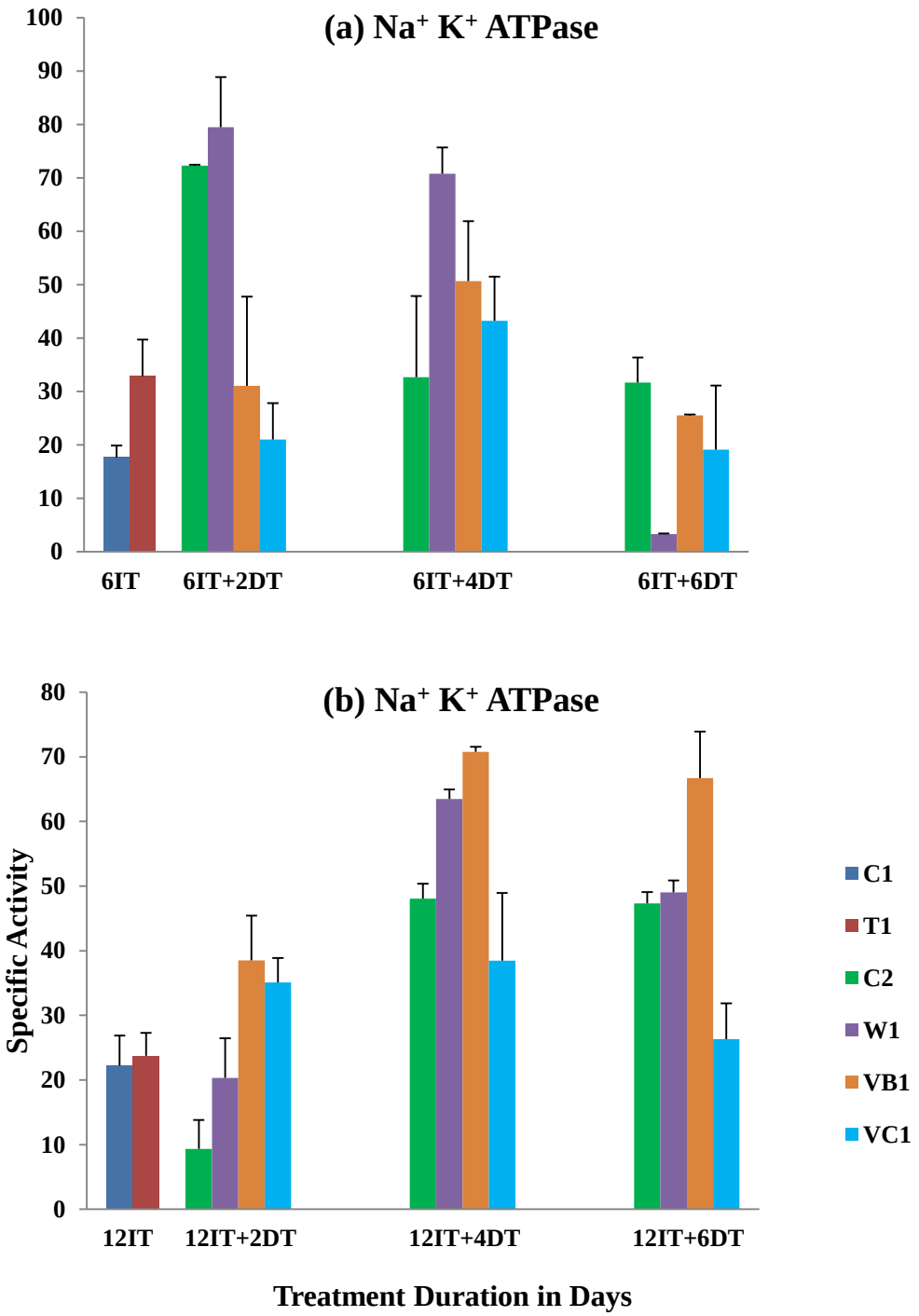


Fig 23 - Changes in the specific activity of **Na^+ K^+ ATPase** of chick **muscle**. (a) TBT dose 0.06 mg kg^{-1}bw d^{-1} exposed for 6 days (b) TBT dose 0.06 mg kg^{-1}bw d^{-1} exposed for 12 days and its possible recovery by natural washing of toxicant (withdrawal), Vitamin B complex and Vitamin C for next 2, 4, 6 days. Data expressed are mean of specific activity ± SD. Abbreviations used in graphs are mentioned in materials and methods chapter.

