PSYCHOTROPIC DRUG AND OXIDATIVE STRESS IN ACUTE POISONING

Thesis

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S.H. KADAR ALI, M.Sc.(Psy)

INSTITUTE OF BIOCHEMISTRY

MADRAS MEDICAL COLLEGE

CHENNAI - 600 003

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CERTIFICATE

This is to certify that the thesis entitled "PSYCHOTROPIC DRUG AND OXIDATIVE STRESS IN ACUTE POISONING" submitted to the **T.N. Dr. M.G.R. MEDICAL UNIVERSITY,** CHENNAI-32 for the degree of doctor of philosophy, is the bonafide record of research work done by **Mr.S.H.KADAR ALI, M.Sc(PSY),** during the period of January 2004-October 2008 under my guidance at the Institute of Biochemistry, Madras Medical College,Chennai-3, India and that the thesis has not formed the basis for the award of any Degree, Diploma, Associateship, Fellowship or any other similar title to the candidate and represents independent and original work on the part of the candidate under my direct supervision.

(Dr.R.VANAJA, M.Sc., Ph.D.,)
Supervisor &
Assistant Professor of Biochemistry
Madras Medical College
Chennai-3

DECLARATION

I hereby declare that the thesis entitled **"PSYCHOTROPIC DRUG AND OXIDATIVE STRESS IN ACUTE POISONING"** is a research work done by me under the supervision and guidance of **Dr. .R.VANAJA, M.Sc., Ph.D.,** Assistant Professor of Biochemistry, Madras Medical College, Chennai-3, from January 2004 to October 2008. Previously, the thesis or any part thereof has not formed the basis for the award to me of any Degree, Diploma, Associateship, Fellowship or any other similar title.

Chennai – 600 003 **(S.H.KADAR ALI)**

CONTENTS

S.no	Title	Page no.
1.	INTRODUCTION	1
2.	AIMS AND OBJECTIVES	9
3.	REVIEW OF LITERATURE	10
4.	PATIENTS AND METHODS	46
5.	RESULTS	62
6.	DISCUSSION	128
7.	CONCLUSION	136
8.	SUMMARY	138
	BIBLIOGRAPHY	

ABBREVIATION

dl	-	Decilitre
g	-	Gram
hr	-	Hours
l	-	Litre
IU/L	-	International units/litre
Kg	-	Kilogram
M	-	Molar
mg	-	Milligram
µ.mole	-	Micromole
min	-	Minute
ml	-	Mililitre
m.mole	-	Millimole
ng	-	Nanogram
n.mole	-	Nanomole
µl	-	Microlitre
%	-	Percent
µg	-	Microgram
U/ml	-	Units/mililitre
AP	-	Acute amitriptyline poisoning
ATP	-	Adenosine triphosphate,
ADP	-	Adenosine diphosphate
NAD^+	-	Nicotinamide adenine dinucleotide oxidized form

NADPH	-	Nicotinamide adenine dinucleotide phosphate reduced from
PMS	-	Phenazine Methosulphate
NBT	-	Nitro Blue Tetrazolium
Vit. E	-	Vitamin E
ALA	-	Alpha lipoic acid
MDA	-	Malonyldialdehyde
LPO	-	Lipid peroxidation
LDH	-	Lactate dehydrogenase
CPK	-	Creatinine phosphokinase
SOD	-	Superoxide Dismutase
ROS	-	Reactive oxygen species
AchE	-	Acetyl cholinestrase
TEEP	-	Tetraetyl pyrophosphate
IV	-	Intravenous
IM	-	Intramusclar
GI	-	Gastro intestinal
CYP	-	Cytochrome P_{450}
TBA	-	Thiobarbituric Acid
TCA	-	Trichloroacetic Acid
AST	-	Aspartate Transaminase
ALT	-	Alanine Transminase
Hb	-	Haemoglobin
GPX	-	Glutathione Peroxidase
GSH	-	Reduced Glutathione

GSSH	-	Oxidised Glutathione
O_2	-	Superoxide
H_2O_2	-	Hydrogen peroxide
OH	-	Hydroxyl radical
DNA	-	Deoxy ribo nucelic acid
GST	-	Glutathione transferase
FRP	-	Ferritin repressor protein
MT	-	Metallothionein
ABTS	-	Azino-dietylbenzthia zoline sulphonate.
TLC	-	Thin layer chromatography
Rf	-	Ratio Front
Gr.I	-	Group I
Gr.II	-	Group II
Gr. III	-	Group III
Gr.IV	-	Group IV
Gr.V	-	Group V
Gr.A	-	Group A
Gr.B	-	Group B
AIDN	-	Amitriptyline induced delayed neuropathy

PSYCHOTROPIC DRUG AND OXIDATIVE STRESS IN ACUTE POISONING

INTRODUCTION

Acute poisoning is a common and urgent medical problem in all developed, and many developing, countries of the world. In Britain it accounts for 15-20% of all acute medical emergency admissions to hospital. The different types of acute poisoning are accidental (10%) and intentional (90%). In the intentional type only 10% are actual attempted suicide cases and 80% are self-poisoning cases. In older age groups the great majority are intentional. Acute poisoning is more common in females than in males in all age groups, the ratio of females to males being about 1.4 : 1.0. The increase has been marked in patients of lower social class. Currently in all European Countries the main drugs causing death of self-poisoning patients admitted to hospital are analgesics, antidepressants and benzodiazepines.[1]

Stress-related diseases cost American Industry billions of dollars a year; several billion (psychotropic drugs and antidepressant) pills are prescribed in the world each year; and although it cannot be quantified, stress seems to be involved in much of our unhappiness, irritability and dissatisfaction.[2]

Drugs used to treat psychiatric disorders are known collectively as psychotropics. They are classified according to their main mode of action. Attempted suicide is in a steady increase. In 1990 there were over 100,000 cases per year in Britain. Most suicide attempts are due to drug overdose, either prescribed or non-prescribed. Suicide attempts are commoner in women than in men and in young adults than in the elderly.[3]

The drugs used to modify or to correct pathological behavior, psychotic disorders, moods or thoughts are known as Psychotropic drugs,[4] which can be classified as follows:

I Antipsychotic drugs (Major Tranquilizers)

II Anti anxiety drugs.

III Psychotogenic or Psychodelic drugs

IV Antidepressant drugs.

The first antipsychotic drugs are further classified as phenothiazines, benzamides, etc. One of the most extensively used antipsychotic drugs in this group is Chlorpromazine.

The second antianxiety drugs can be classified as benzodiazepines, nonbenzodiazepines, etc. Alprazolam and diazepam are widely used in this group.

The third psychedelic drugs could be classified as (i) Those with indole ring e.g lyseric acid diethylamide (LSD i.e with indole ring) (ii) Without indole ring e.g cannabis (marijuana) showing miscellaneous action (iii) Drugs showing anticholinergic action e.g atropine.

The fourth is antidepressant drugs. These drugs are classified as tricyclic, tetracyclic, monoamine oxidase inhibitors (MAOI) and lithium compounds. The tricyclic are divided into two groups :

i. tertiary amines eg) Imipramine, Amitriptyline etc.

ii. secondary amine eg) Desipramine, Nortreptyline and Protriptyline.

Action : Amitriptyline exerts its antidepressant action by blocking the neuronal reuptake of nor adrenaline and serotonin. Amitriptyline has some sedative action. Endogenous depression responds to a greater extent than other types. Amitriptyline has significant anticholinergic activity.

Amitriptyline : 3-(10,11 Dihydro - 5H - dibengo [a,d] cyclohepten- 5 ylidene) - N, N dimethylpropylamine; $C_{20}H_{23}N$; relative moleuclar mass, 277.

Amitriptyline is very widely used tricylic antidepressant. It is metabolized by N-demethylation to nortriptyline, which is an antidepressant in its own right. Protriptyline is an analogue of amitriptyline.[5] The tricylic antidepressant amitriptyline has been easily available and commonly abused for suicidal purposes in developing countries.

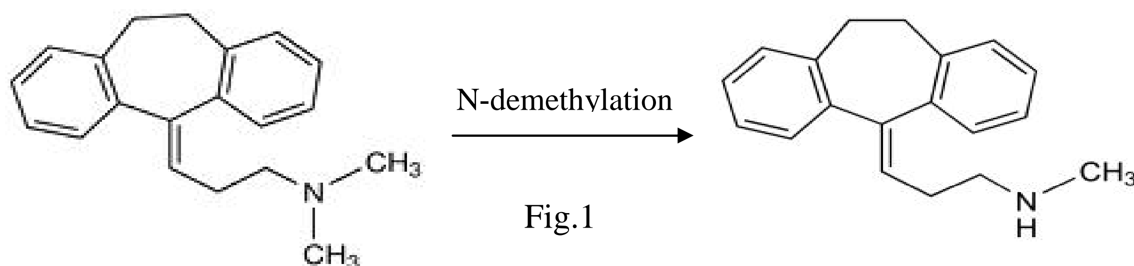


Fig.1

OXIDATIVE STRESS

The term oxidative stress refers to the situation of imbalance between production of free radicals and antioxidant defence[6] principally oxidative stress in human can result in diminished body antioxidant when the free radical production overwhelms the endogenous antioxidant levels, they cause considerable cell damage or death.

All major biomolecules like lipids, proteins, and nucleic acids react with free radicals, but lipids are probably the most susceptible.[7] The oxidative destruction of lipids (lipid peroxidation) is a destructive self-perpetuating chain reaction releasing malonyldialdehyde (MDA) as the end product.[8]

Studies on acute amitriptyline poisoning cases indicate increased level of SOD, MDA and reduced antioxidant capacity of blood (FRAP assay) acute amitriptyline poisoning cases showed increased oxidative stress and reduced antioxidant status.

VITAMIN C

Ascorbic acid or vitamin C is a monosaccharide antioxidant found in both animals and plants. As it cannot be synthesized in humans and must be obtained from the diet, it is a vitamin.[9] Most other animals are able to produce this compound in their bodies and do not require it in their diets.[10] In cells it is maintined in its reduced form by reaction with glutathione, which can be catalysed by protein disulfide isomerase and glutaredoxins.[11,12] Ascorbic acid is a reducing agent and can reduce and their by neutralize reactive oxygen species such as hydrogen peroxide.[13] In addition to its direct antioxidant effects, ascorbic acid is a substrate for the antioxidant enzyme ascorbate peroxide, a function i.e particularily important in stress resistance in plants.[14]

ALPHA LIPOIC ACID

Alpha lipoic acid initially classified as a vitamin discovered three decades earlier possessed potent antioxidant properties. It is more potent than old guard antioxidant vitamin C & Vitamin E, it recycles these vitamins and enhances their effectiveness. It dissolves in both water and fat and thus claimed to be an "universal antioxidant" it can reach tissues composed of fat such as the nervous system as well as those made mainly of water such as heart. Alpha-lipoic acid was effective in various neurological disorders like Parkinsons diseases[15] and Alzhemier's disease. Also intravenous forms of alpha-lipoic acid were administered in hospitals to treat acute mushroom poisoning cases.

Based on the above beneficial effects of vitamin C and alpha-lipoic acid, we have decided to supplement vitamin C and alpha-lipoic acid to acute amitriptyline poisoning cases and study their effects before and after supplementation.

LDH ENZYME

Lactate dehydrogenase (LDH) is a zinc containing enzyme that is part of the glycolcytic pathway. It is found in cytoplasm of all cells and tissues in the body, LDH is a tetramer of two active subunits, H (Heart) and M(Muscle) with a molecular weight of 134 kDa, combinations of these subunits produce five isoenzyme ranging from LDH_1, to LDH_5.

Human LDH can be separated into five different isoenzymes (LDH_1, to LDH_5) based on their electrophoretic mobility.[16]

Another form of LDH of four C subunits is found in spermatozoa and in semen but has never been detected in serum, even in individuals with seminoma[17] rarely, another band detected in electrophoresis and termed LDH_6 can be seen; this probably represents alcohol dehydrogenase, which can also metabolize lactate.[18]

There are many inherited terms of deficiency of H[19] and M[20]sub unit of LDH.

LDH is widely distributed is mamalian tissues, being rich in myocardium, kidney & liver.

The tissue distribution of LDH varies primarily in its isoenzyme composition, not in its content of LDH, in contrast to enzymes such as Aspartrate Transaminase (AST), Alanine Transaminase (ALT) and Creatinine Kinase (CK), which shows marked variation in enzyme activity between tissues.

Most tissues have LDH ratios between plasma and serum of 500 to 1000:1. In plasma, the majority of LDH comes from break down of erythrocytes and platlets, with varying contribution from other organs. LDH is apparently eliminated in bile, as injection of radiolabeled LDH, results in radioactivity in the gall bladder and small intestine.[21]

Isoenzymes of Lactate Dehydrogenase

The LDH of normal human serum has been found to be separable into 5 different components by appropriate electrophoretic techniques. Each of these isoenzymes is distinguishable from the others by electrophoretic and various other chemical procedures. Indeed the great current interest in isoenzymology derives from the observations on the multiple molecular forms of LDH. The isoenzymes of LDH are designated according to their electrophoretic mobility. The fractions with the greatest mobility (anodic) is called LDH_1, that with least mobility is called LDH_5, and the other 3 are designated accordingly as LDH_2, LDH_3 and LDH_4 respectively.

The five LDH isoenzymes have the same molecular weight (135 000) but differ in the charge that they carry. Each isoenzyme is a tetramer made up of four subunits, each of 34,000 daltons. There are two types of these sub units, designated H and M, respectively, for heart polypeptide chain (M). The five isoenzymes of LDH consist of the five possible combinations of monomers H and M. Hence there are two homotetramers (LDH_1 and LDH_5) and three hybrids.

LDH isoenzymes	Subunits
L_1	HHHH
L_2	HHHM
L_3	HHMM
L_4	HMMM
L_5	MMMM

The H and M chains differ significantly in their amino acid composition and thus in their structural and kinetic properties, they are probably under the control of two distinct genes.

Tissue LDH consists of 5 isoenzymes in varying proportions, and the LDH activity of each tissue has a characteristic isoenzyme composition, thus the LDH of myocardium and erythrocytes consists largely of the fastest moving isoenzymes (LDH_1 and LDH_2).

In Liver and skeletal muscle, the principal isoenzymes are LDH_4 and LDH_5. In general tissues exhibiting aerobic metabolism demonstrate predominantly faster moving isoenzymes (LDH_1) with more H subunits, while tissues exhibiting anaerobic metabolism demonstrate predominantly slower moving isoenzymes (LDH_5) with more M. units. A number of tissues (lung, spleen, pancreas, thyroid, adrenals and lymph nodes) consists mainly of LDH_3. The relative concentration of the several isoenzymes in normal serum is LDH_2, LDH_1, LDH_3, LDH_4 and LDH_5 in descending order, normal serum LDH has been presumed to derive mainly from erythrocytes with LDH_2 higher than LDH_1.

CREATININE KINASE

Creatinine Kinase (CK) is involved in energy storage in tissues, primarily muscle, during periods of active muscle contraction, ATP is used up and creatinine phosphate is converted by CK to creatine and ATP. To allow continued contraction, during periods of rest, ATP is converted to creatinine phosphate by CK to serve as an energy reservoir.

CK is predominately found as a dimer of catalytic subunits, each with molecular weight of about 40 kDa; the two sub units are termed M (for muscle) and B (for Brain). The three resulting isoenzymes are CK_1 (BB), CK_2 (MB) and CK_3 (MM). Another structurally different form of CK, with molecular weight of 64 kDa is present in mitochondria, although it is seldom released to the circulation, it may also form oligomers. It is termed as macro CK_2 with molecular weights upto 250 kDa.

CK is found in small amounts throughout the body, but is in high concentration only in muscle and brain, although CK, from brain virtually never crosses the blood-brain barrier to reach plasma.

In skeletal muscle, CK-MB comprises 0% to 1% of the total CK in type 1 fibers and 2% to 6% of CK in type 2 fibers. During regeneration of skeletal muscle, increased amounts of CK-MB are produced relative to CK-MM, similar to the pattern seen in total muscle.[22]

In the normal heart an average of 15% to 20% of the CK is CK-MB; its distribution is not uniform, with CK-MB percentage greater in the right heart than in the left heart.[23] A single study however suggests that CK-MB is not found in normal myocardium.[24] CK-MB is the dominant isoenzymes of CK found in brain and in smooth muscle.

CK is not stable on storage at room temperature for more than a few hours. Activity can be mostly regenerated using N-acetylcysteine, which regenerates sulfhydryl groups in the catalytic site. CK is stable when frozen for several months.

The most commonly used method for measuring CK-MB is mass immunoassay, either using two different antibodies or the "CONON" monoclonal antibody that specific for CK-MB isoenzyme.[25] There are slight but significant differences between different immunoassays.[26]

CK by mass measurements is stable even at refrigerator temperatures, and shows only slight decrease when stored for many days at room temperature.[27]

Measurement of CK isoforms is usually accomplished using high-resolution electrophoresis. Because of the low activity of CK-MB isoforms, precision is generally poor for this purpose and careful control of assay conditions is necessary to achieve reproducible results; an automated, electrophoertic instrument is required for this purpose.[28] Immunoassays have also been used for quantifying the isoforms of CK-MB.[29]

AIMS AND OBJECTIVES

Psychotropic drug overdoses are the most common suicidal poison with high morbidity and mortality and account for a large proportion of patients admitted to intensive care units. It is noted that psychotropic drug poisoning accounted for 15-20% out of the total admission to the medical intensive care unit of Govt. General Hospital, Chennai-3, Tamil Nadu, India. During 2005 and 2006 psychotropic drug overdosages accounted for 36.3% of the total poisoning cases. Many persons young and old consume drugs to end their lives. Though routiene standard treatment (RST) is being given to all the acute drug poisoning patients, the mechanism of toxicity is differ in each case , moreover no detailed study is available with regard to enzyme variations and antioxidant status in acute amitriptyline poisoning (AP) cases,the more widely used psychotropic drug nowadays.

To evaluate antioxidant status in acute amitriptyline poisoning (AP) cases undergoing routine standard treatment (RST) after supplementation with Vitamin C and alpha lipoic acid either alone or in combination, and compare it with that of normal healthy volunteers.

To assess the plasma cholinesterase and RBC cholinesterase levels at the time of admission and at the time of discharge.

To analyse the total and isoenzyme pattern of CPK and LDH in blood samples of acute amitriptyline poisoning cases and to find out the maximum tissue damage involved in the cases on admission.

REVIEW OF LITERATURE

Examination of patients with acute oral poisoning by psychotropic agents has indicated that toxicolypoxic encephalopathy develops, when the high concentrations of toxicants and their active metabolites specifically affect brain structures, resulting in oxidative stress and impaired natural detoxification mechanisms.[30]

In acute poisoning, oxidative stress is a result of tissue inflammation, poor dietary intake of micronutrients due to drowsiness, free radical burst from activated macrophages, and excessive drug. These free radicals may in turn contribute to tissue inflammation if not neutralized by antioxidants.

The total antioxidants status (TAS) of individuals is a function of dietary, enzymatic, and other systemic antioxidants and is therefore an indicator of the free radical load.

The term oxidative stress refers to the situation of imbalance between production of free radicals and antioxidant defence.[31] Principally, oxidative stress in human can result from diminished body antioxidants when the free radical production overwhelms the endogenous antioxidant levels, causing considerable cell damage/ death.

All major biomolecules like, lipids, proteins and nucleic acids may be attacked by free radicals, but lipids are probably the most susceptible.[32]

The oxidative destruction of lipids (Lipid peroxidation) is a destructive self-perpetuating chain reaction releasing malonyl dialdehyde (MDA) as the end product.[33]

This sums up the levels of enzymatic antioxidants (SOD, catalase. GPx, GST) and non-enzymatic antioxidants (Glutathione, Vitamin C, and alpha lipoic acid).

Choice for selecting vitamin C and alpha lipoic acid as antioxidant supplement.

Theory of Antioxidant

One of the most persuasive arguments for the use of extra vitamin C, alpha lipoic acid. and other antioxidants is their ability to absorb free oxygen radicals. Oxygen combines with hydrogen in the free hydroxyl radical - OH or in the highly reactive hydrogen peroxide molecule, H_2O_2. (Alpha lipoic acid, Tocopherols and tocotrienols (Vitamin E) ascorbic acid (vitamin C), and the Carotenoids react with these free radicals, notably peroxyl radicals, and with singlet molecular oxygen (O_2^-). If O_2^- floats free of the energy engines, it may interact vigorously with other molecules. Cell membranes are vulnerable to O_2^- injury; damaged membranes disturb the function of the entire cell. Extra O_2^- reacting with DNA can make the code sticky and can cause mistakes in code reading or replication, resulting in cell mutation. The cumulative damages of trillions of random O_2^-, encounters with critical molecules over many years contributes to accelerated aging and cellular dysfunction, like cancer. Cells contain oxygen detoxification enzymes: peroxidases, superoxide dismutase, and catalase. Several molecules combine harmlessly with O_2^- and are referred to as "antioxidants". Vitamin C is the cheapest, safest, and best antioxidant. If the amount of Vitamin C in cells is raised, enough O_2^- can be soaked up to make a long-term difference. The effect of Vitamin C is enhanced if another nutrient antioxidants alongside, alpha lipoic acid is presented.

Role of vitamin C as antioxidant

Antioxidant Mechanism of Ascorbic Acid.

- Hydrogen donation to reactive oxygen species
- Quenching of singlet oxygen

Vitamin C is a strong and powerful water-soluble antioxidant that efficiently protects important organic and biological molecules against oxidative degradation. Vitamin C is the best known, the most studied, and the most frequently supplemented antioxidant. It works even better in conjunction with other antioxidants, such as alpha lipoic acid and carotenoids, by establishing a peculiar recycling system with synergic effect.

As a water-soluble antioxidant, Vitamin C is in a unique position to "scavenge" aqueous peroxyl radicals before these destructive substances get a chance to damage the lipids. It works along with alpha lipoic acid a fat-soluble antioxidant, and the enzyme glutathione peroxidase to stop free radical chain reactions. As an antioxidant, vitamin C's primary role is to neutralize free radicals. Since ascorbic acid is water soluble, it can work both inside and outside the cells to combat free radical damage. Free radicals seek out an electron to regain their stability. Vitamin C is an excellent source of electrons; it can donate electrons to free radicals such as hydroxyl and superoxide radicals and quench their reactivity.[34]

The versatile vitamin C also works along with glutathione peroxidase (a major free radical-fighting enzyme) to revitalize alpha lipoic acid a fat-soluble antioxidant. In addition to its work as a direct scavenger of free radicals in fluids, vitamin C also contributes to the antioxidant activity in the lipids.

Immune system functions

Vitamin C can enhance the body's resistance to an assortment of diseases, including infectious disorders and many types of cancer. It strengthens and protects the immune system by stimulating the activity of antibodies and immune system cells such as phagocytes and neutrophils. Vitamin C assists the immune system in two of its primary functions to rid the body of foreign invaders and to monitor the systems for any sign of tumor cells. It accomplishes these vital tasks by stimulating the production of white

blood cells, primarily neutrophils, which attack foreign antigens such as bacteria and viruses. It also boosts the body's production of antibodies and interferon, the protein that helps protect us from viral invaders and cancer cells.

Vitamin C prevents free radical damage in the tissues and helps protect the central nervous system from such damage. In a study of guinea pigs, an ascorbic acid pretreatment effectively diminished the acute lung damage caused by the introduction of superoxide anion free oxygen radicals to the trachea.[35] Ascorbic acid was also tested as an antioxidant to inflammatory reaction in mice. Apparently, vitamin C has a low order of toxicity, or intoxications would be common. Determination of vitamin C requirements based on antioxidant functions will require development of more reliable tests for in vivo oxidative damage and further understanding of the interactions of ascorbate with other physiological antioxidants. Additionally, a practical method for measuring the vitamin C body pool is needed as a standard of comparison with proposed functional measures and measures of health of disease endpoints.

Role of Alpha lipoic acid(ALA) as antioxidants

Alpha lipoic acid initially classified as a vitamin discovered three decades earlier possessed potent antioxidant properties. It is more potent than the old guard antioxidants. It is more potent than the old guard antioxidants vitamin C and E, it even recycles these vitamins and enhances their effectiveness.

In addition to functioning as an antioxidant, this hard working nutrient assists B vitamins in producing energy from the proteins carbohydrates and fats consumed through foods.

As it dissolves in both water and fat this is called "UNIVERSAL ANTIOXIDANT". It can reach tissues composed of fat, such as in the nervous system as well as those made mainly of water such as in the heart.

Intravenous forms of alpha lipoic acid are administered in hospitals to treat cases of acute mushroom poisoning and other cases of acute poisoning that affect the liver.

A study was conducted with 600 mgs of alpha lipoic acid given daily to 9 Alzheimer's patients, on an average for 80 days. The treatment lead to stabilization of cognitive functions in the Alzheimer's study group. This was the first indication that alpha lipoic acid might be a successful neuroprotective therapy option for Alzheimer diseases and related dementias.[36]

Alpha lipoic acid benefits anyone whose limbs tend to tingle or become numb or "fall asleep" due to nerve compression. In animal studies, alpha lipoic acid has been seen to increase the blood flow to the nerves and improved transmission of nerve impulses.

Parkinson’s disease (PD) is disorder of the central nervous system. Clinically the disease is characterized by disease in spontaneous movements, gait difficulties, postural instability, rigidity and tremor. PD may appear at any age but it is uncommon in people under 30 years of age.

Oxidative stress appear to play an important role in neuronal degeneration associated with PD.[37]

Depletion of glutathione (GSH) in the brain is the earliest indicator of oxidative stress presymptomatic PD.[38]

Studies both in vitro and vivo models have suggested that pretreatment with alpha lipoic acid increase cellular levels of GSH, probably by preventing its depletion thereby protecting mitochondria integrity.

Results with previous studies suggest that alpha lipoic acid may be an effective neuro protective agent in age associated neuro degeneration utilizing the PC12 cell model system.

Based on the above beneficial effects of alpha lipoic acid on nervous system it has been decided to supplement alpha lipoic acid with acute amitriptyline poisoning cases, which have increased oxidative stress and neuro toxicity, and to study the effect after supplementation of alpha lipoic acid.

The drug usually enter the blood stream after absorption through the G.I tract. However, in studying the effects of chemicals in laboratory animals, other routes of administration may also be used. The most common routes are

Intravenous, Intraperitoneal, Subcutaneous, Intramuscular

The toxicity of a chemical or drug may or may not depend on the route of administration. If a toxicant is injected intraperitoneally, most of the chemical enters the liver via the portal circulation before reaching the general circulation.

Therefore, an intraperitoneally administered compound may be completely extracted and biotransformed by the liver with subsequent excretion into bile without gaining access to the systemic circulation.

Propranolol[39] and lidocaine[40] are two drugs with efficient extraction during the first phase pass through the liver. A chemical with a high first-pass effect that is toxic in an organ other than the liver and G.I tract is likely to be less toxic when administered intraperitoneally than when administered by other routes (IV, IM, Subcutaneously) because the intrapertoneal route favours extraction in the liver to reduce what is available systemically. In contrast, compounds with no appreciable biotransformation in the liver are likely to show similar toxicity independent of the route of administration. Therefore preliminary information as the contribution of biotransformation and excretion of xenobiotics to toxic outcome can be derived by comparing toxic responses after administration by different routes.

Intentional (e.g. sucide) exposure are typically oral, or parenteral injection of a drug. They are readily absorbed by passive diffusion, across the G.I tract, with peak blood concentration occurring within minutes to several hours.[41]

After entering the blood by absorption drug compounds distributes to tissues throughout the body. Distribution usually occur rapidly. The rate of distribution to organs or tissues is determined primarily by blood flow and the rate of diffusion out of the capillary bed into the cells of a particular organ or tissue.

The final distribution depends largely as the affinity of a xenobiotic for various tissues. In general, the initial phase of distribution is dominated by blood flow. Whereas the eventual distribution is determined largely by affinity. Lipid-soluble molecules readily permeate the membrane itself, very polar molecules and ions of even moderate size (molecular wt. of 50 or more) cannot enter cells easily except by special transport mechanism because they are surrounded by a hydration shell, making their actual size much larger.

The concentration of a toxicant in blood depends largely on its volume of distribution. The volume of distribution (Vd) is used to quantify the distribution of a xenobiotic throughout the body. It is defined as the volume in which the amount of drug would need to be uniformly dissolved in order to produce the observed blood concentration.

1 gm of a drug in a 70 Kg human.

Compartment	% of Total	Litres in 70 Kg Human	Plasma Conc. after 1 g of chemical
Plasma water	4.5	3	333 mg/L
Total extra cellular water	200	14	71 mg/L
Total body water	55	38	26 mg/L
Tissue toxicity	-	-	0-25 mg/L

Distribution of toxicants is a complex and under most circumstances cannot be equated with distribution into one of the water compartments of the body. Binding to/or dissolution in various storage sites of the body such as fat, liver and bone, are usually more important factors in determining the distribution of chemicals/drugs.

Liver and kidney have a high capacity for binding many chemicals/drugs. These two organs probably concentrate more toxicants than do all the organs combined, and in most cases, active transport or binding to tissue components are likely to be involved. Excess drugs rapidly and widely distributes to various tissues especially liver, kidney, adipose and tissues rich in lipids.[42]

There are many organic compounds that are highly stable and lipophilic, leading to their accumulation in the environment. The lipophilic nature of these compounds also permits rapid penetration of cell membranes and uptake by tissues.

