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Effect of Anti-Oxidants on Endocrine and Metabolic Parameters of Diabetic Rats

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United Arab Emirates University
Deanship of Graduate Studies

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B.Sc in Biological Sciences
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A Thesis
Submitted to
United Arab Emirates University
In Partial Fulfilment of the Requirements for the
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The Thesis of Mariam Surour Al-Shamsi for the Degree of Master of Science in Environmental is approved.

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It is said "Never settle for less than your dream. Some where, some time, some day, some how, you will find them". Thank you Allah by the names you are known and called. It is only by your grace, I have made it this far.

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ABSTRACT

Abstract

Diabetes mellitus is recognized as one of the leading causes of morbidity and mortality in the world. About 5 to 6% of the world population suffers from this disease and the number of people diagnosed with diabetes is rapidly increasing. Diabetes has been demonstrated to be associated with oxidative stress and hyperglycemia, one of the most important indictors of oxidative stress. Normally the endogenous mechanisms of enzymes and antioxidants are able to destroy the reactive species and create a balance between antioxidant and free radicals. In diabetes, the oxidative stress is increased due to the deficiency in the antioxidant defense. The intake of antioxidants such as vitamin E and C may reduce the oxidative stress associated with diabetes and hence help to restore the antioxidant defense system.

The aim of this study is to investigate the effect of different doses of either vitamin E or C on the metabolic and biochemical parameters of normal and streptozotocin (STZ)-induced diabetic rats. Biochemical analysis, immunohistochemistry, and radioimmunoassay techniques were used to study the effect of these vitamins on the metabolic and biochemical parameters of normal and diabetic rats. The result of this study revealed that the oral administration of vitamin E and C significantly reduced the body weight gain in a dose-correlated manner. Moreover, vitamin E and C significantly ($p<0.0001$) reduced the blood glucose level in normal ($p<0.0008$) and in diabetic ($p<0.0007$) rats. The oral administration of these vitamins significantly enhanced glucose tolerance in normal and diabetic rats. Both vitamin E and C significantly affect the biochemical parameters of both normal and diabetic rats. Both vitamin E and C have an effect on liver function, for example the low dose of vitamin E significantly ($p<0.012$) increased the plasma gamma-glutamyl level in normal rats while the moderate dose of vitamin C significantly ($p<0.0008$) reduced plasma gamma-glutamyl level in diabetic rats.

Moreover, the kidney function of normal and diabetic rats was affected after the oral administration of vitamin E and C. High dose of vitamin C significantly ($p<0.01$) increased the blood urea nitrogen level of diabetic rats, while low dose of vitamin E significantly increased ($p<0.03$) blood urea nitrogen of normal rats. The plasma level of electrolytes such as calcium and sodium also changed significantly ($p<0.00001$) after oral administration of either vitamin E or C.

In an immunohistochemical study of pancreas on the number and distribution of insulin and glucagon cells, a significant increase in the number of insulin positive cells was observed in rats treated with vitamin E and C after the onset of diabetes when compared to control. However, the number was still significantly less than that obtained for control normal rats.

However, both vitamin E and C fail to increase the insulin level of normal and diabetic rats. The level of plasma glucagon increased after the onset of diabetes mellitus and both of vitamin E and C significantly reduced its level in diabetic rats when compared to control.

In conclusion antioxidants such as vitamin E and C may ameliorate the metabolic and biochemical parameters of diabetic rats.

INTRODUCTION

1. Diabetes Mellitus

1.1 History of diabetes mellitus

Diabetes mellitus is a disease which has a very old history. The clinical features of diabetes mellitus that we see today were observed 3000 years ago by the ancient Egyptians. Araetus of Coppodocia described the term diabetes in (81-133 AD) (1). Diabetes is derived from the Greek word "diabaniein" which means to pass through and this mean the large quantity of water taken orally due to 'polydipsia' has passed through the body in the same way 'polyuria' (2). After that, Thomas Willis in 1675 added the word mellitus which mean "honey sweet" after rediscovering the sweetness of urine and blood of the patients. Later on, Claude Bernard discovered the role of the liver in glycogen and that diabetes resulted from the excess of glucose production while the role of pancreas in diabetes was discovered by Mering and Minkowski in 1889 (1). Insulin was eventually discovered by Banting and Best in 1922 (3).

1.2 Epidemiology

Diabetes mellitus, recently, is taking its place as one at the main threats to human health in the 21st century. In the past two decades the number of people with diabetes has increased worldwide. This increase in the incidence and prevalence of diabetes mellitus is due to behavioral and lifestyle changes in the past two decades (4).

Diabetes mellitus affects approximately 6% of the world population. India has the greatest number of diabetic patients, followed by China which has 17 million diabetic patients and then USA which has 15 million in the year 2000 (2). In the year 2000, the total number of people with diabetes mellitus is 151 million and the number is projected to increase by 46% to reach 221 million by the year 2010 and 300 million in 2025 (4). By the year 2025, diabetes will increase by more than 193% in both the Middle East and India and this means that more than 50 million people will have diabetes in each of these regions (2).

1.3 Definition

Diabetes mellitus (DM) is a heterogeneous group of disorders characterized by high blood glucose level (5). DM is associated with abnormal changes in protein, carbohydrate and fat metabolism (6) and induce disturbances in lipid profiles especially, an increased susceptibility to lipid peroxidation (7). These are the result of insufficient insulin action (6).

1.4 Classification

Diabetes mellitus is classified into two main types (8). Recently, the terms insulin dependent diabetes mellitus and non insulin dependent diabetes mellitus were dropped and American Diabetes Association (ADA) and WHO proposed new classification for diabetes based on its etiology. Diabetes mellitus is now classified into four categories type 1, type 2, diabetes due to other specific mechanisms or condition, and gestational diabetes (9).

1.4.1 Type 1 diabetes mellitus

Type 1 diabetes was formally referred to as insulin dependent diabetes or juvenile onset diabetes (10). It is an older disorder, it was found in the ancient Egyptian and Greek writing. Type 1 diabetes affects about 0.5% of the population in developed countries. Type 1 diabetes mellitus is an autoimmune disease resulting from a specific destruction of ß-cell of the islets of Langerhans of the pancreas (11). Recently, the ß-cell destruction has been suggested to be due to islet cell autoantibodies to insulin, glutamic acid decarboxylase and tyrosine phosphatase (12).

Type 1 DM is the most common endocrine disorder among children and young adults and it may be caused by infections (enteroviruses), genetic disorder, and/ or inappropriate diet (13).

Type 1 diabetes has two different phases. The first phase is the insulitis phase. In this phase the islet has leukocytes which infiltrate the islets. The second phase is called the diabetes phase. In this phase, the ß-cell of the islets has been destroyed, and it is unable to produce sufficient insulin, which control glucose levels, resulting in hyperglycemia (11).

1.4.2 Type 2 diabetes mellitus

Type 2 diabetes is the most common form of diabetes. It affects more than 90% of the diagnosed cases (14). It is a heterogeneous syndrome characterized by abnormalities in the metabolism of fat and carbohydrate. Type 2 DM is caused by a multi-factorial mechanism including both genetic and environment factors that affect ß-cell function and tissue insulin sensitivity. Several adipocytokines such as leptin, TNF-alpha, resistin and adiponectin play a role in insulin resistance and possible ß-cell dysfunction (15). The onset of type 2 diabetes begins in middle age (16). It has different subtypes and each one is characterized by different degrees of insulin resistance and ß-cell dysfunction (5).

1.4.3 Gestational diabetes mellitus

Gestational diabetes (GDM) is a carbohydrate intolerance resulting in hyperglycemia during pregnancy. GDM develops in 0.15 to 15% of all pregnant women. It is characterized by preeclampsia in pregnant women and macrosomia and birth trauma in the fetus. According to the latest WHO recommendation, GDM is diagnosed by performing the universal standard 75 g OGTT and evaluating blood glucose level after 2 h (17).

1.5 Complications of diabetes mellitus

Diabetes mellitus is a chronic disease associated with severe late complications. It affects the metabolism of carbohydrates, protein, fat and electrolytes which cause changes in the structure of the vascular system (2). Recently, studies suggest that protein kinase C activation may play an important role in the development of diabetes complication, and the use of its inhibitors can reduce these complications. It is reported that an inhibitor of protein kinase C was able to reduce renal and retinal dysfunction in diabetic animals (18). The end product of the non enzymatic glycation induces cytokines in vascular cells which play role in the development of diabetic complication (19).

Furthermore, oxidative stress is derived from glucose autooxidation, advanced glycation and mitochondrial dysfunction may damage endothelial cell function (20). Hyperglycemia induces the production of superoxide by mitochondria, leading to the formation of a strong oxidant proxy nitrite, which damage DNA. The damage of the DNA activates the nuclear enzyme poly (ADP-ribose) polymerase, which depletes the intracellular concentration of NAD (+), thus slowing glycolysis, ATP formation, and ADP ribosylation of GAPDH. All of these processes contribute to the development of late degenerative complications (21).

Both types of diabetes mellitus are characterized by chronic hyperglycemia which leads to the development of specific microvascular and macrovascular disorders (22). The most common chronic complications of diabetes include retinopathy, nephropathy, neuropathy and atherosclerosis (23). Diabetic nephropathy causes thickening of the glomerular capillary and tubular basement membranes. DM also causes expansion of the mesangial matrix (24).

Diabetic retinopathy is a leading cause of blindness. Other severe complications of diabetes include cardiovascular illness, which is the major cause of morbidity and mortality, and encompasses macrovascular disease with heart attacks, strokes, and gangrene and hardening of the skin and cataract formation (25).

1.6 Management and treatment of diabetes mellitus

Currently, the treatment of type 2 diabetes mellitus involves diet modification, weight reduction, exercise; insulin and oral medications (26). Diet and exercise therapy may be beneficial in the treatment and prevention of type 2 DM. Physical exercise increases the utilization of blood glucose level and decreases its level. It should not be considered as a lone therapy, it should be combined with dietary therapy and medications (27). The diet treatment is the basic therapy of type 2 diabetes. It plays an important role in preventing diabetes from developing, and it is effective in the management of DM. Diet treatment

should include a balanced combination of proteins, carbohydrates, lipid and vegetables (28).

Patients with type 1 diabetes depend on insulin so the usual treatment is by subcutaneous injection of insulin (29). There are many kinds of insulin preparations, such as rapid acting insulin like insulin lisper (homolog) and insulin aspart (novo log), and these insulin products are rapidly absorbed. Insulin lisper is different from human insulin by the exchange of the amino acid lysine and proline at position 28 and 29 while substitution of aspartic acid for proline at position 28 created insulin aspart. This type of insulin is injected at meal time or used in insulin pump. Short acting insulin is a regular insulin with onset of action between 30 to 60 min. Moreover, regular insulin acts almost immediately after intravenous injection. Intermediate insulin, such as neutral protamine hagedorn, is absorbed slowly because of the addition of protamine to regular insulin. Lente insulin which is regular insulin bound to zinc and has longer effective duration than NPH. Long acting insulin such as ultra lente insulin (insulin zinc extended) is absorbed slowly in its zinc crystalline form (30).

Currently, several alternative methods for insulin delivery, instead of injection, have been developed to eliminate pain and disruption of lifestyle, which are associated with insulin injection. Intrapulmonary insulin (inhaled insulin) is an alternative to regular insulin injection. It is good in controlling glucose levels and it had no adverse bronchopulmonary effects (31).

Type 2 DM therapy relies mainly in agents purposed to reduce hyperglycemia (32). Five classes of agents currently exist. Sulfonylureas which lower plasma glucose by acting on the beta cell to stimulate insulin secretion (33). Sulfonylureas bind to ATP-sensitive potassium channels and inhibit its efflux and this lead to efflux of calcium through the voltage-dependent calcium channels. The high intracytosolic calcium concentration resulted in the release of insulin (34). Meglitinides agents are non sulfonylureas which target different sites on beta cells, leading to a similar cascade of events increasing insulin release (35).

Biguanides such as metformin decrease hepatic glucose production by reducing gluconeogenesis. It is not a hypoglycemic agent because it does not increase insulin secretion (36). Thiazolidine enhances glucose uptake and utilization in peripheral tissues, mainly skeletal muscle tissue (34).

Alpha-glycosidase inhibitors such as carobs and miglitol act by delaying carbohydrate absorption in the small intestine (36).

2. Oxidative stress

Oxidative stress refers to the state in which cells are exposed to excessive levels of molecular reactive species (38). It is also defined as a disturbance in the balance between the production of the reactive species and antioxidant defence (39).

These reactive species are generated as a result of metabolic reaction in the form of free radicals or non free radicals and they could be oxygen or nitrogen derived. They are called peroxidants (40).

The free radical molecule which is formed from oxygen is called reactive oxygen species (ROS) such as superoxide and hydroxyl and it is also referred to oxygen derived non radicals like hydrogen peroxide. On the other hand, the free radical molecule which is formed from nitrogen is called reactive nitrogen species (RNS) like nitric oxide and nitrogen dioxide (41).

Most biological molecules contain paired electrons in their outer orbit but in the case of the free radicals the molecule contains an unpair electron in the outer orbit and this results in very reactive molecules which can gain or lose electron. ROS and RNS function as immunological host defence. They are generated by macrophages and neutrophilis to kill microbes and destroy other foreign matter. Nitric oxide plays an important role in neurotransmission and regulation of blood pressure. Moreover, several cytokines, growth factors,

hormones and neurotransmitters induce rapid production of reactive species to act as signalling molecules in many transduction pathways (42).

Some ROS play an important cellular messenger role in the regulation of apoptosis, transcription factor activation, kinase activation and gene expression at low concentration (43). Highly reactive molecules which are called free radicals react with any molecule which they come in contact with causing a kind of chemical havoc. They can cause damage to important biological molecules, like protein, carbohydrates, lipids and DNA (44).

3. Diabetes mellitus and Oxidative stress

Diabetes mellitus can be caused by oxidative stress. The sources of the reactive species in diabetes include free radical reactions related to glycation of proteins, consumption of NADPH through the polyol pathway, glucose autoxidation, hyperglycemia-induced pseudo hypoxia and activation of protein kinase C (45).

In the normal condition, endogenous mechanisms, enzymes and antioxidant molecules are able to destroy reactive molecular species and reduce the harmful effect of oxidants (46) but in diabetes the hyperglycemia and possible free fatty acid induce the reactive molecular species and oxidative stress which play role in causing insulin resistance and ß cell dysfunction. Reactive oxygen and nitrogen species may also play a role in the pathogenesis

of the late diabetes complication because they have the ability to oxidize and damage DNA, protein and lipid (38).