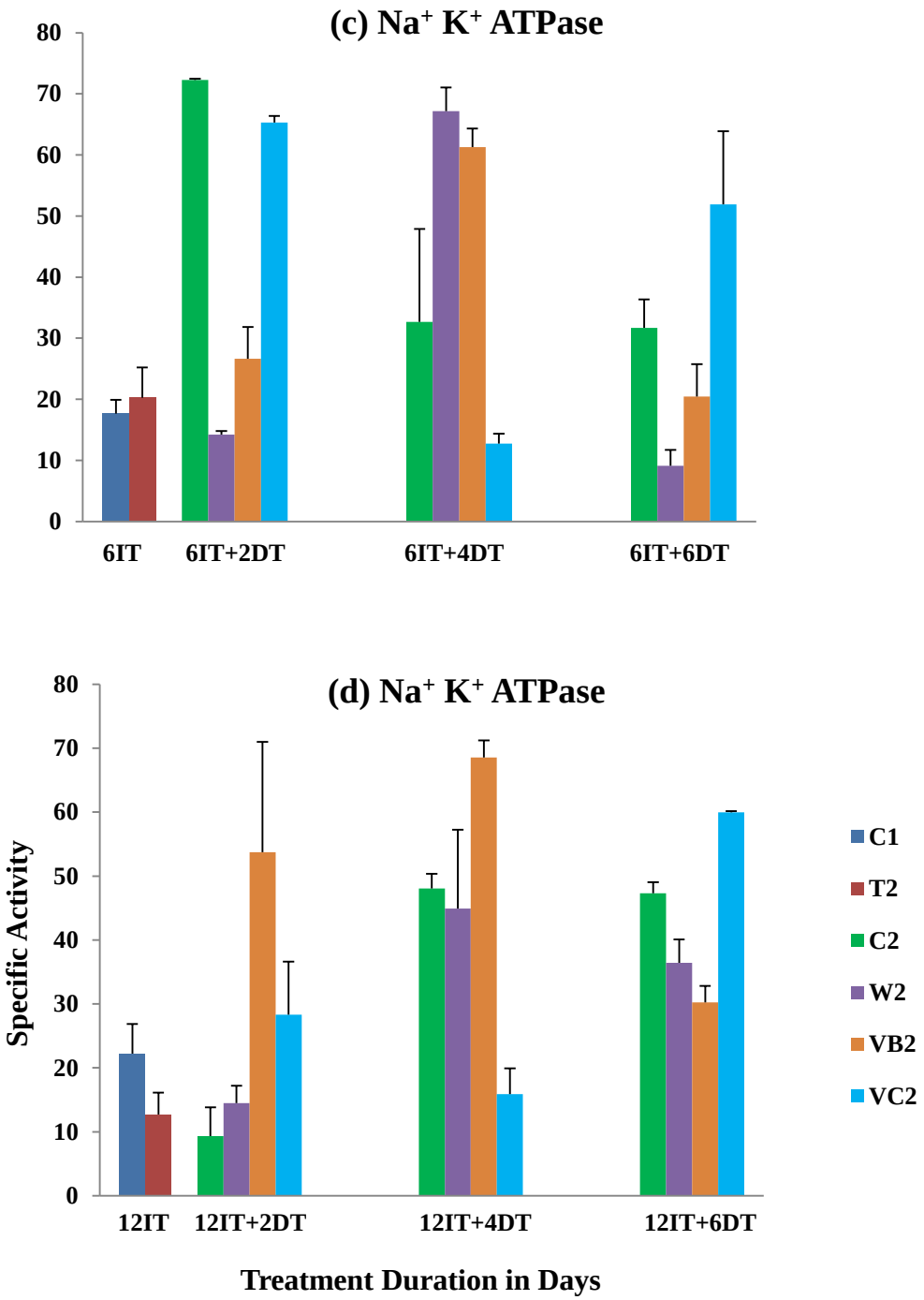


Fig 23 - Changes in the specific activity of **Na^+ K^+ ATPase** of chick **muscle**. (c) TBT dose 0.6 mg kg^{-1}bw d^{-1} exposed for 6 days (d) TBT dose 0.6 mg kg^{-1}bw d^{-1} exposed for 12 days and its possible recovery by natural washing of toxicant (withdrawal), Vitamin B complex and Vitamin C for next 2, 4, 6 days. Data expressed are mean of specific activity ± SD. Abbreviations used in graphs are mentioned in materials and methods chapter.

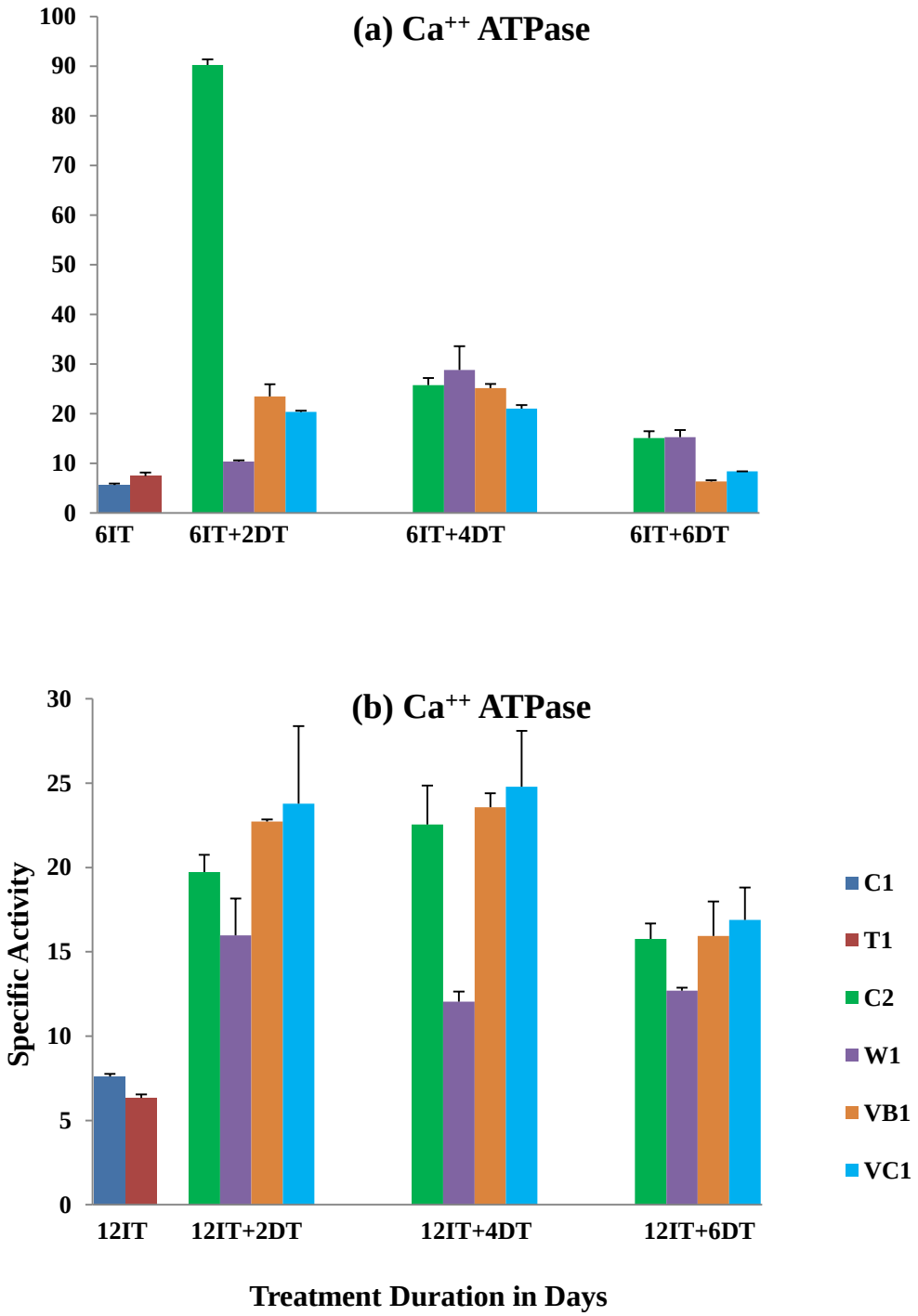
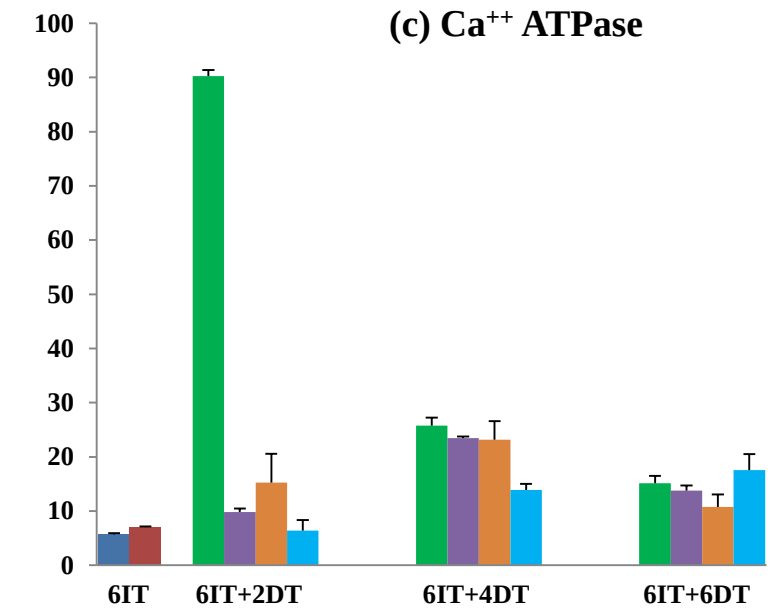