There have been numerous attempts to alter storage of lipothelic toxins in adipose tissues in animal models, in humans changes in body fat composition appear to be the most effective in reducing body burdens and interventions to reduce the absorption of these compounds from the G.I tract appear to be some what helpful in reducing storage depots of persistent organic pollutants in fat.[43]

CHOLINESTERASE

Cholinesterase is an enzyme which has the ability to hydrolyse acetylcholine. Two related enzymes have this ability. One is acetylcholinesterase, which is called the true cholinesterase, or cholinesterase. True cholinesterase is found in erythrocytes, the lungs and spleen, nerve endings, and the gray matter of the brain. It is responsible for the prompt

hydrolysis of acetylcholine released at the nerve endings to mediate transmission of the neural impulse across the synapse. The degradation of acetylcholine is necessary to the depolarization of the nerve so that it can be repolarized in the next conduction event.

The other cholinesterase is acylcholine acylhydrolase, it is usually called pseudocholinesterase, benzoyl cholinesterase, or choline esterase II. Although it is found in the liver, pancreas, heart, white matter of the brain and serum its biological role is unknown. The serum enzymes is the one whose assay is clinically useful.

Structure of Acetylcholinesterase

The enzyme structure depicted in figure 1 exists in two general classes of molecular forms, simple homomeric oliogomers of catalytic subunits (i.e monomers, dimers and tetramers) and heteromeric associations of catalytic subunits with structural subunits. The homomeric forms are found as soluble species in the cell, presumably destined for export, or associated with the outer membrane of the cell through either an intrinsic hydrophobic amino acid sequence or an attached glycophospholipid. One heterologous form the largely found in neuronal synapses, is a tetramer of catalytic subunits disulfide-linked to a 20,000 dalton lipid linked subunit. Similar to the glycophospholipid attached form, it is found in the outer surface of the cell membrane. The other consists of tetramer of catalytic subunits, disulfide linked to each of the three strands of a collagen like structural subunit. This molecular species, whose molecular mass approaches 10^6 daltons, is associated with the basal lamina of junctional areas of skeletal muscle.

STRUCTURE OF ACETYLCHOLINESTERASE

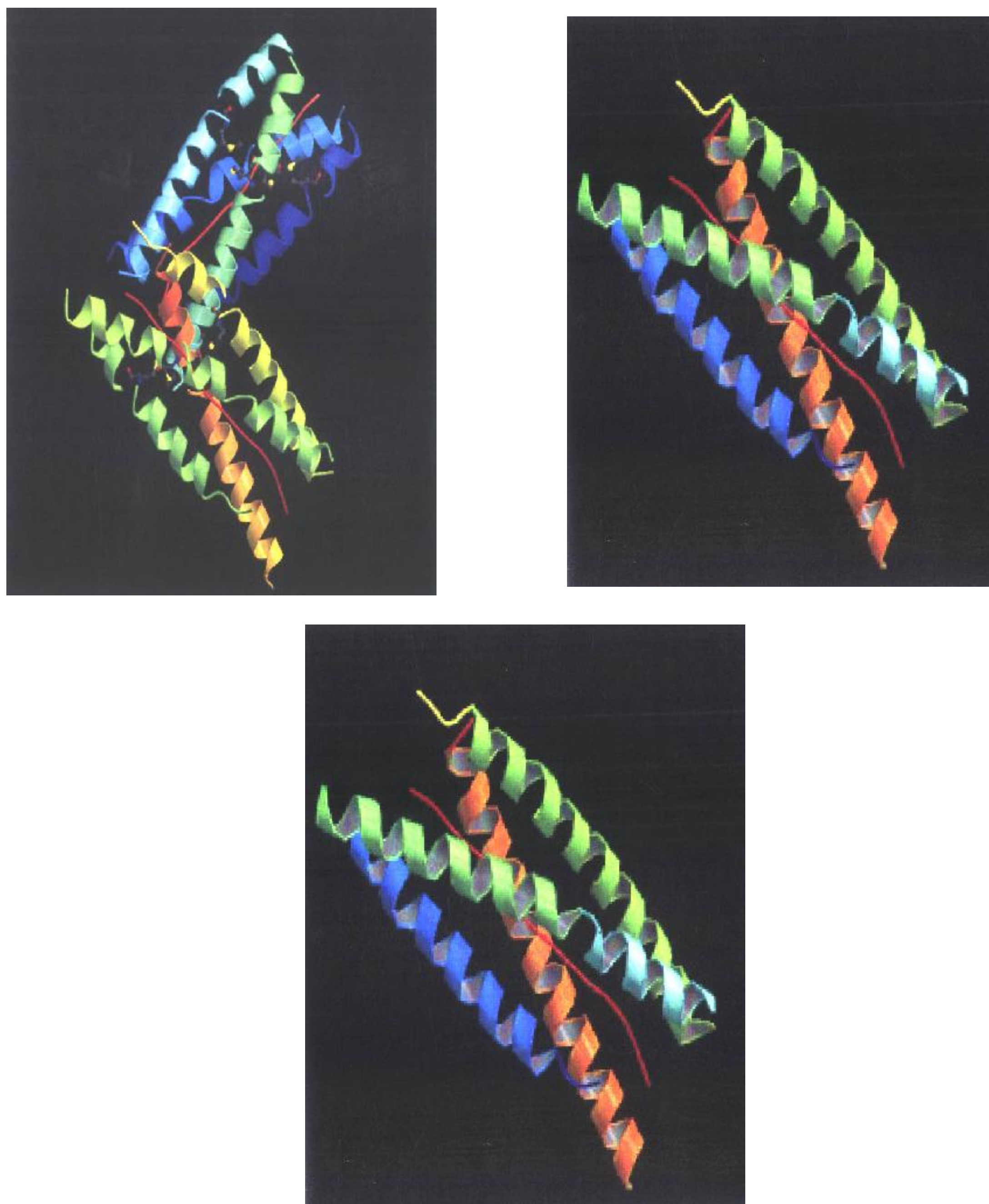


Fig.2

Molecular cloning revealed that a single gene encodes vertebrate AChE. However multiple gene products are found, this diversity arises from alternative processing of the mRNA. The different forms differ only in their carboxyl termini, the portions of the gene encoding the catalytic core of the enzyme is invariant. Hence, the individual AChE species can be expected to show identical substrate and inhibitor specificities.

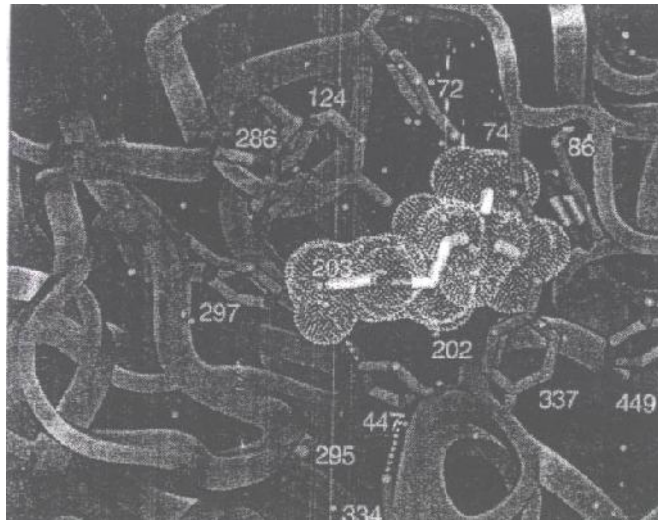


Fig.3

The gene controlling the synthesis of SChE can exist in many allelic forms. Four of the most common forms are designated as $E\frac{u}{1}E\frac{a}{1}E\frac{\mathrm{u}}{1}$ At least 25 others forms exist, and another gene locus is recognized (E2). The normal, most common phenotype is designed as or UU. The gene is referred to as the atypical gene, the sera of people homozygous for this gene are only weakly active toward most substrate for cholinesterase and possess increased resistance to inhibition of enzyme activity of dibucanie. Hence the cholinesterase present in normal sera can be separated by electrophoresis into 7 to 12 bands, the number obtained depending on the experimental technique used.

A separate structurally related gene encodes butyrylcholinesterase, which is synthesized in the liver and is primarily found in plasma. The cholinesterases define a superfamily of proteins whose structural motif is the alpha, beta hydrolase fold. The family includes several esterases, other hydrolases found in the nervous system and surprisingly proteins without hydrolase activity such as thyroglobulin and members of the actin and neurologin families of proteins. The three dimensional structure of AChE shows the active center to be nearly centrosymmetric to each subunit and reside at the base of a narrow gorge about 20 A in depth. At the base of the gorge lie the residues of the catalytic traid, serine 203, histidine 447, and glutamate 334 (Fig. 3).

Action of AChE[44]

The type of reaction catalyzed by both cholinesterase is as follows (Fig.4).

The two enzymes differ in specify towards some substrates while behaving similarly towards other. The serum enzyme acts on benzolycholine but cannot hydrolyze acetyl Beta-methylcholine, the red cell enzyme acts on the latter but not on the former. Only choline esters are split by the red cell enzyme, aryl or alkyl esters are not attacked. The red cell enzyme is inhibited by its substrate, acetylcholine, if present at concentrations about 102 mol/L, the serum enzyme is not inhibited by this substrate.

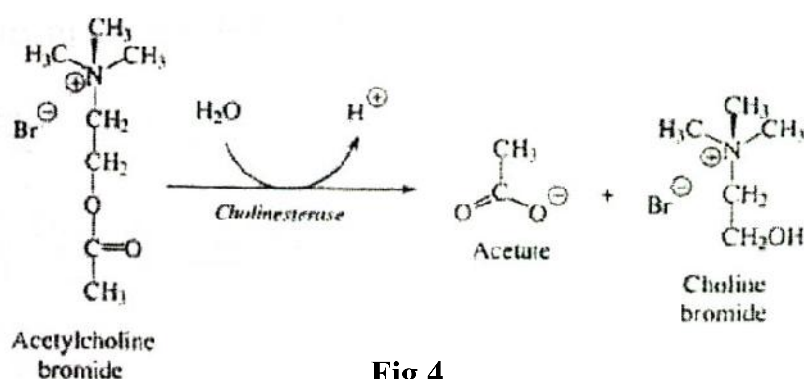


Fig.4

The catalytic mechanismn of AChE resembles that of other hydrolases, where the serine hydroxyl group is rendered highly nuecleophilic through a charge relay system involving the carboxyl from glutamate, the imidazole on the histidine, and the hydroxyl of the serine.

During enzymatic attack of acetylcholine, an ester with trigonal geometry, a tetrahedral intermediate between enzyme and substrate is formed that collapses to an acetyl enzyme conjugate with the concomitant release of choline. The acetyl enzyme is very liable to hydrolysis, which results in the formation of acetate and active enzyme. AChE is one of the most efficient enzymes known and has the capacity to hydrolyze $6x10^5$ ACh molecules per molecule of enzyme per minute, this yields a turnover time of 150 microseconds.

AChE Inhibition[45]

Both enzyme are inhibited by the alkaloids, prostigmine and physostigmine, both of which contain quaternary nitrogen (also present in choline) in their structures. These two compounds are typical competitive inhibitors, competing with the choline residue of acetylchoine for is binding site on the enzyme surface. Both enzymes are irreversibly inhibited by some organic phosphorous compounds, such as diisopropylfluorophosphate. The phosphoryl group binds very tightly to the enzymes site at which binding of the acyl group normally occurs, thus preventing attachment of the acetylcholine. Both enzymes are also inhibited by a large variety of other compounds, among which are morphiate, quinine, tertiary amines, phenothiazines, pyrophosphate, bile salts, citrate, fluoride, and borate.

The mechanism of hydrlosis of ACh by AChE and its inhibition and reactivation are depicted in (Fig. 5).

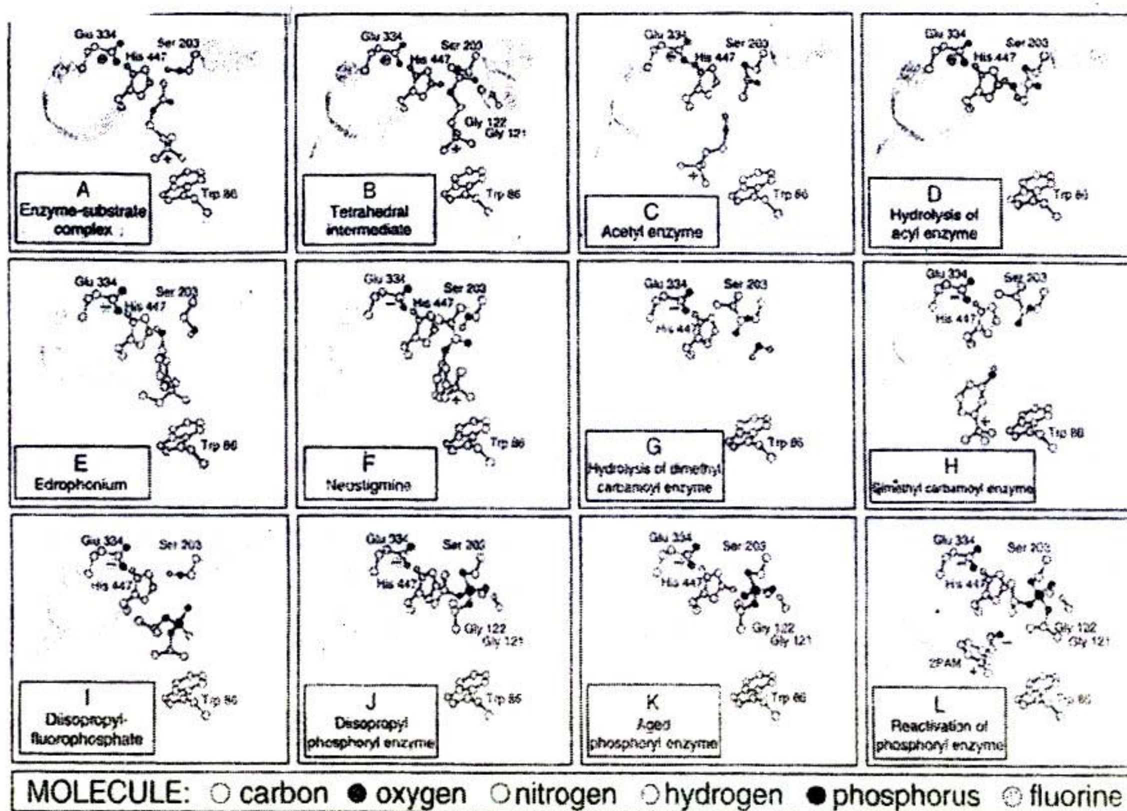


Fig.5

Three distinct domains on AChE constitute binding sites for inhibitory ligands and form the basis for specifity differences between AChE and butyrylcholinesterase, the acyl pocket of the active center, the choline subsite of the active center, and the peripheral anionic site. Reversible inhibitors such as edrophonium and tacrine bind to the choline subsite in the vicinity of tryptophan 86 and glutamate 202 Edrophonium has a brief duration of action owing to its quaternary structure and the reversibility of its binding to the AChE active center. Additional reversible inhibitors, such as denopezil, bind with higher affinity to the active center. Other reversible inhibitors such as propidium and the peptide toxin fasciculin, bind to the peripheral anionic site on AChE. This sites resides at the lip of the gorge and is defined by tryptophan 286 and tyrosines 72 and 24.

Drugs that have a carbamoyl ester linkage, such as physotigamine and neostigimne, are hydrolyzed by AChE but much more slowly than is ACh. Both the quaternary amine neostigmine and the tertiary amine physostigmine exist as cations at physiological PH. By serving as alternate substrate with a similar binding orientation as acetylcholine, the attack by the active center serine gives rise to the carbomylates enzymes.

The carbamoyl moiety resides in the acyl pocket outlined by phenylalanines 295 and 297. In contrast to the acetyl enzyme, methylcarbamoyl AChE and dimethylcarbamoyl AChE are far more stable for hydrolysis of the dimethylcarbamoyl enzyme is 15 to 30 minutes. Sequestration of the enzyme in its carbamoylate form thus precludes the enzyme catalyzed hydrolysis of ACh for extended periods of time. In vivo, the duration of inhibition by the carbamolylating agents is 3 to 4 hours.

Biotransformation of Amitriptyline

The group as a whole is rapidily absorbed and the drugs enter the tissues leaving only a small quantity in circulation. They are readily metabolized in the liver and the conjugated metabolites are rapidly cleared by the kidney. An important step in metabolism is demethylation. Thus imipramine is converted to desipramine and amitriptyline to nortriptyline. The urine contains mainly metabolites.

The rate of metabolism shows wide individual variation so that the plasma concentration may vary widely from one patient to another on a constant dose. Thus Braithwaite and Widdop (1971) found a 15-fold range for amitriptyline and a 12-fold range for nortriptyline under such conditions. In view of this, methods have been developed for monitering plasma concentrations to achieve better therapeutic control. It is claimed (Kragh-Sorensen et al., 1973) that for nortriptyline the opitimal concentration in

plasma is 175 µg/l with less satisfactory effects at higher or lower figures. The prescription of these substances to depressed patients is associated with the risk of deliberate self-poisoning. The ingestion of more than 1 g is likely to have serious effects. The clinical feature are especially centered on the nervous and cardiovascular systems. There may be hallucinations, hyper-reflexia, convulsions and varying degrees of unconsciousness but rarely profound coma. Depression of respiration, dilation of pupils, dryness of the mouth and urinary retention are seen. The cardiovascular effects include tachycardia, hypotension and a tendency to develop severe arrhythmias leading to cardiac arrest. These feature become apparent within a hour or two after ingestion and severe symptoms rarely persist for longer than 18 to 24 hours. Intensive supportive theraphy may be needed during the time and the rapid detection of the metabolites in the urine may aid in the detection of the cause of poisoning and the need for such care.

Antidepressants are administrated for different types of defensive illness although a satisfactory relationship between plama concentration and effect exists only for uncomplicated endogeneous depression. As a consequence, therapeutic ranges are not clearly defined. Some of these drugs, e.g.imipramine and desipramine, have a linear plasma concentration-response curve while with others, e.g. amitriptyline and nortriptyline, the curve is of an ‘inverted U’ type. This means that the antidepressant effects improves as plasma concentrations increase but beyond a certain concentration further increases cause a lessening of the effect.

Amitriptyline exerts its antidepressant action by blocking the neuronal reuptake of nor adrenalin and serotonin. Amitriptyline has some sedative action. Endogeneous depression response to a greater extend than other types. Amitriptyline has significant anticholinergic activity.

Literature for antioxidants

Free radicals are produced as part of normal cellular metabolism[46] as well as the result of abnormal reactions stimulated by some disease process or xenobiotic. Due to their ubiquitous nature, and because numerous studies have shown that free radicals have the potential to produce most of the tissue changes associated with the expression of a variety of toxicities and disease processes[47], a role has been suggested for these reactive species in numerous disorders[48]. However, the strength of the links between free radicals and over 100 different diseases and toxic syndromes is often tenuous since it is usually dependent on measuring modifications of tissue molecules that are believed to result from free radical reactions, but which may have other origins.

A radical is an atom or molecule that contains one or more unpaired electrons. Radicals may be charged or uncharged. Since oxygen is so prevalent in biological systems, oxygen radicals are the most common. This quantitative consideration, as well as the ready reactivity of carbon-centered radicals with oxygen, have resulted in the terms radicals and oxidants often being used interchangeably even though they are clearly not equivalent.

A free radical is one that has moved out of the immediate location of its generation and is no longer "controlled" by that environment. Radicals that are retained within their sites of generation have been called "caged" radicals[49]. The distinction between free and caged radicals is important since radicals produced by some enzyme reactions are controlled and the loss of this control has significant toxicologic implications including the effects of antioxidants or other radical traps on these processes.

Oxidation is defined as the removal of one or more electrons from a molecule. Conversely, reduction is defined as the addition of one or more electrons. A balance between oxidation and reduction is required in all chemical reactions. Thus, redox balance is, at equilibrium, inviolable and when

one substance is oxidized some other must be reduced. Within biological systems, this balance is a steady state and can be shifted between different classes of molecules that have different impacts on cell functions, or between compartments. As a result, cells can experience oxidant imbalances.

The term oxidative stress was initially defined as "a disturbance in the prooxidant-antioxidant balance in favour of the former."[50] This definition was later extended to include the detrimental effects of such a change on the function as "a disturbance in the prooxidant-antioxidant balance in favour of the former, leading to potential damage."[51] Widely accepted as a satisfactory definition of the term "oxidative stress", it leaves open the possibilities of the disturbance resulting from increases in oxidant production or decreases in tissue reductive capacity, or a combination of these processes.

Mechanisms leading to cellular injury

The ability of free radicals and other reactive species to induce cellular damage has been demonstrated in a variety of experimental systems. The reactive nature of these species makes all cellular macromolecules potential targets, and a vast array of changes have been identified that could provide mechanistic explanations for the observed injury.[46]

Protein Binding

Binding to proteins is the most common fate of reactive intermediates. Although the binding of a xenobiotic to critical macromolecules may disrupt their function and be directly related to the cell injury, the relationship between covalent binding to proteins and toxicity is significantly more complex than once thought. In recent years, several compounds have been identified that bind extensively to biological molecules without causing observable cellular damage.[52]

Specific amino acid residues within proteins are receiving increasing attention as targets for free radical-induced oxidation reactions. Modifications include reversible processes such as the loss of critical sulfhydryl groups on cysteine through simple thiol oxidation or the formation of mixed disulfides, or the oxidation of methionine to its sulfoxide. Irreversible oxidation of amino acids also occurs such as the formation of carbonyls, hydroperoxides, and their reduced hydroxide products, or ring cleavage in histidine or tryptophan. These modifications can impair both functional and structural activities of proteins and are potentially serious problems for a cell.

Lipids Role

Lipids have a critical structural and functional role in membranes. Any disruption of this role, through either covalent binding or oxidation, can lead to cell death. The double bonds found in many of the fatty acids ester-ified to membrane phospholipids are ready targets for free radical reactions. The abstraction of a hydrogen atom from one of these double bonds yields a radical species whose double bonds can rearrange to the conjugated configuration and that can readily interact with molecular oxygen. The resultant lipid peroxyl radical can abstract a hydrogen atom from another fatty acid yielding a new radical and a lipid hydroperoxide. The lipid hydroperoxide is unstable and can decompose or be reduced to the more stable enol form. As these reactions progress, ionic channels may be affected, membrane transport proteins or enzymes may be inactivated, or the lipid bilayer itself may become more permeable.

Membrane lipids become much more susceptible to peroxidation as cells die. Thus, when assessing lipid peroxidation as a marker of oxidative or free radical mediated injury, it is not possible to say whether the process was a cause or effect of the observed damage. Despite this limitation, measurements of peroxidation products can be useful in assessing the potential for free radicals to be involved in the observed effects.

THE INTERACTION OF ROS WITH DNA

The interaction of reactive oxygen species (ROS) with deoxyribonucleic acid (DNA) may involve direct modifications of DNA (i.e. the oxidation of DNA bases or sugars, and strand breaks), or they may be mediated through changes in transcription factors or enzymes involved in regulating gene expression. It has been suggested that ROS modulate the efficiency of the overall process of signal transduction at many sites.[53] ROS-induced alterations in gene regulation can ultimately induce changes that are either beneficial or detrimental to cells.[54,55]

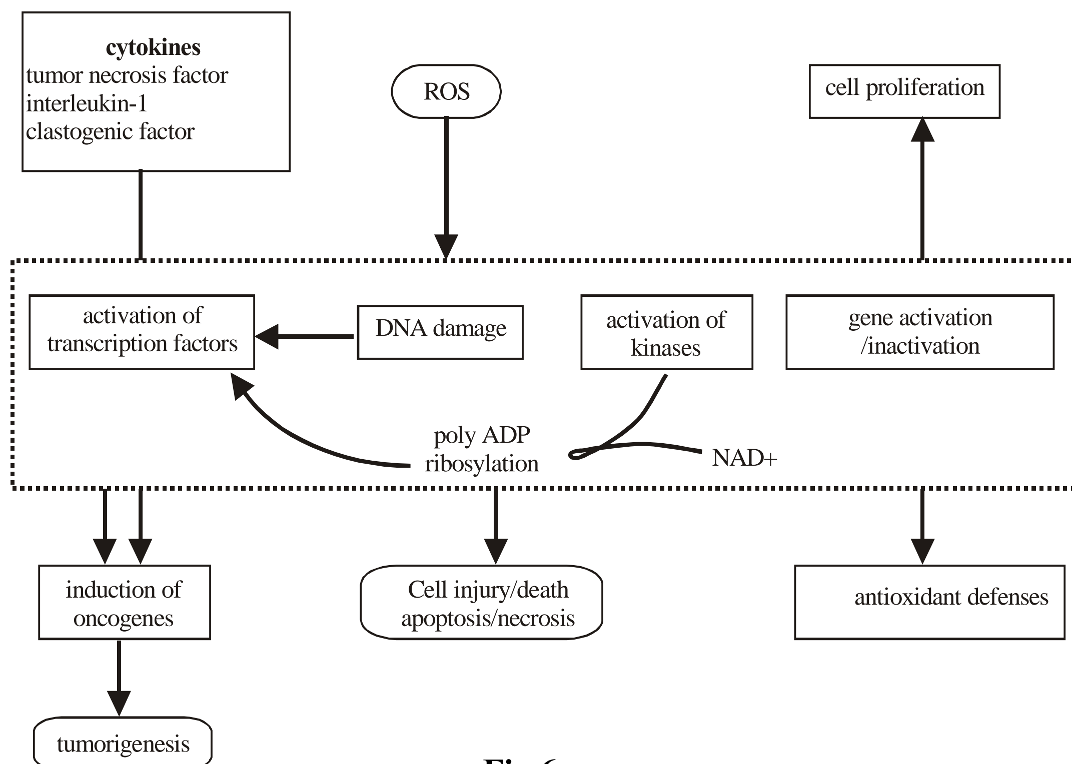


Fig.6

Other effects induced by ROS (Fig. 6) may be more direct and are usually associated with toxicity or carcinogenesis. For example, damage to DNA (whether from ROS or other mechanisms) can lead to the activation of various repair enzymes such as poly (ADP-ribose) polymerase. This enzyme, when activated, cleaves NAD+ to produce poly (ADP-ribose) on a variety of nuclear proteins.

The liver is the largest solid organ in the body, performing a wide variety of vital metabolic functions, glycogen storage, synthesis of plasma proteins, bile formation and metabolism and excretion of xenobiotics and endogenous metabolities. These reactions require a substantial amount of energy in the form of ATP, which is generated mainly by oxidative phosphorylation in mitochondria. The energy for ATP synthesis is derived from the four-electron reduction of molecular oxygen to water. However, a small fraction of the oxygen is reduced to superoxide (O_2^-) by a one-electron reduction. Several enzymatic reactions generate other reactive oxygen species such as hydrogen peroxide. It is estimated that, under physiological conditions, about 5% of molecular oxygen is converted to reactive oxygen species. Chemicals can increase the formation either directly (redox-cycling agents) or indirectly by inducing mitochondrial injury. In addition, certain phagocytic cells in the liver (Kupffer cells, infiltrating neutrophils, and monocytes) can generate large amounts of reactive oxygen species. These oxygen metabolities can react with lipids, proteins, carbohydrates, or DNA and thereby cause cell dysfunctions or even cell death.[56,57]

SOURCE OF REACTIVE OXYGEN SPECIES

In order to understand antioxidant defense strategies in the liver, it is important to recognize the variety of potential prooxidants and the location where they can be generated. Molecular oxygen can be reduced in one-electron steps to superoxide (O_2^-), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($OH^\bullet$). In addition to a spontaneous dismutation, superoxide can react with nitric oxide to form peroxynitrite and peroxynitrous acid, which can decompose to nitrogen dioxide and "hydroxyl radical-like" species.[58] Hydrogen peroxide, when generated in the presence of ferrous iron, is reduced to the extremely reactive hydroxyl radical. However, in the presence of myeloperoxidase from neutrophils, hydrogen peroxide forms the strong oxidant hypochlorous acid (HOCl). Singlet oxygen ($^1O_2$) can be generated as a result of

the spontaneous dismutation of superoxide, reaction of hypochlorous acid with hydrogen peroxide or direct activation of triplet oxygen by radiation. In addition to the described primary reactive oxygen species, a variety of secondary reactive oxygen species such as alkoxyl- ($RO^{\bullet}$) and peroxyl radicals ($ROO^{\bullet}$) can be formed (e.g., oxidation of fatty acids). Reactive oxygen species are generated at multiple sites within the cell and vary greatly in their reactivity as indicated by their half-lives.[59] The spectrum ranges from highly reactive species such as hydroxyl radicals (10^{-9}s), moderately reactive radicals such as the peroxyl radical (7s), to the stable hydrogen peroxide.[59]

FORMATION OF INTRACELLULAR REACTIVE OXYGEN

Mitochondria

Hydrogen peroxide formation by liver mitochondria was reported initially by (Boveris and Chance.[60] However, it was quickly recognized that actually superoxide was formed. Mitochondrial oxidant stress in the liver was demonstrated during hypoxia-recoxygeneration injury[61] acetaminophen toxicity,[62] and chemical hypoxia.[63]

Microsomes

The microsomal cytochrome P450 system catalyzes reactions which introduce oxygen into molecules (phase I reactions). This requires partially reduced oxygen species, which may dissociate from cytochrome P450.[64] Isolated microsomes generate superoxide and hydrogen peroxide when supplemented with NADH or NADPH.