4. Antioxidants

An antioxidant is defined as any substance that when present at low concentration compared to those of an oxidisable substrate, significantly delays or prevents oxidation (47). Antioxidant can be produced endogenously or provided from exogenous sources such as vitamins C and E (40). Fruits and vegetables are good sources of antioxidants (48).

Actually endogenous antioxidants include enzymes like superoxidase dismutase. superoxidase dismutase is metalloenzymes, which catalyzes the dismustation of superoxide radicals into H_2O_2 and oxygen. Catalase catalyzes the decomposition of hydrogen peroxide into molecular oxygen and water. They are located in peroxisomes and in the cytosol (49). The antioxidant glutathione peroxidase is an enzyme with much greater affinity for hydrogen peroxide than catalase, and it is located in both mitochondria and the cytosol where it serves as a cellular protectant against free radical induced damage to membrane lipids, protein and nucleic acid (50).

Melatonin is another example of endogenous antioxidant. It can neutralize oxygen and nitrogen reactive species, acts as indirect antioxidant by stimulating the synthesis of glutathione and it can preserve the functional integrity of superoxide dismutase and catalase (51).

Also, the endogenous antioxidant system includes some minerals such as selenium, copper and zinc. These trace elements are linked together in cytosolic defense against free radicals. Copper and zinc ions stimulate protective cellular stress signaling pathway. Selenium exists in the cell as selenocysteine and selenomethionine which catalyzes the reduction of peroxynitrite at the expense of glutathione (52). Some compounds which also act as antioxidant include flavonoids, bilirubin and uric acid (40).

5. Vitamin E (α-tocopherol)

In 1920, Evans and co-workers described a dietary substance, which is important in rat's reproduction and they called it vitamin E. It is naturally found in oils from vegetable and plants. Synthetic vitamin E can be made from the reaction of trimethylhydroquinone with isophytol (53).

Vitamin E is defined as a lipid soluble, chain breaking radical scavenger (54). It is a highly viscous oil, insoluble in water and rapidly oxidized by atmospheric oxygen (Fig 1). These properties of α-tocopherol limit its therapeutic application. Because of its high instability to oxidation, the acetate and acid succinate esters of the vitamin are commonly used for clinical uses (43). It is also characterized by low molecular weight and lipid solubility, which help it to scavenge reactive species in lipid laden compartments like cell membrane (55).

Vitamin E has the ability to scavenge a wide spectrum of free radicals including singlet oxygen, superoxide and hydroxyl radicals (56). Also, it is believed that vitamin E can act as membrane stabilizer by forming complexes with the products of membrane lipid hydrolysis such as lysophospholipids and free fatty acids (57).

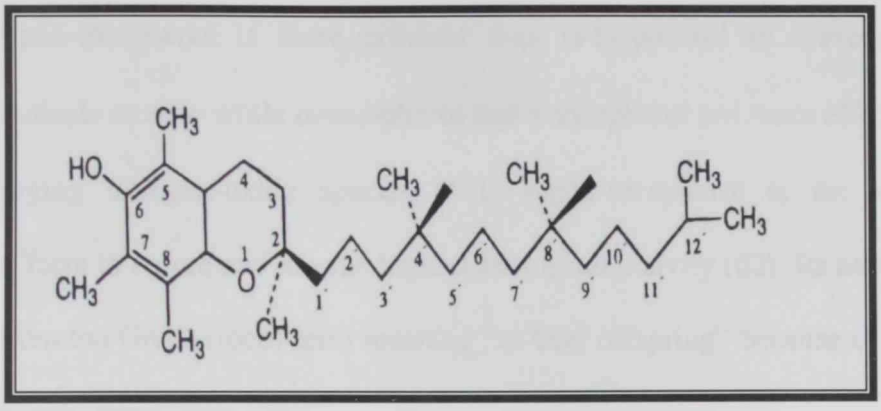

Figure 1: Chemical structure of α-tocopherol. (58).

Vitamin E dose not function as an antioxidant only, it has a great benefit against several disorders such as atherosclerosis, ischemic heart disease and tumors (59), because it acts as a transcriptional regulator for gene expression via a transcription factor TAP (60). It also down regulates protein kinase-C activity, thus decreasing smooth muscle cell proliferation (57)

Vitamin E occurs naturally in four isomers (α, ß, γ and δ) of both tocopherols and tocotrienols (61). The difference between tocopherols and tocotrienols resides in the phytyl-chain saturation. Tocopherols are saturated, while tocotrienols are unsaturated but they both scavenge lipid peroxyl radicals before reacting with other lipids, thereby ending the propagation of lipid peroxidation in membranes.

Alpha-tocotrienol is more efficient than α-tocopherol in scavenging peroxyl radicals *in vitro* while α-tocopherol and γ-tocopherol are more effective in scavenging nitrogen-oxide species (57). Alpha-tocopherol is the most important form in nature and has the highest biological activity (62). Its name is derived from the Greek (toco) term meaning "to bear offspring" because of it is role in reproduction of rats (63).

6- Vitamin C (Ascorbic acid)

Vitamin C is a water soluble antioxidant that was firstly isolated and characterized by Szent-Gyorgyi in 1928 (64). It is an abundant component of plants (Fig 2). In plants, it reaches a concentration of over 20 mM in chloroplast and occurs in the cell compartments including cell wall. It plays a role in photosynthesis as an enzyme cofactor and in the control of cell growth (65).

Ascorbate is synthesized by most vertebrates excluding humans, monkeys, guinea pigs, the Indian fruit bat and in some fish it is synthesized in the liver (55).

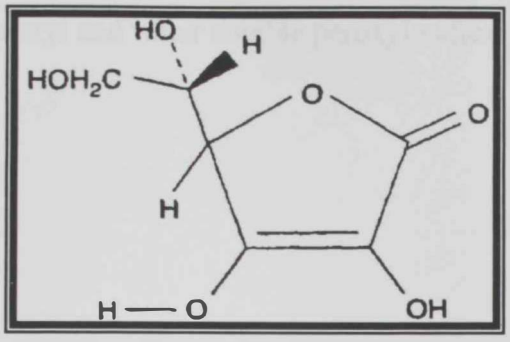

Figure 2: Chemical structure of Ascorbic acid Vitamin C (66).

Vitamin C is important for many enzymatic reactions and also acts as a free radical scavenger. Specific non overlapping transport protein mediate the transport of the oxidised form of vitamin C, dehydroascorbic acid, and the reduced form, L-ascorbic acid across biological membranes. Dehydroascorbic acid uptake across the membrane occurs via the facilitated diffusion through glucose transporters Glut 1, 3 and 4, while L-ascorbic acid enters cell via Na dependent system (SVCT 1 and SVCT 2) (67) .

The cellular uptake of vitamin C is promoted by insulin and inhibited by hyperglycemia (68). Ascorbic acid has several antioxidant properties (69). It is an essential cofactor involved in many biochemical functions, and it acts as an electron donor or reducing agent, it is said to have ascorbate oxidant activity (62).

It has greater roles in the aqueous interstitial and intracellular fluid compartments (55). Ascorbate effectively scavenge singlet oxygen, superoxide, hydroxyl and water soluble peroxyl radical, and hypochlorous acid (63).

Objectives of this study

The main objective of this study is to determine which antioxidants have anti-diabetic effects, examine the effect of antioxidant on metabolic and biochemical parameters of both normal and diabetic rats, and determine the mechanism of action of these antioxidants.

Specific aims of this study:

1. To study the hypoglycemic effect of vitamin E and C in normal and diabetic rats.
2. To investigate the effect of vitamin E and C on metabolic parameters such as body weight, plasma glucose level, and oral glucose tolerance test in normal and diabetic rats.
3. To examine the effect of vitamin E and C on biochemical parameters such as liver enzymes, kidney parameters, and plasma electrolytes level in normal and diabetic rats.
4. To investigate the effect of vitamin E and C on plasma insulin and glucagon level in normal and diabetic rats.
5. To examine the effect of vitamin E and C on the morphology of pancreatic islets of Langerhans in normal and diabetic rats.

MATERIALS AND METHODS

1. Experimental animals

Male Wistar rats aged seven to eight weeks and weighting 200-300 g were used in this study. All rats were obtained from the Faculty of Medicine and Health Sciences, United Arab Emirates University. All rats were housed in a temperature (25 °C) and humidity controlled rooms and 12 hours light and dark periods. The animals were fed on a standard rat chow and tap water *ad libitum.*

2. Induction of experimental diabetes

Diabetes was induced in the rats by a single intraperitoneal injection of streptozotocin (STZ) (Sigma, St Louis, MO) at a dose of 60 mg/kg body weight (70). The STZ was freshly dissolved in citrate buffer (0.5 M, pH4.5). The rats were considered diabetic if the fasting blood glucose level values were more than 250 mg/dl.

3. Experimental design

Rats were randomly divided into three groups according to three different doses of both vitamin E and vitamin C (low dose 0.2 mg, moderate dose 0.4 mg, and high dose 0.8 mg per animal of vitamin E) and (low dose 10 mg/kg, moderate dose 50 mg/kg and high dose 100 mg/kg of vitamin C). Rats were orally treated with either vitamin E and vitamin C by using intubation loop. Each of these groups was divided into five subgroups of six animals each:

1- Group 1 served as normal untreated group, where the rats have not been treated either with STZ or with vitamin E or C.

2- Group 2 served as diabetic control. STZ-induced diabetic rats that was not treated with either vitamin E or C.

3- Group 3 served as diabetic treated group. This group was treated with three different doses (low, moderate and high) of either vitamin E or vitamin C for ten days prior to the STZ-induction of diabetes.

4- Group 4 also served as a diabetic treated group. This group was induced with STZ to develop diabetes and it was treated with the three different doses low, moderate and high of either vitamin E or vitamin C ten days after the induction of diabetes.

5- Group 5 served as normal non diabetic treated group. This group was treated with the three different doses of either vitamin E or vitamin C only.

4. Weight measurement

The weights of normal and diabetic rats were recorded on a weekly basis using a 9001 Scale (Satorius, UK). The mean ± standard deviation of the weight for each of the experimental groups was calculated for each week of the experimental period.

5. Plasma glucose measurement

The blood glucose level was measured weekly using (One Touch II Glucometer, Life Scan, Johnson and Johnson, USA) for each individual animal of all the groups. The blood samples for glucose measurement were drawn from the tail vein, and rats were considered diabetic if their blood glucose levels exceeded 250 mg/dl (71). The mean ± standard deviation of the blood glucose for each of the experimental groups was calculated for each week of the experimental period.

6. Oral glucose tolerance test (OGTT)

At the end of the four weeks from the commencement of the treatment, rats were subjected to an oral glucose tolerance test, after an overnight fast of 18 h. On the day of the OGTT the animals were given an oral dose of glucose 10 ml/kg body weight, 30% w/v. The blood glucose measurements were made at 0, 30, 60, 120, and 180 min after glucose challenge.

7. Tissue collection and tissue processing

At the end of the experiment, normal and treated rats were anaesthetized with diether. A long abdominal incision was made and the pancreas was quickly removed, trimmed of connective tissue and fixed overnight in Zamboni's fixative (72). The tissue samples were later dehydrated in graded concentration

of ethanol. The specimens were changed every 2 hours in 70% and 95% and 3 changes in absolute ethanol for 2 hours. After dehydration the specimens were cleared in xylene and subsequently embedded in paraffin wax at 55°C. Section of 6 µm thickness were cut on a microtom (Shandon AS325, USA), and placed in water bath at 49°C. Thereafter, they were transferred onto microscopic slides, which were dried in an oven at 60°C for 30 min to enhance attachment of sections.

8. Immunohistochemistry

6 µm thickness paraffin sections were deparaffinised with xylene two times (5 min each) and then transferred into absolute ethanol two times (5 min each). After that the sections were incubated in 0.3% of hydrogen peroxide solution in methanol for 30 min to block the activity of endogenous peroxidase. The tissues were then hydrated in descending concentration of ethanol and washed 3 times in PBS (phosphate buffered saline) for 5 min each. After washing in PBS the tissues were marked around with a Dako pen to prevent solutions draining away from the tissue section. The staining procedure was started by incubating the sections with blocking reagent for 30 min. After that, the blocking reagent was drained off and appropriate dilution of the primary antibodies were applied and incubated at 4°C for 24 hr. On the following day the sections were incubated at the room temperature for 1 hr. The slides were then washed 3 times in PBS for (5 min each) and incubated with prediluted biotinylated anti-guniea pig or anti-mouse or anti-rabbit IgG (Sigma, St Louis, MO, USA) for 1 hr, then washed in PBS 3 times (5 min each) and subsequently incubated in streptavidin peroxidase conjugate (Sigma) for 1 h. After a final

wash in PBS 2 times (5 min each), the peroxidase activity was revealed by incubating the sections in 3,3-diaminobenzidine tetrahydrochloride DAB (Sigma) in PBS for (5 min). The slides were then washed for 5 min under running tap water, and counterstained with haematoxylin for 15 s and washed briefly in tap water. Then they were differentiated in acid ethanol and washed for 5 min under running tap water, then dehydrated in ascending grades of ethanol, and subsequently cleared in xylene for longer time to dissolve the Dako pen mark. The tissues were subsequently mounted in DPX. Slides were examined under microscope and immunopositive areas of the tissue section were photographed.

9. Immunofluorescenece

Isolated pancreatic tissues were retrieved, fixed and embedded in paraffin as described in section 7. Sections of about 6μm thickness were deparaffinised in xylene, hydrated in descending concentration of ethanol (3 min each) and washed 3 times in PBS for (5 min each). After washing in PBS, the tissue was marked around with a Dako pen to prevent solutions draining away from the tissue section. The staining procedure was started by incubating the sections with blocking reagent for 30 min. After that, the blocking reagent was drained off and appropriate dilution of primary antibodies were applied and incubated at 4°C for 24 h. On the following days the sections were incubated at the room temperature for 1 hr. The slides were then washed 3 times in PBS (5 min each) and incubated with secondary antibodies conjugated with either FITC or TRITC (Jackson Laboratory, USA) for 1 h and washed in PBS (3 times 5 min

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each). Sections were then mounted in CITI-Fluore mounting media, and viewed and photographed under Nikkon Fluorescence microscope.