Fig 24 - Changes in the specific activity of **Ca^{++} ATPase** of chick **muscle**. (a) TBT dose 0.06 mg kg^{-1}bw d^{-1} exposed for 6 days (b) TBT dose 0.06 mg kg^{-1}bw d^{-1} exposed for 12 days and its possible recovery by natural washing of toxicant (withdrawal), Vitamin B complex and Vitamin C for next 2, 4, 6 days. Data expressed are mean of specific activity ± SD. Abbreviations used in graphs are mentioned in materials and methods chapter.



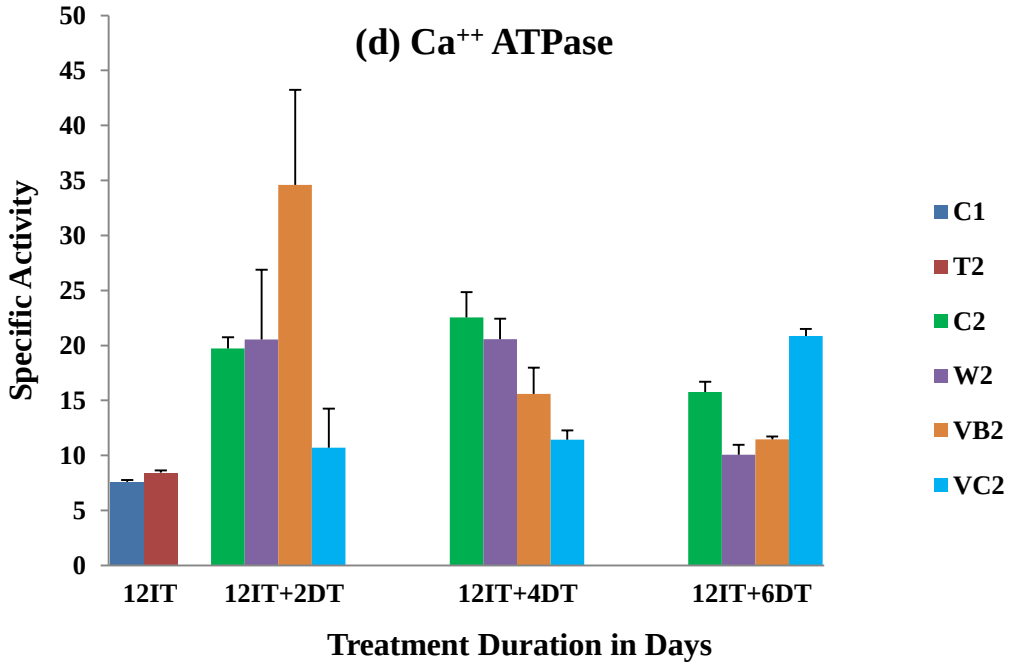


Fig 24 - Changes in the specific activity of **Ca^{++} ATPase** of chick **muscle**. (c) TBT dose 0.6 mg kg^{-1}bw d^{-1} exposed for 6 days (d) TBT dose 0.6 mg kg^{-1}bw d^{-1} exposed for 12 days and its possible recovery by natural washing of toxicant (withdrawal), Vitamin B complex and Vitamin C for next 2, 4, 6 days. Data expressed are mean of specific activity ± SD. Abbreviations used in graphs are mentioned in materials and methods chapter.