A selective group of compounds, for example, parquat,[65] diquat[66,67] and menadione[68] can be reduced by cytochrome b_5 or cytochrome P450-reductase directly, forming an unstable organic radical. In the presence of molecular oxygen, this radical transfers its electron to oxygen thereby generating

superoxide. Because the original compound can go through this redox cycle numerous times, such chemicals can generate enormous amounts of superoxide intracellularly.[66,67,68]

Peroxisomes

Peroxisomes of hepatocytes contain several oxidases, that is, enzymes that generate hydrogen peroxide, including D-amino acid oxidase, fatty acyl-CoA oxidase and, in nonprimates, urate oxidase.[69] High-fat diet or administration of hypolipidemic drugs cause peroxisome proliferation and an increase in fatty acyl-CoA oxidase activity.[70,71] The degradation of long-chain fatty acids by fatty acyl CoA oxidase is responsible for a continuous generation of hydrogen peroxide in peroxisomes. Fasting and peroxisome proliferators can potentiate the oxidant stress in peroxisomes, thereby overwhelming the capacity of catalase.[72]

Xanthine oxidase and aldehyde dehydrogenase are cytosolic enzymes which contribute to reactive oxygen formation in the cytosol. Xanthine dehydrogenase and xanthine oxidase activities are present in sinusoidal endothelial cells, kupffer cells, and hepatocytes.[73] Prolonged hepatic ischemia[74] or drug toxicity can induce a conversion of the enzyme to the oxidase, which uses oxygen as electron acceptor to generate superoxide.[75,76] Xanthine oxidase was shown to contribute to the postischemic oxidant stress in the liver.[77] However, during severe cell injury, xanthine oxidase can be released into the extracellular space, bind to vascular endothelial cells, and may contribute to the oxidant stress outside hepatocytes.[78]

Formation Of Extracellular Reactive Oxygen

There is increasing experimental evidence indicating that hepatic nonparenchymal cells (kupffer cells, infiltration neutrophils) can be a relevant source of oxidant stress in the extracellular space. Enhanced reactive oxygen

formation by Kupffer cells was shown during endotoxemia,[79,80] ischemia-reperfusion injury[81,82] and administration of a wide variety of chemicals such as galactosamine[83], retinol[84] phenobarbital[85] acetaminophen[86] carbon tetrachloride,[84] and dichlorobenzene.[87] In most of these cases, the Kupffer cell-induced oxidant stress contributes to liver injury. However, the chemical may not directly activate macrophages but enhance intestinal permeability and thus increase exposure of Kupffer cells to endotoxin as was shown for galactosamine,[88,89] retinol[90] and chronic ethanol consumption.[91] Direct evidence for a neutrophil-induced oxidant stress was found during the later reperfusion phase after ischemia[92] and endotoxemia.[93,94]

Antioxidant defense mechanisms in the liver

A significant amount of reactive oxygen species on a continuous basis. In addition, reactive oxygen formation can be drastically increased upon exposure to endotoxin and a variety of chemicals or during reoxygenation after subjecting the liver to ischemia. Becuase of the variety of oxygen metabolites formed, their vastly different reactivity and their localization, a complex network of antioxidant defense mechanisms is necessary to control these unavoidable reactive oxygen metabolities.

INTRACELLULAR ANTIOXIDANT DEFENSE MECHANISMS

Enzymes

The first line of defense against superoxide are superoxide dismutases (SOD)[95,96]. In hepatocytes, Cu^{2+}/Zn^{2+}-SOD is located in the nuclear and cytosolic matrix and Mn^{3}+SOD is present in mitochondria.[97] the redox reactive metal in each enzyme (Cu^{2+}or Mn^{3+}) is reduced by the first superoxide molecule which yields oxygen. A second superoxide molecules is then reduced to hydrogen peroxide by the metal ion. The reaction is only limited by the diffusion of superoxide to the enzyme. Although the half-life of superoxide

anion is estimated to be about 5s at physiological pH,[98] the high intracellular activities of SOD keep steady-state concentrations of superoxide in the range of $10^{-11} – 10^{-12}$M.

Intracellular hydrogen peroxide can be a dismutation product of superoxide or is directly generated by oxidases. Two different enzymes, catalase[99] and glutathione peroxidase,[100.101] are available to detoxify hydrogen peroxide. The bulk of catalase activity in liver cells is localized in peroxisomes; however, some activity of immunologically distinct forms is found in the cytosol and intracellular granules.[102] Mammalian catalase is a hemeprotein, which reduces hydrogen peroxide to water by utilizing electrons from either hydrogen peroxide (catalase reaction) or from other small molecules such as methanol or ethanol (peroxidase reaction).[103] The reaction mechanism includes the divalent oxidation of the ferriheme prosthetic group to an FeIV-cation radical. In a subsequent reaction with either hydrogen peroxide or a low molecular weight reductant, this compound is reduced back to the ferriheme state.[104] Catalase activity is inducible in the lung by hyperoxia and in the liver by caloric restriction or phenobarbital[104]. Hypolipidemic drugs such as clofibrate increase the number of peroxisomes in the liver thereby increasing total catalase activity.[105] Although catalase has been used therapeutically in many experimental models of liver injury[106], the intracellular delivery of catalase was not confirmed in most cases. Increased intracellular catalase activity in combination with SOD protected against hyperoxic lung injury.[107] The main function of catalase is to detoxify endogenously generated reactive oxygen species in peroxisomes. Little hydrogen peroxide escapes this compartment under normal conditions.[108] However, enhanced fatty acid metabolism, for example, during fasting, in combination with induction of fatty acyl CoA oxidase by peroxisome proliferators, can exceed the capacity of catalase. In this case, cytosolic gluthathione peroxidase detoxifies the escaping reactive oxygen.[109] On the other hand, during a high oxidant stress in the cytosol some of the hydrogen peroxide can diffuse into peroxisomes and be metabolized by catalase.[110]

The second enzyme metabolizing hydrogen peroxide is glutatione peroxidase.

This enzyme contains selenium in the form of selenocysteine[111] which was confirmed by cloning of the gene.[112] Selenium is critical for the catalytic function of the enzyme.[113] Glutathione peroxidase reduces hydrogen peroxide to water utilizing reducing equivalents from glutathione. In addition to hydrogen peroxide, the enzyme can metabolize a wide variety of organic peroxides.[114] In contrast, the enzyme depends almost exclusively on GSH as its cofactor.[114] Because of the limited hepatocellular supply of GSH, GSSG is reduced immediately by glutathione reductase using reducing equivalents from NADPH. This reaction is the rate-limiting step of the glutathione redox cycle. In order to avoid excessive accumulation of GSSG during high oxidant stress, GSSG can also be excreted from hepatocytes.[115] In mitochondria, the glutathione redox cycle operates in a similar fashion to the cytosolic compartment; however, GSSG can not be transported out of mitochondria.[116] This means that GSSG can only be reduced or it accumulates. An oxidant stress inside mitochondria may therefore induce higher hepatic GSSG levels than cytosolic reactive oxygen formation.[117]

In addition to the high selenium-dependent glutathione peroxidase activity in the liver, a lower, selenium-independent enzyme activity was identified.[118] This enzyme, which is present only in the cytosol, was identified as a member of the glutathione-S-transferase family (GST-B) GSH-S-Transferase also accepts a broad spectrum of organic hydroperoxides but does not accept hydrogen peroxide as a substrate.[119] During selenium deficiency with critically reduced activities of the selenium-dependent peroxidase, GST-B is substantially induced.[120] Despite this adaptation, selenium-deficient animals are more susceptible to oxidant stress-induced liver injury.[121]

Low molecular weight antioxidant

This category include α-tocopherol (vitamin E), ß-carotene, ascorbate (vitamin C), and glutathione as most relevant examples. These compounds have two major objectives. Firstly, they intercept radical chain reactions and prevent the further formation of damaging radicals and secondly, they transfer the radical function away from sensitive targets, for example, from hydrophobic membranes to the aqueous phase. The most effective chain-breaking compound in membranes is α-tocopherol.[122] It effectively reduces peroxyl radicals to the hydroperoxide, which can be metabolized by the phospholipid hydroperoxide glutathione peroxidase. α-Tocopherol prevents the propagation of the radical chain by avoiding the foration of new alkyl radicals. On the other hand, the α--tocopherol radical can be reduced by ascorbate or thiols, for example, cysteine and glutathione. Ascorbate can be regenerated in the aqueous phase by a GSH-dependent dehydro-ascorbate reductase or a NADH-dependent semidehydro-ascorbate reductase. In support of this hypothesis, it was shown that during diquat-induced oxidant stress in hepatocytes the depletion of glutathione and ascorbate precedes depletion of tissue vitamin E levels, onset of lipid peroxidation and cell injury.[123] On the other hand, recent data suggest that during peroxide-induced lipid peroxidation vitamin E and ascorbate can act independently as anti-oxidants.[124] There is also evidence for a glutathione-dependent regeneration of vitamin E; however, this appears not be a direct reaction. It is hypothesized that a GSH - dependent microsomal enzyme maintains protein thiols which then can reduce vitamin E. The maintenance of thiol groups of membrane proteins may provide a significant antioxidant capacity to membranes.

The importance of vitamin E as a defense system in hepatic membranes is supported by the increased susceptibility of vitamin E-deficient animals to lipid peroxidation and hepatocellular injury induced by a variety of chemicals.[125] In fact, acetaminophen or allyl alcohol cause liver necrosis in

normal animals by glutathione depletion and subsequent binding of reactive metabolites to vital intracellular proteins. The development of the injury takes 10-24 hrs and there is little or no lipid peroxidation detectable in vivo. However, in vitamin E-deficient animals, both compounds can induce dramatic lipid peroxidation and destroy the liver within 1-3 h.[126] Consequently, vit. E administration inhibited lipid peroxidation and protected against acetaminophen and allyl alcohol in the deficient animals. Protection by vit. E supplementation against hepatotoxicity was found after carbon tetrachloride administration, in chronic iron overload, and during ischemia-reperfusion injury.[127]

Carotenoids, for example, ß-carotene, are efficient quenchers of the highly reactive singlet oxygen (O_2). The quencing reaction includes the transfer of excitation energy from O_2 to the carotenoid resulting in the formation of a carotenoid triplet. Subsequently, the excitation energy is dissipated physically through rotational and vibrational interactions between the carotenoid and solvent molcules, regenerating the original molecule. In a competing chemical reaction, the carotenoid molecule can be destroyed. ßCarotene can react with other reactive oxygen species, for example, peroxyl radicals.[128] However, in vitro studies demonstrated that ß-carotene is an effective antioxidant only at low oxygen tension, but even under these conditions, α-tocopherol and bilirubin are superior antioxidants. Evidence for the antioxidant effects of carotenoids in vivo are limited. In vitamin C-deficient guinea pigs, ß-carotene reduced CCl_4-induced lipid peroxidation.[129]

Metal-binding proteins

Most of the reactive oxygen species are generated after reductive cleavage of peroxides, for example, hydroxyl radicals or alkoxyl radicals. This reaction can be catalyzed by redox-active transition metals (Fenton reaction). Under physiological conditions, metal ions mainly involved in this reaction are

iron (Fe^{2+}) and copper (Cu^{+}). To prevent the initiation of radical chain reactions, these metals are transported and stored bound to proteins. Metal chelation is a very effective measure to prevent lipid peroxidation and oxidative DNA damage. Metal-binding proteins are ferritin, transferrin, and lactoferrin for iron and metallothionein for other metals.

Ferritin is the most important intracellular storage protein for iron. It is a 24-subunit protein with a central core where iron is stored as ferric oxohydroxide. Two isoforms with heavy (H) or light (L) subunits are found in various tissues; the liver contains mainly the L-forms, which is the dominant form for cells where iron is stored.[130] After iron administration, there is an increase of transcription of the L-gene; however, the main regulation of ferritin synthesis under translational control.[131] A ferritin repressor protein (FRP) in the cytosol binds to ferritin mRNA and prevents translation. In the presence of chelated iron or heme, the FRP is inactivated and protein synthesis proceeds. One ferritin molecule can store up to 4500 atoms of iron; however, the protein is normally only 20% saturated. Because of the high efficiency of iron-binding proteins, the free iron concentration is extremely low, for example, 10^{-14} M for Fe^{2+} and $<10^{-17}$ M for Fe^{3+}. This suggests that iron has to be released from ferritin, transferrin, or lactoferrin to participate in Fenton reactions. All storage proteins have a much higher affinity for ferric iron compared to ferrous iron and therefore have to oxidize any Fe^{2+} for binding (ferrioxidase activity). In contrast, for iron to be released from a binding protein it has to be reduced. Superoxide, reduced flavins, NADH, and a long list of organic compounds can mobilize iron from ferritin in vitro and there is evidence for the diquat radical to release iron from ferritin in vivo[132]. A critical role for iron in the induction of lipid peroxidation and liver injury was shown in different experimental models after administration of acetainophen, allyl alcohol, diquate, t-butyl hydroperoxide, halothane, carbon tetrachloride, ethanol, and ischemia-reperfusion injury. In most cases, the experimental evidence involves the protective effect of an iron chelator, for example, desferrioxaamine, in the

pathophysiology. Additional support comes from observations that iron pretreatment aggravates lipid peroxidation and injury.[133]

Metallothionein (MT) is a low molecular weight intracellular protein that contains 20 cysteines to bind metal ions, for example, zinc, copper, cadmium, with high affinity. MT is highly inducible, especially in the liver, by metals and endotoxin. Its main biological function may be the chelation and detoxification of metal ions[134], however, several studies have suggested that it may also have an antioxidant effect. Zinc-metallothionein inhibited lipid peroxidation in vitro, the protective effect appeared to be not only due to thiol oxidation but more importantly due to the release of Zn^{2+} and its antioxidant effect. During stress-induced hepatic lipid peroxidation in vivo, MT levels increased; vitamin E treatment prevented lipid peroxidation and MT induction.[135] These data support the hypothesis that MT can participate directly and indirectly in antioxidant functions within liver cells.

EXTRACELLULAR ANTIOXIDANT DEFENSE MECHANISMS

Reactive oxygen formation in the extracellular space is potentially more harmful than intracellular oxidant stress because of the limited antioxidant defense mechanisms in plasma. Significant antioxidants in plasma include albumin, transferrin, lactoferrin, ceruloplasmin, haptoglobin, urate, vitamin E, biliurubin, extracellular superoxide dismutase, and glutathione peroxidase. Serum albumin can bind copper ions and scavenge hypochlorous acid and other reactive oxygen species with its sulfhydryl groups.[136] The acute phase protein ceruloplasmin inhibits lipid peroxidation by oxidizing Fe^{2+} to Fe^{3+} (ferrioxidase activity) without release of oxygen radicals. Transferrin and lactoferrin can bind iron with high affinity and therefore virtually eliminate free iron from plasma. On exposure to peroxides, free heme or hemoglobin release iron and induced lipid peroxidation, heptoglobin/ hemopexin in plasma binds heme/hemoglobin and prevents the release of redox-active iron. Vitamin E is

the major lipid - soluble, chain-breaking antioxidant in plasma. It is important in protecting plasma lipoproteins against oxidation.[137] Uric acid is a direct scavenger of oxygen radicals and can also tightly bind iron and copper.[138] Ascorbate levels in plasma are 50-200μM. It is an important antioxidant in the extracellular space due to its direct scavenig properties for a number of oxygen-derived free radicals and its capacity to regenerate α-tocopherol. Its prooxidant activity in the presence of iron or copper ions is less relevant in vivo because transition metal ions are bound effectively. However in certain disease states, for example, iron overload, high plasma levels of ascorbate may be detrimental. Bilirubin bound to serum albumin may protect unsaturated fatty acids from peroxidation.[139]

In addition to the described metal binding proteins and free radicals scavengers, plasma also contains Cu/Zn-superoxide dismutase and Se-glutathione peroxidase activity but no catalase.[140] The function of these enzymes in the extracelllular space is not clear. The low enzyme activities in combination with low plasma levels of glutathione suggest that the extracellular detoxification of superoxide and hydrogen peroxide is much less effective than inside cells. Both extracellular enzymes are clearly distinct from the intracellular enzymes. For example, the extracellular SOD (EC-SOD) has a higher molecular mass and possesses attached carbohydrates. This enable the enzyme to bind to heparin sulfate proteoglycan in the glycocalyx of cell surfaces and connective tissue.[141] Therefore, it was proposed the EC -SOD, when localized on the luminal side of endothelial cells as well as in the interstitial space, may protect locally against excessive superoxide formation. In support of this hypothesis it has been shown that recombinant EC-SOD can protect against ischemia-reperfusion injury in the heart; the beneficial effect requires tissue binding of the EC-SOD and is independent of circulating SOD activity.[142] In contrast to EC-SOD, plasma gluthathione peroxidase is dependent on a cofactor. Average plasma GSH concentrations of 5-25μM are well below the millimolar K_m of the enzyme. In addition, plasma peroxidase

exhibits a slow GSH rate constant, which may be responsible for the slow metabolism of several peroxides. The enzyme functions best when free fatty acid hydroperoxides are the substrates in a GSH-rich microenvironment. Thus, extracellular glutathione peroxidase is not an efficient peroxide-metabolizing enzyme in plasma.

A more liver - specific antioxidant defense system has recently been recognized. An enhanced extracelluar oxidation of GSH was observed during hepatic ischemia reperfusion and also during endotoxemia reflecting a Kupffer cell-induced oxidant stress. It was also shown that hepatocytes released enhanced amounts of GSH into the vascular space under these conditions. Depletion of hepatocelluar GSH with phorone decreased the vascular GSH levels and aggravated liver injury.[143] On the other hand, reinfusion of GSH into the portal vein increased plasma GSH levels and protected against the Kupffer cell-mediated injury. These data suggest that GSH released from hepatocytes into the space of Disse can trap reactive oxygen species and therefore protect at least in part the sinusoidal lining cells and hepatocytes from oxidant-induced injury.

LITERATURE FOR VITAMIN C

Choice for selecting vitamin C as antioxidant supplement.

Antioxidants that are reducing agents can also act as pro-oxidants. For example, vitamin C has antioxidant activity when it reduces oxidizing substances such as hydrogen peroxide,[144] however, it will also reduce metal ions that generate free radicals through the Fenton reaction.

$2Fe^{3+}$ + Ascorbate $2Fe^{2+}$ + Dehydroascorbate

$2Fe^{2+} + 2H_2O_2$ $2Fe^{3+} + 2OH. + 2OH^-$

The relative importance of antioxidant and pro-oxidant activites of antioxidants are an area of current research, but vitamin C, for example,

appears to have a mostily antioxidant action in the body.[145,146] However, fewer data are available for other dietary antioxidants, such as vitamin E,[147] or the polyphenols.[148] Vitamin C or L-ascorbate is an essential nutrient for a large number of primate species, a small number of other mammalian species (notably guinea pigs and bats), few species of birds and some fish.[149] The presence of ascorbate is required for a range of essential metabolic reactions in all animals and plants. It is made internally by almost all organisms, humans being the most well-known exception. It is widely known as the vitamin whose deficiency causes scurvy in humans.[150,151,152] It is also widely used as a food additive. The pharmocophore of vitamin C is the ascorbate ion. In living organisms, ascorbate is an antioxidant since it protects the body against oxidative stress,[153] and is a cofactor in several vital enzymatic reactions.[154] The uses and the daily requirement amounts of vitamin C are matters of on-going debate. People consuming diets rich in ascorbate from natural foods, such as fruits and vegetables, are healthier and have lower mortality from a number of acute and chronic illnesses.

LITERATURE FOR ALPHA LIPOIC ACID

Choice for selecting alpha lipoic acid as antioxidant supplement

Alpha lipoic acid initially classified as a vitamin discovered three decades earlier possessed potent antioxidant properties. It is more potent than the old guard antioxidants vitamin C and E, it even recycles these vitamins and enhances their effectiveness.

In addition to functioning as an antioxidant, this hard working nutrient assists the B vitamins in producing energy from the proteins, carbohydrates and fats consumed through foods.

As it dissolves in both water and fat this is so called "UNIVERSAL ANTIOXIDANT" it can reach tissues composed of fat such as the nervous system as well as those made mainly of water such as heart.

Intravenous forms of alpha lipoic acid are administered in hospitals to treat the case of acute mushroom poisoning and other cases of acute poisoning that affects liver.

Study conducted with 600 mgs of alpha lipoic acid given daily to 9 Alzheimer's patients, on an average for 80 days, the treatment lead to stabilization of cognitive functions in the Alzheimer's study group. This was the first indication that alpha lipoic acid might be successful neuroprotective therapy option for Alzheimer diseases and related dementias.[155]

Alpha lipoic acid will benefit anyone whose limbs tend to tingle or become numb or "fall asleep" due to nerve compression. In animal studies, alpha lipoic acid increases the blood flow to the nerves and improved transmission of nerve impulses.

Parkinson's disease (PD) is the disorder of the central nervous system. Clinically the disease is characterized by disease in spontaneous movements, gait difficulties, postural instability, rigidity and tremor. PD may appear at any age but it is uncommon in people younger than 30 years.

Oxidative stress appear to play an important role in neuronal degeneration associated with PD.[156]

The depletion of gluthathione (GSH) in the brain is the earliest indicator of oxidative stress presymptomatic PD.[157]

Studies both in vitro and in vivo models have suggested that pretreatment with alpha lipoic acid increased cellular levels of GSH. Probably by preventing its depletion thereby protecting mitochondria integrity.

Results with previous studies suggests that alpha lipoic acid may be an effective neuro protective agent in age associated neuro degeneration utilizing the PC 12 cell model system.

Based on the above beneficial effects of alpha lipoic acid on nervous system it has been decided to supplement alpha lipoic acid to acute amitriptyline poisoning cases, which have increased oxidative stress and neuro toxicity, and to study the effect after supplementation of alpha lipoic acid.

LITERATURE FOR ISOENZYMES

LDH Isoenzymes

Elevated serum levels of LDH are observed in variety of conditions. The highest values (2 to 40 fold elevations) are seen in patients with megaloblastic anemia, in those with extensive carcinoma and in those with severe shock and hypoxia. Moderate elevation (2 to 3 fold) occur in patients with myocardial infarctions, pulmonary infarctions, granulocytic or acute leukemia, hemolytic anaemia infections mononucleosis, and progressive muscular dystrophy. Patients with chronic renal diseases, especially those with nephrotic syndrome or hemolytic anaemia, also have increased values.[158]

The pattern of elevated serum LDH levels in patients with myocardial infarction is quite characteristic. High levels are observed in almost all patients within 24 hours of the apparent onset of infarction. Although the degree of elevation is not so striking as that of AST, the elevated levels persist longer (10 to 14 days).

The characteristically prolonged period of elevated LDH values with an increase of LDH isoenzymes (ie LDH) higher than LDH_2 (Flipped LDH) yields a pattern that is useful in the Laboratory diagnosis of mycocardial infarction.[159] The "Flipped" LD pattern usually appears within 12 to 24 hours and is present within 48 hrs in sera of 80 per cent of patients with a myocardial infarction[160].

Most patients with pulmonary infarction have elevated levels of LDH, usually within 24 hours of the onset of pain. The pattern of normal AST and elevated LDH levels within one to two days after an episode of chest pain provides suggestive evidence for pulmonary infarction.

Based on the report of elevated LDH in acute amitriptyline poisoning cases, it has been decided to study the isoenzyme patterns of LDH, and find out the maximum tissue or organ damage in acute amitriptyline cases.

Creatinine Kinase

The major cause of increased CK is cardiac or skeletal muscle damage, CK-MB is increased in chronic myopathies[161]and in chronic renal failure[162] in both situations, CK-MB typically does not show a rise and fall as in myocardial infarction, but remains relatively stable over many days.

CK-BB is increased with injury to smooth muscle, such as may occur with intestinal ischemia, CK-BB is often found in patients with malignancies, especially prostate cancer, small cell carcinoma of the lung and intestinal malignancies. CK-BB may be present transiently after cardiac arrest, presumably reflecting intestinal ishemia.

Relative percentage of CK isoenzyme in various tissues

	CK 1(BB)	CK 2 (MB)	CK 3 (MM)
Skeletal muscle, leg	0	2-3	97-98
Skeletal muscle, arm	0	0-1	99-100
Skeletal muscle, respiratory	0	3-7	93-97
Cardiac muscle, normal	0	2-3	97-98
Cardiac muscle, abnormal	0	10-15	85-80
Lung	20-50	0-5	30-60
Brain	97-98	2-3	0
Intestinal smooth muscle	90-95	0	5-10
Prostate	95-100	0-2	0-5
Placenta	100	0	0

PATIENTS AND METHODS

A total of 132 subjects were enrolled for the study and were divided into 5 groups. They were selected from IMCU and Toxicology Ward, Govt. General Hospital between Sept. 2005 and March 2008. Consent was obtained from the attendants of the patients. The study was approved by the ethical committee of Madras Medical College, Chennai-3.

Selection of subject : The patients were randomly selected. The Edinburgh scale was used to classify the depth or grade of coma of poisoned patients, graded as under:-

Grade 1 : Patient drowsy but responding to verbal commands.

Grade 2 : Patient unconscious but responding to minimal stimuli (for example, shaking, shouting)

Grade 3 : Patient unconscious and responding only to painful stimuli (for example, rubbing the sternum)

Grade 4 : Patient unconscious with no response to any stimuli.

The study was restricted only to the grade I. patients from IMCU.

The groups were classified as follows.

Group I : Consisted of 30 healthy volunteers (15 males and 15 females) mean age 32 years.

Group II : Consisted of 30 patients (18 males and 12 females), mean age 34 years. These patients received only routine standard treatment (RST)

Group III : Consisted of 21 patients (12 males and 9 females), mean age 32 years. These patients received routine standard treatment (RST) + Vitamin C supplementation.

Group IV : Consisted of 27 patients (13 males and 14 females), mean age 31 years. These patients received routine standard treatment (RST) + alpha lipoic acid supplementation.

Group V : Consisted of 24 patients (14 males and 10 females), mean age 34 years. These patients received routine standard treatment (RST) + Vitamin C and alpha lipoic acid supplementation.

Basal level of oxidative stress markers and enzymatic and non–enzymatic antioxidants were measured at the beginning of the treatment and followed up until the day of discharge from IMCU.

Exclusion criteria:

Less than 18 years and more than 60years are not included in this study. Patients those who have taken other drugs along with amitriptyline are not included in this study. Patients with TLC positive and spectra(uv-vis) negative are not included in this study.

Sample Collection

From each experimental subject 10 ml of venous blood was drawn from the antecubital vein. 5 ml of blood collected in a plain tube for enzyme analysis and 5 ml of blood collected in sterile heparin vacutainer tubes. All samples were kept in ice till they were brought to the laboratory. Plasma separated by centrifugation at 1500 x g for 10 minutes and this plasma was stored in a new clean storage vials and stored at - 80°C and used for analysis of antioxidants and plasma cholinesterase. The cells were separated and washed with normal saline and RBCs were subjected to lysis and used for RBC cholinesterase estimation.

50ml of gastric aspirate was collected from all patients who are directly admitted to IMCU and poison centre GGH, Chennai-3. This gastric aspirate is taken for TLC(thin layer chromatography) identification.

Methods

Plasma Cholinesterase estimation The cholinesterase levels were determined in plasma and RBC lysate. Estimation was done by colorimetric method using acetyl choline (SD fine chemicals) as substrate (Venkatarakan et al. 1993)[163]. Both true and pseudo cholinesterase would hydrolyse the substrate and produce choline and acetic acid. The change in colour of the indicator bromothymol blue (SD fine chemicals) caused by the liberated acetic acid from cholinesterase was read by spectrophotometer at 620 nm.

Bromothymol blue 0.5ml solution was diluted with 3.8 ml of distilled water and 0.2 ml of 15% acetyl choline chloride was added. To it 100µl of plasma was added and the change in colour was read at 620 nm at 37 degree c after 30 minutes. A standard graph was plotted using acetic acid 0.15 N in concentration of 10,20,50,100 and 200 micro moles.

In RBC cholinesterase estimation RBCs were extracted by first adding distilled water 3 ml followed by precipitation of hemoglobin with acetone 2 ml and centrifugation at 3000 rpm. The supernatant was used for estimation of RBC cholinesterase.

Lipid peroxidation assay

Lipid peroxidation levels in plasma was assayed by measuring the TBARS by the method of Ohkawa et al. 1979[164]. In brief 0.5 ml of plasma was treated with 3 ml of ice cold 10% TCA, mixed well and 2 ml of TBA was added. The tubes were covered with glass marbles and kept in the boiling water bath for 20 min. After cooling, the tubes were centrifuged and the absorbance of the supernatant was read at 532 nm. 1, 1', 3, 3' – tetramethoxypropane was used as a standard. The level of lipid peroxides is expressed as n moles of TBARS/mg protein.

Catalase activity

Catalase was assayed according to the method of Takahara et al., 1960.[165] To 1.2 ml of phosphate buffer (0.05M, pH 7.0), 0.2 ml of the hemolysate was added and the enzyme reaction was started by the addition of 1.0 ml of H_2O_2 (0.03M in phosphate buffer) solution. The decrease in bsorbance was measured at 240 nm at 30 sec. intervals for 3 min. The enzyme blank was run simultaneously with 1.0ml of distilled water instead of hydrogen peroxide. The enzyme activity is expressed as μ moles of H_20_2 decomposed/min/ mgHb.

Superoxide dismutase activity

SOD was assayed by the method of Misra and Fridovich, 1972[166]. 0.1 ml of hemolysate was added to tubes containing 0.75 ml ethanol and 0.15ml chloroform (chilled in ice) and centrifuged. To 0.5ml of supernatant, added 0.5 ml of EDTA (0.6 mM) solution and 1 ml of Carbonate- bicarbonate buffer (0.1 M, pH 10.2). The reaction was initiated by the addition of 0.5 ml of epinephrine (1.8 mM) and the increase in absorbance at 480 nM was measured with UV spectrophotometer. The enzyme activity is expressed as 50% inhibition of epinephrine autooxidation/min/mgHb.

Glutathione peroxidase activity

The activity of glutathione peroxidase was determined by the method of Rotruck et al. 1973[167]. In brief, 0.4ml of buffer, 0.1ml of sodium azide, 0.2 ml of reduced glutathione, an aliquot of hemolysate, 0.1 ml of H_20_2 and distilled water were taken to make a final volume of 2.0ml. The tubes were incubated at 37°C for 10 min. The reaction was stopped by adding 0.56ml of 10% TCA. To determine the residual glutathione content, the supernatant was removed by centrifugation; 3.0ml of disodium hydrogen phosphate and 1 ml of DTNB reagent were added and read at 412 nm. A blank was treated with only disodium hydrogen phosphate and 1.0ml of DTNB reagent. Suitable aliquot of standards were taken and treated similarly. The activity of glutathione was expressed as μg of GSH utilized/min/mg/Hb.