10. Radioimmunoassay

10.1 Insulin assay

Insulin was determined by using a modified method of Herbert (73). All test samples and controls were assayed in duplicates. Insulin measurement was performed using LINCO Research, Inc (Missouri, USA) radioimmunoassay kits. The procedure was conducted as described in the kit manual. Briefly, a volume of 200 µl of either calibrator controls or test samples were pipetted to previously labeled tubes. After this, 100 µl of hydrated ^{125}I-insulin was added to all tubes. 100 µl of rat insulin antibody was added to all tubes except the total count and NSB tubes and then vortexed. After vortexing, the tubes were covered with parafilm and incubated at 4°C for 24 h. On the following day a volume of 1 ml of cold (4°C) precipitating reagent was added to all tubes except the total count tubes. Then the tubes were vortexed and incubated at 4°C for 20 min. After incubation, all tubes except the total count tube were centrifuged for 20 min at 2,000 xg at 4°C. The tubes were decanted gently and radioactivity was counted for 1 min using a gamma counter (Beckman). Results were analyzed by using Beckman Immunofit EIA/RIA analysis software, version 2.00 and values were expressed as ng/ml.

10.2 Glucagon assay

Glucagon was determined by double antibody technique of Nishio (74). All test samples and controls were assayed in duplicates. Glass tubes were used for this assay because it has been shown that glucagon adheres onto plastic surfaces. Glucagon measurement was performed using DPC® (Los Angeles, USA) radioimmunoassay kits. The procedure was conducted as described in the kit manual. Briefly, a volume of 200 µl of calibrator, control, or sample was pipetted into previously labeled tubes. After this, 100 µl of glucagon antiserum was added to all tubes except the NSB (non-specific binding) and total count tubes and vortexed. After vortexing the tubes were covered with parafilm and incubated at 4°C for 24 h. After the first incubation, 100 µl of ^{125}I-glucagon was added to all tubes and vortexed. The samples were incubated at 4 °C for 24 h. On the following day a volume of 1 ml of cold (4°C) precipitating reagent was added to all tubes except the total count and centrifuged at 4°C for 15 min at 1500 xg. The tubes were decanted gently and radioactivity was counted for 1 min using a gamma counter (Beckman). Results were analyzed by using Beckman Immunofit EIA/RIA analysis software, version 2.00 and values were expressed as pg/ml.

11. Biochemical analysis

Blood urea nitrogen (BUN), Creatinine (CRE), alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactic dehydrogenase (LDH), gamma-glutamyl transferase (GGT), calcium (Ca), phosphorus (PHOS), sodium (Na), potassium (K) and magnesium (Mg) were performed in Al-Qattara Veterinary Laboratory by using Beckman coulter (Synchron Lx20 PRO clinical system).

12. Statistical analyses

Data are expressed as mean ±SD. Student's *t*-test was used to analyze the significance of differences between mean values and different groups were analyzed by analysis of variance using Duncan's multiple range tests. A p value of less than 0.05 was considered statistically significant.

RESULTS

1. Effect of Vitamin E on metabolic parameters of normal and diabetic rats

1.1 Body weight

Figure 3 shows the effect of different doses of vitamin E on body weight of normal and diabetic rats. The oral administration of low dose (0.2 mg) of vitamin E to rats treated 10 days after the onset of diabetes resulted in weight gain compared to untreated diabetic rats, during the experimental period. However, this weight gain was still significantly smaller compared to normal untreated and normal treated rats. Also, the weight gain by normal treated rats with low dose of vitamin E was smaller compared to untreated normal rats. On the other hand, the administration of low dose of vitamin E to rats treated 10 days before the onset of diabetes resulted in high weight loss.

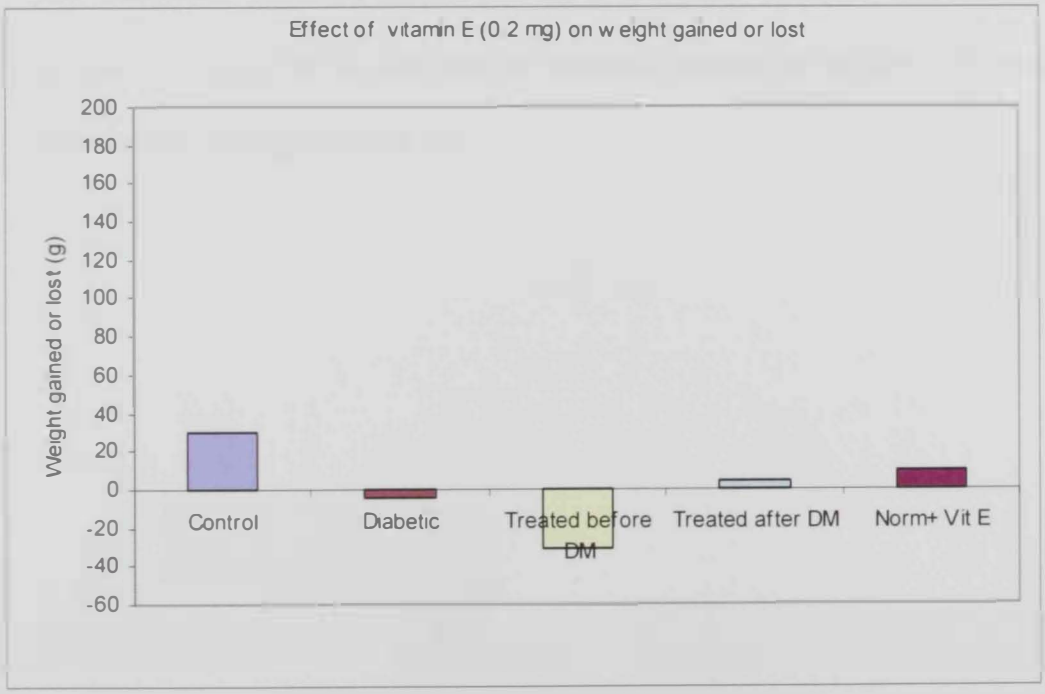

Figure 3: Histograms showing the effect of vitamin E (0.2 mg) on body weight gained or lost of normal and diabetic rats. DM= diabetes mellitus

Rat receiving moderate dose (0.4 mg) of vitamin E 10 days after the onset of diabetes were subjected to more weight loss compared to untreated diabetic rats. Moreover, the weight loss observed in rats treated 10 days before the onset of diabetes rats was the highest when compared to normal and diabetic controls. However, oral administration of (0.4mg) of vitamin E failed to increase weight of treated normal rats in the same was, as level of untreated rats (Fig 4).

The high dose (0.8mg) of vitamin E given to diabetic rats also resulted in weight loss in both diabetes rats treated before and after the onset of diabetes. Normal rats, which received high dose of vitamin E failed to gain weight in the same manner as untreated normal rats Fig 5. It is clear, however, that rats which received vitamin E before the onset of diabetes showed the highest weight loss compared to other groups Fig 3-5

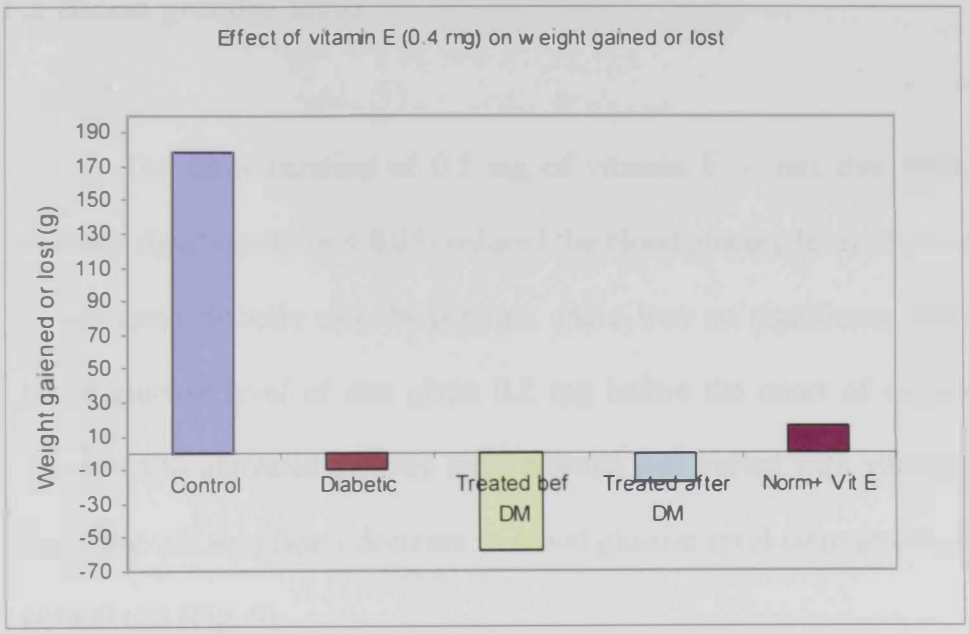

Figure 4: Histograms showing the effect of vitamin E (0.4 mg) on body weight gained or lost in normal and diabetic rats. DM= diabetes mellitus.

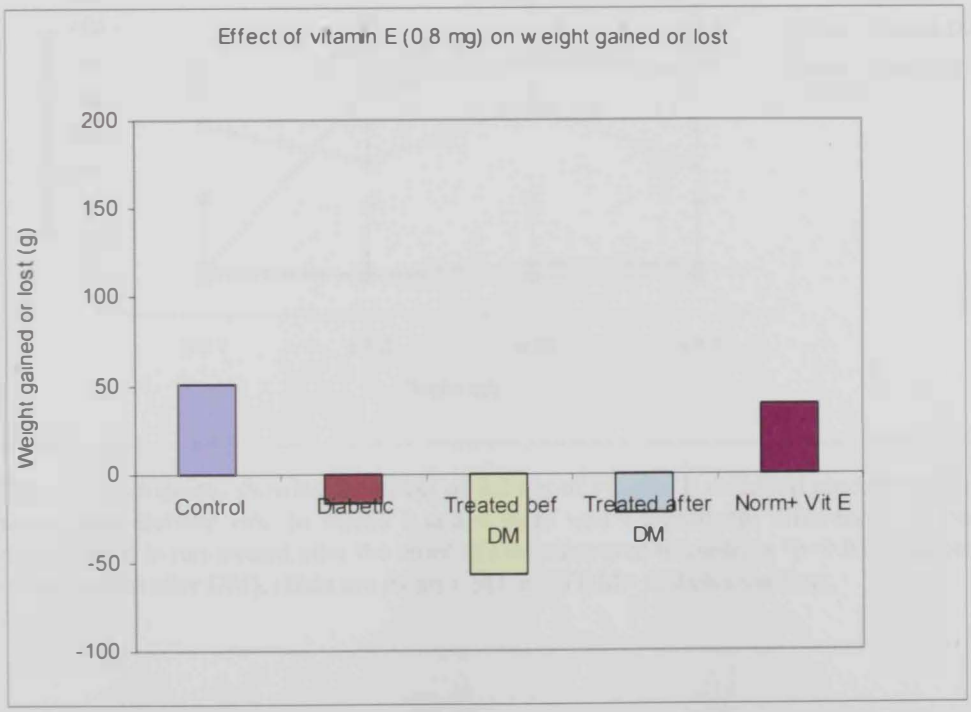

Figure 5: Histograms showing the effect of vitamin E (0.8 mg) on body weight gained or lost in normal and diabetic rats. DM= diabetes mellitus.

1.2 Blood glucose level

The administration of 0.2 mg of vitamin E to rats that were already diabetic significantly ($p < 0.05$) reduced the blood glucose level when compared to untreated diabetic rats. In contrast, there was no significant difference in blood glucose level of rats given 0.2 mg before the onset of diabetes when compared to untreated diabetic rats. Normal rats treated with vitamin E had a small but not significant decrease in blood glucose level compared to untreated normal rats (Fig. 6)

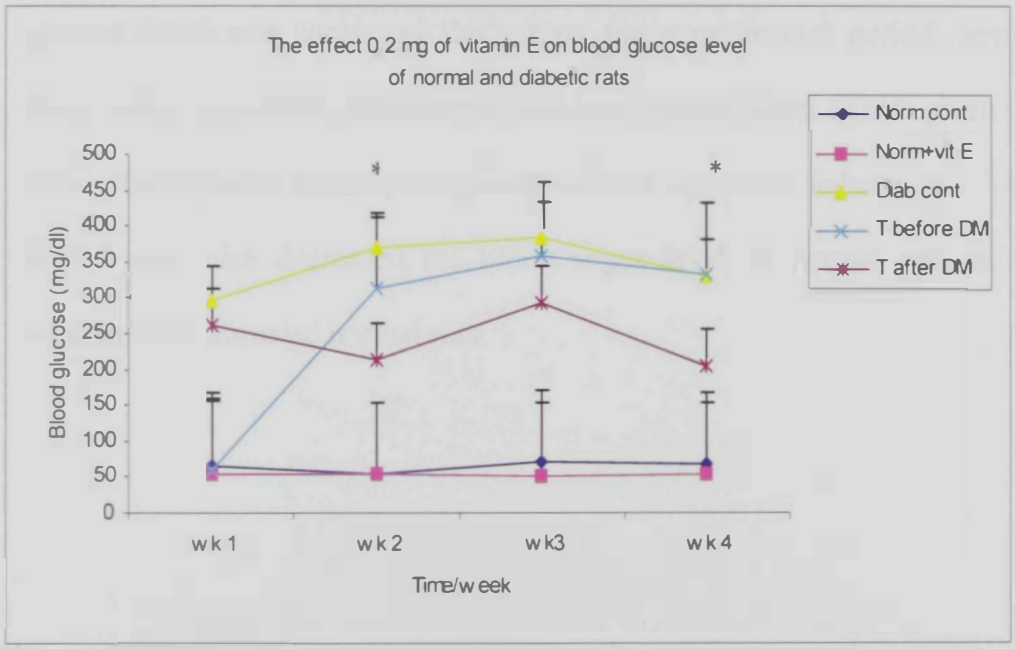

Figure 6: Histograms showing the effect of 0.2 mg of vitamin E on blood glucose level of normal and diabetic rats. In weeks 2 and 4 there was a significant difference in blood glucose level in rats treated after the onset of DM compared to controls *$p<0.05$ (diab cont versus treated after DM). (Data are mean ± SD, n=6) DM= diabetes mellitus.

The blood glucose level was slightly reduced in diabetic rats treated with a higher dose of vitamin E (0.4 mg) before and after the onset of diabetes compared to untreated diabetics. However, the reduction in blood glucose level was not statistically significant Fig 7. The blood glucose level of normal rats treated with vitamin E was comparable with that of untreated normal rats.