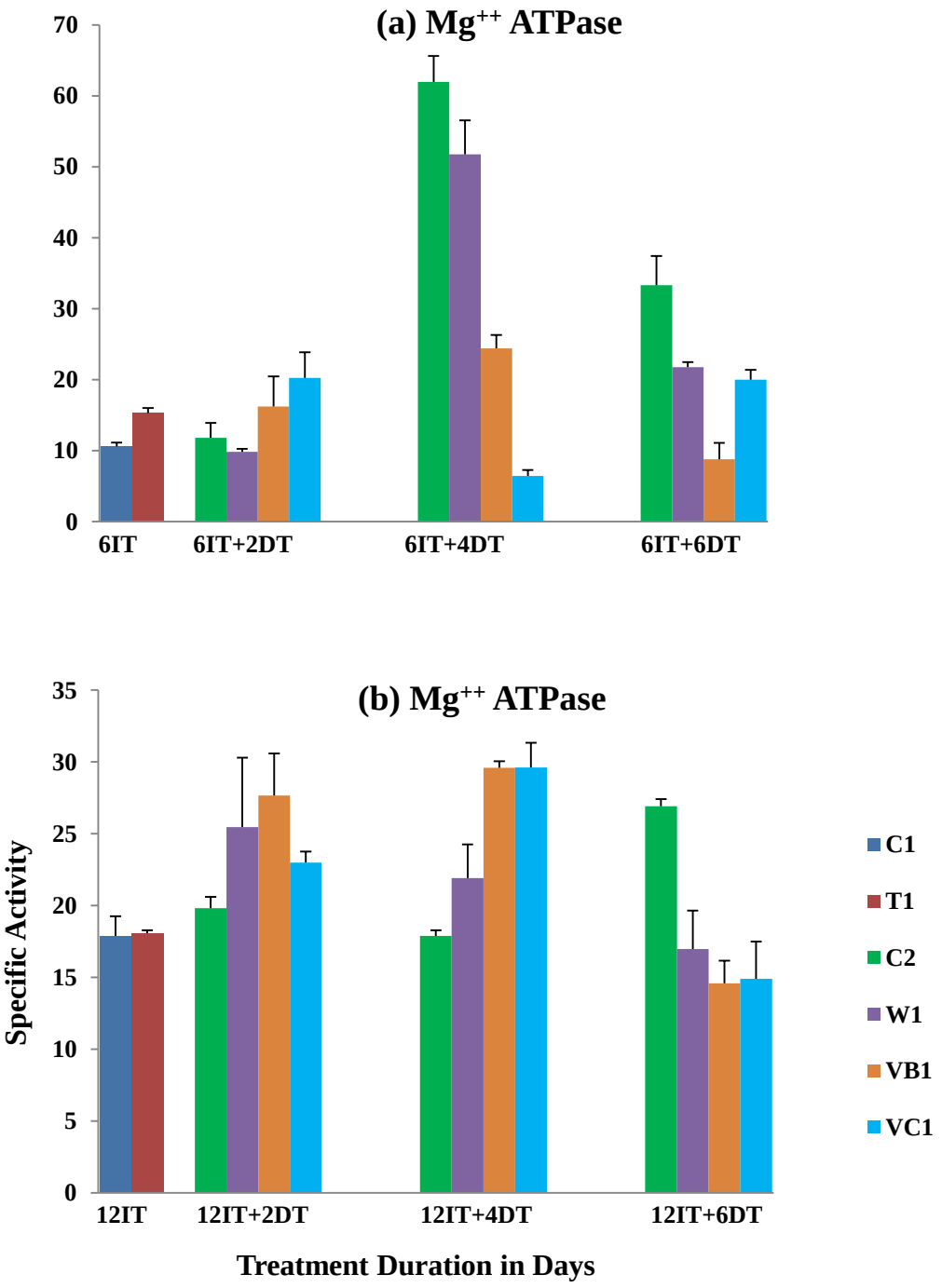


Fig 25 - Changes in the specific activity of **Mg^{++} ATPase** of chick **muscle**. (a) TBT dose 0.06 mg kg^{-1}bw d^{-1} exposed for 6 days (b) TBT dose 0.06 mg kg^{-1}bw d^{-1} exposed for 12 days and its possible recovery by natural washing of toxicant (withdrawal), Vitamin B complex and Vitamin C for next 2, 4, 6 days. Data expressed are mean of specific activity ± SD. Abbreviations used in graphs are mentioned in materials and methods chapter.

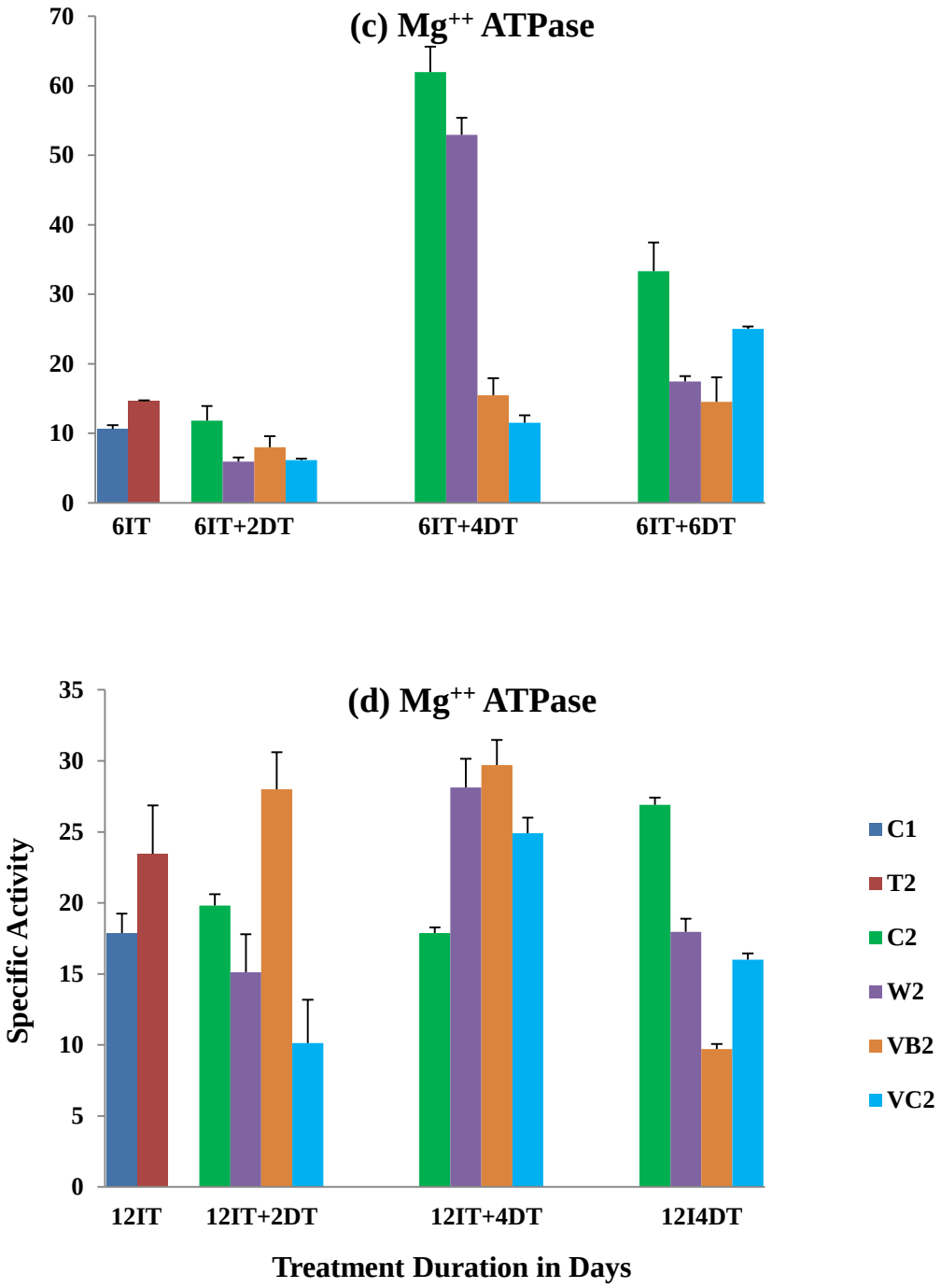


Fig 25 - Changes in the specific activity of **Mg^{++} ATPase** of chick **muscle**. (c) TBT dose 0.6 mg kg^{-1}bw d^{-1} exposed for 6 days (d) TBT dose 0.6 mg kg^{-1}bw d^{-1} exposed for 12 days and its possible recovery by natural washing of toxicant (withdrawal), Vitamin B complex and Vitamin C for next 2, 4, 6 days. Data expressed are mean of specific activity ± SD. Abbreviations used in graphs are mentioned in materials and methods chapter.