Glutathione-S-Transferase activity

The enzyme was assayed by the method of Habig et al. 1974[168]. To 1 ml of phosphate buffer (0.5M, pH 6.5), 0.1 ml of serum, 1.7 ml of water and 0.1 ml of 1-Chloro-2, 4-dinitrobenzene (CDNB), in 95% ethanol (0.03M) were added and incubated at 37°C for 15 minutes. After incubation, 0.1ml of reduced glutathione (0.03M) was added. The increase in optical density was measured against of the blank at 340nm. The enzyme activity is expressed as nmoles of CDNB conjugated/min/mg protein.

Reduced Glutathione activity

The total reduced glutathione content was determined by the method of using Moron et al. 1979[169]. In brief, proteins were precipitated with 5% TCA. The solution was mixed well and centriguged. To 0.5 ml of supernatant, 2 ml of 0.2 M phosphate buffer was added followed by 0.5 ml of DTNB reagent and read at 412 nm in a spectrophotometer against a blank of 5% TCA. A series of standards were treated in a similar manner. The amount of glutathione in the hemolysate was expressed as µg of GSH/mg Hb.

Vitamin C estimation

Ascorbic acid in plasma was oxidized by Cu^{2+} to form dehydroascorbic acid that reacts with acidic 2,4 dinitrophenyl hydrazine to form red bis-hydrazone, which was measured spectrophotometrically by 2,4 dinitrophenylhydrazine method.[170] In short, 0.5 ml of plasma was added to 2 ml of freshly prepared MPA (6g/dl in water), mixed well and centrifuged at 2500xg for 10 min. to 2 ml of the supernatant or similar volume of MPA blank, 0.4 ml of a reagent containing thiourea (5g/l), copper sulphate (0.6 g/dl) and 2,4 dinitrophenyl hydrazine (2g/l) in 4.5 mol/l sulphuric acid solutions mixed in a ratio of 1:1:20 was added and incubated in a 37°C water bath for 3 hrs, followed by cooling for 10 min. in an ice bath. To all tubes, 2 ml of cold H_2S0_4 (conc) was added and the optical density recorded at 520 nm. The concentration of ascorbic acid in plasma was determined using a standard curve.

Total Antioxidant Status[171]

Total antioxidant status of the sample was measured by the commercial kit. Supplied by Randox.

Principle

$ABTS^R$ (2-2' – Azino-di-[ethylbenzthiazoline sulphonate] incubated with a peroxidase (metmyoglobin) and H_2O_2 to produce the radical cation $ABTS^R$. This has a relatively stable blue green colour, which was measured at 600 nm. Antioxidants in the added sample cause suppression of this colour production to a degree which was proportional to their concentrations.

20 µl of plasma was added to 1 ml of chromogen and incubated at RT for 1 mt. The initial Absorbance (A_1) measured at 600 nm. 200 µl of substrate added to it and incubated at RT for 3 mts. The final absorbance A_2 was measured at 600 nm.

A Blank and a standard were run simultaneously the initial absorbance A_1 and final absorbance A_2 was measured at 600nm for both blank and standard.

A_2-A_1 =ΔA of the sample

A_2-A_1= ΔA of the blank

A_2-A_1= ΔA of the standard

$$\text{Factor} = \frac{\text{Concentration of the Standard}}{\Delta A \text{ blank} - \Delta A \text{ standard}}$$

Total Anti oxidant status in m.mol/I = Factor X (ΔA Blank-ΔA sample)

THIN LAYER CHROMATOGRAPHY[172,173,174,175]

Amitriptyline drugs are detected by means of thin layer chromatography(TLC). In the recent year technique, thin precoated silica gel of absorbents or thin aluminium sheets have been developed to produce superior results when compared to conventional chromatography. In this studies silica gel coated aluminium sheets had been used which were 20 x 20 cms in dimension, commercially available plates.

Drug extraction from stomach wash contents:

The stomach wash contents obtained from the patient who have been suspected to have consumed drug was taken for TLC analysis, 10 ml of the collected stomach wash contents was taken in the vortex tube of 11 cm length and 2.5 cm diameter and to it was added equal volume of 10 ml of chloroform : propane-2-ol (9:1) mixture which was allowed to stand for 5 minutes. The solution was throughly mixed by the vortex mixer (cyclomixer) for about 5 to 10 minutes.

The mixed solution was poured into a separating funnel where it was allowed to stand for 30-45 minutes for the aqueous and organic layer to separate. The drug compound suspected get extracted into the chloroform layer, which remained at the bottom of the separating funnel. The separated organic layer containing chloroform and drug was removed from the separating funnel by slowely opening the knob where by the lower layer was allowed to run into a funnel with filter paper 2 gms of sodium sulphite present on the filter paper removes the water molecules and polar substances from the layer and the rest of the solution poured into a beaker, where it is evaporated to near dryness in a water-bath at 60°c. Few drops of chloroform was added in the breaker and the extract was ready for application.

TLC application

About 5 µl of the extracted sample was spotted to the TLC plate at a position 2.0 to 2.5 cm from the bottom edge of the plate with a help of a specialized capillary tube. The circular spot about 2 to 6 mm in diameter was spotted on a line parallel to the standard drug substance. The two spots of standard and test sample were plotted at a distance of more than 1.5 cm distance with much to prevent any smearging.

Methodology of TLC development

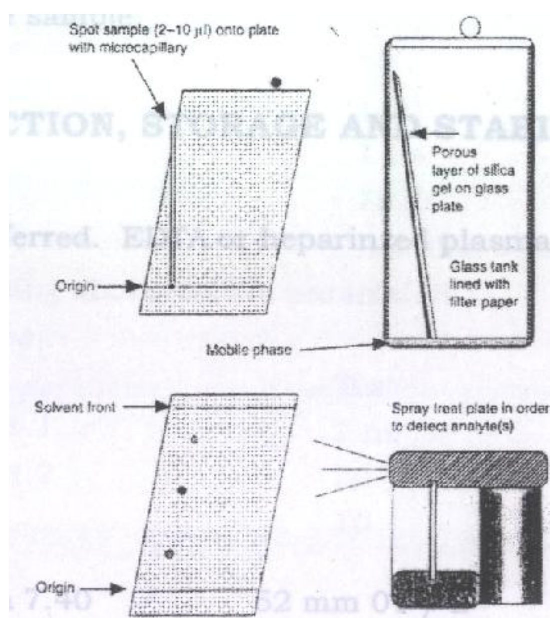
The atmosphere of the TLC rectangular glass tank was saturated completely with the mobile phase of the solvent mixture which was prepared by mixing Ethyl acetate, Menthnol, Ammonium hydroxide concentrated(EMA) in the proportion of 85: 10: 5. The prepared solvent mixture was poured in the TLC developing tank for a depth of 0.5 to 1 cm in which the TLC plate spotted with sample and standard was allowed to stand for their development for 60 to 70 minutes. The plate, was kept as nearly vertical as possible and it was seen that the spots were above the levels of the solvent mixture. The tank was closed with the lid and was allowed to stand at room temperature until the mobile phase had ascended for nearly 15 cms carrying the test sample and the standard.

The TLC plate was removed from the tank and dried at 60°C to 70°C in an oven. After drying the TLC plate was examined under an ultra violet light which had a maximum output at about 254 nm to 366 nm. The separated compound from the test sample if it was a drug show as blue or pale blue spot along with that of the standard against the fluorescent background of the TLC plate. After examining under UV light the TLC plate sprayed with 1% Mandelin's reagent by TLC sprayer, drug compounds appear as blue spot at the center and yellow colour at the outer spot at a distance of 10 to 12 cms from the point of spotting.

Calculation of Rf: (Relative front or ratio front): The distance travelled by solvent mixer is marked and this distance is first measured in cms next the distance travelled by the solute is marked as a rounded spot. The distance from the centre of the spot to the point of its spoting, which was measured in cms is the distance travelled by the solute. Rf value for the drug was calculated using the following formula.

$$Rf = \frac{\text{Distance travel by solute in cms}}{\text{Distance travel by solvent in cms}}$$

Basic apparatus for thin layer Chromatography



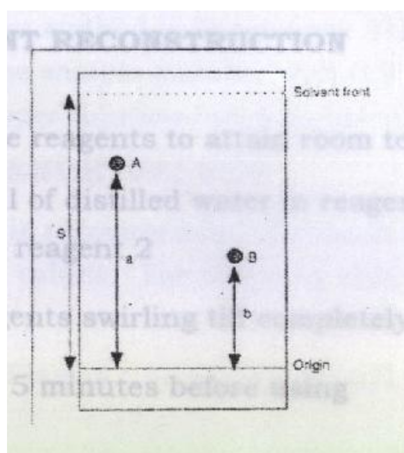


Fig.7

METHODS FOR TOTAL AND ISOENZYMES ANALYSIS

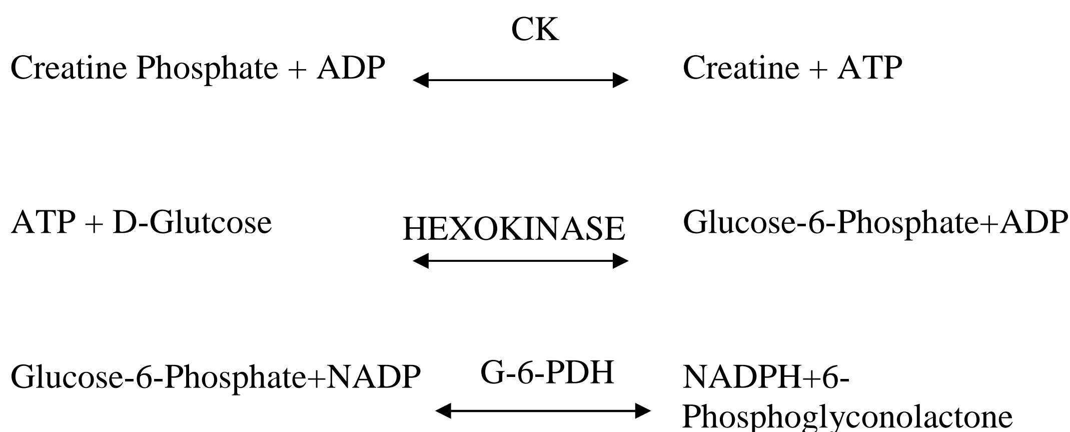
Twenty cases were selected randomly among all the groups and divided into two groups as group A and group B for LDH and CPK study. The blood samples were collected on admission and analysed for total and isoenzymes of CK and LDH.

Total CK analysis

Total CK was analysed by semi auto analyser Micro lab 200 by UV kinetic IFCC method.

Principle

CK catalyzes the conversion of Creatine Phosphate and ADP to Creatine and ATP. The ATP produced and glucose are converted to ADP and glucose-6-phosphate by hexokinase (HK) in the second reaction. In the final reaction, glucose-6-phosphate dehydrogenase (G-6-PD) oxidizes the glucose-6-phosphate produced in the second reaction and reduces NADP. The NADPH produced in the final reaction is proportional to the creatine produced in the initial reaction. The rate of increase in NADPH absorption at 340 nm is thus directly proportional to the CK activity.



REAGENTS

Reagent 1 (Enzymes)

N-Acetyl-L-Cysteine 20 mmol/L, ADP – 2mmol/L, AMP-5mmol/L, NADP-2mmol/L, D-Glucose-20mmol/L, Diadenosine Pentaphosphate-10µmol/L, EDTA-2 mmol/L, Hexokinase-≥3500 U/L, G-6-PDH-≥2000 U/L, Creatine Phosphate-30mmol/L.

Reagent 1A (Buffer)

Imidazole buffer, pH 7.10 – 100 mmol/L, Magnesium Acetate-10 mmol/l.

Procedure

3ml of Reagent 1A into Reagent 1 bottle and mix by gentle swirling till completely dissolved. This is working Reagent. 1000µl of working Reagent and 20µl of serum sample are added and the analysis is carried out in semi auto analyser by kinetic mode at 340nm. Initial absorbance A_0 after 10 minutes and repeat the absorbance change per minute (ΔA/min).

Calculation

Total CK activity in µ/l at 37°C = ΔA/min x 6666

Total LDH Analysis

Serum total LDH was analyzed by semi auto analyzer Micro lab 200 by Mod. IFCC method.

Principle

Lactate dehydrogenase catalyzes the reduction of pyruvate with NADH to form NAD. The rate of oxidation of NADH to NAD is measured as a decrease in absorbance which is proportional to the LDH activity in the sample.

Reagent

L_1 – Buffer Reagent, L_2 Starter Reagent, 4 Parts of L_1 and 1 part of L_2 is added and this is working reagent.

Procedure

1000 µl of working reagent 20 µl of sample is added and the analysis is carried out in semi auto analyzer by kinetic method of 340nm. Initial absorbance A0 after 1 minute and final absorbance reading after every 1, 2 & 3 minutes mean absorbance change per minute (ΔA/min) is calculated.

Calculation

Total LDH activity in µ/l at 37°C = ΔA/min x 8095

CK Isoenzyme analysis

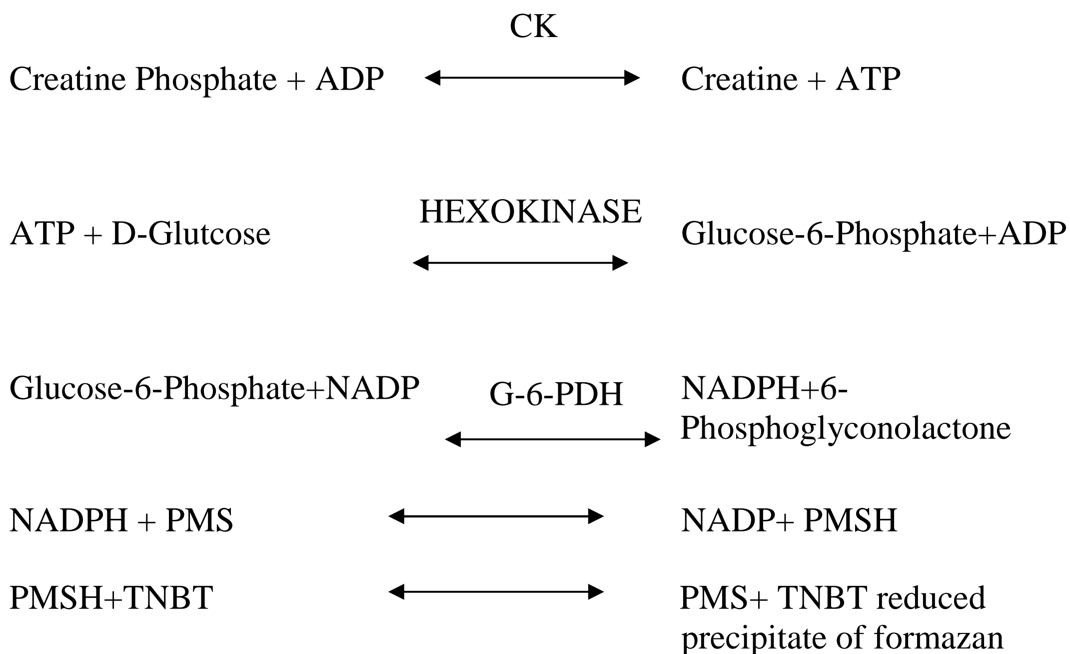
Analysis of Iso enzyme of CPK was carried out by HYDRASYS system SEBIA, PN 1210.

The HYDRASYS SEBIA system is a semi automated multi-parameter. Electrophoresis system. Commercial kits hydragel 7 Iso-CK are available for the analysis. The test was carried out as per the instruction provided in the kit by the manufacturer.

Principle

Creatine kinase isoenzymes consist of two subunits: M ("muscle") and B ("brain"), assembled in dimers. The three resulting combinations constitute the three isoenzymes. MM, principally located in cardiac and skeletal muscles, MB in cardiac muscle and BB in cerebral tissues. Each submit has a specific electric charge which confers characteristics mobility to the individual CK isoenzymes. On HYDRAGEL 7 ISO CK and HYDRAGEL ISO CK 15/30 gels, the BB fraction is the most anodic, the MM fraction is the most cathodic and the MB is intermediary.

All CK isoenzymes catalyze the same reaction that is utilized in their visualization. In the HYDRAGEL, 7 ISO-CK kit, the serum samples are electrophoresed and the separated CK isoenzymes are visualized using a specific chromogenic substrate according to the following reactions:



The amount of resulting formazan precipitate is proportional to the CK enzymatic activity.

Sample for Analysis

Fresh serum samples are taken for analysis. Sample can be stored at 2 to 8°C upto one week. 500 µl serum sample with 5 µl of activation solution are mixed and incubated for 10 mts at room temperature.

Procedure: The steps include processing of Hydragel agrose gels in the following sequences.

Sample application, Electrophoresis migration, Incubation with substrate, Stopping the enzymatic reaction, Blotting and final drying of the gel, Gel Scanning.

By identifying the pattern we can evaluate the fraction of CPK isoenzyme which is elevated.

LDH iso enzyme analysis

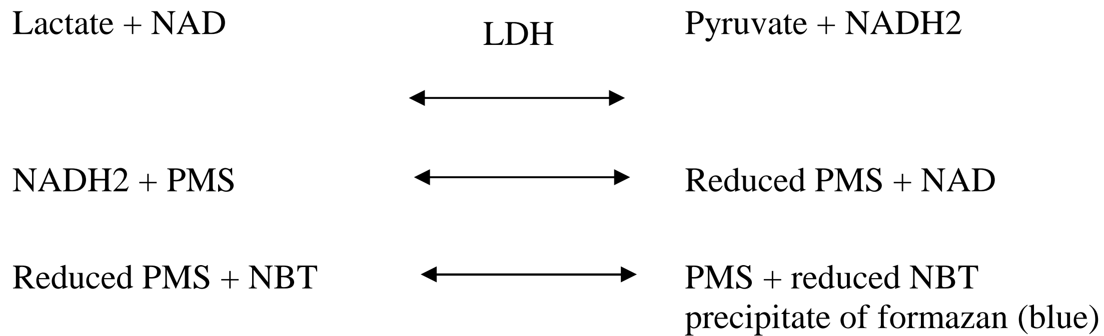
Iso enzyme analysis of LDH was carried out by HYDRASYS system SEBIA, PN 1210.

The HYDRASYS SEBIA system is a semi automated multi-parameter Electrophoresis system.

Commercial kits hydragel 7 ISO-LDH are available for the analysis. The test was carried out as per the instruction provided in the kit by the manufacturer.

Principle

All LDH isoenzymes catalyze the same reversible reaction which is utilized in their visualization. In the HYDRAGEL 7 ISO-LDH kit, the visualization of LDH isoenzymes is performed according to the following reactions.



The amount of resulting formazan precipitate is proportional to the LDH enzymatic activity.

NOTES:

NAD : Nicotinamide Adenine Dinucleotide

PMS : Phenazine Moethosulfate

NBT : Nitro Blue Tetrazolium.

Sample for analysis

Fresh serum samples are taken for analysis. Samples can be stored at 2°C to 8°C after collection and can be kept for one week.

Procedure

500 µl of serum sample with 5 µl of activation solution are mixed and incubated for 10 mts at room temperature.

The steps include processing of Hydragel agrose gels in the following sequence.

Sample application, Electrophoretic migration, Incubation with substrate, Stopping the enzymatic reaction, Blotting & final drying of the gel, Gel Scanning.

By identifying the pattern we can evaluate the fraction of iso enzyme LDH which is elevated.

STATISTICAL ANALYSIS

Statistical evaluation was carried out using SPSS (Version 14.0) Data obtained from the study groups were compared by the parametric student's t-test; correlation analysis between variables were made by Pearson test; P value <0.001 was considered statistically significant. All the results were expressed as means with their standard deviation (mean ± SD). Statistical analysis was also performed by using standard deviation and ANOVA.

The effect of vitamin C, alpha lipoic acid and both comdined were analysed for each groups and expressed as percentage of benefit with and without supplementation of vitamin C, alpha lipoic acid and both combined. Multiple comparison of each group with the normal was carried out using Bonferoni-t-test.

RESULTS

Identification of amitrirtyline overdoses in gastric aspirate in all cases admitted directily to intensive medical care unit(IMCU) and toxicology ward, Government General hospital between September 2005 and march 2008 are carried out by thin layer chromatography(TLC). The samples along with controlsare run simultaneously and based on their rf values in the TLC chromotogram(fig 7),the amitriptyline overdoses are detected and confirmed in all the groups.

Tables and charts

Table1: Comparison of RBC Cholinesterase in μ/ml

Groups	On admission		On discharge		Student's Paired t-test
	Mean	SD	Mean	SD	
Normal-Gr I	317.33	27.65	317.33	27.65	t=0.00 P=1.00 Not Significant
RoutineTreatment-Gr II	142.23	9.71	175.37	13.39	t=11.69 P=0.001 Significant
RoutineTreatment+VitC-Gr III	182.62	25.79	223.19	34.31	t=6.46 P=0.001 Significant
RoutineTreatment+ALA-Gr IV	150.81	32.09	198.52	42.98	t=5.88 P=0.001 Significant
RoutineTreatment+VitC+ALA-Gr V	146.71	9.88	232.08	47.89	t=9.06 P=0.001 Significant

The mean levels of RBC cholinesterase on admission compared with men levels of RBC cholinesterase on discharge. All groups showed the significant increase.

Table2: Percentage of benefit of different method of treatment on RBC Cholinesterase

Groups	On admission	On discharge	RBC gain
	Mean	Mean	Mean
Normal	317.33	317.33	.00
RoutineTreatment	142.23(44.8%)	175.37(55.3%)	33.13(10.5%)
RoutineTreatment+VitC	182.62(57.5%)	223.19(70.3%)	40.57(12.8%)
RoutineTreatment+ALA	150.81(47.5%)	198.52(62.5%)	47.70(12.7%)
RoutineTreatment+VitC+ALA	146.71(46.2%)	232.08(73.1%)	85.38(26.9%)

The percentage of benefit of treatment on supplementation with vitamin c and alpha lipoic acidshowed a slight increase in group III(12.8%) and group IV(12.7%),when compared with groupII(10.5%) without supplementation. But marked increase was observed in group V(26.9%) cases after supplementation with vitamin c and alpha lipoic acid in combination.

Table3: Comparison of RBC Cholinesterase gain

Groups	N	Mean	Std. Deviation	Oneway ANOVA F-test	Multiple comparison by Bonferroni t-test
RoutineTreatment	30	33.13	15.51		4 Vs 3
RoutineTreatment+VitC	21	40.57	28.78	F=11.14 P=0.001 significant	1 Vs 3
RoutineTreatment+ALA	27	47.70	42.16		2 Vs 3
RoutineTreatment+VitC+ALA	24	85.38	46.16		3 Vs 1,2,4

The multiple comparison of each group with the routiene standard treatment(RST) groupII, showed significant increase in values in all groups.

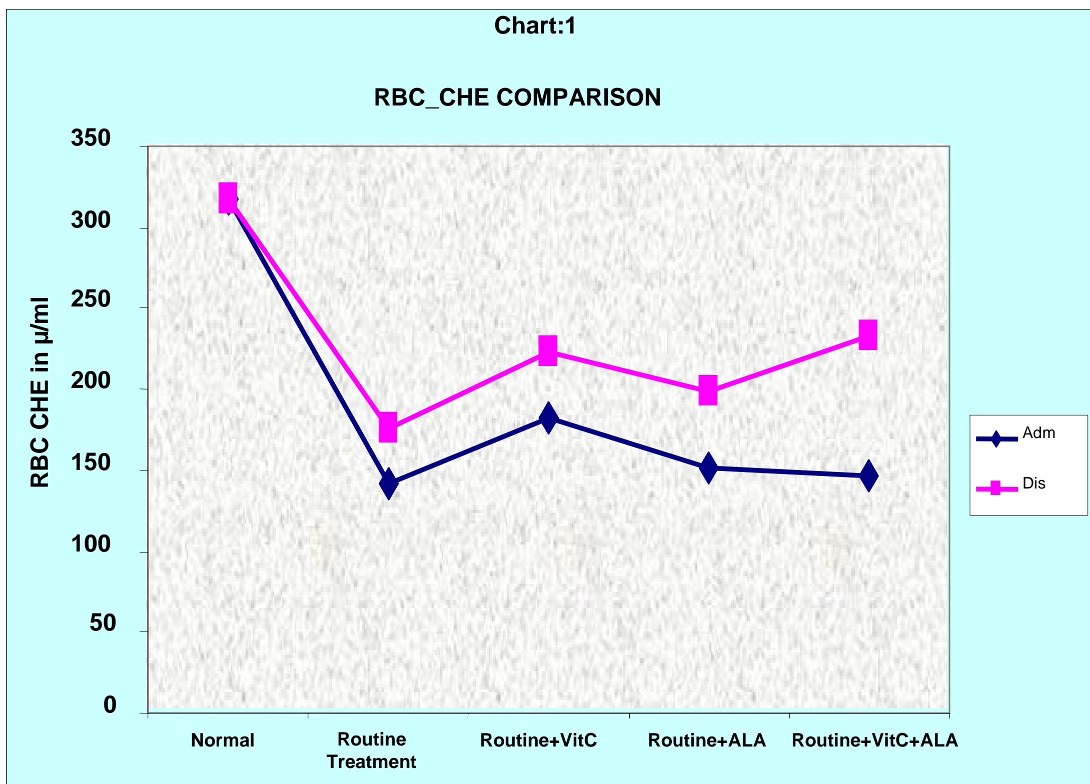


Chart:1
RBC_CHE COMPARISON
RBC CHE in µ/ml
350
300
250
200
150
100
50
0
Normal
Routine Treatment
Routine+VitC
Routine+ALA
Routine+VitC+ALA
Adm
Dis

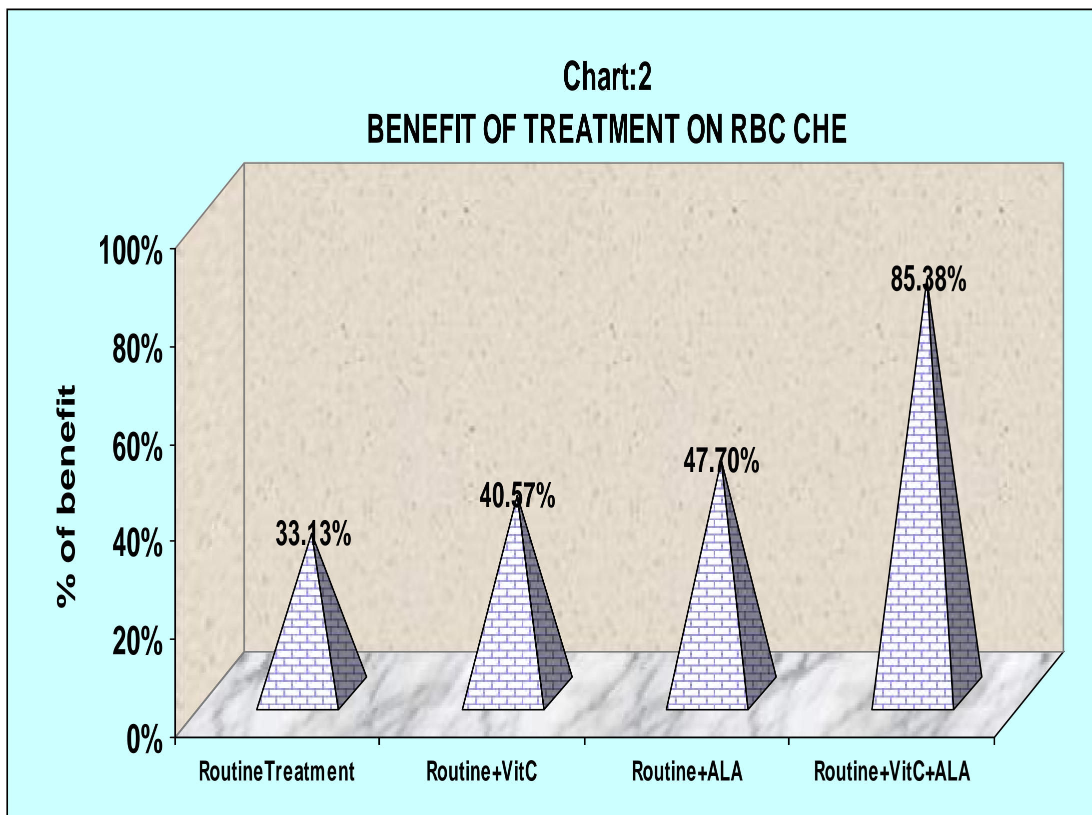


Chart:2
BENEFIT OF TREATMENT ON RBC CHE
100%
80%
60%
40%
20%
0%
% of benefit
33.13%
40.57%
47.70%
85.38%
RoutineTreatment
Routine+VitC
Routine+ALA
Routine+VitC+ALA

Table4: Comparison of plasma cholinestrase in µ/ml

Groups	On admission		On discharge		Student's Paired t-test
	Mean	SD	Mean	SD	
Normal	174.00	8.93	174.00	8.93	t=0.00 P=1.00 Not Significant
RoutineTreatment	70.07	8.31	115.47	11.59	t=16.73 P=0.001 Significant
RoutineTreatment+VitC	90.71	13.71	130.38	23.39	t=7.81 P=0.001 Significant
RoutineTreatment+ALA	82.81	7.39	116.07	13.01	t=12.16 P=0.001 Significant
RoutineTreatment+VitC+ALA	62.54	12.18	132.54	23.24	t=12.89 P=0.001 Significant

The mean levels of plasma cholinesterase on admission compared with mean levels of plasma cholinesterase on discharge in all groups, showed significant higher values.