Figure 8 shows that the administration of 0.8 mg of vitamin E in rats treated after the onset of diabetes significantly reduced the blood glucose level when compared to untreated diabetic rats. The reduction in the level of blood glucose level was consistent throughout the experimental period. Similarly, there was a significant reduction in the blood glucose level of rats given 0.8mg before the onset of diabetes when compared to untreated diabetic rats. Vitamin E (0.8 mg) also decreased the blood sugar level of normal rats markedly compared to untreated normal rats.

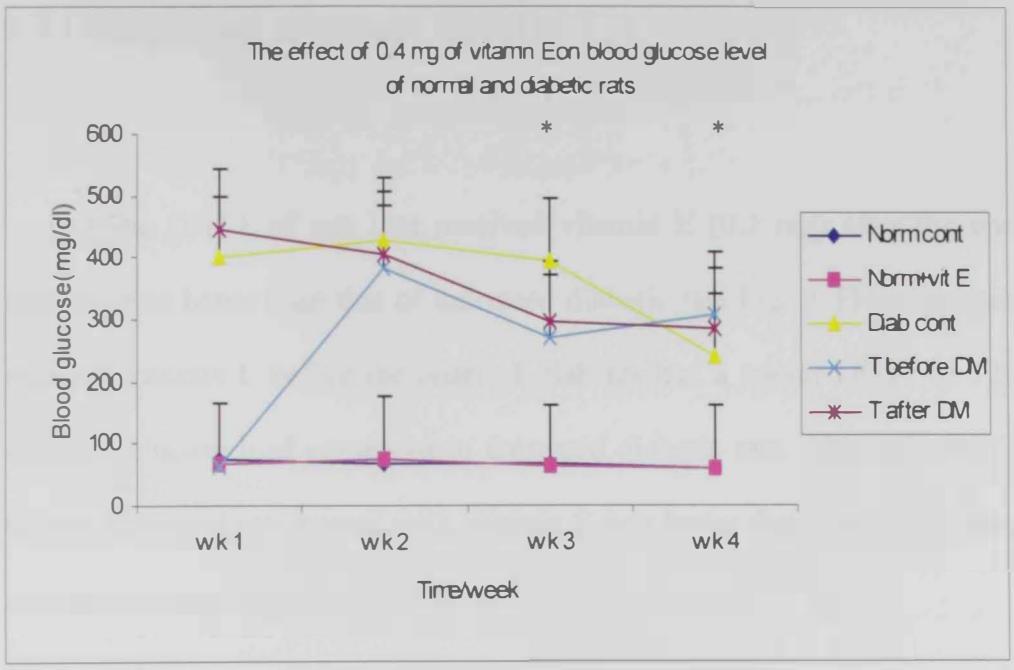

Figure 7: Histograms showing the effect of 0.4 mg of vitamin E on blood glucose level of normal and diabetic rats. In week 3 and 4 there was a significant difference in blood glucose level in rats treated before the onset of DM compared to controls *p<0.05 (diab cont versus treated before DM). (Data are mean ± SD, n=6) DM= diabetes mellitus.

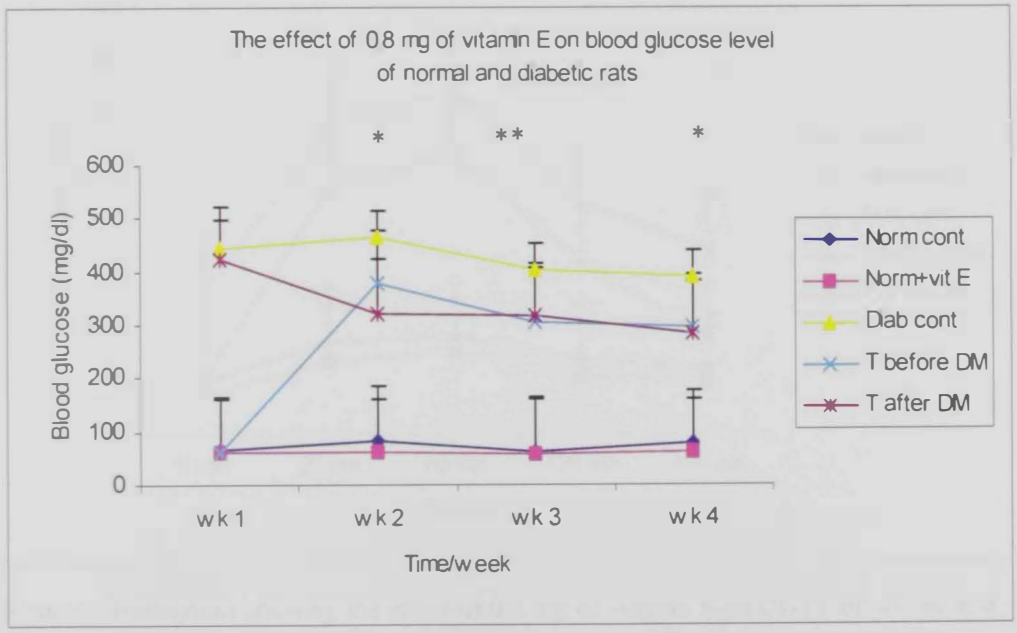

Figure 8: Histograms showing the effect of 0.8 mg of vitamin E on blood glucose level of normal and diabetic rats. In weeks 2, 3 and 4 there was a significant difference in blood glucose level in rats treated after the onset of DM compared to controls *p<0.05 (diab cont versus treated after DM) and rats treated before the onset of DM compared to controls in week 3 **p<0.05 (diab cont versus treated after DM). (Data are mean ± SD, n=6) DM= diabetes mellitus.

1.3 Oral glucose tolerance test (OGTT)

The OGTT of rats that received vitamin E (0.2 mg) after the onset of diabetes was better than that of untreated diabetic rats Fig. 9. However, rats that received vitamin E before the onset of diabetes had a poorer OGTT at 120 min after the glucose load compared to untreated diabetic rats. Although, the OGTT values of normal rats treated with vitamin E was better than that of the untreated normal rats, they were nonetheless normal.

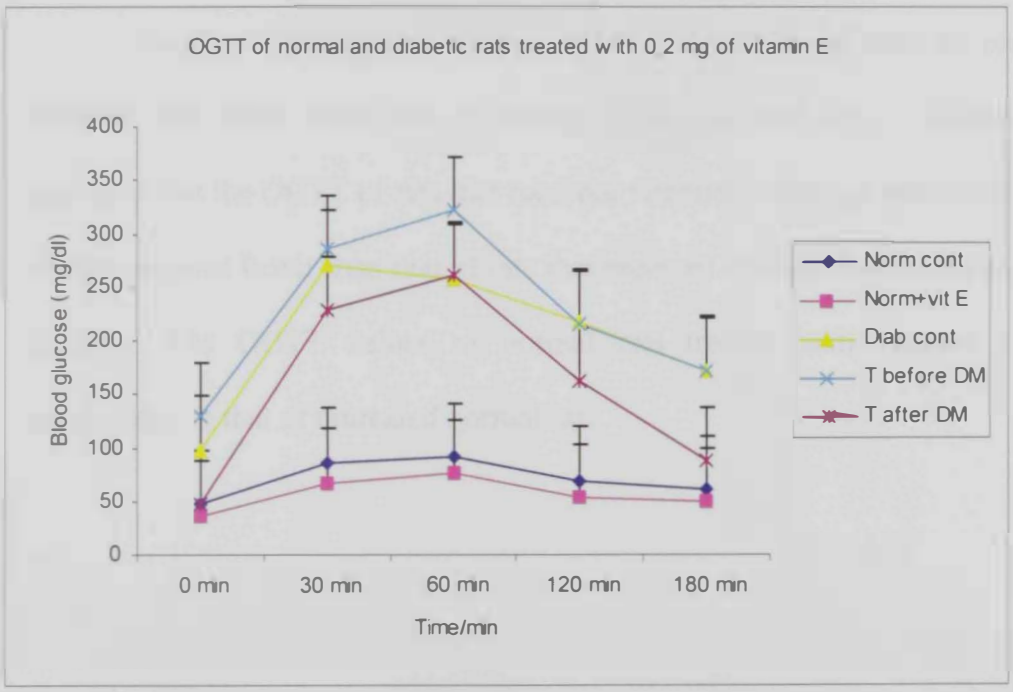

Figure 9: Histograms showing the effect of 0.2 mg of vitamin E on OGTT of normal and diabetic rats. (Data are mean ± SD. n=6) DM= diabetes mellitus.

The OGTT of rats that received vitamin E (0.4 mg) before the onset of diabetes was better than that of untreated diabetic rats Fig.10. Moreover, rats that received vitamin E after the onset of diabetes had a better OGTT in the first 60 min compared to untreated diabetic rats. The handling of glucose was poorly done in rats of this group after 60 min. However, it appeared that the OGTT of rats that received vitamin E (0.4mg) before the onset of diabetes was better than that of rats that received vitamin E after the onset of diabetes. The OGTT values of normal rats treated with vitamin E was comparable to that of untreated normal rats.

The OGTT of rats that received vitamin E before and after the onset of diabetes was better than that of untreated diabetic rats Fig.11. However, it appeared that the OGTT of rats that received vitamin E (0.8mg) before the onset of diabetes was better than that of rats that received vitamin E after the onset of diabetes. The OGTT values of normal rats treated with vitamin E was comparable to that of untreated normal rats.

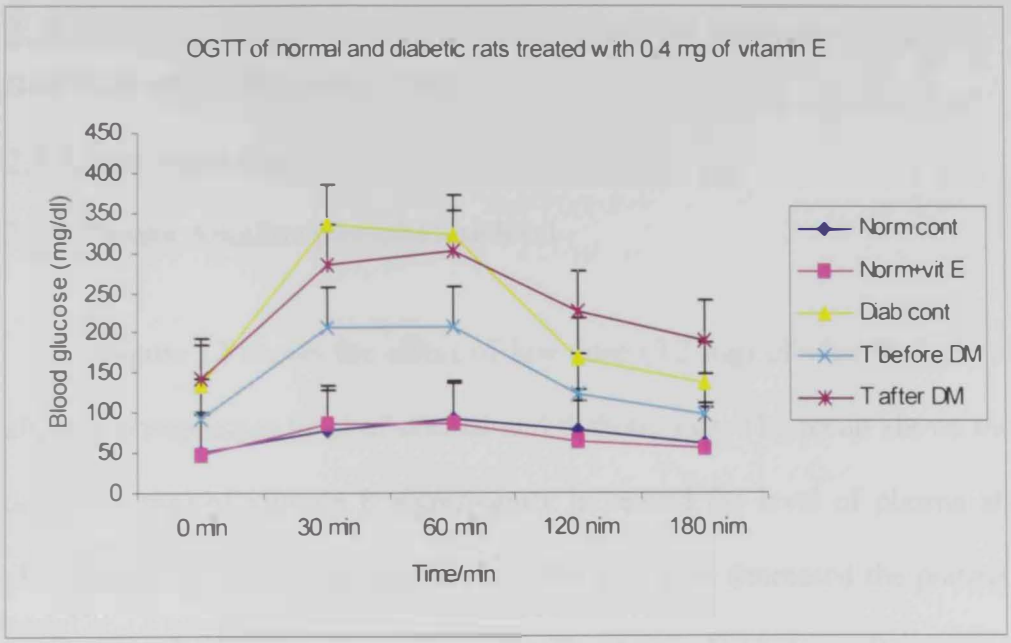

Figure 10: Histograms showing the effect of 0.4 mg of vitamin E on OGTT of normal and diabetic rats. (Data are mean ± SD, n=6) DM= diabetes mellitus.

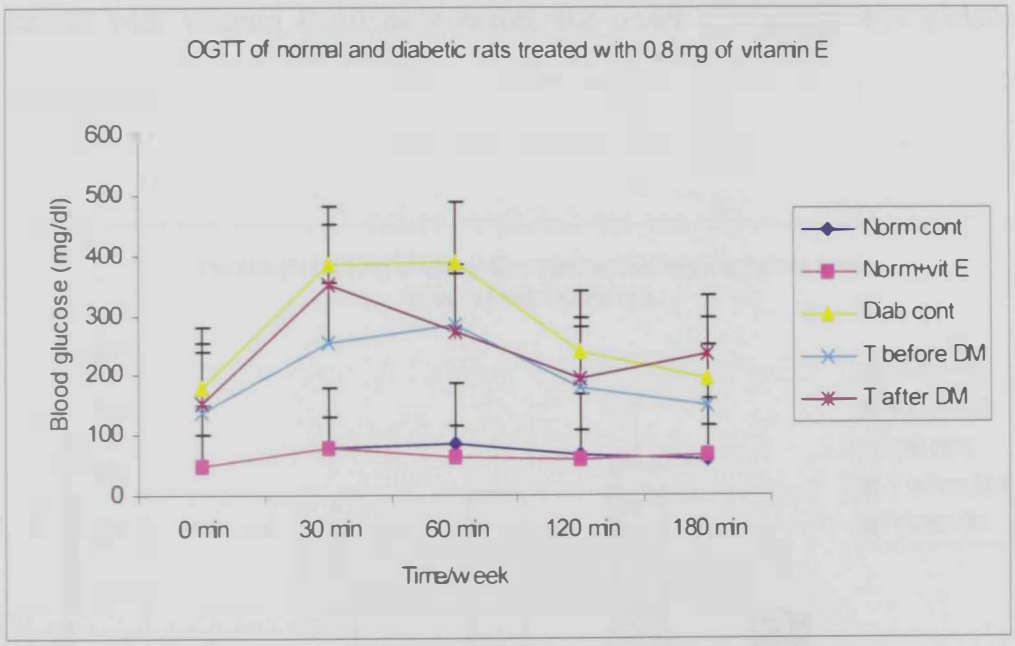

Figure 11: Histograms showing the effect of 0.8 mg of vitamin E on OGTT of normal and diabetic rats. (Data are mean ± SD, n=6) DM= diabetes mellitus.

2. Effect of Vitamin E on biochemical parameters of normal and diabetic rats

2.1 Liver enzymes

2.1.1 Plasma Alkaline phosphatase level

Figure 12 shows the effect of low dose (0.2 mg) of vitamin E on plasma alkaline phosphatase level of normal and diabetic rats. The result shows that low dose (0.2 mg) of vitamin E significantly increased the level of plasma alkaline phosphatase ($p< 0.035$) in normal rats. The low dose decreased the plasma level of alkaline phosphatase in rats treated with vitamin E 10 days after the onset of diabetes. However, the alkaline phosphatase plasma level slightly increased in rats treated with vitamin E 10 days before the onset of diabetes but without any significance.