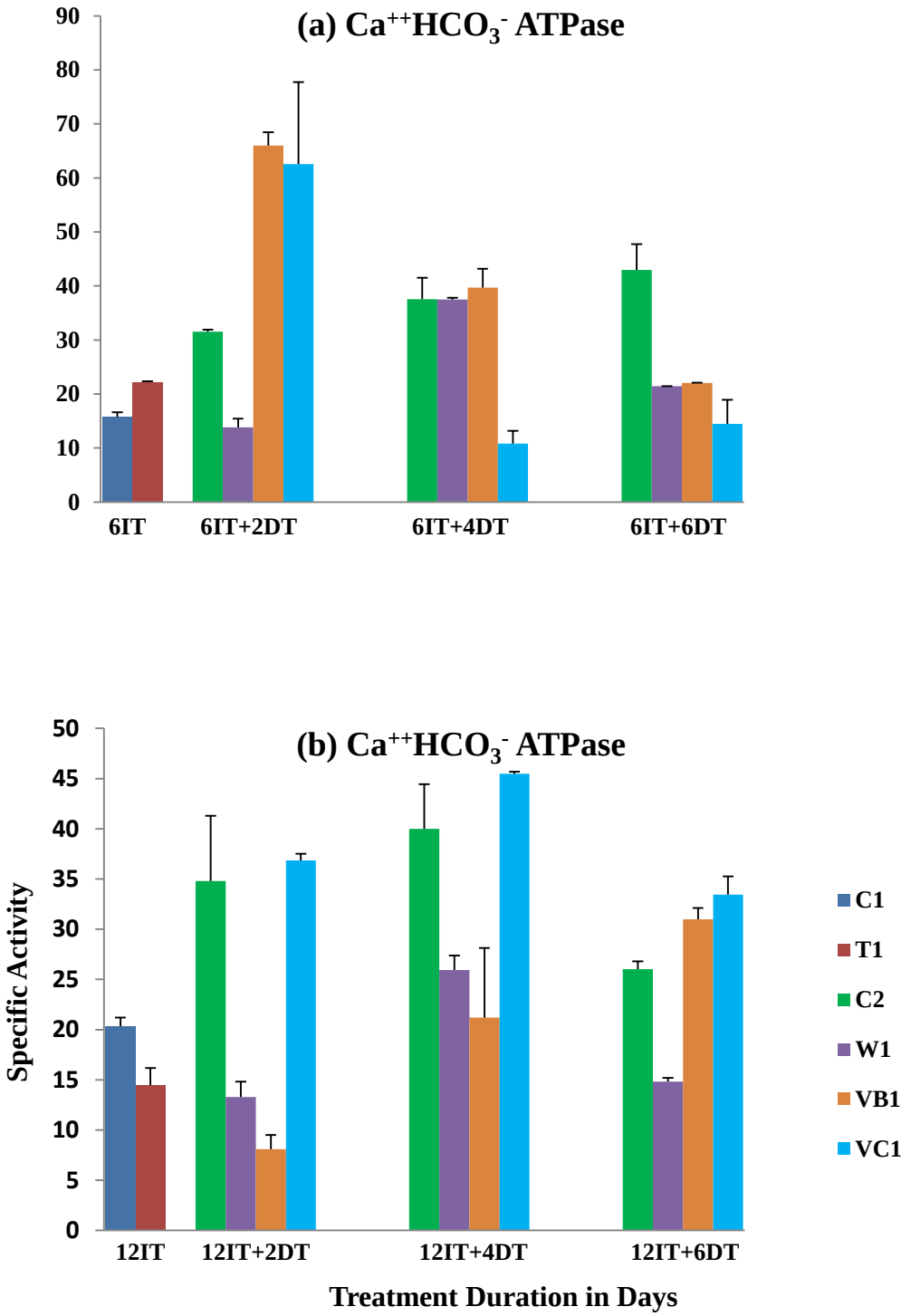


Fig 26 - Changes in the specific activity of **$Ca^{++}HCO_3^-$ ATPase** of chick **muscle**. (a) TBT dose 0.06 mg kg^{-1}bw d^{-1} exposed for 6 days (b) TBT dose 0.06 mg kg^{-1}bw d^{-1} exposed for 12 days and its possible recovery by natural washing of toxicant (withdrawal), Vitamin B complex and Vitamin C for next 2, 4, 6 days. Data expressed are mean of specific activity ± SD. Abbreviations used in graphs are mentioned in materials and methods chapter.