Table5: Percentage of benefit of different method of treatment on plasma cholinesterase

Groups	On admission	On discharge	Pl.Che gain
	Mean	**Mean**	**Mean**
Normal	174.00	174.00	.00
RoutineTreatmen	70.07(40.3%)	115.47(66.4%)	45.40(26.1%)
RoutineTreatment+VitC	90.71(52.1%)	130.38(74.9%)	39.67(22.8%)
RoutineTreatment+ALA	82.81(47.6%)	116.07(66.7%)	33.26(33.3%)
RoutineTreatment+VitC+ALA	62.54(35.9%)	132.54(76.2%)	70.00(40.3%)

The percentage of benefit of treatment before and after supplementation with vitamin C showed a significant decrease in group IIII ie 22.8%, when compared with group II(26.1%). the group V showed marked improvement (ie 40.3%), when compared with the group II(ie 26.1%). Eventhough the values of plasma cholinesterase increased on discharge it was not upto the levels ofnormal individuals(group I).

Table6: Comparison of plasma cholinesterase gain

Groups	N	Mean	Std. Deviation	Oneway ANOVA F-test	Multiple comparison by Bonferroni t-test
RoutineTreatment	30	45.40	14.86		4 Vs 3
RoutineTreatment+VitC	21	39.67	23.26	F=57.16 P=0.001 significant	1 Vs 3
RoutineTreatment+ALA	27	33.26	14.21		2 Vs 3
RoutineTreatment+VitC+ALA	24	70.00	26.60		3 Vs 1,2,4

Multiple comparison with normal individuals showed significant decrease in values for all the groups.

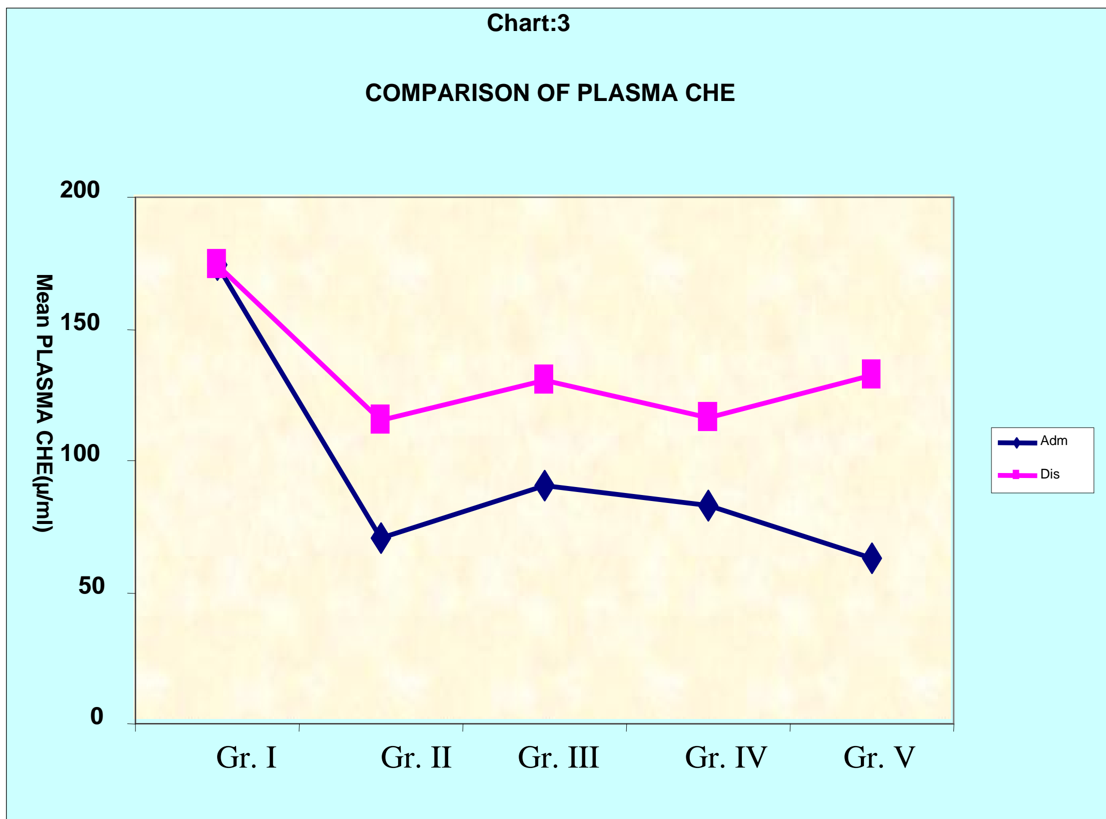


Chart:3
COMPARISON OF PLASMA CHE
Mean PLASMA CHE(µ/ml)
200
150
100
50
0
Gr. I
Gr. II
Gr. III
Gr. IV
Gr. V
Adm
Dis

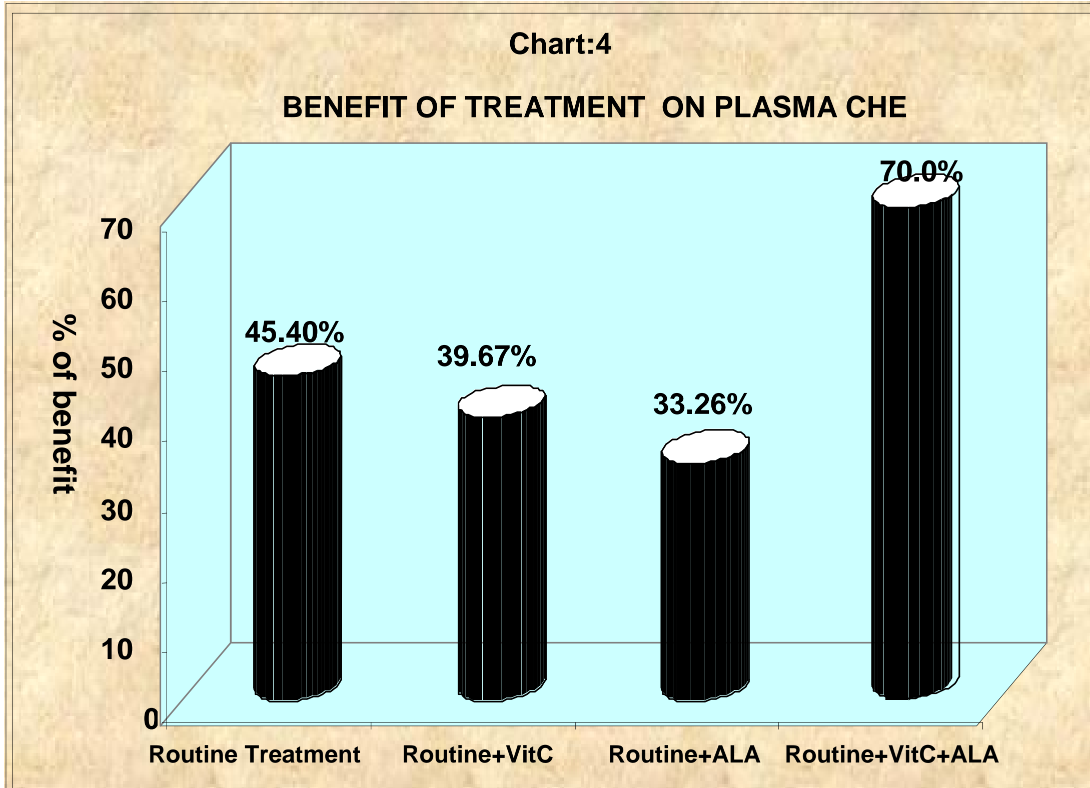


Chart:4
BENEFIT OF TREATMENT ON PLASMA CHE
% of benefit
0
10
20
30
40
50
60
70
45.40%
39.67%
33.26%
70.0%
Routine Treatment
Routine+VitC
Routine+ALA
Routine+VitC+ALA

Table7: comparison of superoxide dismutase in µmoles/mg Hb

Groups	On admission		On discharge		Student's Paired t-test
	Mean	SD	Mean	SD	
Normal	4.50	.74	4.50	.74	t=0.00 P=1.00 Not Significant
RoutineTreatment	6.34	.78	5.95	.84	t=2.01P=0.05 Significant
RoutineTreatment+VitC	6.33	.87	6.21	.50	t=0.15 P=0.90 not Significant
RoutineTreatment+ALA	6.29	.10	6.47	.68	t=0.22 P=0.92 not Significant
RoutineTreatment+VitC+ALA	6.52	.94	4.85	.57	t=5.11 P=0.001 Significant

The mean values of SOD on admission compared with mean values of SOD on discharge, showed significant lower values in group II and group V. The level of SOD was more decreased in group V ie with the combined supplementation of antioxidants

Table 8 Percentage of benefit of different method of treatment on superoxide dismutase

Groups	On admission	On discharge	SOD reduction
	Mean	Mean	Mean
Normal	4.50	4.50	.00
RoutineTreatment	6.34(140.9%)	5.95(132.2%)	0.39(8.7%)
RoutineTreatment+VitC	6.33(140.7%)	6.21(138.0%)	0.12(2.7%)
RoutineTreatment+ALA	6.29(139.7%)	6.47(143.7%)	-0.16(4.0%)
RoutineTreatment+VitC+ALA	6.52(144.9%)	4.85(107.8%)	1.67(37.0%)

The percentage of benefit of treatment in SOD after supplementation with vitamin C (group III) and alpha lipoic acid (group IV) did not show much improvement.In our study we observed that though the levels of SOD on discharge were almost equal when compared with the values on admission, it remained higher when compared with the normal individuals.

Table 9 Comparison of superoxide dismutase reduction

Groups	N	Mean	Std. Deviation	Oneway ANOVA F-test	Multiple comparison by Bonferroni t-test
RoutineTreatment	30	0.39	0.89		4 Vs 3
RoutineTreatment+VitC	21	0.12	0.88	F=7.11 P=0.001 significant	1 Vs 3
RoutineTreatment+ALA	27	-0.16	0.68		2 Vs 3
RoutineTreatment+VitC+ALA	24	1.67	0.93		3 Vs,1,2,4

Multiple comparisons of SOD in all groups with normal individuals showed significant decrease values.

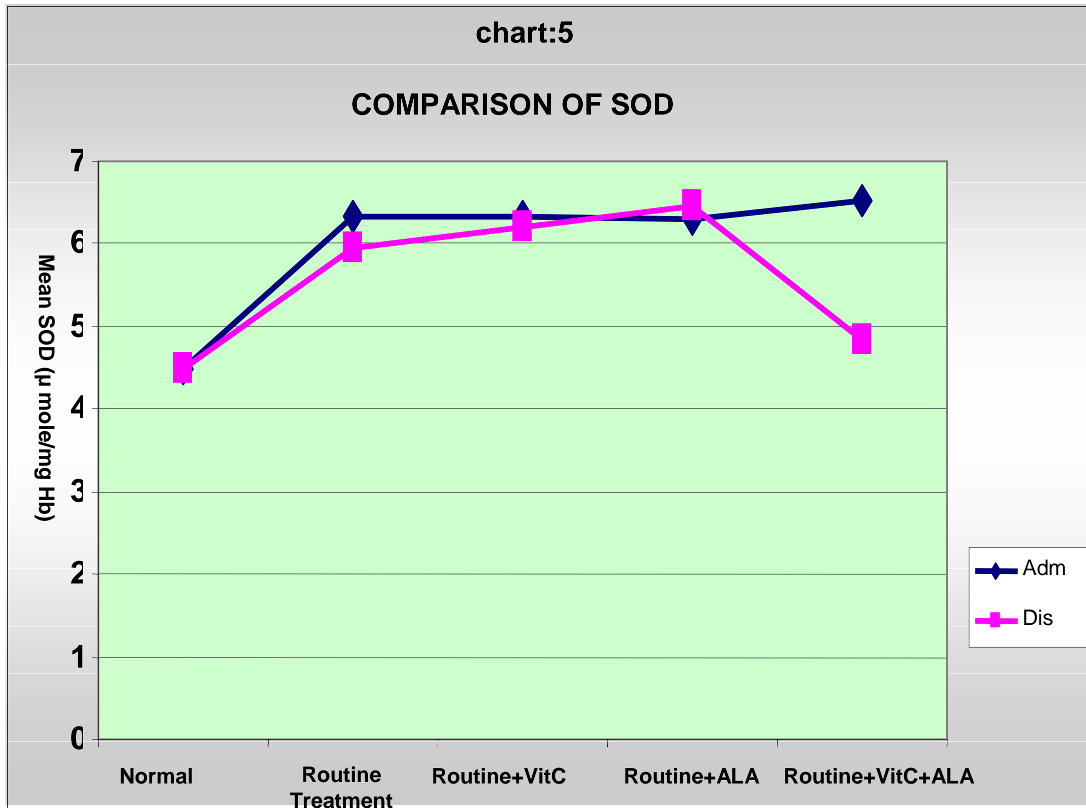


chart:5
COMPARISON OF SOD
Mean SOD (µ mole/mg Hb)
7
6
5
4
3
2
1
0
Normal
Routine Treatment
Routine+VitC
Routine+ALA
Routine+VitC+ALA
Adm
Dis

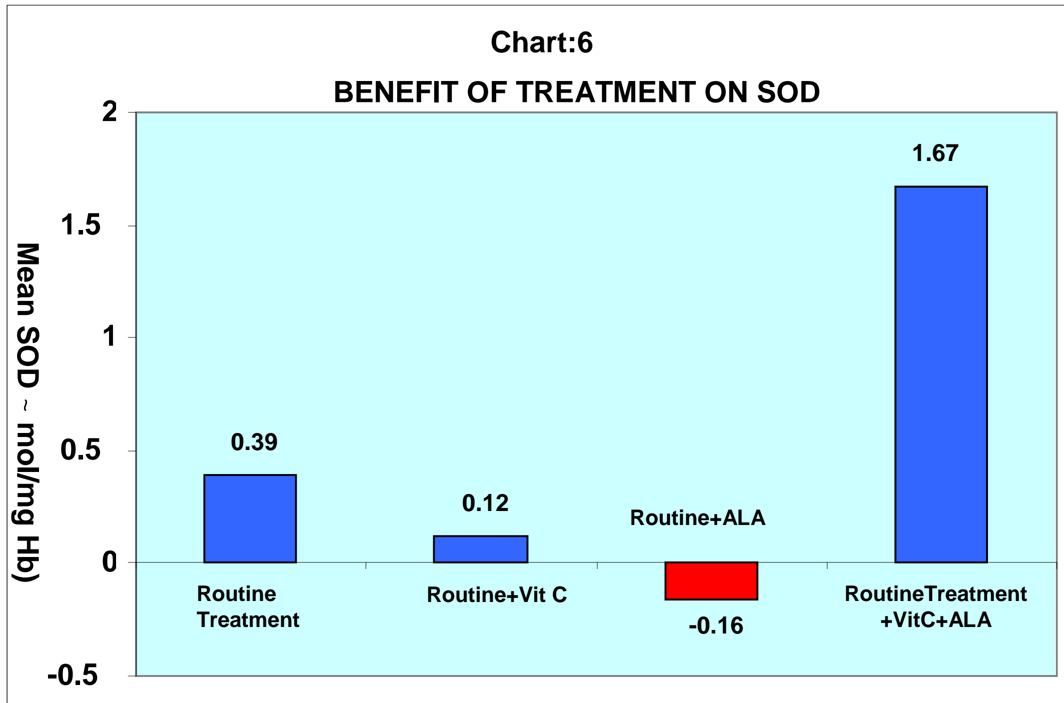


Chart:6
BENEFIT OF TREATMENT ON SOD
Mean SOD ~ mol/mg Hb)
2
1.5
1
0.5
0
-0.5
0.39
0.12
-0.16
1.67
Routine Treatment
Routine+Vit C
Routine+ALA
RoutineTreatment +VitC+ALA

Table 10 Comparsion of catalase in µ.moles/mg Hb

Groups	On admission		On discharge		Student's Paired t-test
	Mean	SD	Mean	SD	
Normal	2.98	.58	2.98	0.58	t=0.00 P=1.00 Not Significant
RoutineTreatment	5.09	.71	4.97	0.73	t=0.14 P=0.89 not Significant
RoutineTreatment+VitC	5.58	.67	3.27	0.63	t=4.08 P=0.001 Significant
RoutineTreatment+ALA	4.98	.22	3.82	0.55	t=4.80 P=0.001 Significant
RoutineTreatment+VitC+ALA	4.76	.69	4.43	0.49	t=15.11 P=0.001 Significant

The mean levels of catalase on admission compared with the mean levels of catalase on discharge, showed significant change in groupIII, Group IV, Group V, but in group II no significant change was noticed. There was a significant decrease noticed in-group III, group IV, group V after supplementation of vitamin C, alpha lipoic acid and both, together respectively.

Table 11 Percentage of benefit of different method of treatment on catalase

Groups	On admission	On discharge	Catalase reduction
	Mean	Mean	Mean
Normal	2.98	2.98	.00
RoutineTreatment	5.09(171%)	4.97(167%)	0.12(4.0%)
RoutineTreatment+VitC	4.76(159%)	4.43(149%)	0.33(10%)
RoutineTreatment+ALA	4.98(167%)	3.82(128%)	0.84(28%)
RoutineTreatment+VitC+ALA	5.58(189%)	3.27(110%)	2.31(79%)

The percentage of benefit of treatment before and after supplementation of antioxidants showed gradual improvement in group III, group IV, group V.In the above groups, group Vcases showed maximum improvement (ie 79%).

Table 12 Comparison Of Catalase Reduction

Groups	N	Mean	Std. Deviation	Oneway ANOVA F-test	Multiple comparison by Bonferroni t-test
RoutineTreatment	30	.1215	.72324		4 Vs 1,3
RoutineTreatment+VitC	21	.335	.90388	F=9.45 P=0.001 significant	1 Vs3, 4
RoutineTreatment+ALA	27	.843	.57683		2 Vs 1,3
RoutineTreatment+VitC+ALA	24	2.315	.95622		3 Vs1 ,2,3

Multiple comparison of each groups with the normal individuals showed significant higher values in all groups with or without supplementation of antioxidants.

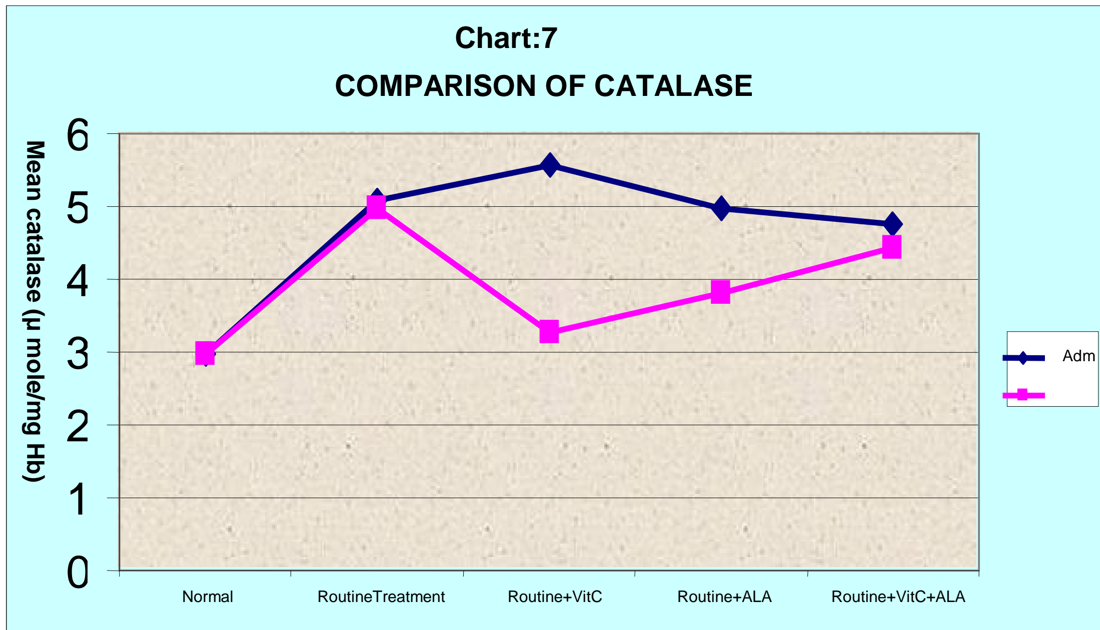


Chart:7
COMPARISON OF CATALASE
Mean catalase (µ mole/mg Hb)
6
5
4
3
2
1
0
Normal
RoutineTreatment
Routine+VitC
Routine+ALA
Routine+VitC+ALA
Adm
Dis

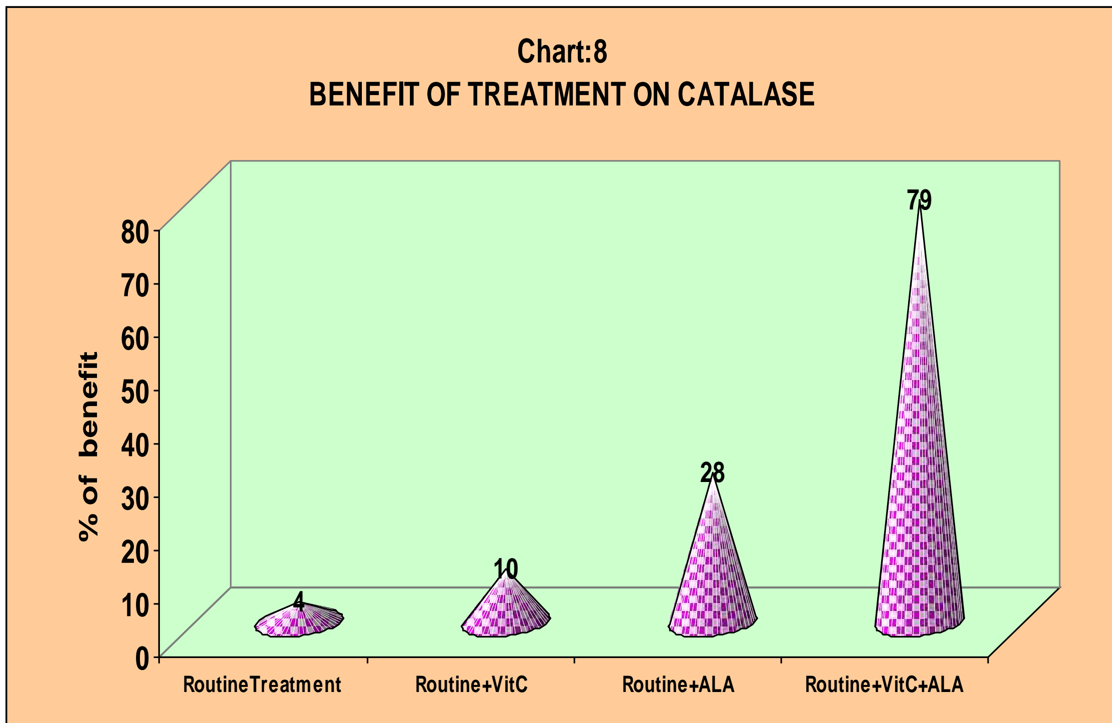


Chart:8
BENEFIT OF TREATMENT ON CATALASE
% of benefit
0
10
20
30
40
50
60
70
80
4
10
28
79
RoutineTreatment
Routine+VitC
Routine+ALA
Routine+VitC+ALA

Table 13 Glut-peroxidasecomparison in µg/ mg Hb

Groups	On admission		On discharge		Student's Paired t-test
	Mean	SD	Mean	SD	
Normal	5.53	.74	5.53	.74	t=0.00 P=1.00 Not Significant
RoutineTreatment	6.46	.98	6.04	.65	t=2.21 P=0.03 Significant
RoutineTreatment+VitC	6.58	.65	6.10	.61	t=2.20 P=0.02 Significant
RoutineTreatment+ALA	5.88	.65	5.83	.61	t=0.25 P=0.80 Not Significant
RoutineTreatment+VitC+ALA	6.62	.74	5.65	.51	t=5.69 P=0.001 Significant

The mean levels of glutathione peroxidase on admission was compared with mean levels on discharge showed decrease in levels for all groups). In fact the values for group IV cases showed no significant. But the decrease in the levels of glutathione peroxidase was significant in group III and group V cases on supplementation with antioxidants.

Table 14 Percentage of benefit of different method of treatment on Glutathione peroxidase

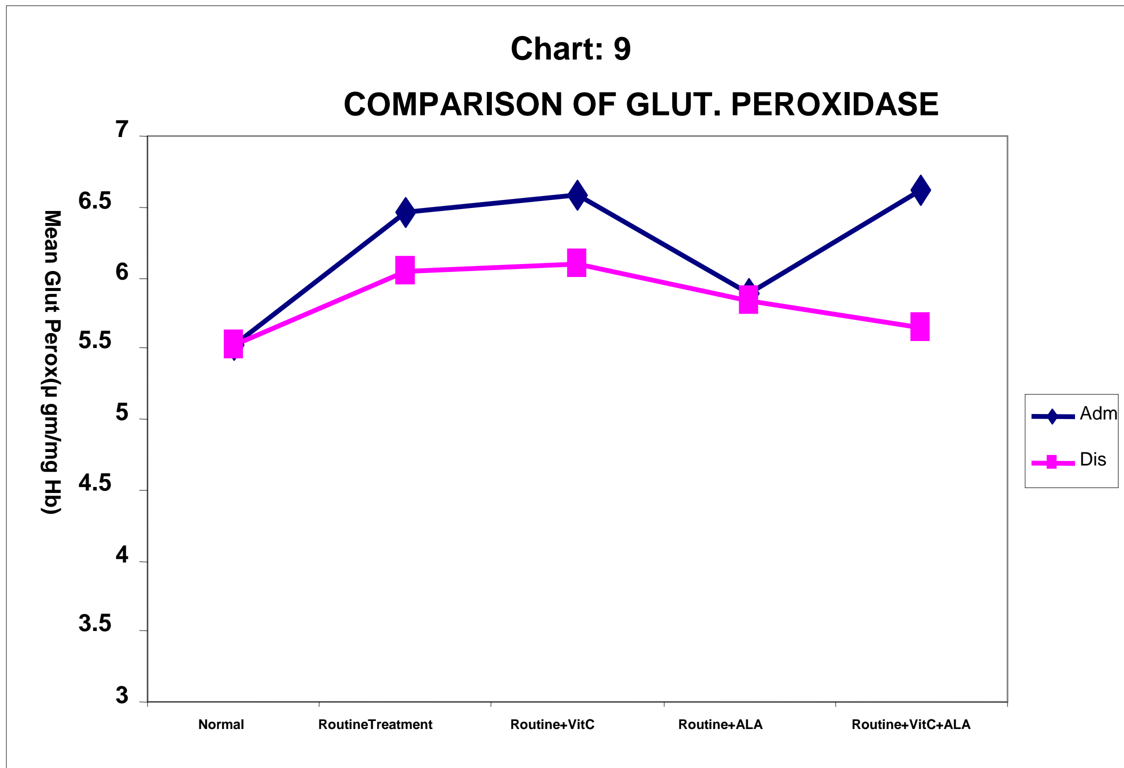
Groups	On admission	On discharge	Glut_ peroxidase reduction
	Mean(%)	Mean(%)	Mean(%)
Normal	5.53	5.53	5.53
RoutineTreatment	6.46(116.8%)	6.04(109.2%)	-0.41(7.4%)
RoutineTreatment+VitC	5.58(100.9%)	5.10(92.2%)	-0.48(8.1%)
RoutineTreatment+ALA	5.88(106.3%)	5.83(105.4%)	-0.05(0.9%)
RoutineTreatment+VitC+ALA	6.62(119.7%)	5.65(102.1%)	-0.96(11.4%)

The percentage of benefit of treatment for glutathione peroxidase showed not much improvement for group IV cases. Whereas group III and group V cases showed marked improvement(ie 8.1% and 11.4% respectively).

Table 15 Comparison of Glutathione peroxidase reduction

Groups	N	Mean	Std. Deviation	Oneway ANOVA F-test	Multiple comparison by Bonferroni t-test
RoutineTreatment	30	-.4103	1.015		4 Vs 3
RoutineTreatment+VitC	21	-.4814	1.002	F=3.78 P=0.03 significant	1 Vs3
RoutineTreatment+ALA	27	-.0488	1.008		2 Vs 3
RoutineTreatment+VitC+ALA	24	-.9612	.826		3 Vs1,2,4

Multiple comparison of glutathione peroxidase with group II showed increased values for group III and group V and decreased values for group IV cases.