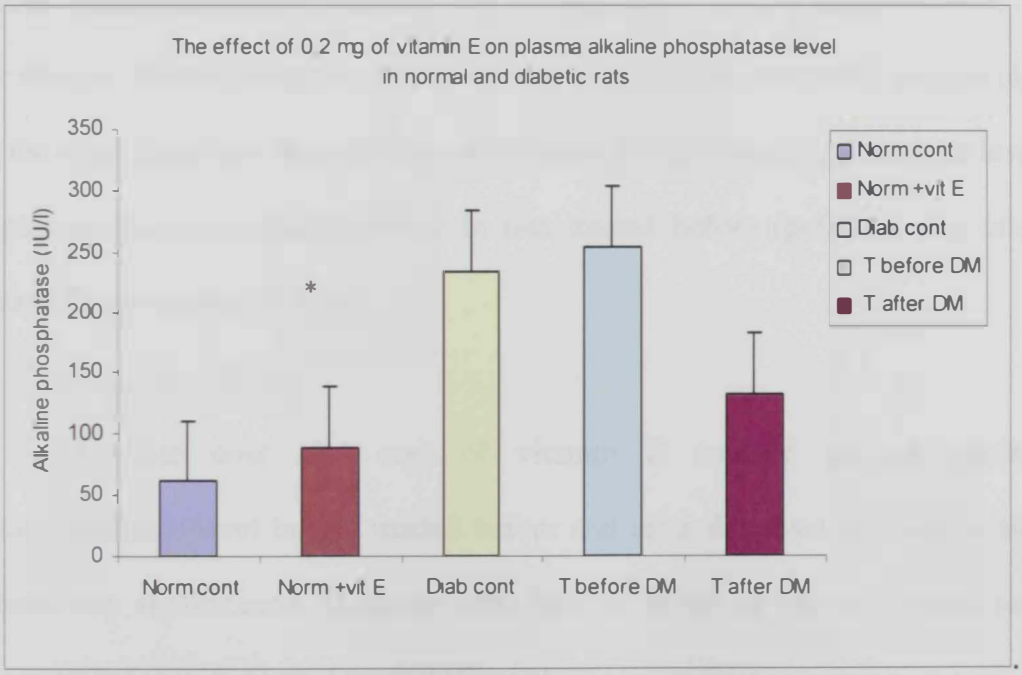

Figure 12: Histograms showing the effect of 0.2 mg of vitamin E on plasma alkaline phosphatase level of normal and diabetic rats. 0.2 mg of vitamin E induced significant increases in plasma alkaline phosphatase level in normal non diabetic rats * $p < 0.05$ (norm cont versus norm+ vitamin E). (Data are mean ± SD, n=6) DM= diabetes mellitus.

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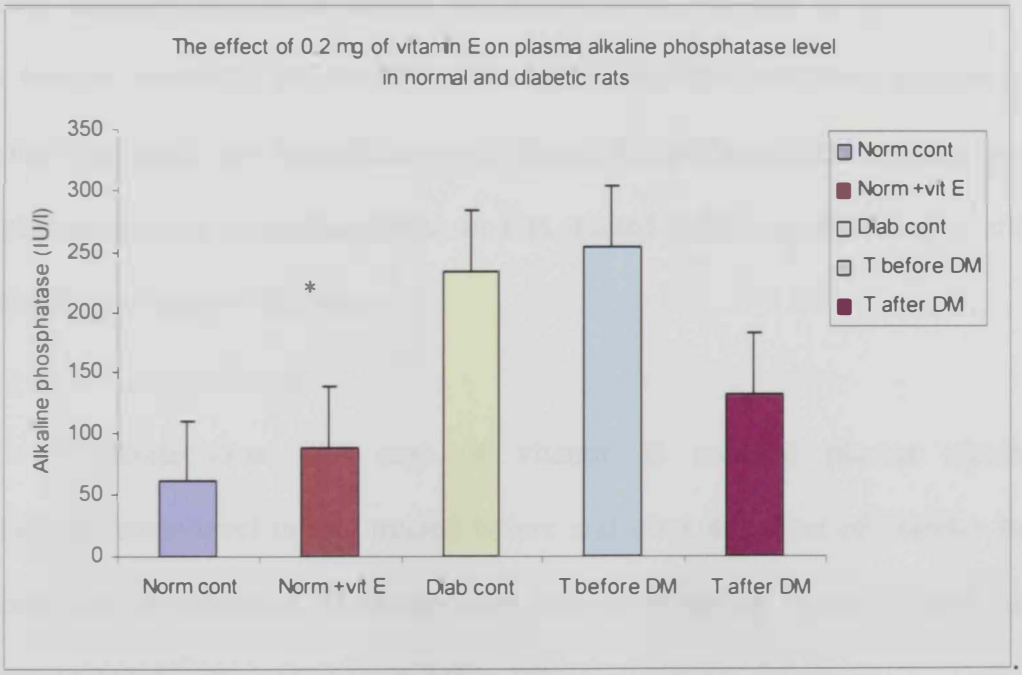

Figure 12: Histograms showing the effect of 0.2 mg of vitamin E on plasma alkaline phosphatase level of normal and diabetic rats. 0.2 mg of vitamin E induced significant increases in plasma alkaline phosphatase level in normal non diabetic rats * $p < 0.05$ (norm cont versus norm+ vitamin E). (Data are mean ± SD, n=6) DM= diabetes mellitus.

The moderate dose (0.4 mg) of vitamin E had no significant effect on the plasma alkaline phosphatase level of normal treated rats. On the other hand, the moderate dose increased the plasma alkaline phosphatase level in the treated before DM group. This increase is not statistically different when compared to control. No significant change was observed in plasma alkaline phosphatase level of rats treated after the onset of diabetes (Fig.13). The oral administration of high dose (0.8 mg) of vitamin E had no effect on plasma alkaline phosphatase level of normal and treated diabetic rats (Fig.14).

2.1.2 Plasma alanine aminotransferase level

Figure 15 shows the effect of low dose (0.2 mg) of vitamin E on plasma alanine aminotransferase of normal and diabetic rats. The oral administration of low dose of vitamin E had no effect on normal rats when compared to controls. On the other hand, low dose (0.2 mg) of vitamin E significantly reduced the level of plasma alanine aminotransferase in rats treated before ($p<0.014$) and after ($p<0.006$) the onset of diabetes.

Moderate dose (0.4 mg) of vitamin E reduced plasma alanine aminotransferase level in rats treated before and after the onset of diabetes but without any significance. Moderate dose had no effect on normal treated rats when compared with normal control (Fig 16).

The oral administration of high dose (0.8 mg) of vitamin E increased plasma alanine aminotransferase level without any significance compared to diabetics control (Fig.17). No significant change was observed in the alanine aminotransferase level of normal rats after oral administration of high dose of vitamin E.

2.1.3 Plasma aspartate aminotransferase

Figure 18 shows that low dose (0.2 mg) of vitamin E had no effect on plasma aspartate aminotransferase level of normal rats when compared with normal control. In diabetic rats, the low dose reduces plasma aspartate aminotransferase level of rats treated 10 days after the onset of diabetes rats with significance ($p<0.019$) and without any significance in rats treated 10 days before the onset of diabetes.

After the oral administration of moderate dose (0.4 mg) of vitamin E a decrease in the plasma aspartate aminotransferase level of normal treated rats was observed but without any significance (Fig.19). The moderate dose can increase the plasma aspartate aminotransferase level of rats treated before and after the onset of diabetes. This increase had no statistical significance when compared with diabetic control.

The high dose (0.8 mg) of vitamin E had no effect in the plasma aspartate aminotransferase level of normal treated rats (Fig. 20). The high dose increased the plasma aspartate aminotransferase level of rats treated before and after the

onset of diabetes. This increase has no statistical significance when compared to diabetics control.

2.1.4 Plasma lactic dehydrogenase level

The oral administration of low dose (0.2 mg) of vitamin E significantly ($p<0.033$) reduced the level of plasma lactic dehydrogenase in untreated normal rats. However, the low dose also increased plasma lactic dehydrogenase level but without any significance (Fig. 21).

No change in plasma lactic dehydrogenase level was observed in normal rats after oral administration of moderate (0.4 mg) of vitamin E. While the moderate dose increased the lactic dehydrogenase plasma level in rats treated 10 days after the onset of diabetes without any significance (Fig. 22), and it had no effect on the plasma lactic dehydrogenase level of rats treated before the onset of diabetes.

The high dose (0.8 mg) of vitamin E had no effect on the level of plasma lactic dehydrogenase in normal rats and rats treated 10 days before the onset of diabetes rats. On the other hand, there was a significant ($p<0.03$) increase in plasma lactic dehydrogenase level of rats treated 10 days after the onset of diabetes (Fig. 23).

2.1.5 Plasma gamma-glutamyl transferase

The result of the effect of different low dose (0.2 mg) of vitamin E on plasma gamma-glutamyl transferase level of normal and diabetic rats is shown in Fig. 24. In normal rats with low dose of vitamin E, there was a significant increase in plasma gamma-glutamyl transferase level ($p<0.012$). Diabetic rats exhibited high plasma gamma-glutamyl transferase level. The low dose of vitamin E successfully reduced this level in rats treated before and after the onset of diabetes but without any statistical significance.

However, after the oral administration of the moderate dose (0.4 mg) of vitamin E, the plasma gamma-glutamyl transferase level of rats treated 10 days before and after the onset of diabetes was increased compared to the plasma gamma-glutamyl transferase level of diabetic control rats (Fig.25).

Figure 26 shows the effect of high dose (0.8 mg) of vitamin E on plasma gamma-glutamyl transferase of normal and diabetic rats. The result showed that high dose of vitamin E had no significant effect on the plasma gamma-glutamyl transferase level of normal and diabetic rats.

2.1.5 Plasma gamma-glutamyl transferase

The result of the effect of different low dose (0.2 mg) of vitamin E on plasma gamma-glutamyl transferase level of normal and diabetic rats is shown in Fig. 24. In normal rats with low dose of vitamin E, there was a significant increase in plasma gamma-glutamyl transferase level ($p<0.012$). Diabetic rats exhibited high plasma gamma-glutamyl transferase level. The low dose of vitamin E successfully reduced this level in rats treated before and after the onset of diabetes but without any statistical significance.

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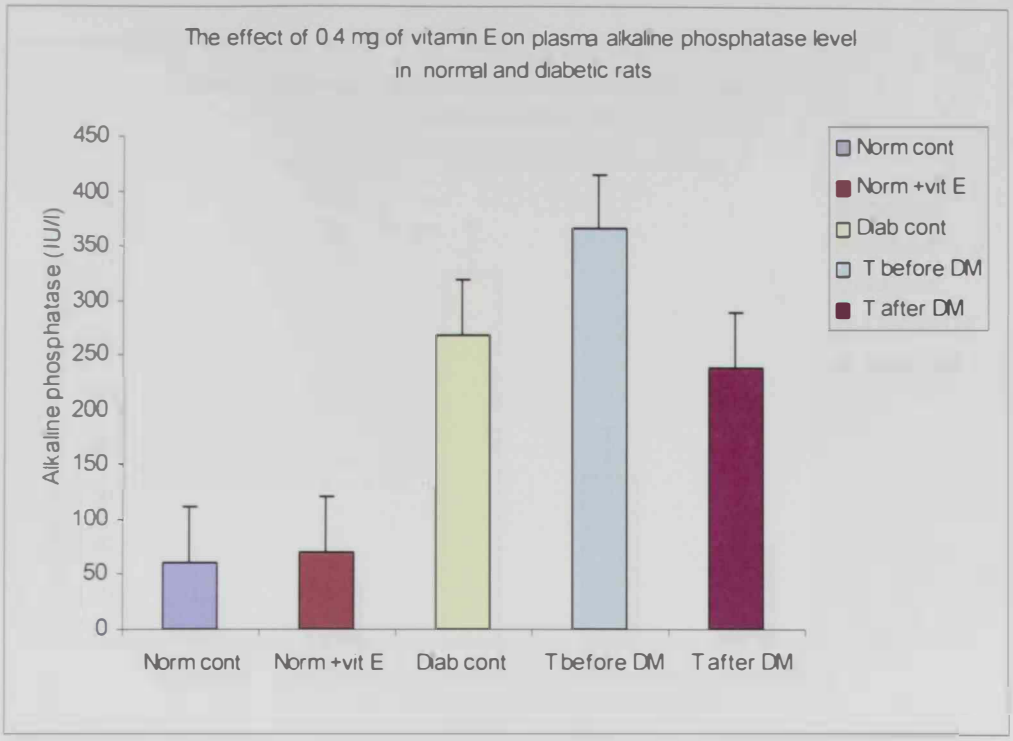


Figure 13: Histograms showing the effect of 0.4 mg of vitamin E on plasma alkaline phosphatase level of normal and diabetic rats. (Data are mean ± SD, n=6) DM= diabetes mellitus.

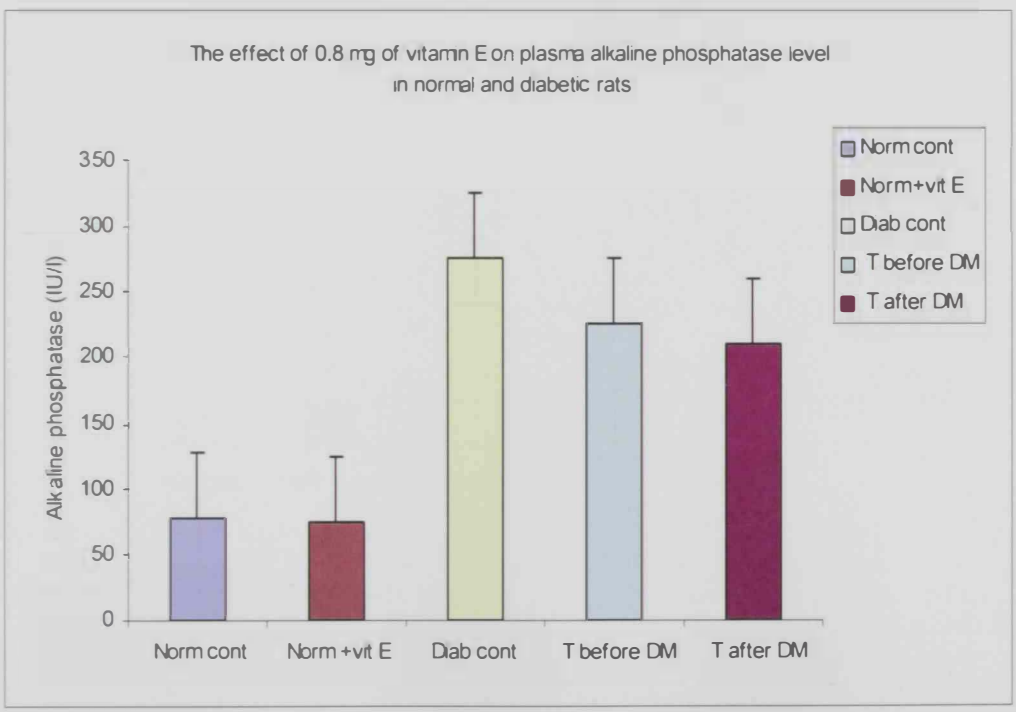


Figure 14: Histograms showing the effect of 0.8 mg of vitamin E on plasma alkaline phosphatase level of normal and diabetic rats. (Data are mean ± SD, n=6) DM= diabetes mellitus.