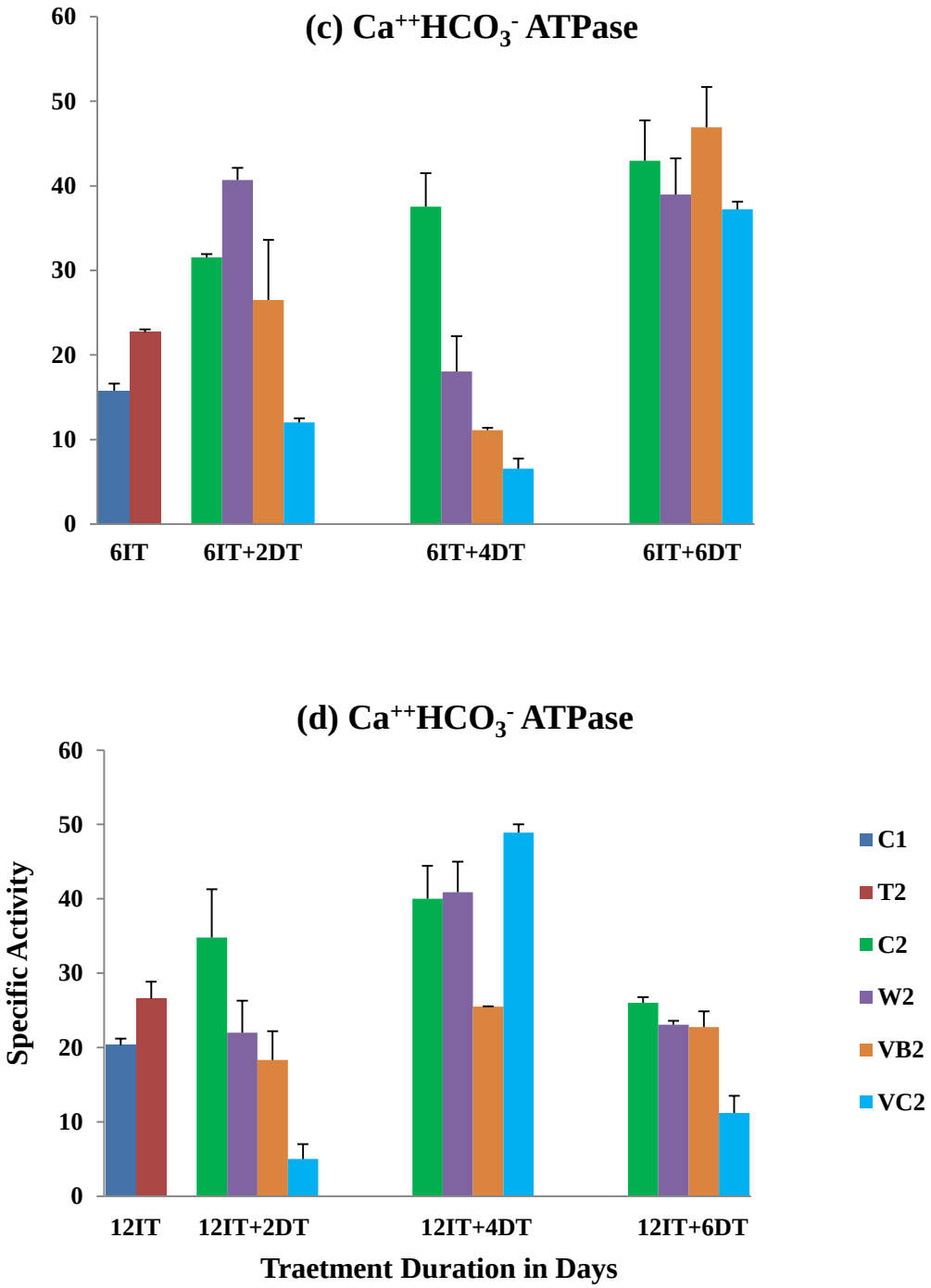


Fig 26 - Changes in the specific activity of **$Ca^{++}HCO_3^-$ ATPase** of chick **muscle**. (c) TBT dose 0.6 mg kg^{-1}bw d^{-1} exposed for 6 days (d) TBT dose 0.6 mg kg^{-1}bw d^{-1} exposed for 12 days and its possible recovery by natural washing of toxicant (withdrawal), Vitamin B complex and Vitamin C for next 2, 4, 6 days. Data expressed are mean of specific activity ± SD. Abbreviations used in graphs are mentioned in materials and methods chapter.

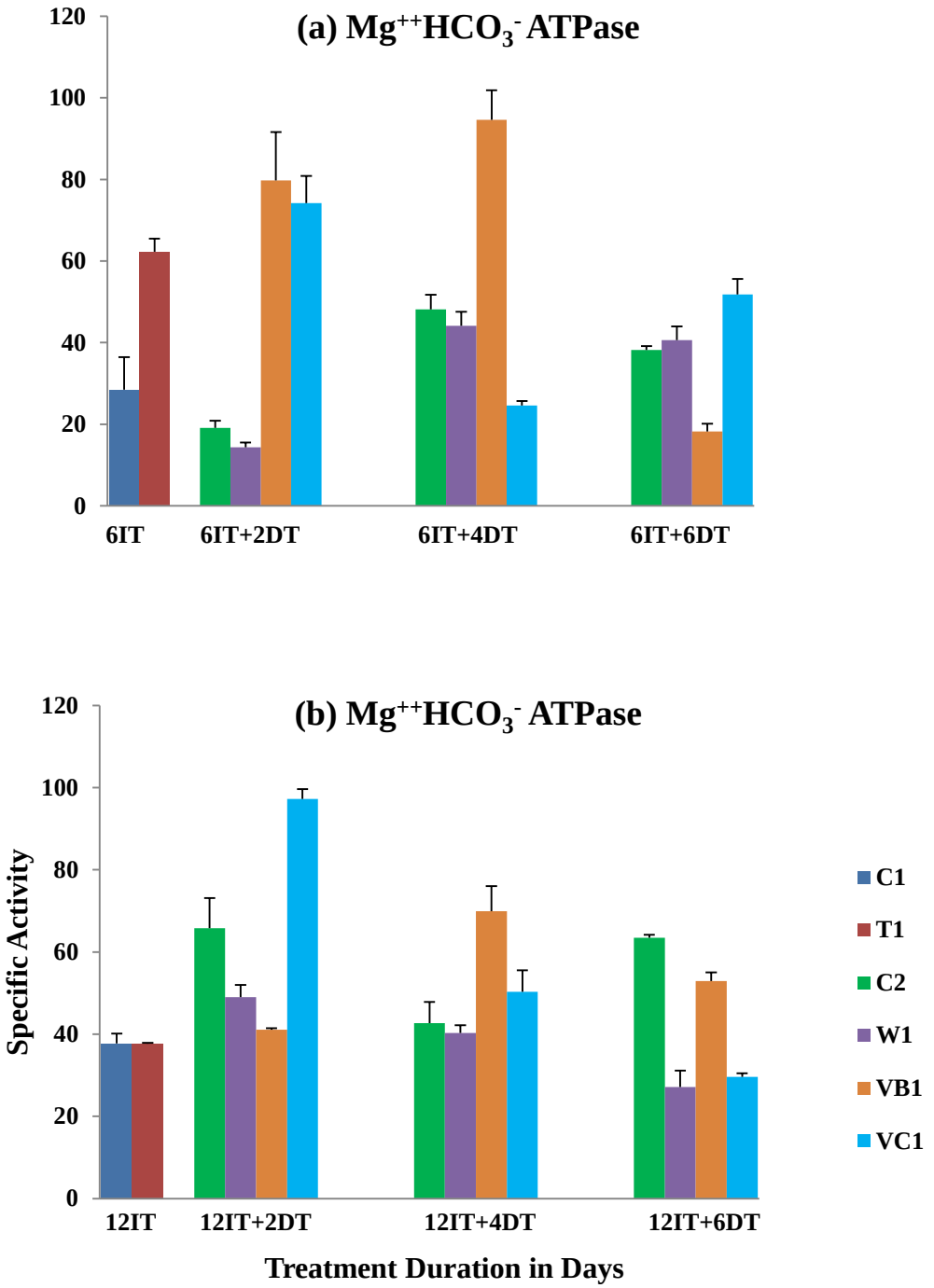


Fig 27 - Changes in the specific activity of **$Mg^{++}HCO_3^-$ ATPase** of chick **muscle**. (a) TBT dose 0.06 mg kg^{-1}bw d^{-1} exposed for 6 days (b) TBT dose 0.06 mg kg^{-1}bw d^{-1} exposed for 12 days and its possible recovery by natural washing of toxicant (withdrawal), Vitamin B complex and Vitamin C for next 2, 4, 6 days. Data expressed are mean of specific activity ± SD. Abbreviations used in graphs are mentioned in materials and methods chapter.