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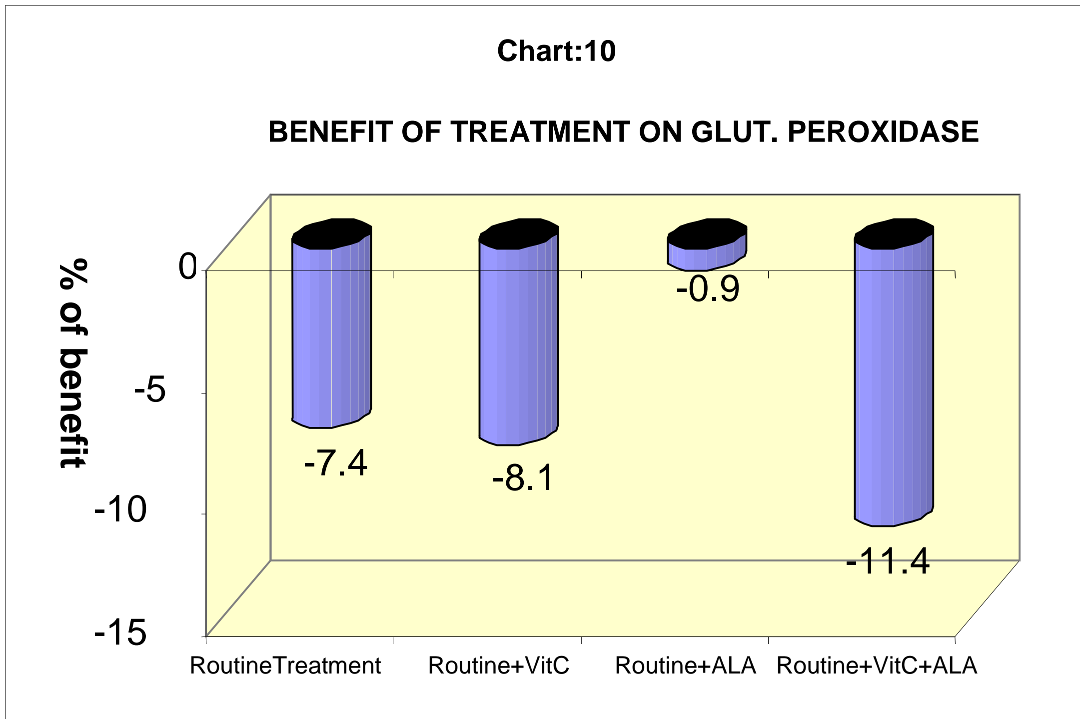


Chart:10
BENEFIT OF TREATMENT ON GLUT. PEROXIDASE
% of benefit
0
-5
-10
-15
-7.4
-8.1
-0.9
-11.4
RoutineTreatment
Routine+VitC
Routine+ALA
Routine+VitC+ALA

Table 16 comparison of Glutathione transferase n moles/ml

Groups	On admission		On discharge		Student's Paired t-test
	Mean	SD	Mean	SD	
Normal	5.02	.82	5.02	.82	t=0.00 P=1.00 Not Significant
RoutineTreatment	6.16	.61	5.65	.59	=3.29 P=0.003 Significant
RoutineTreatment+VitC	5.26	.63	5.10	.70	t=0.88 P=0.38 Not Significant
RoutineTreatment+ALA	5.51	.89	5.26	.51	t=1.14 P=0.26 Not Significant
RoutineTreatment+VitC+ALA	5.84	.66	5.16	.49	t=3.96 P=0.001 Significant

The mean levels of glutathione transferase on admission was compared with the levels on discharge showed significant decrease in values for all groups. In group II cases we noticed the values were very high on admission when compared to all other groups (ie 6.16%).

Table 17 Percentage of benefit of different method of treatment on Glutathione transferase

Groups	On admission	On discharge	Glut_transfer reduction
	Mean(%)	**Mean(%)**	**Mean(%)**
Normal	5.02	5.02	5.02
RoutineTreatment	6.16(117.8%)	5.65(106.1%)	-0.50(9.9%)
RoutineTreatment+VitC	5.26(112.5%)	5.10(103.7%)	-0.15(2.9%)
RoutineTreatment+ALA	5.51(111.6%)	5.26(107.4%)	-0.25(4.9%)
RoutineTreatment+VitC+ALA	5.84(113.3%)	5.16(96.1%)	-0.68(13.5%)

The percentage of benefit of treatment for glutathione transferase showed no improvement for group III and group IV cases. But group V cases showed marked improvement(ie 13.5%).

Table 18 Comparison of Glutathione transferase reduction

Groups	N	Mean	Std. Deviation	Oneway ANOVA F-test	Multiple comparison by Bonferroni t-test
RoutineTreatment	30	-.5053	0.84117		-
RoutineTreatment+VitC	21	-.1543	0.79915	F=1.57 P=0.20 Not significant	-
RoutineTreatment+ALA	27	-.2519	1.14464		-
RoutineTreatment+VitC+ALA	24	-.6758	0.83520		-

Multiple comparison of each group with the normal showed significant increase in values for all groups on admission and on discharge.

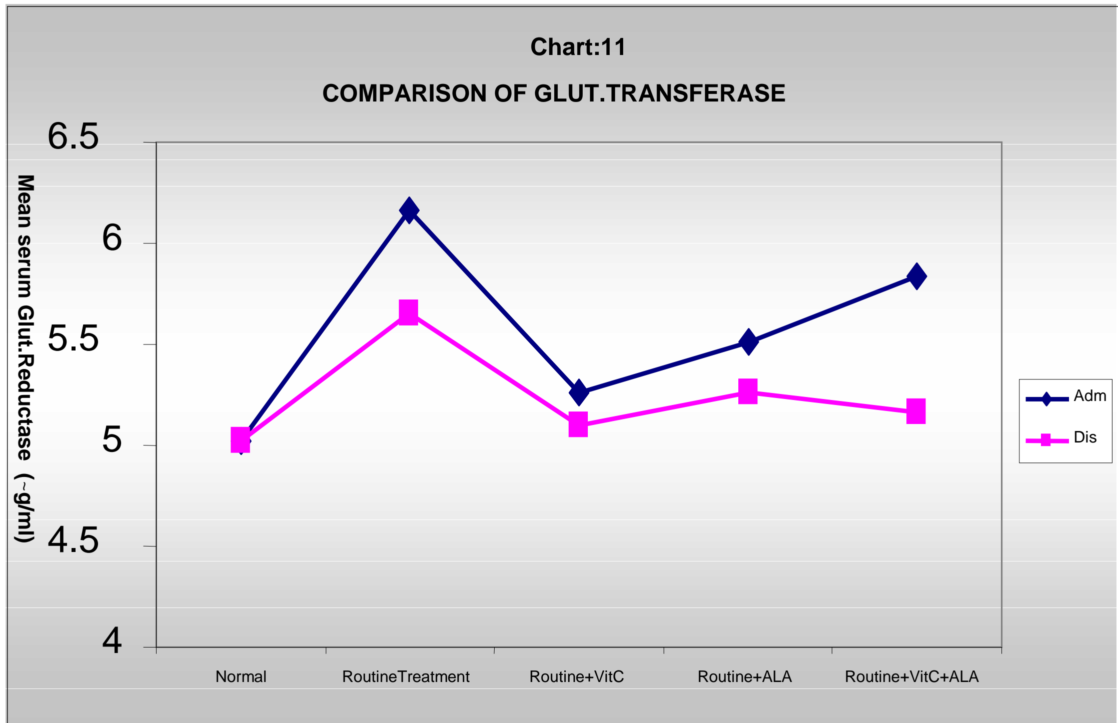


Chart:11
COMPARISON OF GLUT.TRANSFERASE
Mean serum Glut.Reductase (~g/ml)
6.5
6
5.5
5
4.5
4
Normal
RoutineTreatment
Routine+VitC
Routine+ALA
Routine+VitC+ALA
Adm
Dis

Error!

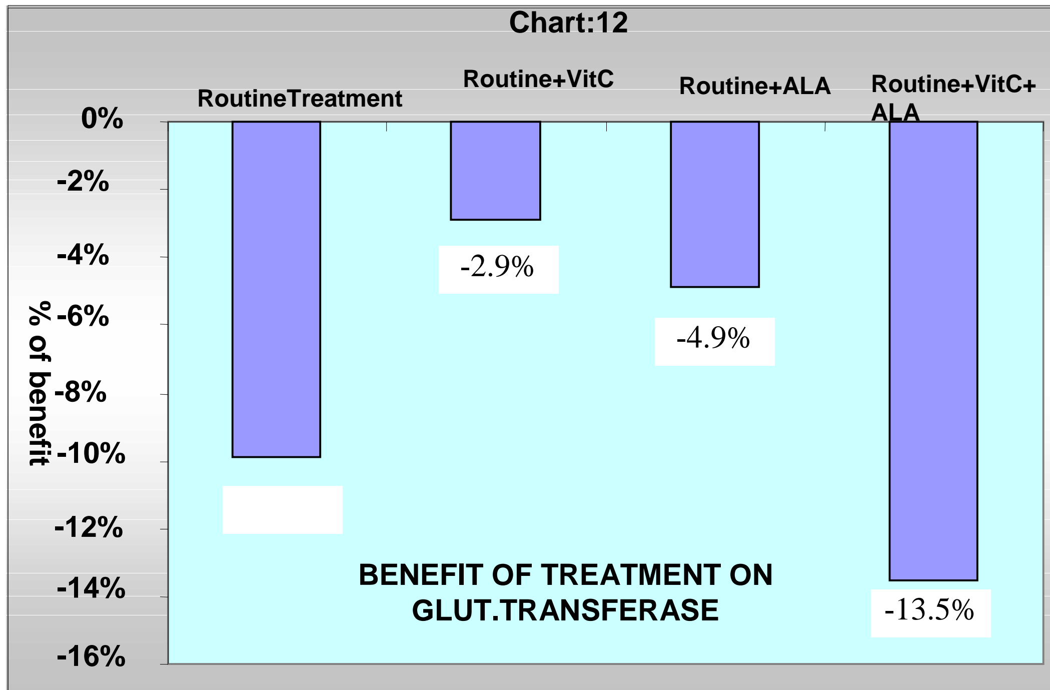


Table 19 serum glutathione reductase in µg /ml

Groups	On admission		On discharge		Student's Paired t-test
	Mean	SD	Mean	SD	
Normal	9.58	.57	9.58	.57	t=0.00 P=1.00 Not Significant
RoutineTreatment	11.29	1.66	10.17	1.58	t=2.21 P=0.03 Significant
RoutineTreatment+VitC	10.78	1.22	9.94	1.21	t=7.20 P=0.04 Significant
RoutineTreatment+ALA	10.69	.36	10.29	.65	t=0.25 P=0.80 Not Significant
RoutineTreatment+VitC+ALA	10.85	1.04	9.16	.73	t=5.69 P=0.001 Significant

The mean levels of serum glutathione reductase in all groups on admission compared with the levels on discharge, showed significant decrease in values in all groups except in group IV cases. In group IV cases we observed that the values remained almost the same on admission(group IV=10.69) as well as on discharge(group IV =10.29, At the same time the group V cases showed marked decrease(ie from 10.85% to 9.16%).

Table 20 Percentage of benefit of different method of treatment on serum glutathione reductase

Groups	On admission	On discharge	SR-GLU-RED reduction
	Mean(%)	Mean(%)	Mean(%)
Normal	9.58	9.58	.00
RoutineTreatment	11.29(117.8%)	10.17(106.1%)	-1.12(4.0%)
RoutineTreatment+VitC	10.78(112.5%)	9.94(103.7%)	-0.84(8.76%)
RoutineTreatment+ALA	10.69(111.6%)	10.29(107.4%)	-0.40(5.6%)
RoutineTreatment+VitC+ALA	10.85(113.3%)	9.16(96.1%)	-1.68(1.69%)

In the same way the percentage of benefit on serum glutathione reductase showed significant decrease in group V(groupV=36.4%).

Table 21 Comparison of serum glutathione reductase reduction

	N	Mean	Std. Deviation	Oneway ANOVA F-test	Multiple comparison by Bonferroni t-test
RoutineTreatment	30	-1.1187	1.18897		-
RoutineTreatment+VitC	21	-.8457	1.59649	F=5.52 P=0.002 significant	-
RoutineTreatment+ALA	27	-.4019	.80200		2 Vs 2
RoutineTreatment+VitC+ALA	24	-1.6867	.94950		3 Vs2

Multiple comparison of each groups with the normal individuals showed increase in values on admission and on discharge except in group V cases which showed reverse trend.

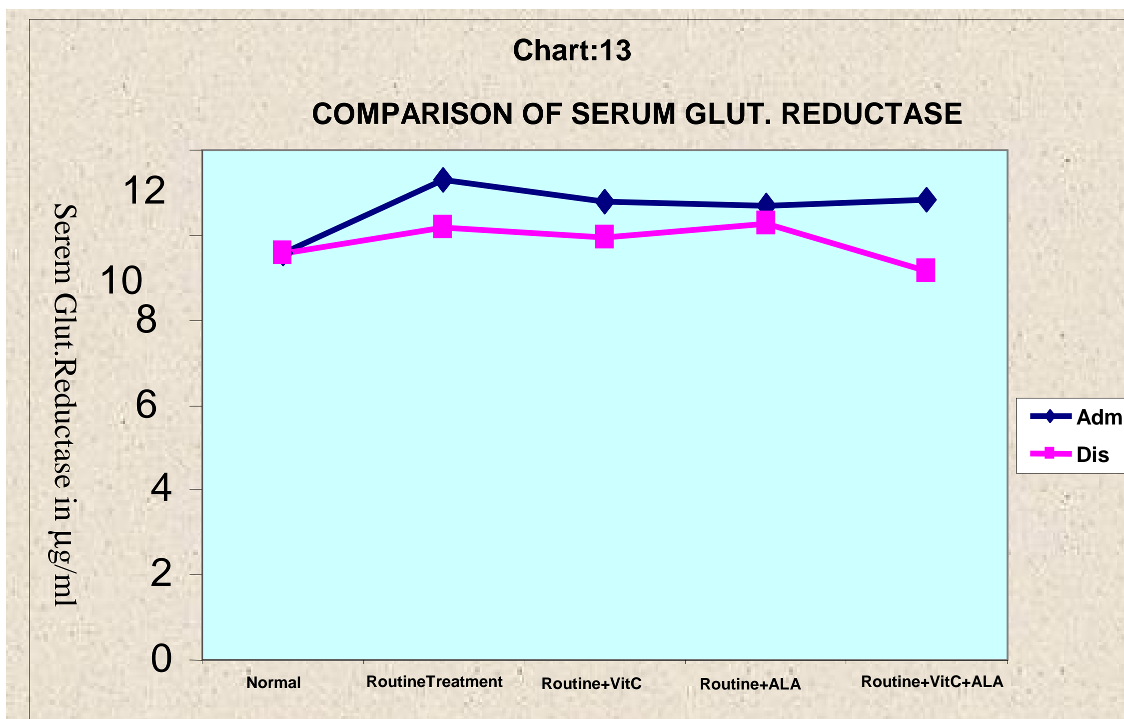


Chart:13
COMPARISON OF SERUM GLUT. REDUCTASE
Serem Glut.Reductase in µg/ml
12
10
8
6
4
2
0
Normal
RoutineTreatment
Routine+VitC
Routine+ALA
Routine+VitC+ALA
Adm
Dis

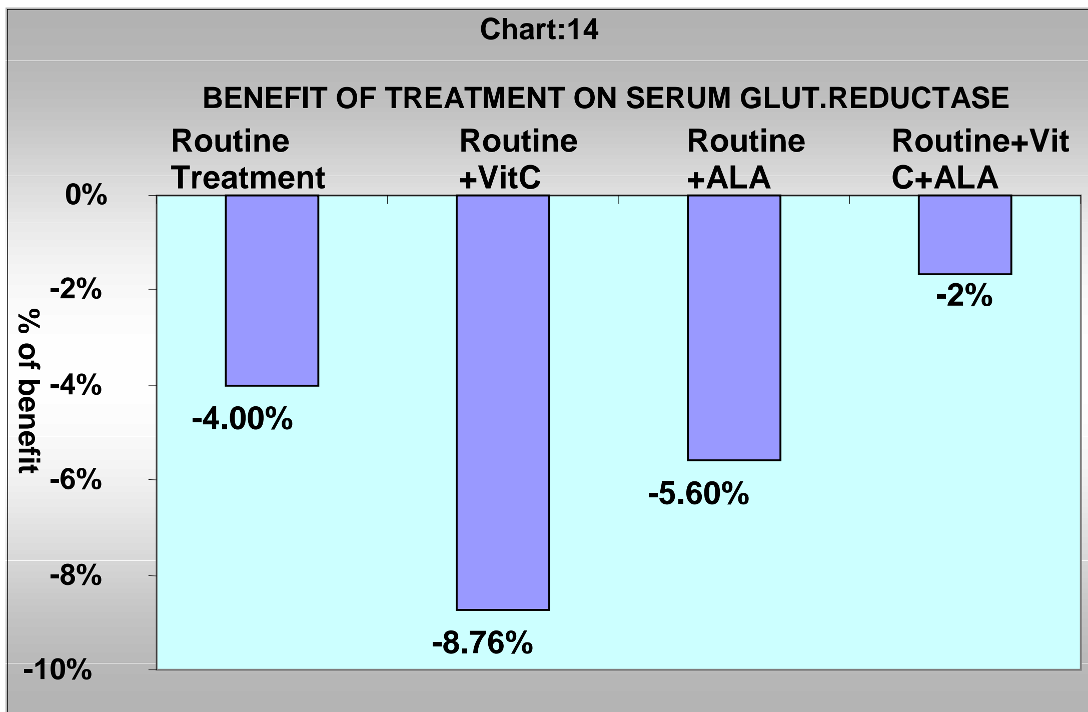


Chart:14
BENEFIT OF TREATMENT ON SERUM GLUT.REDUCTASE
Routine Treatment
Routine +VitC
Routine +ALA
Routine+Vit C+ALA
0%
-2%
-4%
-6%
-8%
-10%
% of benefit
-4.00%
-8.76%
-5.60%
-2%

Table 22 comparison of RBC Glutathione reductase in µg/mg Hb

Groups	On admission		On discharge		Student's Paired t-test
	Mean	SD	Mean	SD	
Normal	1.95	.48	1.95	.48	t=0.00 P=1.00 Not Significant
RoutineTreatment	3.24	.81	2.88	.45	=2.76 P=0.01 Significant
RoutineTreatment+VitC	2.30	.63	2.53	.80	t=1.52 P=0.14 Not Significant
RoutineTreatment+ALA	2.31	.53	2.52	.60	t=1.59 P=0.12 Not Significant
RoutineTreatment+VitC+ALA	2.95	.52	2.05	.59	t=6.49P=0.001 Significant

The mean values of RBC glutathione reductase in all groups on admission compared with the values on discharge, showed increase in RBC glutathione reductase activity on admission and on discharge when compared with the normal individuals(In group III and group IV cases the values increased on admission and continued to increase till discharge (ie 2.30% to 2.53% and 2.31 to 2.52% respectively), but in case of group II and group V, the values increased on admission and decreased on discharge

Table 23 Percentage of benefit of different method of treatment on RBC G-lutathione reductase

	On admission	On discharge	RBC-GLUT-RED gain
Groups	**Mean(%)**	**Mean(%)**	**Mean(%)**
Normal	1.95	1.95	1.95
RoutineTreatment	3.24(166.2%)	2.88(151.5%)	-0.35(16.7%)
RoutineTreatment+VitC	2.30(118.0%)	2.53(`129.7%)	0.23(11.8%)
RoutineTreatment+ALA	2.31(118.5%)	2.52(`129.2%)	0.21(10.7%)
RoutineTreatment+VitC+ALA	2.95(151.3%)	2.05(105.1%)	-0.90(46.2%)

The percentage of benefit on RBC glutathione reductase showed marked improvement in all groups but the effect was maximum in group V cases when compared to group II cases.

Table 24 Comparison of RBC Glutathione reductase reduction

Groups	N	Mean	Std. Deviation	Oneway ANOVA F-test	Multiple comparison by Bonferroni t-test
RoutineTreatment	30	-.3567	.70683		4 Vs 1,2,3
RoutineTreatment+VitC	21	0.2338	.70471	F=14.6	1 Vs 3 ,4
RoutineTreatment+ALA	27	0.2026	.65844	P=0.001	2 Vs 3 ,4
RoutineTreatment+VitC+ALA	24	-.8929	.67395	significant	3 Vs1,2,4

The multiple comparison of each group with the normal individuals showed decrease in the levels of total antioxidants on admission and the levels increased on discharge.

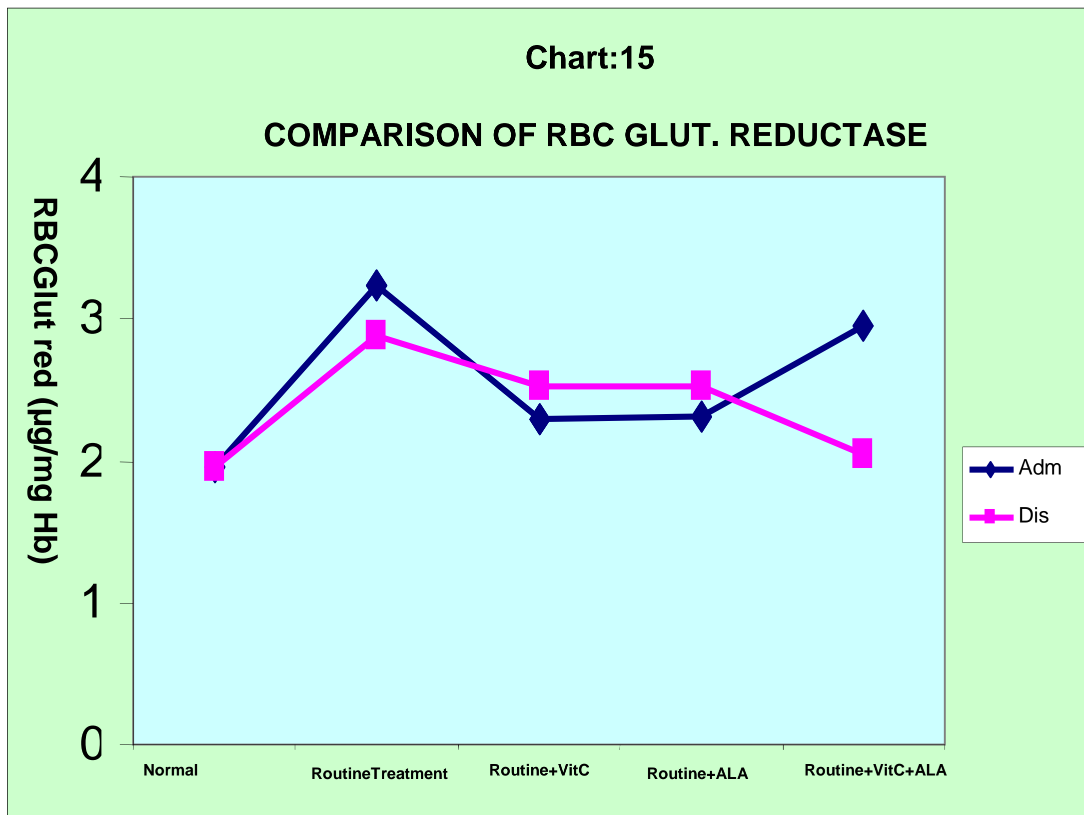


Chart:15
COMPARISON OF RBC GLUT. REDUCTASE
RBCGlut red (µg/mg Hb)
4
3
2
1
0
Normal
RoutineTreatment
Routine+VitC
Routine+ALA
Routine+VitC+ALA
Adm
Dis

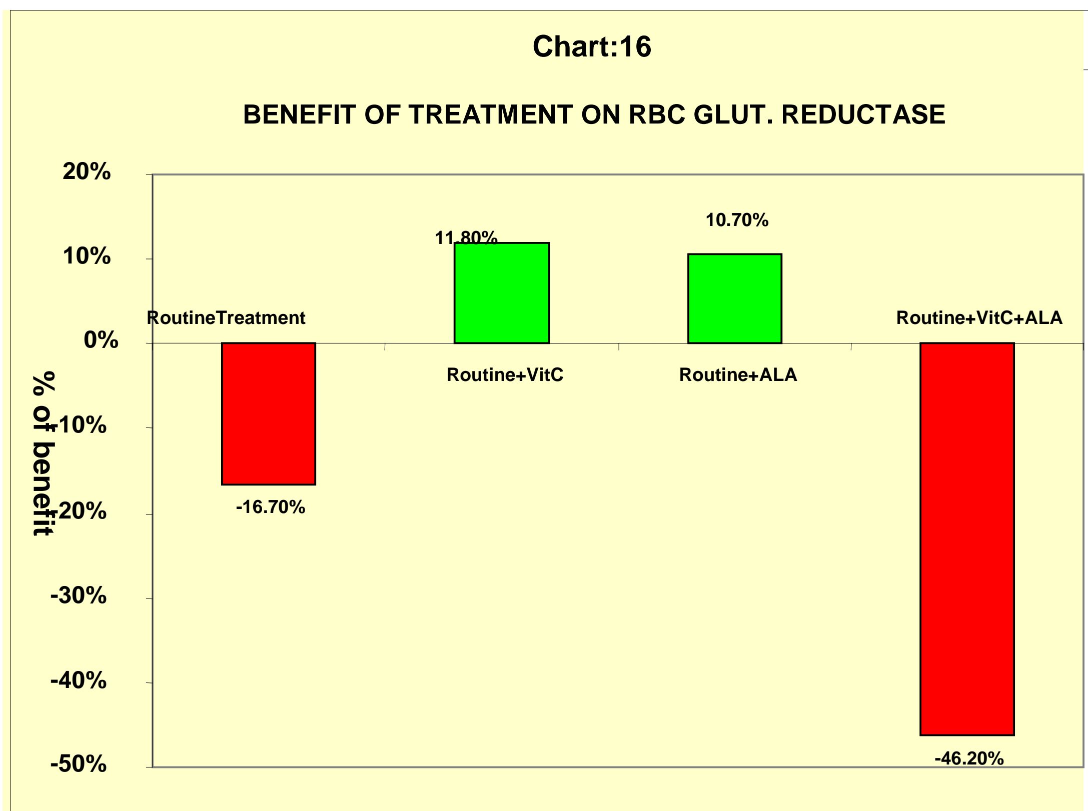


Chart:16
BENEFIT OF TREATMENT ON RBC GLUT. REDUCTASE
% of benefit
20%
10%
0%
-10%
-20%
-30%
-40%
-50%
RoutineTreatment
Routine+VitC
Routine+ALA
Routine+VitC+ALA
-16.70%
11.80%
10.70%
-46.20%

Table 25 Malonyldialdehyde(MDA) in n moles/ml

Groups	On admission		On discharge		Student's Paired t-test
	Mean	SD	Mean	SD	
Normal	1.26	.08	1.26	.08	t=0.00 P=1.00 Not Significant
RoutineTreatment	2.03	.89	2.37	.63	t=3.27 P=0.003 Significant
RoutineTreatment+VitC	2.20	.41	2.54	.56	t=3.63 P=0.002 Significant
RoutineTreatment+ALA	1.87	.11	2.10	.23	t=6.05 P=0.001 Significant
RoutineTreatment+VitC+ALA	2.10	.74	2.01	.56	t=1.74 P=0.09 not Significant

The mean levels of MDA in all groups on admission compared with mean levels on discharge, showed increase in MDA levels on admission and the increase continued till discharge in all groups except in group V cases. In group V cases though the levels at the time of discharge decreased, the decrease was not significant(ie 2.10% to 2.01%).

Table 26 Percentage of benefit of different method of treatment on MDA

Groups	On admission	On discharge	MDA gain
	Mean(%)	**Mean(%)**	**Mean(%)**
Normal	1.26	1.26	1.26
RoutineTreatment	2.03(161%)	2.37(188%)	0.34(26.9%)
RoutineTreatment+VitC	2.20(175%)	2.54(201%)	0.34(26.9%)
RoutineTreatment+ALA	1.87(148%)	2.10(167%)	0.23(18.3%)
RoutineTreatment+VitC+ALA	2.10(167%)	2.01(159%)	-0.08(6.3%)

The percentage of benefit of treatment on MDA showed improvement in group V cases and the levels of MDA decreased on combined supplementation with antioxidants in this group ie 2.10% to 2.01 %.

Table 27 Comparison of MDA reduction

Groups	N	Mean	Std. Deviation	Oneway ANOVA F-test	Multiple comparison by Bonferroni t-test
RoutineTreatment	30	0.3380	.56497		4 Vs 3
RoutineTreatment+VitC	21	0.3381	.42663	F=6.35 P=0.001 significant	1 Vs 3
RoutineTreatment+ALA	27	0.2311	.19846		2 Vs 3
RoutineTreatment+VitC+ALA	24	-0.0883	.24761		3 Vs2 ,1

Multiple comparison of MDA in each group with normal individuals showed increase values both on admission and on discharge .