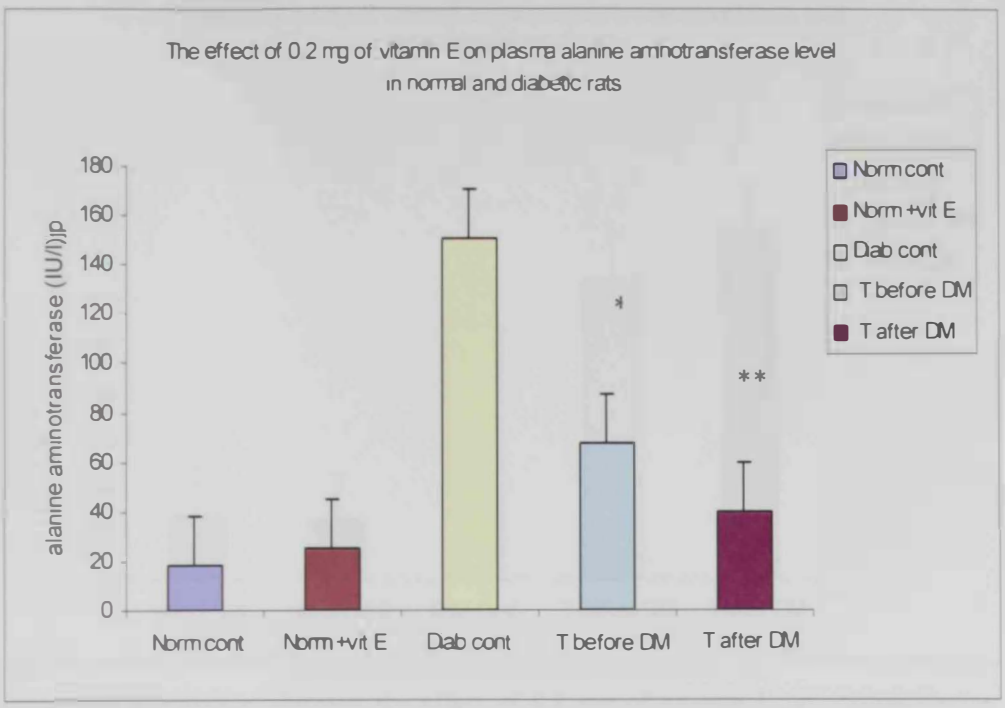


Figure 15: Histograms showing the effect of 0.2 mg of vitamin E on plasma alanine aminotransferase level of normal and diabetic rats. 0.2 of vitamin E induced significant decreases in plasma alanine aminotransferase level in rats treated before and after the onset of DM *p <0.05 (treated before DM versus diab cont), **p<0.01(treated after DM versus diab cont). (Data are mean ± SD, n=6) DM= diabetes mellitus.

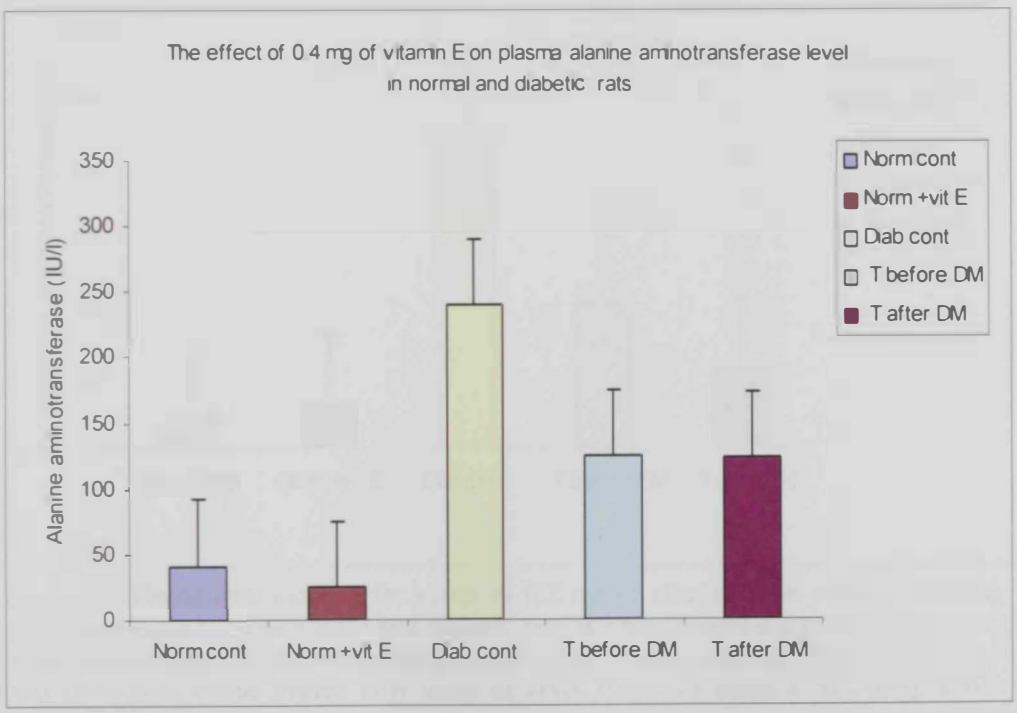


Figure 16: Histograms showing the effect of 0.4 mg of vitamin E on plasma alanine aminotransferase level of normal and diabetic rats. (Data are mean ± SD, n=6) DM= diabetes mellitus

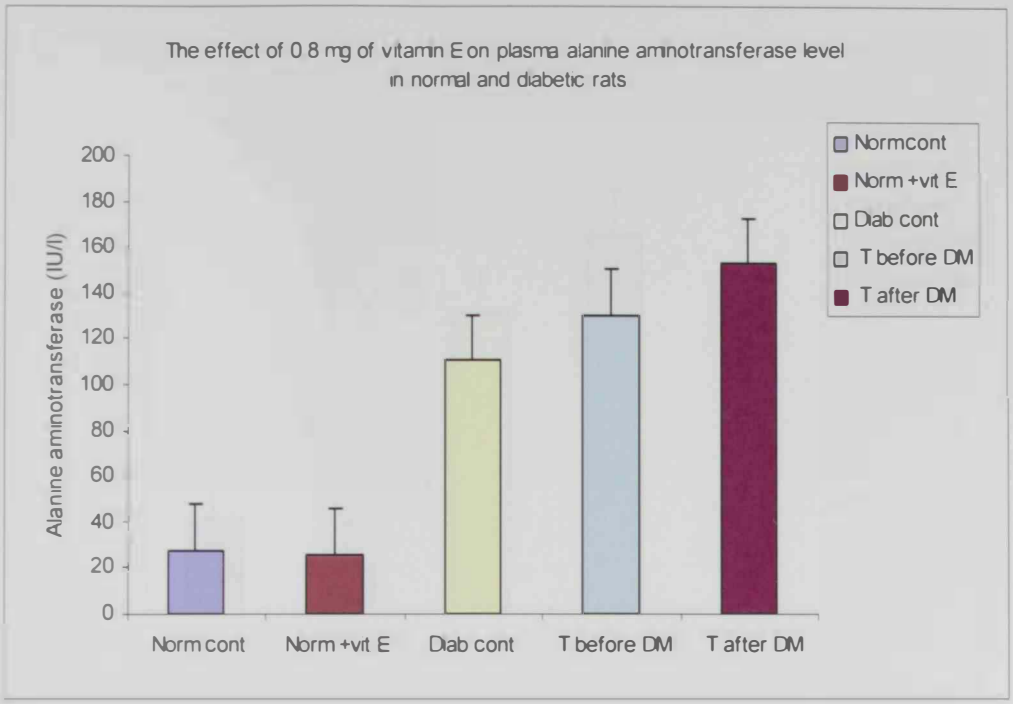


Figure 17: Histograms showing the effect of 0.8 mg of vitamin E on plasma alanine aminotransferase level of normal and diabetic rats. (Data are mean ± SD, n=6) DM= diabetes mellitus

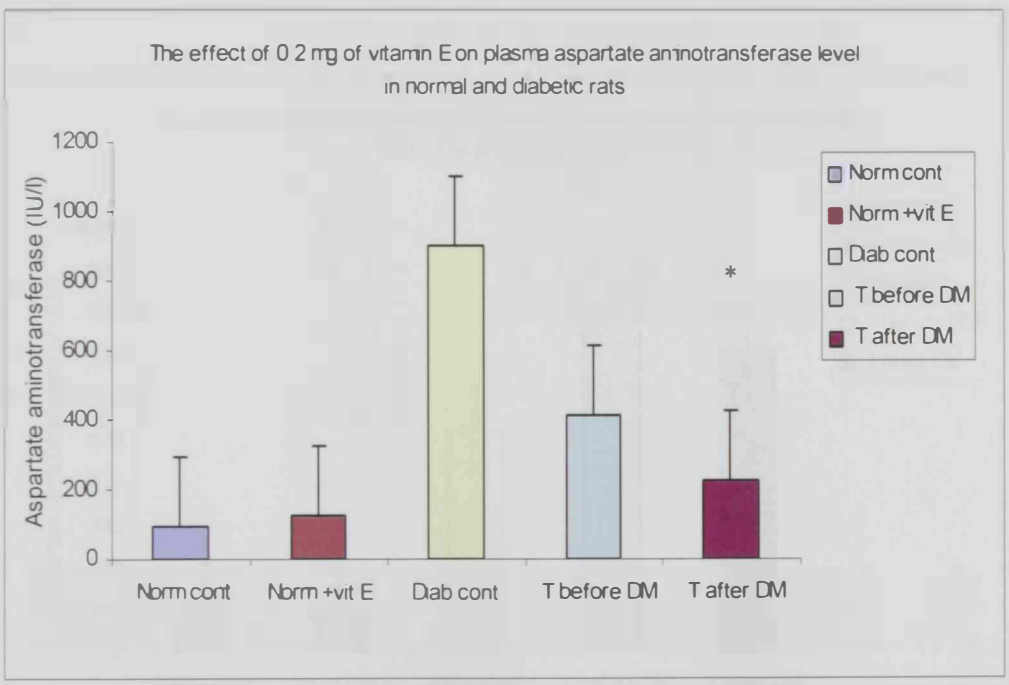


Figure 18: Histograms showing the effect of 0.2 mg of vitamin E on plasma aspartate aminotransferase level of normal and diabetic rats. 0.2 mg induced a significant decrease in the plasma aspartate aminotransferase level in rats treated after the onset of DM *p< 0.05 (diab cont versus treated after onset of DM). (Data are mean ± SD, n=6). DM=

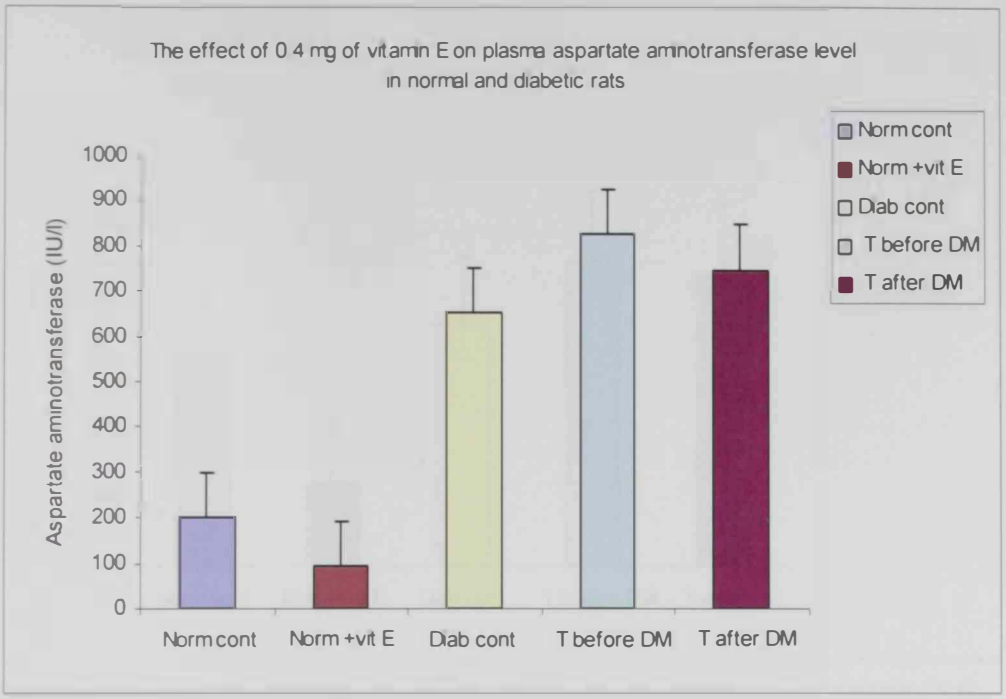


Figure 19: Histograms showing the effect of 0.4 mg of vitamin E on plasma aspartate aminotransferase level of normal and diabetic rats. (Data are mean ± SD, n=6). DM= diabetes mellitus