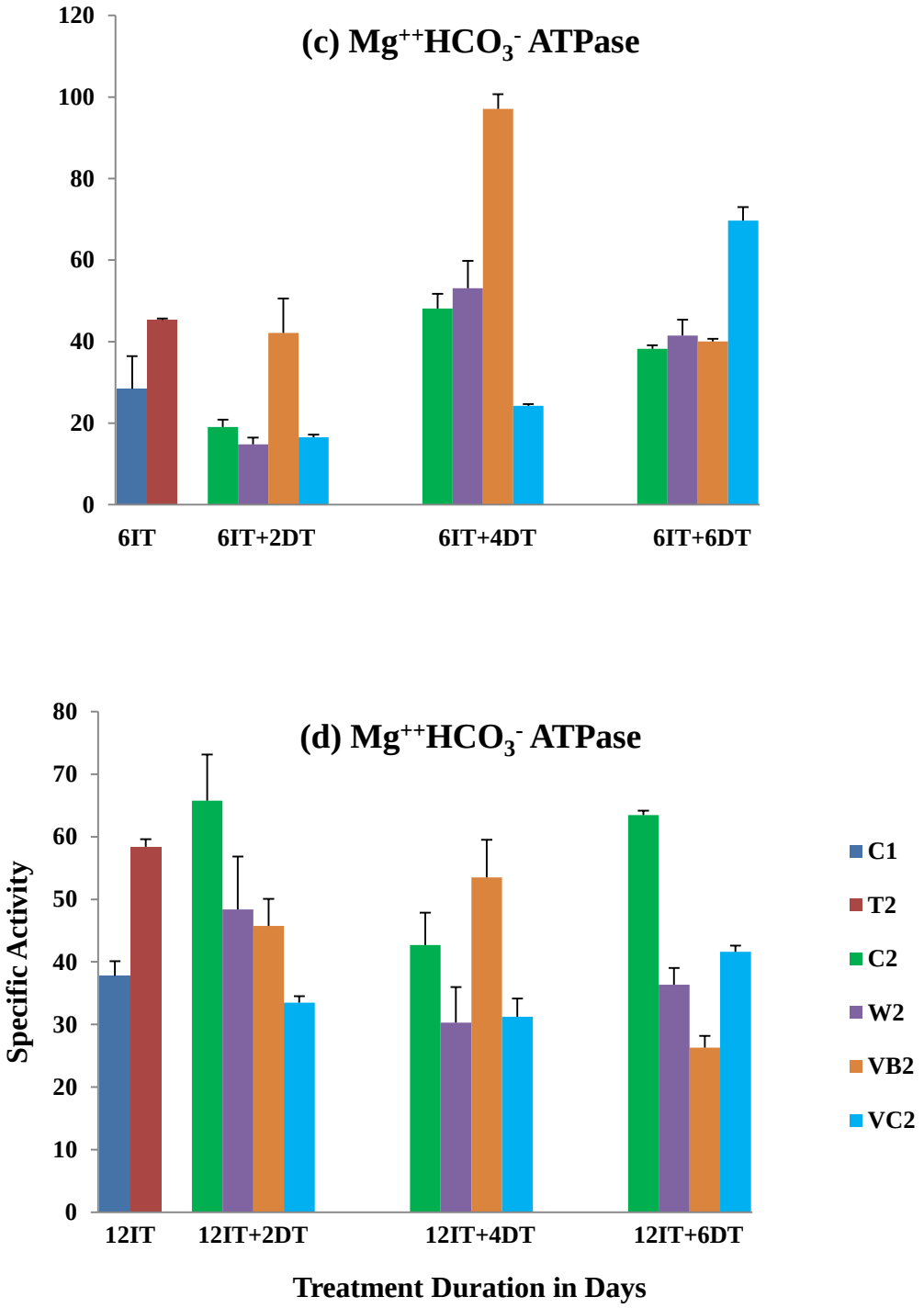


Fig 27 - Changes in the specific activity of **$Mg^{++}HCO_3^-$ ATPase** of chick **muscle**. (c) TBT dose 0.6 mg kg^{-1}bw d^{-1} exposed for 6 days (d) TBT dose 0.6 mg kg^{-1}bw d^{-1} exposed for 12 days and its possible recovery by natural washing of toxicant (withdrawal), Vitamin B complex and Vitamin C for next 2, 4, 6 days. Data expressed are mean of specific activity ± SD. Abbreviations used in graphs are mentioned in materials and methods chapter.