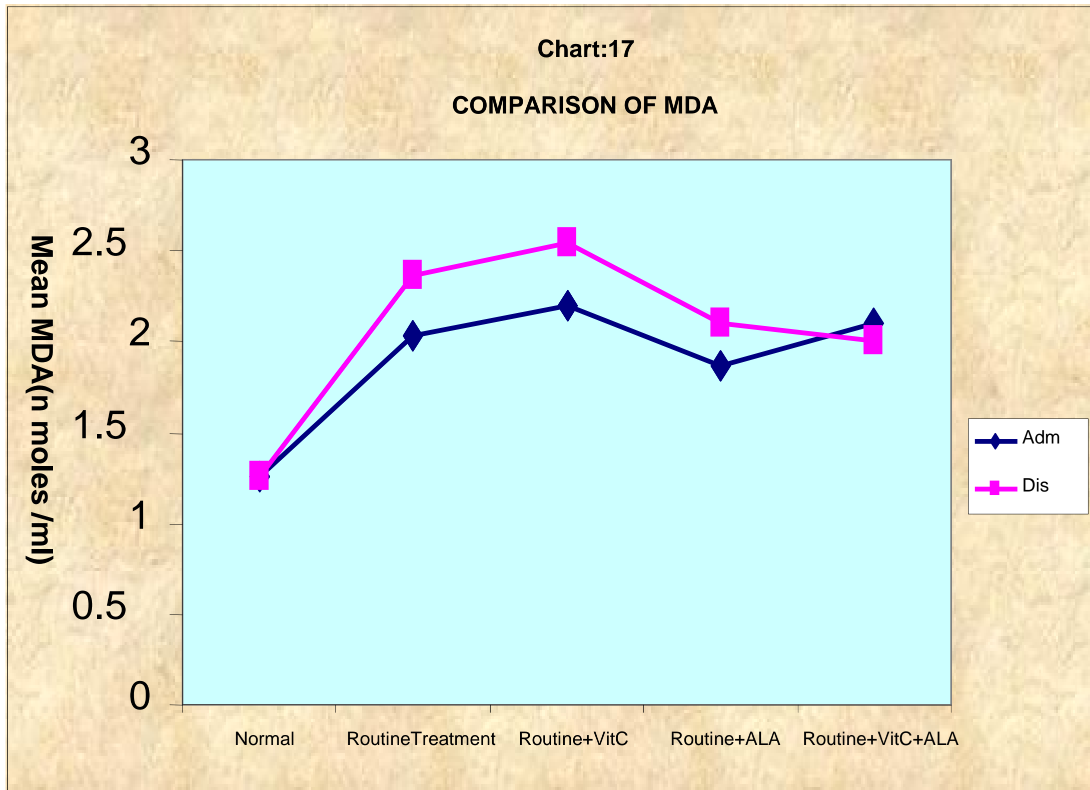


Chart:17
COMPARISON OF MDA
Mean MDA(n moles /ml)
3
2.5
2
1.5
1
0.5
0
Normal
RoutineTreatment
Routine+VitC
Routine+ALA
Routine+VitC+ALA
Adm
Dis

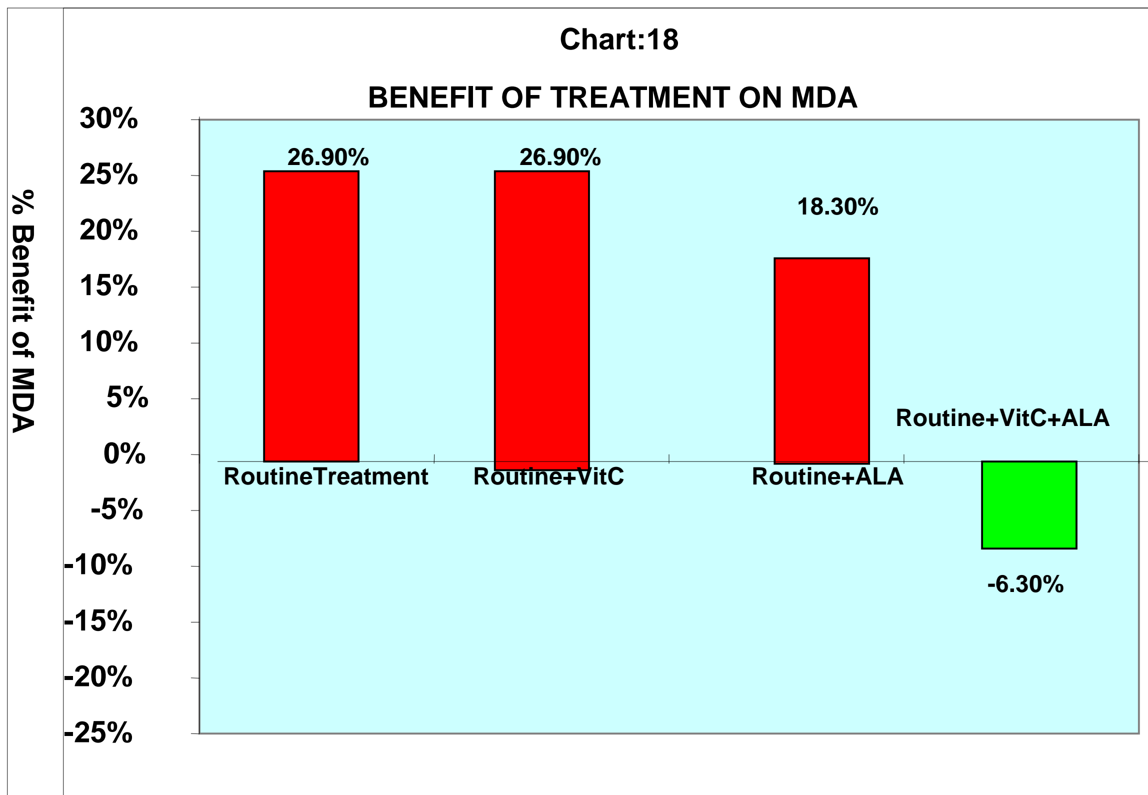


Chart:18
BENEFIT OF TREATMENT ON MDA
% Benefit of MDA
30%
25%
20%
15%
10%
5%
0%
-5%
-10%
-15%
-20%
-25%
26.90%
26.90%
18.30%
-6.30%
RoutineTreatment
Routine+VitC
Routine+ALA
Routine+VitC+ALA

Table 28 comparison of total_ anitioxidant in m.mol/l

Groups	On admission		On discharge		Student's Paired t-test
	Mean	SD	Mean	SD	
Normal	1.67	.16	1.67	.16	t=0.00 P=1.00 Not Significant
RoutineTreatment	.97	.15	.99	.16	=1.90 P=0.07 Not Significant
RoutineTreatment+VitC	1.36	.30	1.55	.30	t=9.30 P=0.001 Significant
RoutineTreatment+ALA	1.27	.09	1.27	.09	t=0.01 P=0.98 Not Significant
RoutineTreatment+VitC+ALA	.92	.12	1.44	.47	t=4.89 P=0.001 Significant

The mean levels of total antioxidants on admission compared with the mean levels on discharge, showed decrease in levels on admission in all groups and the levels improved significantly in group III and group V cases after supplementation of vitamin C and the combination(vitamin C +ALA) respectively. Group II and group IV showed no significant improvement.

Table 29 Percentage of benefit of different method of treatment on total antioxidant

Groups	On admission	On discharge	total_ anitioxidant gain
	Mean(%)	Mean(%)	Mean(%)
Normal	1.67	1.67	1.67
RoutineTreatment	0.97(58.9%)	0.99(59.3%)	0.02(1.1%)
RoutineTreatment+VitC	1.36(81.4%)	1.55(76.0%)	0.19(11.3%)
RoutineTreatment+ALA	1.27(76.0%)	1.27(76.0%)	0.00(0.0%)
RoutineTreatment+VitC+ALA	0.92(55.1%)	1.44(86.2%)	0.52(31.1%)

The percentage of benefit of treatment on total antioxidants showed marked improvement in group III and group V cases, when compared with the routiene standard treatment group (group II) cases , group III 1.1% to 11.3%, group V 1.1% to31.1%.

Table 30 Comparison of total_ antioxidant gain

Groups	N	Mean	Std. Deviation	Oneway ANOVA F-test	Multiple comparison by Bonferroni t-test
RoutineTreatment	30	0.0240	.06911		4 Vs 3
RoutineTreatment+VitC	21	0.1943	.09569	F=20.8	1 Vs 3
RoutineTreatment+ALA	27	0.0000	.12279	P=0.001	2 Vs 3
RoutineTreatment+VitC+ALA	24	0.5217	.52307	significant	3 Vs2 ,1

The multiple comparison of each group with the normal individuals showed decrease in the levels of total antioxidants on admission and the levels increased on discharge.

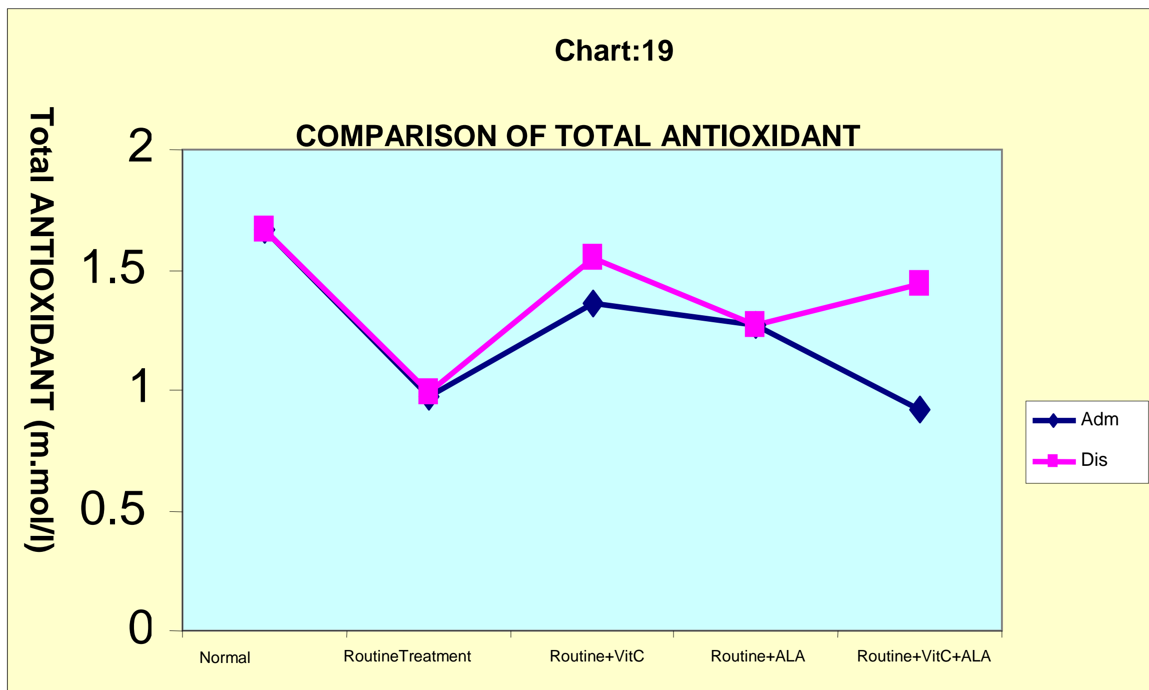


Chart:19
COMPARISON OF TOTAL ANTIOXIDANT
Total ANTIOXIDANT (m.mol/l)
2
1.5
1
0.5
0
Normal
RoutineTreatment
Routine+VitC
Routine+ALA
Routine+VitC+ALA
Adm
Dis

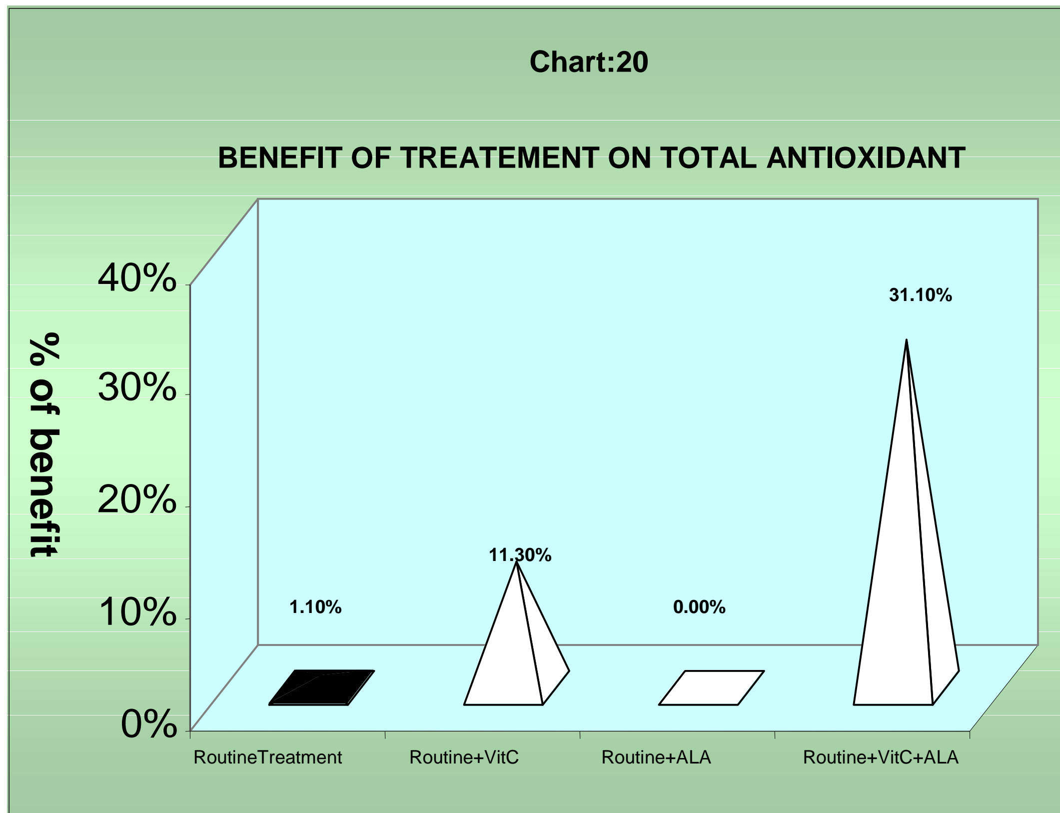


Chart:20
BENEFIT OF TREATEMENT ON TOTAL ANTIOXIDANT
% of benefit
40%
30%
20%
10%
0%
1.10%
11.30%
0.00%
31.10%
RoutineTreatment
Routine+VitC
Routine+ALA
Routine+VitC+ALA

Table 31 The Percentage effect of antioxidants in each group

S.NO	PARAMETERS	GR.III	GR.IV	GR.V
1	RBC cholinesterase	12.8	12.7	26.9
2	Plasma cholinesterase	22.8	33.3	40.3
3	SOD	2.7	4.0	37.0
4	Catalase	10.0	28.0	79.0
5	Glutathione peroxidase	8.1	0.9	11.4
6	Glutathione transferase	2.9	4.9	13.5
7	Serum glutathione reductase	8.8	5.6	36.4
8	RBC glutathione reductase	11.8	10.7	46.2
9	MDA	26.9	18.3	6.3
10	Total antioxidant	11.3	0.0	31.1

The percentage effect of vitamin C (Gr.III), Alphalipoic acid(Gr IV) and in combination (Gr V) of acute amitriptyline poisoning cases is shown.

Table 32 Age distribution

Groups	MALE		FEMALE	
	Mean	**SD**	**Mean**	**SD**
Normal	30.92	8.46	33.56	16.87
RoutineTreatment	34.13	9.54	30.00	6.53
RoutineTreatment+VitC	31.85	10.96	30.93	12.40
RoutineTreatment+ALA	33.00	11.41	29.40	11.25
RoutineTreatment+VitC+ALA	32.67	11.87	34.75	15.62

Out of 132 acute amitriptyline poisoning cases studied, age distribution of male and female showed almost similar pattern and the mean average age in all groups for male was 33 and female was 32.

Table 33 Sex distribution

Group	MALE	FEMALE	Total
Normal	16	14	30
RoutineTreatment	18	12	30
RoutineTreatment+VitC	12	9	21
RoutineTreatment+ALA	13	14	27
RoutineTreatment+VitC+ALA	14	10	24
TOTAL	73	59	132

Out of 132 acute amitriptyline poisoning cases studied, male outnumbered the female, males 73(55.3%) against females 59(44.7%). This distribution shows that the amitriptyline poisoning is seen more in males.

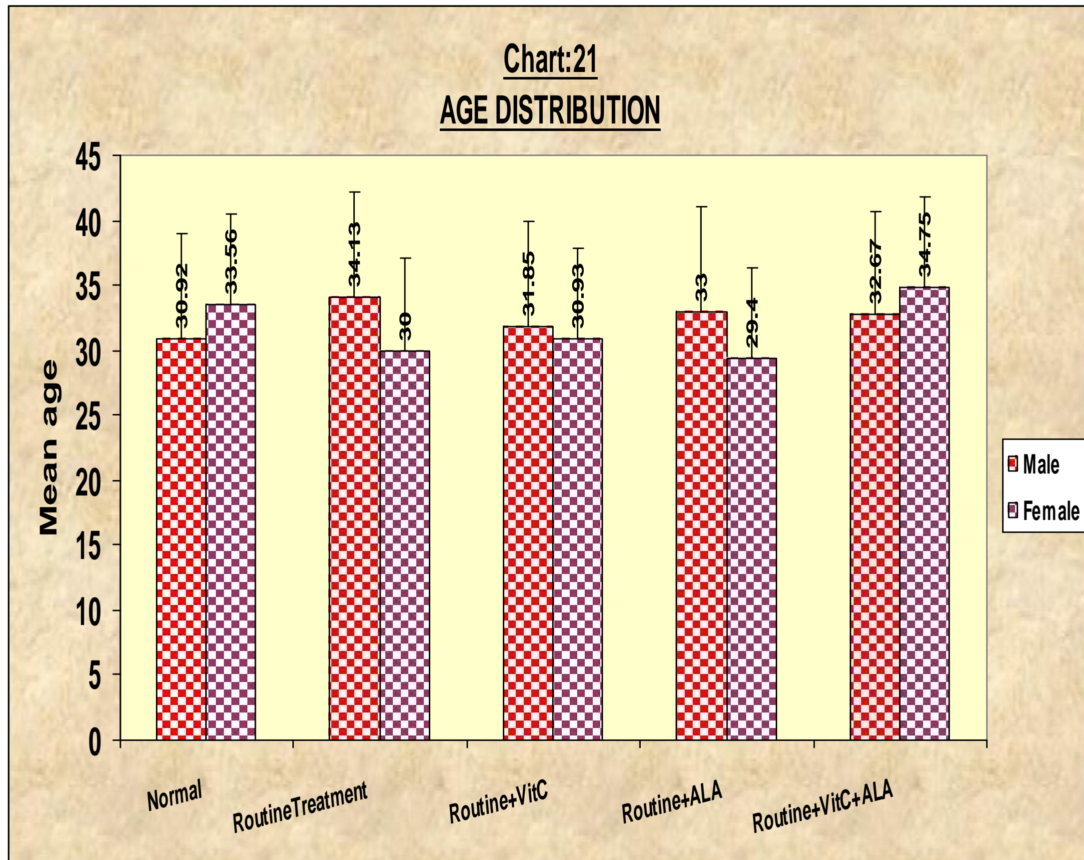


Chart:21
AGE DISTRIBUTION
Mean age
45
40
35
30
25
20
15
10
5
0
30.92
33.56
34.13
30
31.85
30.93
33
29.4
32.67
34.75
Normal
RoutineTreatment
Routine+VitC
Routine+ALA
Routine+VitC+ALA
Male
Female

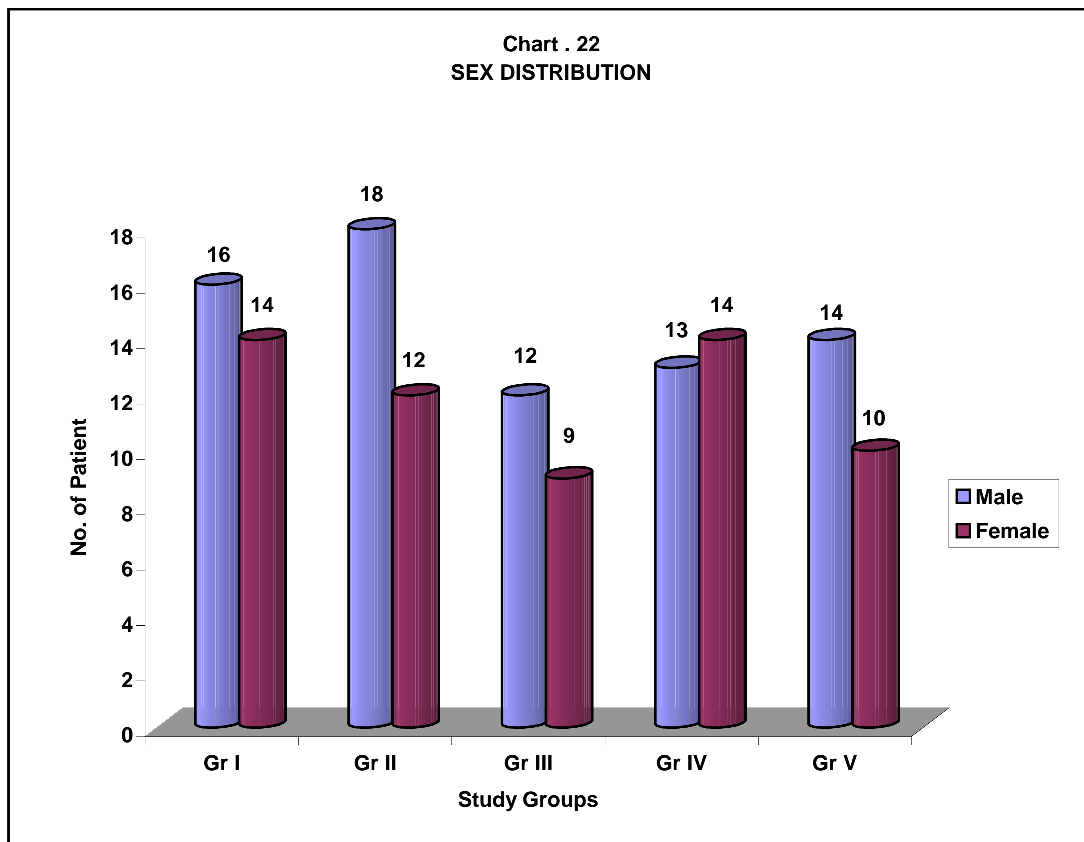


Chart . 22
SEX DISTRIBUTION
No. of Patient
Study Groups
Gr I
Gr II
Gr III
Gr IV
Gr V
Male
Female
0
2
4
6
8
10
12
14
16
18
16
14
18
12
12
9
13
14
14
10

Identification of amitriptyline overdoses in gastric aspirate in all cases admitted directily to intensive medical care unit(IMCU) and toxicology ward,Government General Hospital between September 2005 and march 2008 are carried out by thin layer chromatography(TLC). The samples along with controls are run simultaneously and based on their rf values in the TLC chromotogram(fig 9),the amitriptyline overdoses are detected and confirmed in all the groups.

The mean levels of RBC cholinesterase on admission compared with mean levels of RBC cholinesterase on discharge in all groups, showed significantly higher values on discharge(table 1, P=0.001). Though the levels of RBC cholinesterase showed increase at the time of discharge it was not upto the level of normal individuals. In table 2 the percentage of benefit of treatment on supplementation with vitamin C and alpha lipoic acidshowed a slight increase in group III(12.8%) and group IV(12.7%),when compared with groupII(10.5%) without supplementation. But marked increase was observed in group V(26.9%) cases after supplementation with vitamin C and alpha lipoic acid in combination. The multiple comparison of each group with the routiene standard treatment(RST) groupII, showed significant increase in values in all groups(table.3)

The mean levels of plasma cholinesterase on admission compared with mean levels of plasma cholinesterase on discharge in all groups, showed significant higher values (table.4). The percentage of benefit of treatment before and after supplementation with vitamin C showed a significant decrease in group IIII ie 22.8%, when compared with group II(26.1%) in table .5. The group V showed marked improvement (ie 40.3%), when compared with the group II(ie 26.1%). Eventhough the values of plasma cholinesterase increased on discharge it was not upto the levels of normal individuals(group I).

Multiple comparison with normal individuals showed (table.6) significant decrease in values for all the groups. The results also showed that the regeneration or reactivation of plasma cholinesterase was more rapid than RBC cholinesterase(chart 1,chart.3).

The base levels of both RBC and plasma cholinesterase remained higher in all groups(table 1,4 chart 1,3).

The mean values of SOD on admission compared with mean values of SOD on discharge, showed significant lower values in group II and group V.(table 7,chart 5). The level of SOD was more decreased in group V ie with the combined supplementation of antioxidants. The percentage of benefit of treatment in SOD after supplementation with vitamin C (group III) and alpha lipoic acid (group IV) did not show much improvement(table 8,chart 6). In our study we observed that though the levels of SOD on discharge were almost equal when compared with the values on admission, it remained higher when compared with the normal individuals (table. 8). Multiple comparisons of SOD in all groups with normal individuals showed significant decrease values (table .9).

The mean levels of catalase on admission compared with the mean levels of catalase on discharge, showed significant change in group III, group IV, group V, but in group II no significant change was noticed. There was a significant decrease noticed in group III, group IV, group V after supplementation of vitamin C, alpha lipoic acid and both, together respectively(table 10, chart 7). The percentage of benefit of treatment before and after supplementation of antioxidants showed gradual improvement in group III, group IV, group V. (Table 11, chart 8). In the above groups, group Vcases showed maximum improvement (ie 79%). Multiple comparison of each groups with the normal individuals (Table 12) showed significant higher values in all groups with or without supplementation of antioxidants.

The mean levels of serum glutathione reductase in all groups on admission compared with the levels on discharge, showed significant decrease in values in all groups except in group IV cases.(table 13, chart 9). In group IV cases we observed that the values remained almost the same on admission(group IV=10.69) as well as on discharge(group IV =10.29, table 13, chart 9). At the same time the group V cases showed marked decrease(ie from 10.85% to 9.16%). In the same way the percentage of benefit on serum glutathione reductase showed significant decrease in group V(groupV=36.4%, table 14, chart 10). Multiple comparison of each groups with the normal individuals showed increase in values on admission and on discharge except in group V cases which showed reverse trend (table 15).

The mean levels of glutathione peroxidase on admission was compared with mean levels on discharge showed decrease in levels for all groups (table 16, chart 11). In fact the values for group IV cases showed no significant. But the decrease in the levels of glutathione peroxidase was significant in group III And group V cases on supplementation with antioxidants. The percentage of benefit of treatment for glutathione peroxidase showed not much improvement for group IV cases(table 17, chart 12). Whereas group III and group V cases showed marked improvement(ie 8.1% and 11.4% respectively). Multiple comparison of glutathione peroxidase with group II showed increased values for group III and group V and decreased values for group IV (table 18) cases.

The mean levels of glutathione transferase on admission was compared with the levels on discharge showed significant decrease in values for all groups(table 19, chart 13). In group II cases we noticed the values were very high on admission when compared to all other groups(ie 6.16%, table 19, chart 13). The percentage of benefit of treatment for glutathione transferase showed no improvement for group III and group IV cases (table 20.chart 14).

But group V cases showed marked improvement(ie 13.5%). Multiple comparison of each group with the normal showed significant increase in values for all groups (table 21) on admission and on discharge.

The mean levels of MDA in all groups on admission compared with mean levels on discharge, showed increase in MDA levels on admission and the increase continued till discharge in all groups except in group V cases.(table 22, chart 15). In group V cases though the levels at the time of discharge decreased, the decrease was not significant(ie 2.10% to 2.01%). The percentage of benefit of treatment on MDA showed improvement in group V cases and the levels of MDA decreased on combined supplementation with antioxidants in this group(table 23. chart 16,ie 2.10% to 2.01 %). Multiple comparison of MDA in each group with normal individuals showed increase values both on admission and on discharge (table 24).

The mean levels of total antioxidants on admission compared with the mean levels on discharge, showed decrease in levels on admission in all groups and the levels improved significantly in group III and group V cases after supplementation of vitamin C and the combination (vitamin C +ALA) respectively(table 25, chart 17). Group II and group IV showed no significant improvement. The percentage of benefit of treatment on total antioxidants showed marked improvement in group III and group V cases, when compared with the routiene standard treatment group (group II) cases (table 26, chart 18, group III 1.1% to 11.3%, group V 1.1% to31.1%). The multiple comparison of each group with the normal individuals showed decrease in the levels of total antioxidants on admission and the levels increased on discharge (table 27).

The mean values of RBC glutathione reductase in all groups on admission compared with the values on discharge, showed increase in RBC glutathione reductase activity on admission and on discharge when compared with the normal individuals(table 28, chart 19). In group III and group IV cases

the values increased on admission and continued to increase till discharge (ie 2.30% to 2.53% and 2.31 to 2.52% respectively), but in case of group II and group V, the values increased on admission and decreased on discharge(ie 3.24% to 2.88% and 2.95 to 2.05% respectively). The percentage of benefit on RBC glutathione reductase showed marked improvement in all groups but the effect was maximum in group V cases when compared to group II cases(ie 16.7% to 46.2%,table 29, chart 20). Multiple comparison of each groups with the normal individuals (groupI) showed increase in levels of RBC glutathione reductase on admission and on discharge(table 30).

In our study we observed that the effect of supplementation of vitamin C was higher in group III and the effect of supplementation of the combination (ie vitamin C +ALA) was maximum in group V (table 31).

RESULTS FOR ISOENZYME

The LDH showed a elevation of Total LDH activity in Gr.A and Gr.B (Table 34) acute amitriptyline poisoning cases .This was in accordance with the earlier studies. These samples were analysed for isoenzymes.

Gr.A and Gr.B showed significant bands in LDH_1 region (Fig. 8,9).

The results of CK isoenzyme showed, elevation of 100% CK-MM fraction for Group A, & B of acute amitriptyline poisoning cases. (Table 35; Fig. 10,11).