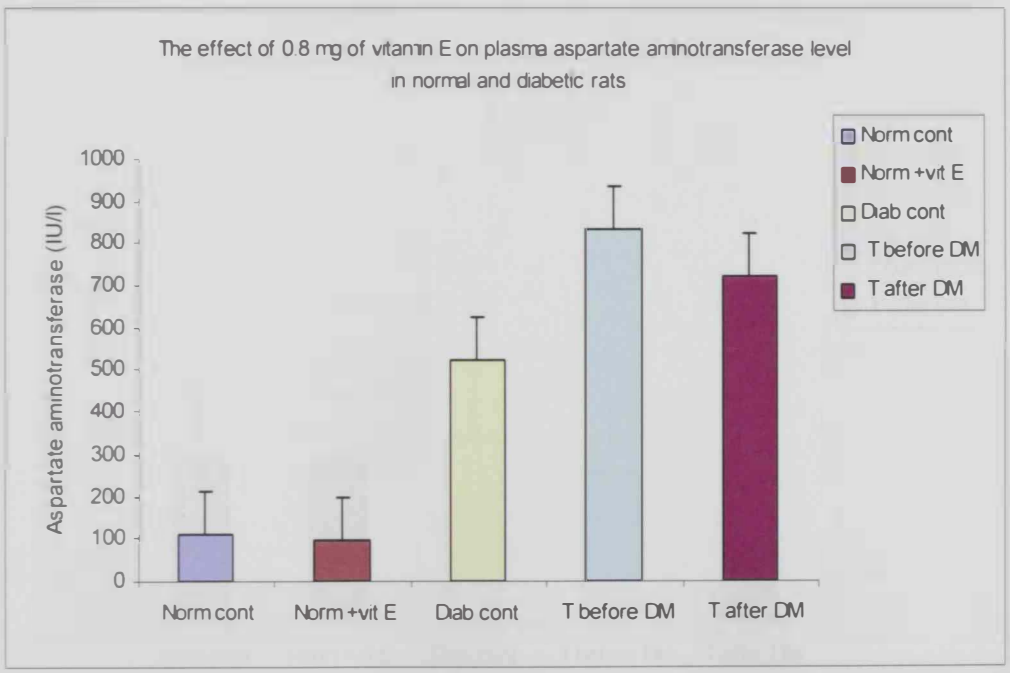


Figure 20: Histograms showing the effect of 0.8 mg of vitamin E on plasma aspartate aminotransferase level of normal and diabetic rats. (Data are mean ± SD, n=6). DM= diabetes mellitus

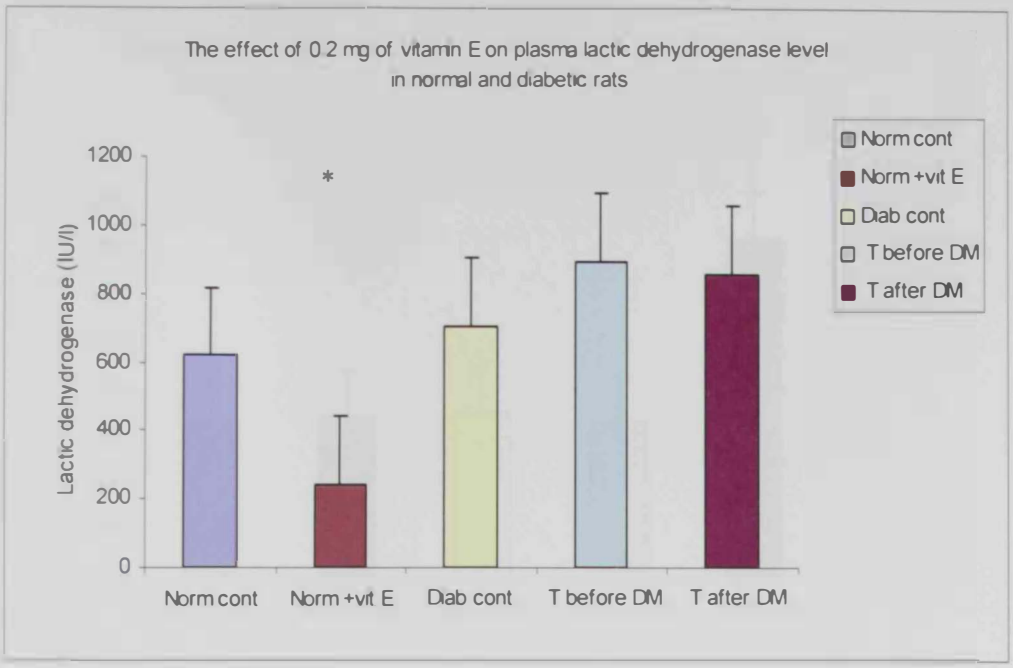


Figure 21: Histograms showing the effect of 0.2 mg of vitamin E on plasma lactic dehydrogenase level of normal and diabetic rats. 0.2 mg of vitamin E induced a significant decrease in the plasma lactic dehydrogenase level in normal non diabetic rats *$p < 0.05$ (Norm cont versus norm cont + vitamin E). (Data are mean ± SD, n=6). DM= diabetes mellitus

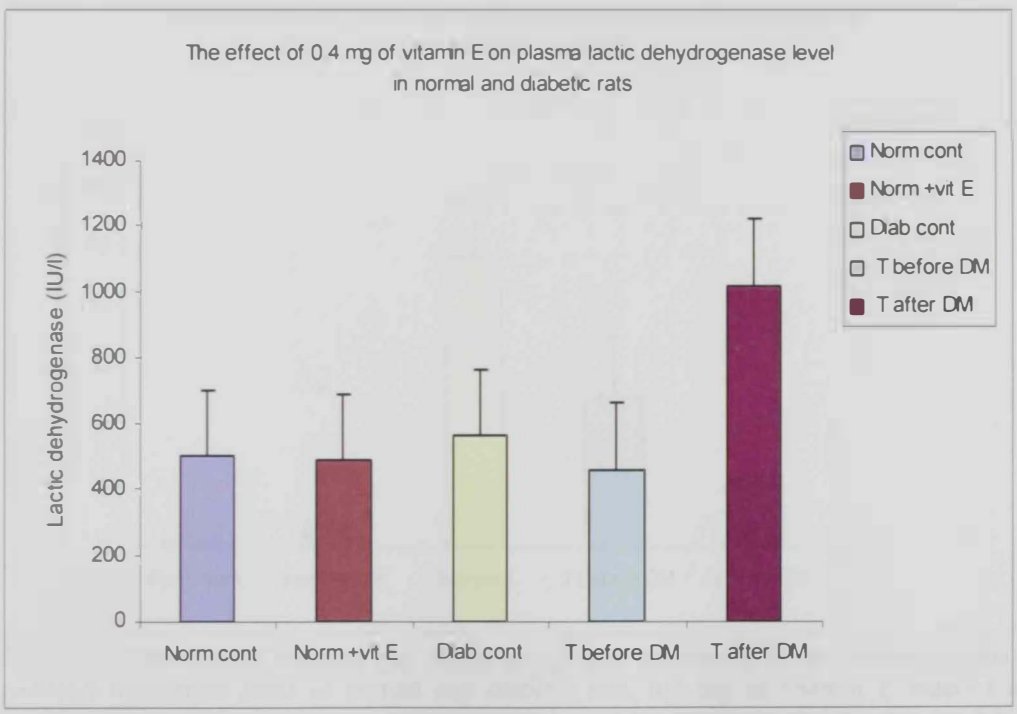


Figure 22: Histograms showing the effect of 0.4 mg of vitamin E on plasma lactic dehydrogenase level of normal and diabetic rats. (Data are mean ± SD, n=6) DM= diabetes mellitus

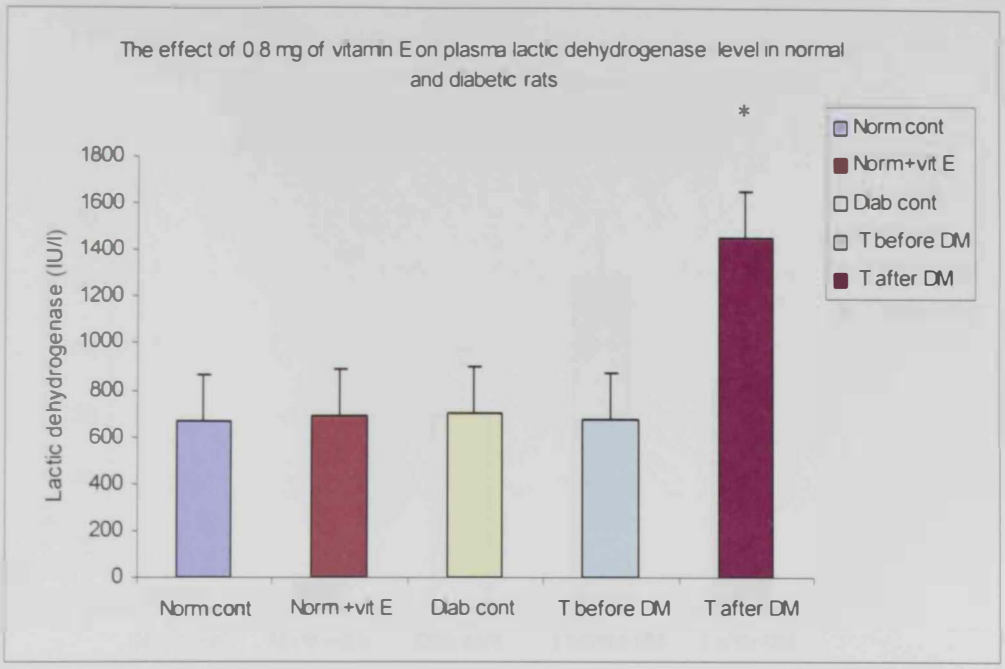


Figure 23: Histograms showing the effect of 0.8 mg of vitamin E on plasma lactic dehydrogenase level of normal and diabetic rats. 0.8 mg of vitamin E induced a significant increase in plasma lactic dehydrogenase level of rats treated after the onset DM *p<0.05 (diab cont versus treated after onset of DM). (Data are mean ± SD, n=6) DM= diabetes mellitus

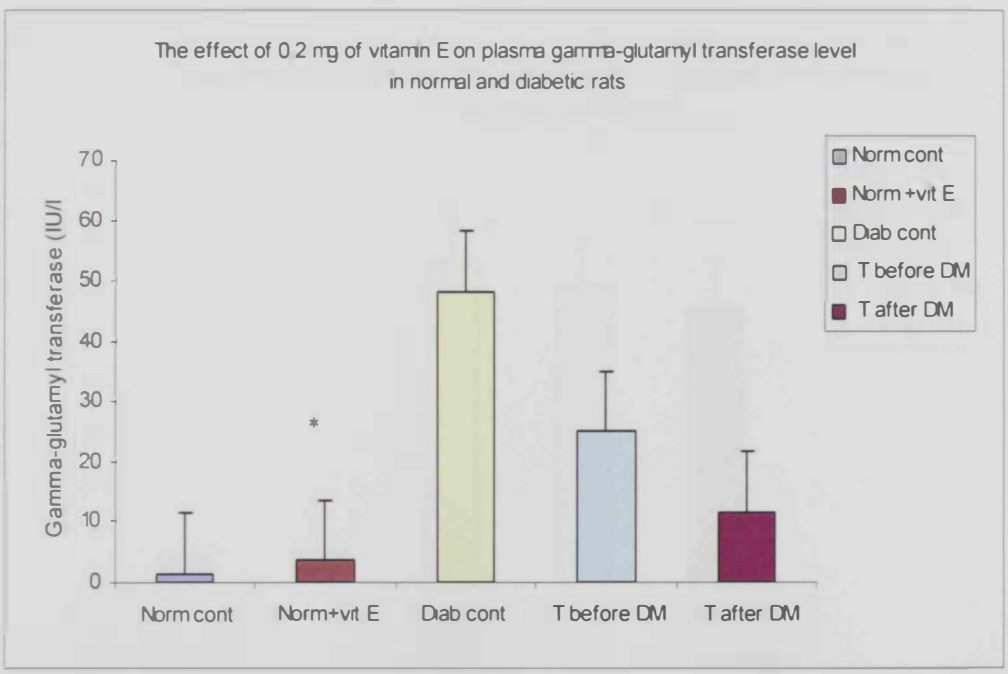


Figure 24: Histograms showing the effect of 0.2 mg of vitamin E on plasma gamma-glutamyl transferase level of normal and diabetic rats. 0.2 mg of vitamin E induced a significant increase in the plasma gamma-glutamyl level in normal non diabetic rats *p<0.05 (norm cont versus norm + vitamin E). (Data are mean ± SD, n=6) DM= diabetes mellitus.

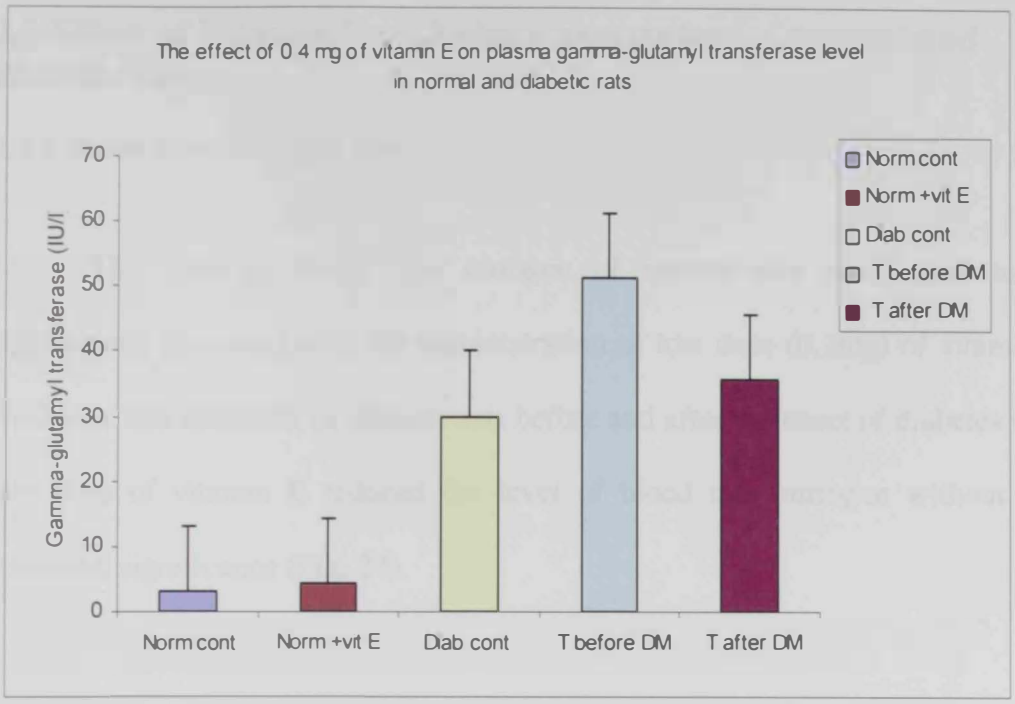


Figure 25: Histograms showing the effect of 0.4 mg of vitamin E on plasma gamma-glutamyl transferase level of normal and diabetic rats. (Data are mean ± SD, n=6) DM= diabetes mellitus.