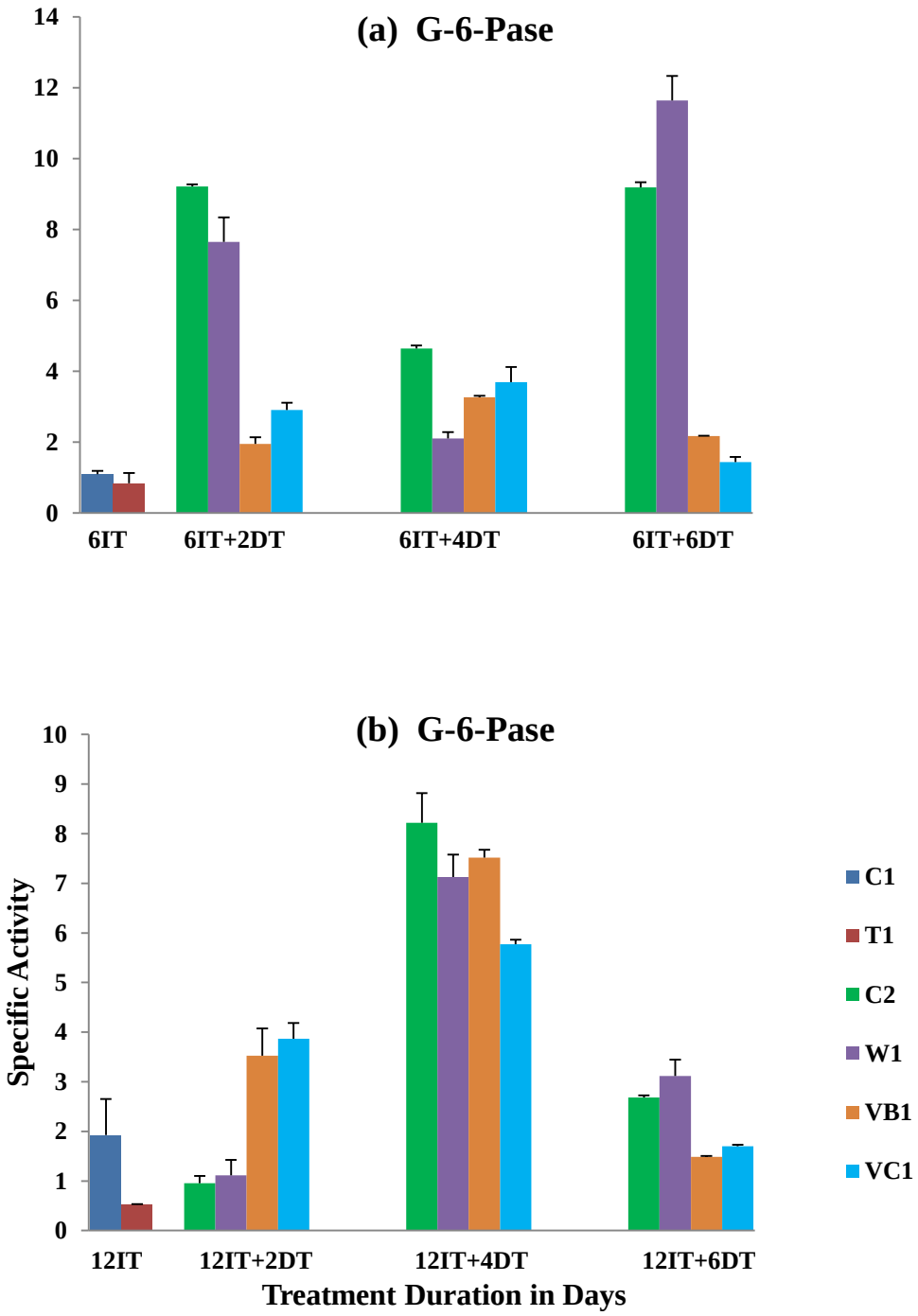


Fig 28 - Changes in the specific activity of **Glucose-6-Phosphatase** of chick **muscle**. (a) TBT dose 0.06 mg kg^{-1}bw d^{-1} exposed for 6 days (b) TBT dose 0.06 mg kg^{-1}bw d^{-1} exposed for 12 days and its possible recovery by natural washing of toxicant (withdrawal), Vitamin B complex and Vitamin C for next 2, 4, 6 days. Data expressed are mean of specific activity ± SD. Abbreviations used in graphs are mentioned in materials and methods chapter.

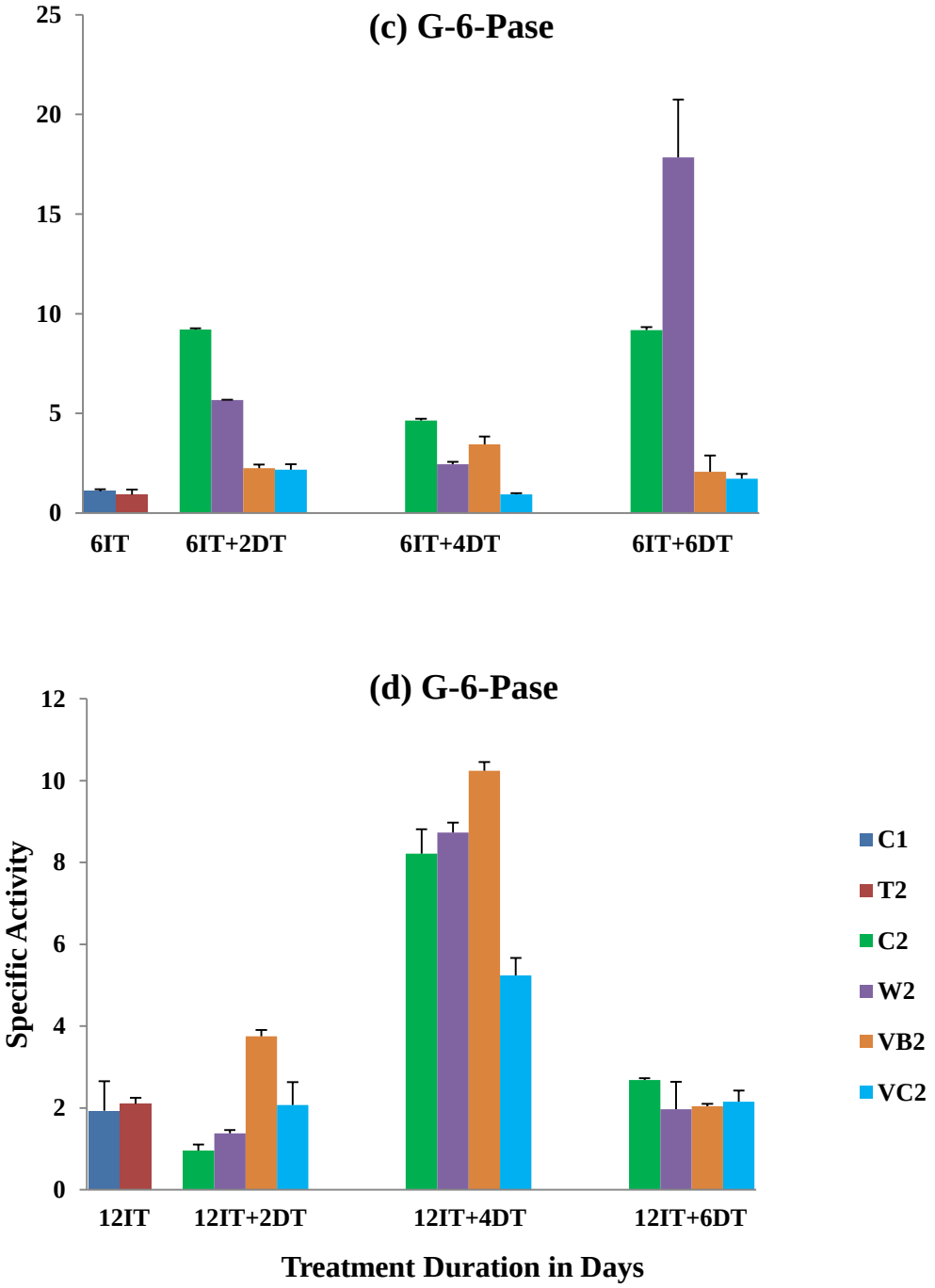


Fig 28 - Changes in the specific activity of **Glucose-6-Phosphatase** of chick **muscle**. (c) TBT dose 0.6 mg kg^{-1}bw d^{-1} exposed for 6 days (d) TBT dose 0.6 mg kg^{-1}bw d^{-1} exposed for 12 days and its possible recovery by natural washing of toxicant (withdrawal), Vitamin B complex and Vitamin C for next 2, 4, 6 days. Data expressed are mean of specific activity ± SD. Abbreviations used in graphs are mentioned in materials and methods chapter.