Table 34 Results for LDH in ~/l

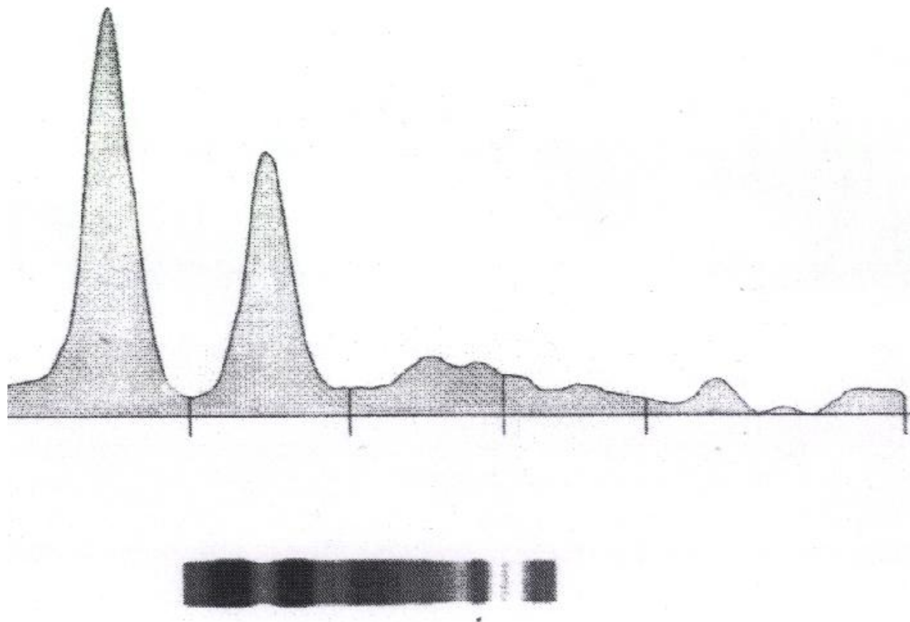
Sl.No.	Gr.A	Gr.B
1.	510	642
2.	605	596
3.	584	512
4.	526	585
5.	661	616
6.	508	554
7.	632	596
8.	610	636
9.	607	588
10.	613	585

Table 35 Results for CPK in µ/l

Sl.No.	Gr.A	Gr.B
1.	396	384
2.	284	310
3.	405	426
4.	494	426
5.	505	584
6.	356	610
7.	410	436
8.	477	296
9.	402	429
10.	408	423

GROUP A- ACUTE AMITRIPTYLINE POISONING CASES

ISO - LDH ENZYME ELECTROPHORESIS



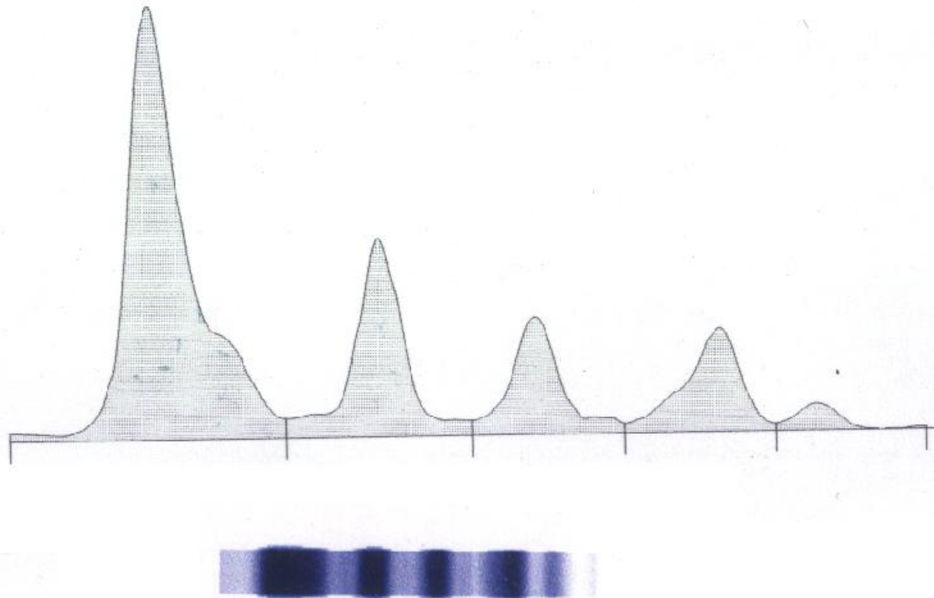
Total LDH - 610 u/l

Fractions	Conc.	Percent	Ref. Range %
LDH - 1	316.0	51.8	16.0 - 31.0
LDH - 2	153.7	25.2	29.0 - 42.0
LDH - 3	73.2	12.0	17.0 - 26.0
LDH - 4	46.4	7.6	6.0 - 12.0
LDH - 5	20.7	3.4	3.0 - 17.0

Fig.8

GROUP B -ACUTE AMITRIPTYLINE POISONING CASES

ISO - LDH ELECTROPHORESIS



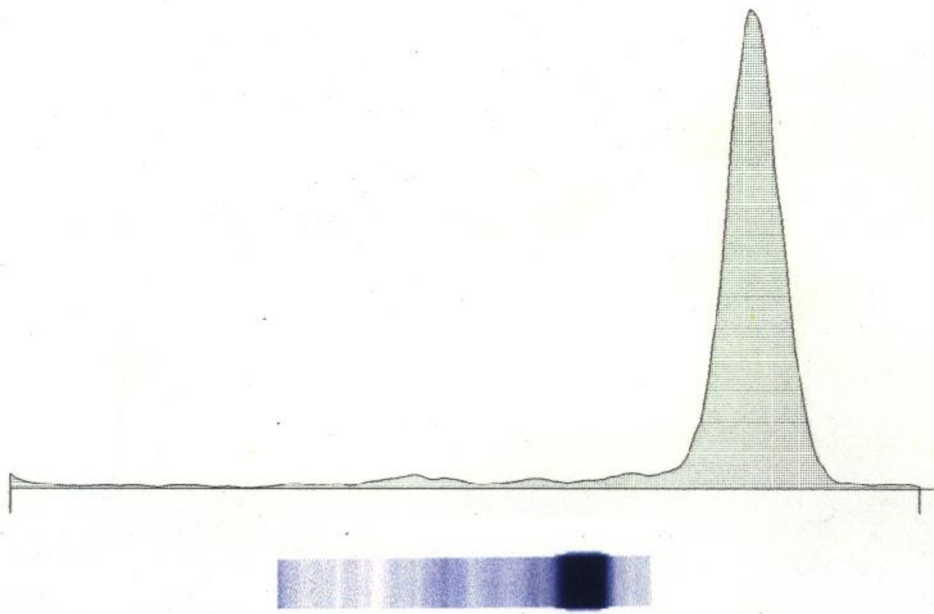
Total LDH - 588 u/l

Fractions	Conc.	Percent	Ref. Range %
LDH - 1	314.7	53.8	16.0 - 31.0
LDH - 2	118.17	20.2	29.0 - 42.0
LDH - 3	70.2	12.0	17.0 - 26.0
LDH - 4	67.8	11.6	6.0 - 12.0
LDH - 5	14.04	2.4	3.0 - 17.0

Fig.9

GROUP A- ACUTE AMITRIPTYLINE POISONING CASES

CK ISO ENZYME ELECTROPHORESIS



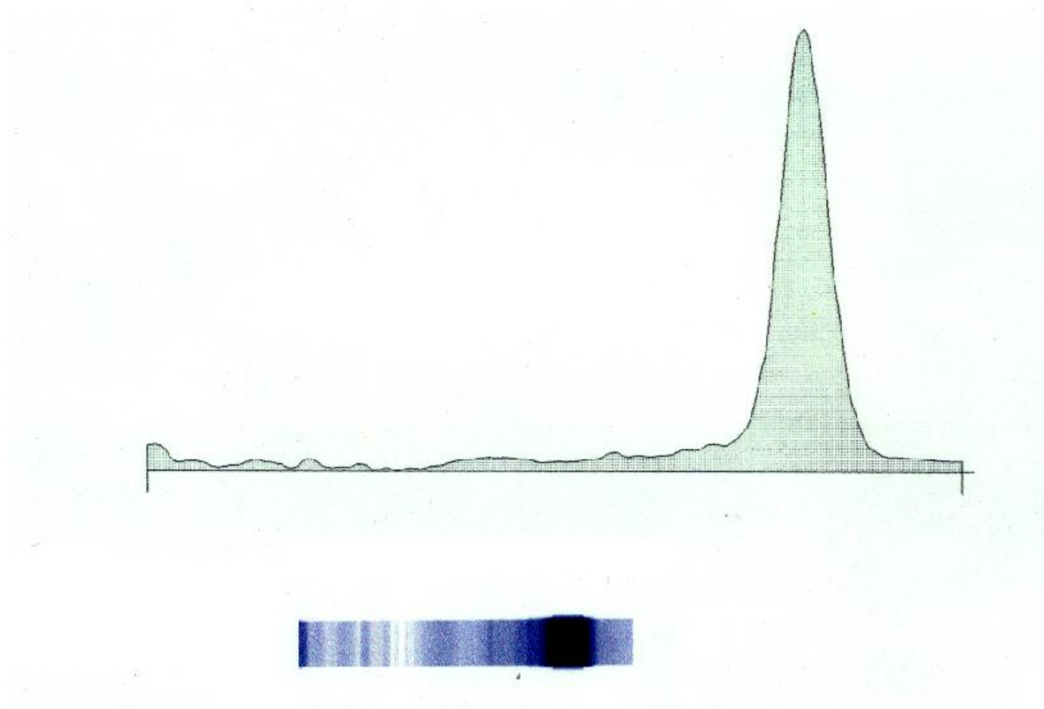
Total CK - 405 u/l

Fractions	Conc.	Percent
CK - MM	405	100

Fig.10

GROUP B -ACUTE AMITRIPTYLINE POISONING CASES

ISO - CK ELECTROPHORESIS



Total CK - 426 u/l

Fractions	Conc.	Percent
CK - MM	426	100

Fig.11

DISCUSSION

The main objective of the present study was to determine the efficacy of Vitamin C and alphalipoicacid for acute AP cases. The presence of increased oxidative stress was noticed in this study. We choose to supplement vitamin c and Alpha lipoic acid for all AP patients. Antioxidants fall into 2 classes of preventive antioxidants, which reduce the rate of chain initiation, (e.g) catalase and other peroxidases; Chain breaking antioxidants which interfere with chain propragation (eg) Vitamin C, Vitamin E. This interaction between Vitamin C and Vitamin E radicals takes place not only in homogenous solutions but also in liposomal membranes systems where Vitamin C and Vitamin E reside separately outside and within the membranes respectively, and Vitamin C and act as a synergist[176] Vitamin E and Vitamin C react rapidly with oxygen free radicals, and are widely accepted antioxidants compounds for their biological activity.[177,178]

Alpha lipoic acid in addition to its own antioxidant properties, also recycles the Vitamin C and Vitamin E there by enhancing their availability in both aquous phase (cytosol) and lipid phase (cell membrane). Also Alpha lipoic acid dissolves both in lipid and water and it can also effectively act as antioxidants.

RBC and Plasma cholinestrase showed significant improvement in all groups. (Table 1, 2, 3, 4, 5; Chart 1, 2, 3, 4). The effect was maximum in Group V (Table 31). The mechanism of Amitriptyline with acetylcholinesterase (AchE) indicates that amitriptyline with a moiety an hydroxyl group on serine in the active (esteratic) site of the enzyme, thus impeding its action on the physiological substrate.

Hence the increase in the RBC and plasma cholinesterase may be due the effect of treatment of oximes, in all groups of Acute AP cases.The supplement of Vitamin c and alpha lipoic acid may not have direct effect in the

improvement in RBC and plama cholinestrase enzymes, it would be interesting to further study whether the reduction in oxidative stress and increase in total antioxidant status of acute AP patients have any influence in reactivation or new synthesis of RBC and plasma cholinestrase.

Our results for SOD showed increased levels at baseline (at time of admission) for all groups of acute AP cases (Table 7 Chart 5).

On supplementation with Vitamin c and alpha lipoic acid we observed significant decrease in SOD for all groups except group IV (Table 8 Chart 6). The maximum reduction in SOD was noticed in Group V.

The increased SOD levels on admission indicate elevated antioxidant status and more oxidative stress in all groups of Acute AP cases. On supplement of alpha lipoic acid decrease in RBC SOD is noticed indicating the reduction in oxidative stress.

The Cu-Zn SOD is usually considered to be a noninducible enzyme. The levels of the protein and mRNA in mammalian tissue remain constant in the face of a number of potentially inducing conditions, including oxidative stress[179] and cytokines such as tumor necrosis factor-Alpha (TNF-Alpha) and interleukin I (IL-I). Some reports however, demonstrate an induction of the enzyme by oxidative stress in endothelial cells[180] and Chinese hamster fibroblasts.[181] This modulation, however is relatively small in comparison to the changes in expression of the Mn-SOD. The greatest changes in activity seem to arise during development and differentiation.[182] Other evidence suggests that the human enzyme is regulated at the post-transcriptional level by a balance of translation and degradation of the two transcripts.[183] this study on SOD for acute AP cases indicates the decrease of oxidative stress on supplement with Vitamin c and alpha lipoic acid and also we observed significant raise in total antioxidant levels of these cases. The induction and expression of SOD enzyme depends on various factor, like toxicity, oxidative stress, detailed and specific study is required with regard to specific cytoplasmic Cu-Zn SOD and mitochondrial Mn-SOD.

We observed significant decrease in catalase enzyme activity after supplementation of Vitamin c and Alpha-lipoic acid in all groups of acute AP patients (Table 10; Chart 7). Mammalian Catalase is a heme-containing protein found in peroxisomes, which are most abundant in liver cells and renal proximal tubules. Catalase detoxifies hydrogen peroxide by converting it to water and oxygen. Studies in the past have shown that the catalase activity is inducible in the lung by hyperoxia and in the liver by caloric restriction or some drugs.[184] Hypolipidemic drugs such as clofibrate increases the number of peroxisomes in the liver there by increasing total catalase activity.[185] The main function of catalase is to detoxify endogenously generated reactive oxygen species in peroxisomes. Hence the reduction in catalase activity in all groups of acute AP cases after supplementation of Vitamin c and alpha lipoic acid may be either due to decreased activity of Reactive Oxygen Species particularly in peroxisomes of liver, and renal proximal tubles or decreased pulmonary activity, which is a prominent clinical symptoms for acute AP cases, the exact mechanism as to how Vitamin c and alpha lipoic acid supplementation reduces the ROS activity in peroxisomes and change in pulmonary status of acute AP cases is not known.

The results for glutathione activity (GST, GPx, SrGSH, RBC-GSH) showed decrease in activity before and after supplementation of Vitamin c nd alpha lipoic acid. Even without supplementation of Vitamin c and alpha lipoic acid there was reduction in the activity of Glutathione in all groups of acute AP cases (Table 13, 17, 20, 24; Chart 9, 11, 13, 15). Marked decrease in glutathione activity was noticed in Group V acute AP cases aftersupplementation of Vitamin c and alpha lipoic acid (Chart 10, 12, 14, 16). Glutathione (GSH) is a tripeptide containing L-glutamate, L-cysteine, and glycine, is the predominant non protein thiol in all mammalian cells, and is found at concentrations of 1-5nM.

The reaction of GSH that are important for cellulardefense against oxidative stress can thereby be divided into functions of GSH on a nucleophile and as a reductant. Glutathione conjugates many Xenobiotics and endogenous chemicals and there by effectively participates in detoxifying process. Also glutathione S-transferase which in the kidney are a family of isoenzymes that are believed to be localized exclusively in the cytosol catalyse the formation of thio ether conjugates of GSH, with a vast number of electrophilic compounds. Although the tissue with the highest activity of GST is the liver, where the isoenzymes comprise upto 5% of total cytosolic protein, the kidneys also contain significant amounts of the enzyme. In general, GSH-S-conjugates are converted by additional renal enzymes to mercapturic acids, (ie N-acetyl cysteine conjugates), which are highly polar and are excreted readily into the urine there by completing a detoxification process. The reduction of glutathione activity in this study proves the reduction of Amitriptyline toxicity in all groups and the maximum effect was reflected in Group V AP cases, after supplementation of Vitamin c and alpha lipoic acid, however several other oxidised terms of glutathione are present in cells at relatively low concentrations like GSOH-sulfenic acid, G-SO-S-R-. This sulfinate. GSO_2^- - sulfinic acid. G-SO-R-sulfoxide G-S-CO-R-thiolester also play a critical role in detoxicifing process.

In this study glutathione peroxidase enzyme showed significance in Group III and Group V cases (Table 13). No significant changes in Group I and Group IV cases were observed, decrease in GPx for Group II and Group III was minimum [Gr. II 116.8% to 109.2%; Gr.III 100.9% to 92.5%] (Table 14; Chart 10) when compared to catalase [Gr. II 171% to 167%; Gr. III 159% to 149%] (Table 11; Chart 8). In humans H_2O_2 is detoxified by catalase and GPx. Although both remove the same substrate, only GPx can effectively remove organic hydroperoxides, being the major source of protective against low levels of oxidative stress.[186]

In vitro studies shows a significant increase in GPx activity, but no change in catalase activity was observed in cells transfected with a copper-Zinc superoxide dismutase expression vector.[187] Alteration of endogenous glutathione peroxidase, maganese superoxide dismutase and glutathione transferase activity in cells transfected with a copper-zinc super oxide dismutase expression vector. Explanation for variations in paraquat resistance[188] in mice with age related oxidative stress. Melatonin reduces oxidative stress in erythrocytes and plasma of senescence-accelerated mice[189] and in other models of oxidative stress.[190] Also mitochondrial respiratory chain deficiency leads to over expression of antioxidant enzymes.[191] These literature suggest that the GPx but not catalase would be modulated by increased reactive species formation in various models of oxidative stress, and this could explain our results.

The increased GPx activity observed in Gr.II and Gr.V (Table 13; Chart 9) cases was due to increased oxidative stress. This stress can increase the rate of GPx, mRNA transcription leading to increased GPx activity. With the progression and severity of the toxicity we would expect our increased oxidative stress that could lead to an inhibition of GPx. Hence we observed decreased GPx activity in Gr.IV cases. In fact the same type of Biphoric response of GPx activity was previously reported after oxidative stress induced by hyperbasic oxygen.[192] Time-dependent course of hyper basic oxygen-induced oxidative effects in rat lung and erythrocytes.[193] and due to inflammation during period and wound healing.[194]

We observed that the lipid peroxidation (LPO) activity was high in all groups of acute AP cases (Table 25; Chart 17), when compared to normal individual indicating oxidative damage. Even after supplementation of Vitamin c and alpha lipoic acid the MDA levels remained high, except for Group V cases (Table 26; Chart 18). These results suggests that there is considerable oxidative damage occurring in all acute AP cases, our results are in agreement

with earlier study which reported increased LPO activity in acute AP cases. It has been established in animals as well as humans that serum concentrations of lipid peroxidation products (LPPs) increase in pulmonary inflamation.[195] The clinical manifestation of acute AP cases were pulmonary odema, pulmonary infarction, hence the increased MDA may be due to pulmonary disorder or manifestations of acute AP cases.

The results for total antioxidants showed significant increase in Group III and Group V acute AP cases (Table 28, 29; Chart 19, 20) these group received the maximum supplementation of alpha lipoic acid. The increase in total antioxidant levels in these patients is a good sign as these antioxidants will neutralise the ROS, and there by reduce the oxidative stress. The exact mechanism for increase in total antioxidants after supplementation of Vitamin C and alpha lipoic acid is unclear, further study is required to investigate whether Vitamin C and alpha lipoic acid enhances, or recycles the endogenous antioxidants or the increase in total antioxidant levels were due to supplementation of Vitamin C and alpha lipoic acid. As the total antioxidant levels significantly improve this will improve the recovery of patients, and also will reduce long term side effects such as Amitriptyline induced delayed polyneuropathy (AIDP).

The data of this study suggests that the effect of supplementation of Vitamin C and alpha lipoic acid were maximum in Group V acute AP cases (Table 31), there was significant improvement in the levels of RBC and plasma cholinestrase, similarly the SOD, catalase and GPx and transferase showed significant lower values, indicating the reduction of oxidative stress, total antioxidants levels showed a maximum elevation in the same group and also the LPO levels was minimum in this group. Though each case of acute AP poisoning is unique, the onset, severity and duration of poisoning, are determined by the dose, rate of exposure, physical and chemical properties of the amitriptyline compound. In this study Group V cases showed maximum

effect, the reasons probably would be the combination of antioxidant and stay of these patients in IMCU and poison centre was maximum (5-13 days). Hence they received the symptomatic treatment more than other groups, and also the supplementation of Vitamin C and alpha lipoic acid were maximum in this group. Oral supplementation of alpha lipoic Acid for longer period will enhance the total antioxidant levels, and decrease the oxidative stress. It would be interesting to study the supplement of Vitamin C and alpha lipoic acid to chronic Amitriptyline poison cases. Also we suggest that I.V. administration of Vitamin c and alpha lipoic acid would be more beneficial to acute AP cases.

LDH is widely distributed in mammalian tissues, being rich in myocardium, kidney and liver. Since the LDH_1 fraction of isoenzyme is elevated for Gr.A and Gr.B (Fig.8,9) cases it indicates the tissue damage from myocardium, pulmonary or even from renal cells as there is no significant raise in LDH_5 region indicating the absence of hepatic cell involvement.

To evaluate for myocardial Infarction, the "Flipped LDH" pattern is useful. No such clear "flipped LDH pattern" were noticed in the Gr.A and Gr.B (Fig. 8,9) acute amitriptyline poisoning cases. Also to diagnosis for myocardial infarction along with the LDH we have to correlate with aspartrate transaminase (AST) values. Elevated AST values with persistent longer levels of LDH (10 to 14 days) suggests myocardial infarction. In this study we observed elevated LDH for 3 to 4 days maximum and LDH returning to normal after 4 days, also not very high AST value were noticed in Gr.A and Gr.B Acute amitriptyline poisoning cases.

Literature for LDH enzymes indicates that, patients with pulmonary infarction have elevated levels of LDH levels usually within 24 hours of the onset of pain. The pattern of normal AST and elevated LDH levels within one to two days after an episode of chest pain provides suggestive evidence for pulmonary infarction also the sign and symptoms for acute amitriptyline

poisoning cases suggests the presence of pulmonary edema and pulmonary infarction.

If the release of LDH enzyme is from renal cells, the elevation of LDH should continue till the entire toxicity is excreted, which may take few days. But in this study for Gr.A and Gr.B cases we observed the increase in LDH for 3 to 4 days. In Gr.A and Gr. B drug toxicity detoxification takes place in liver, and detoxified drug compounds will reach the kidney and may not induce much damage to renal cells. Based on all the observation we arrive at a conclusion that the increased fraction of LDH for Gr.A and Gr.B is not from Liver, myocardium, or renal tissues it may be primarily from pulmonary infarction.

In Group A,B cases we observed only CK-MM muscle fraction (Fig. 10,11) indicating the damage of skeletal muscle of leg, arm, respiratory and lung. As the total CPK was only 2 to 3 fold increase we assume that the CK isoenzymes release may be primarily from lung or respiratory skeletal muscle. If the involvement of skeletal muscle of leg and arms are involved we will observe manifold increase in total CPK as seen in person doing exercise. Exercise is a major variable in CK-levels. There is a direct relationship between intensity of exercises in men and CK,[196] particularly with strength exercise[197]. Respiratory muscles contain more CK-MB than most muscles (3 to 7%) an increased percentage of CK-MB is often seen in those with acute respiratory exertion due to lung disease exacerbation.[197] Also increased use of respiratory muscle in those with obstructive lung diseases can cause elevation of CK[197]. In this study we observed a increase of CK-MM fraction in Group A, B cases (Fig. 10,11) indicating the involvement of respiratory & pulmonary disorders. Also acute amitriptyline poisoning patients exhibit the sign and symptoms of respiratory and pulmonary disorders, we suggest that the increase in CPK may be due to respiratory and pulmonary disorders and not from cardiac or skeletal muscle of the body.

CONCLUSION

Antioxidants levels were increased during treatment after supplementation with vitamin C and alpha lipoic acid. we suggest that antioxidant status of acute amitriptyline poisoning cases should be considered for more effective recovery and that diets low in antioxidants may render slow in recovery.

This study provides quantitative recommemdations for the intake of vitamin C and alpha lipoic acid in fast recovery and in increasing the antioxidant status of glutathione.

This study shows that by oral supplementation with Vitamin c and alpha lipoic acid to acute Amitriptyline poisoning cases considerable oxidative stress was reduced, and it also enhances the total antioxidant levels of these patients. This will help the acute Amitriptyline poisoning patient to recover faster, and stay in intensive care will be reduced, also supplementation of vitamin C and alpha lipoic acid reduces the long term side effects such as Amitriptyline induced delayed Neuropathy (AIDN).

The effect of supplementation of vitamin C and alpha lipoic acid was maximum in Group V(Routine standard treatment+ vitamin C+ alpha lipoic acid) acute Amitriptyline poisoning cases, on these group received the maximum dosage of vitamin C and alpha lipoic acid (5-13 days). It is clear that the supplementation should be for longer period to have the maximum beneficial effect (reduced oxidative stress and increased total antioxidant status). In our opinion oral supplementation will be more effective for chronic Amitriptyline poison cases for a longer duration and for acute Amitriptyline poisoning cases I.V. form of vitamin C and alpha lipoic acid will be more effective.

The recovery and regeneration of plasma cholinesterase was rapid when compared to RBC cholinesterase in all groups of acute Amitriptyline poisoning cases.

We noticed significant decrease in catalase activity in all groups of acute Amitriptyline poisoning cases on supplementation with vitamin C and alpha lipoic acid.

We observed lower values of glutathione activity on admission and increase in values on discharge for Gr. V (Routine standard treatment+ vitamin C+ alpha lipoic acid) acute Amitriptyline poisoning cases.

On the above discussion it could be noticed incidently that the elevated LDH and CPK in Group A(on admission randomily selected 10 cases for LDH and CPK study) and Group B(on admission randomily selected 10 cases for LDH and CPK study) which may be due to pulmonary infarction not due to cardiac or skeletal muscle involvement.

The average age of acute Amitriptyline poisoning cases were 33 for male and 32 for female, and this study shows that the Amitriptyline poisoning poisoning is seen more in male [55.3%] than female [44.7%].

SUMMARY

Psychotropic drug poisoning is an important cause of mortality and morbidity in many countries. It has been estimated that there are 3 million cases of severe poisoning occur in developing countries.

Amitriptyline is the most common suicidal poison with high morbidity and mortality and account for a large proportion of patients admitted to intensive care units. It is noted that acute amitriptyline poisoning accounted for 15-20% out of total admission to the medical intensive care units of Government Hospitals. Though routine standard treatment (RST) is being given to all acute amitriplyline poisoning (AP) cases, the exact mechanism of toxicity still remain elusive.

Studies on acute amitriptylene poisoning patients indicate that besides their inhibiting effect on acetylcholinesterase, acute amitriptyline poisoning may induce oxidative stress in humans.

The main objective of this study was to find out the antioxidant status, enzyme variations in acute amitriptyline poisoning cases.

Vitamin C or ascorbic acid is a monosaccharide antioxidant found in both animals and plants. As it cannot be synthesized in humans and must be obtained from the diet, it is a vitamin.[9] It is a water -soluble antioxidant that protects organic and biological molecules against oxidative degradation. It also works along with glutathione peroxidase (a major free radical - fighting enzyme) to revitalize alpha lipoic acid a fat-soluble antioxidant. Due to its versatile properties it is decided to supplement to acute amitriptyline poisoning cases.

Alpha lipoic acid initially classified as a vitamin possessed potent antioxidant properties. It dissolves in both water and fat and thus claimed to be

an "Universal antioxidant" it can reach tissues composed of fat such as the nervous systems as well as those made mainly of water such as heart. Alpha lipoic acid is seen to be very effective in various neurological disorders like Parkinson's diseases, Alzhmeir's diseases. Since acute amitriptyline poisoning causes considerable neurotoxicity, we have dicided to supplement alpha lipoic acid to acute amitriptyline poisoning cases and study its effect before and after supplementation.

During the course of this study, observed elevated LDH and CPK activity in all acute amitriptyline poisoning cases, we were interested in knowing the exact fraction of isoenzyme elevation so as to find out the maximum tissue or organ damage in acute amitriptyline poisoning cases.

A total of 132 acute amitriptyline cases were selected for study from IMCU and poison centre, Government General Hospital between September 2004 to March 2008, informed consent was obtained from the attendants of the patients. This study was approved by the ethical committee of Madras Medical College, Chennai - 3.

The severity of poisoning was graded according to the method of Edinburgh Scale. Venous blood was drawn from each experimental subjects, antioxidant levels, enzymes and isoenzyme patterns of CPK and LDH were carried out for patients. 50ml of gastric aspirate was collected from all patients and this is taken for identification of amitriptyline drugs by TLC.

Blood investigations were carried out by standard procedures by semi autoanalyzer, spectrophotometer, chromatographic techniques and semiautomated electrophoresis system.

Statistical evaluation was carried out using SPSS (Version 14.0). Data obtained from the study groups were compared with variable by student `t' test, standard deviation, mean, ANOVA and percentage of benefit with and without

supplementation of Vitamin C, alpha lipoic acid and in combination. Multiple comparison of each group with normal and without antixoidants was carried out using Bonferroni t-test. Standard tables and charts are depicted whenever required.

Resutls were disucssed in detail for all parameters with the literatrue avaialble and observations were recorded.

Based on the study the conclusions are arrived as follows.

1. Supplementation of Vitamin C, alpha lipoic acid and their combination decrease the oxidative stress and increase total antioxidant status of acute amitriptyline poisoning cases.

2. The effect of supplementation of Vitamin C and alpha lipoic acid combination was maximum in Group V acute amitriptyline poisoning cases. The logner supplementation of antioxidant combination will help the recovery of acute amitriptyline poisoning cases and also to reduce their long term side effects such as amitriptyline induced delayed neuropathy (AIDN). I.V. form of supplementation will be more beneficial for acute amitriptyline poisoning cases, also supplemntation of these antioxidants to chronic amitriptyline poisoning cases will reduce their long term side effects.

3. The recovery and regeneration of plasma cholinesterase was more rapid than RBC cholinesterase.

4. Significant decrease in catalase activity was noticed in all groups of acute amitriptyline poisoning cases on supplementation with antioxidants.

5. The study shows only lower values of RBC Glutathione reductase activity on admission and increase in values on discharge for Group III and Group IV acute amitriptyline poison cases.

6. The average age of acute amitriptyline poisoning cases was 33 for male and 32 for female, and this study shows that the amitriptyline poisoning is seen more in male (55.3%) than female (44.7%).

7. The isoenzyme studies show that the increased LDH in Gr.A (ten cases were selected randomly among the groups only on admission for LDH and CPK study) and Gr.B (ten cases were selected randomly among the groups only on admission for LDH and CPK study) cases may be due to pulmonary infarction and may be moderately due to hepatic cell damage.

8. The increase of CK in acute amitriptyline poisoning cases in Gr.A and Gr.B are primarily due to pulmonary infarction not due to cardiac or skeletal muscle involvement.

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