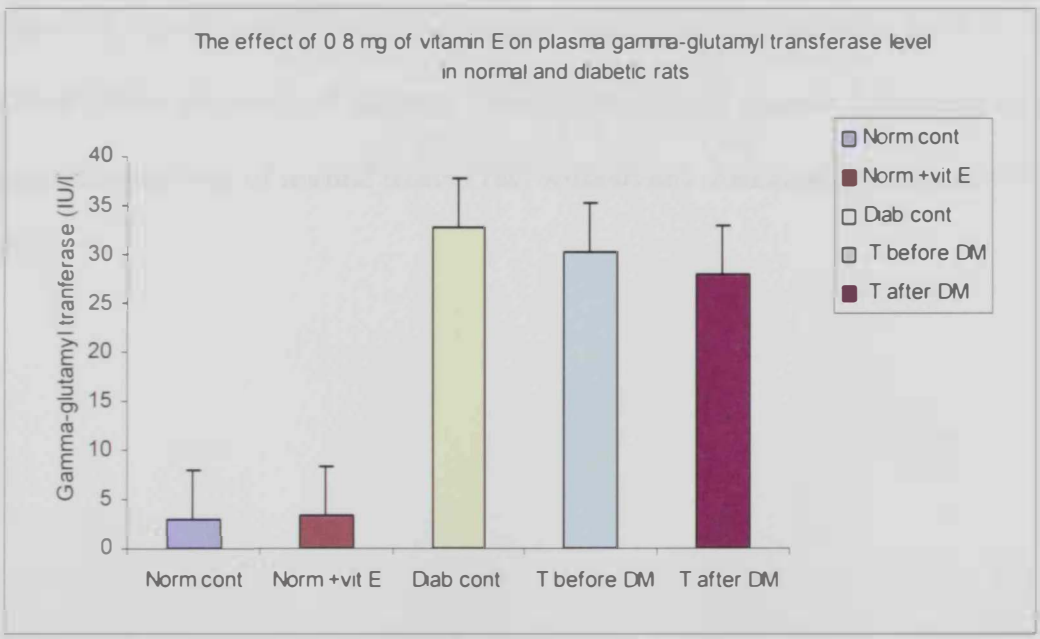


Figure 26: Histograms showing the effect of 0.8 mg of vitamin E on plasma gamma-glutamyl transferase level of normal and diabetic rats. (Data are mean ± SD, n=6) DM= diabetes mellitus.

2.2 Effect of Vitamin E on Kidney parameters of normal and diabetic rats

2.2.1 Blood urea nitrogen level

The level of blood urea nitrogen of normal rats was found to be significantly increased after the administration of low dose (0.2mg) of vitamin E ($p<0.03$). The treatment of diabetic rats before and after the onset of diabetes with low dose of vitamin E reduced the level of blood urea nitrogen without any statistical significance (Fig. 27).

Treatment with moderate dose (0.4 mg) of vitamin E resulted in increase in the level of blood urea nitrogen of normal and diabetic rats without any statistical significance (Fig. 28). The oral administration of high dose (0.8 mg) of vitamin E significantly ($p<0.03$) increased the blood urea nitrogen level of rats treated before the onset of diabetes. Also, high dose of vitamin E increase blood urea nitrogen level of normal treated rats without any statistical significance (Fig. 29).

2.2.2 Plasma creatinine level

Figure 30 shows that low does of vitamin E can significantly increase the plasma creatinine level in normal rats when compared with control rats. No significant changes were observed in rats treated before and after the onset of diabetes.

No change was observed in the plasma creatinine level of normal and diabetic rats after oral administration of moderate dose of vitamin E (Fig. 31).

Also, after the oral administration of high dose of vitamin E no significant change was observed in the plasma creatinine level of normal rats. There was a decrease in the plasma creatinine level of rats treated before and after the onset of diabetes. However, this decrease was not statistically significant level (Fig. 32).

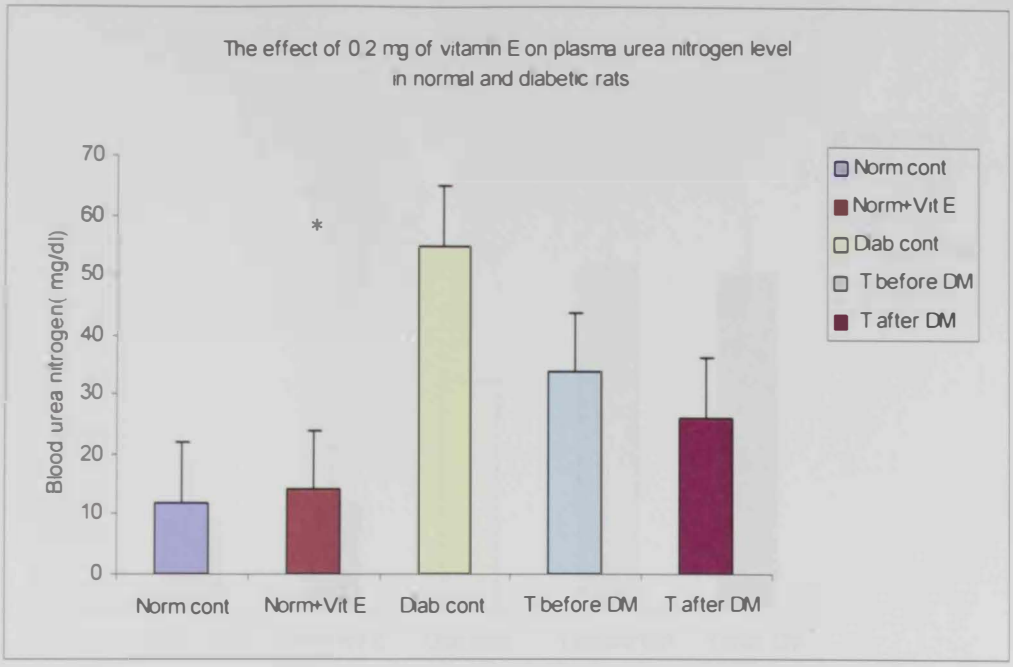


Figure 27: Histograms showing the effect of 0.2 mg of vitamin E on plasma urea nitrogen level of normal and diabetic rats. 0.2 mg/kg of vitamin E induced a significant increase in the plasma urea nitrogen level of normal non diabetic rats *p<0.05 (norm cont versus norm + vitamin E). (Data are mean ± SD, n=6) DM= diabetes mellitus.

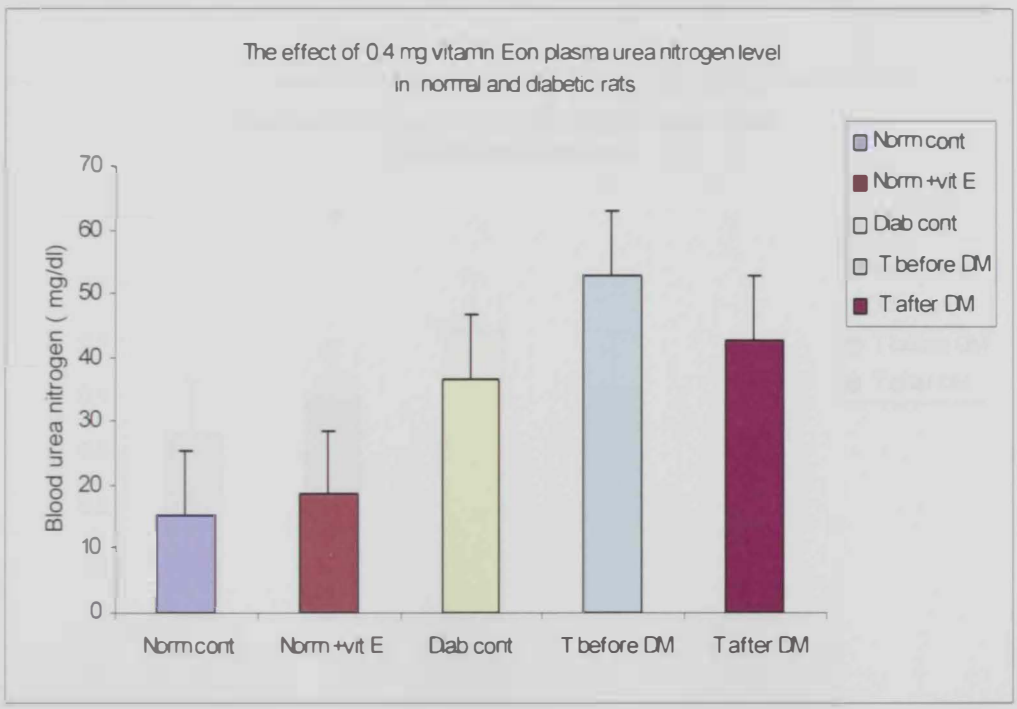


Figure 28: Histograms showing the effect of 0.4 mg of vitamin E on plasma urea nitrogen level of normal and diabetic rats. (Data are mean ± SD, n=6) DM= diabetes mellitus.

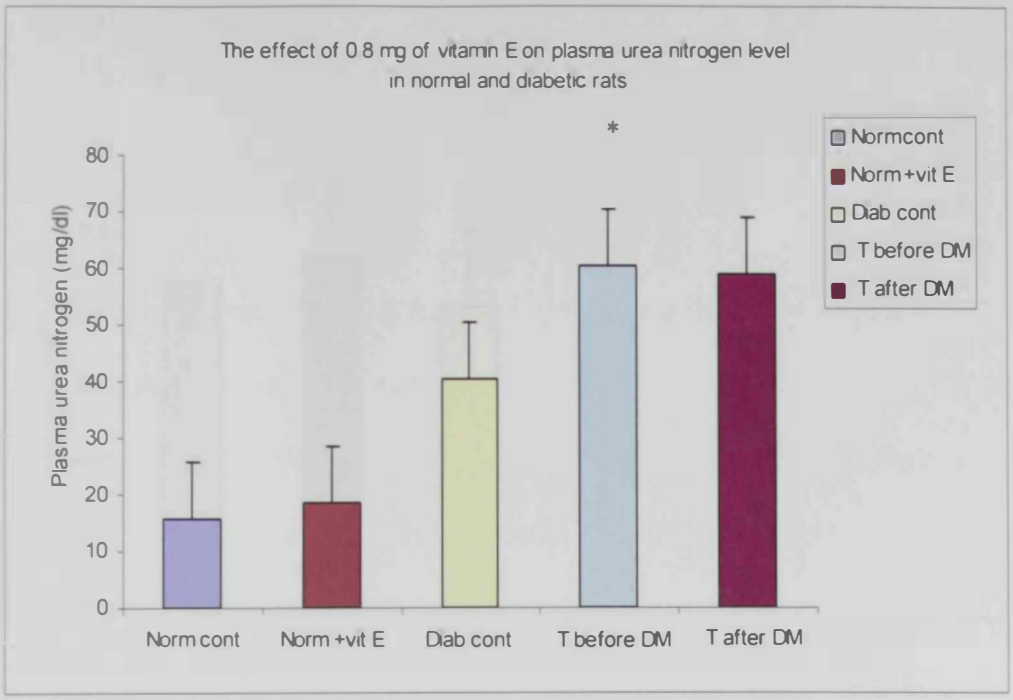


Figure 29: Histograms showing the effect of 0.8 mg of vitamin E on plasma urea nitrogen level of normal and diabetic rats. 0.8 mg of vitamin E induced a significant increase in the plasma urea nitrogen level of rats treated before the onset of DM rats *p<0.05 (diab cont versus treated before DM). (Data are mean ± SD, n=6) DM= diabetes mellitus.

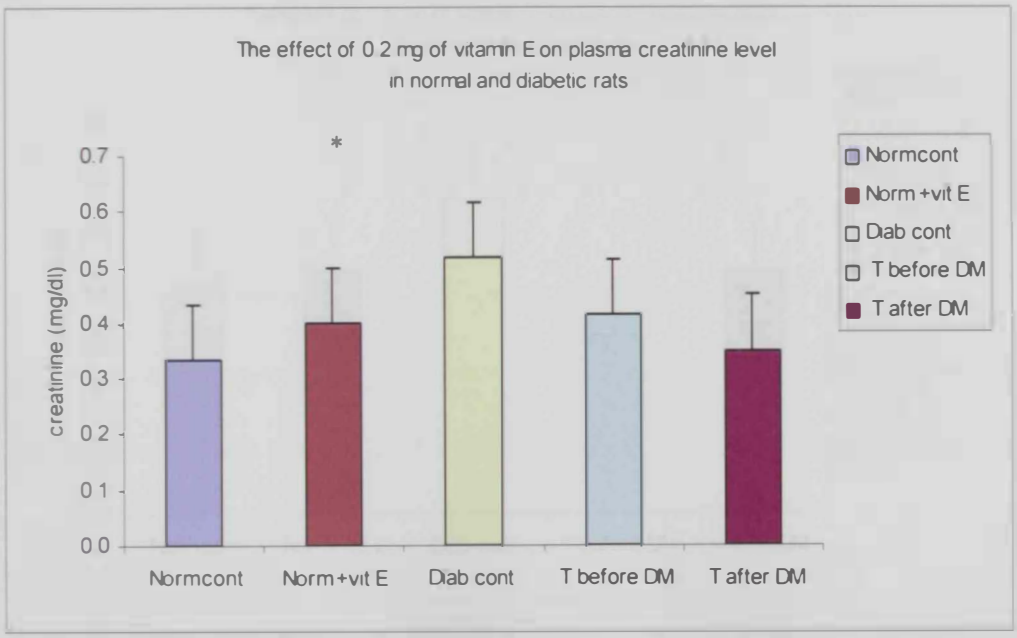


Figure 30: Histograms showing the effect of 0.2 mg of vitamin E on plasma creatinine level of normal and diabetic rats. 0.2 mg of vitamin E induced a significant increase in the plasma creatinine level of normal non diabetic rats *p<0.05 (norm cont versus norm+ vitamin E). (Data are mean ± SD, n=6) DM= diabetes mellitus.

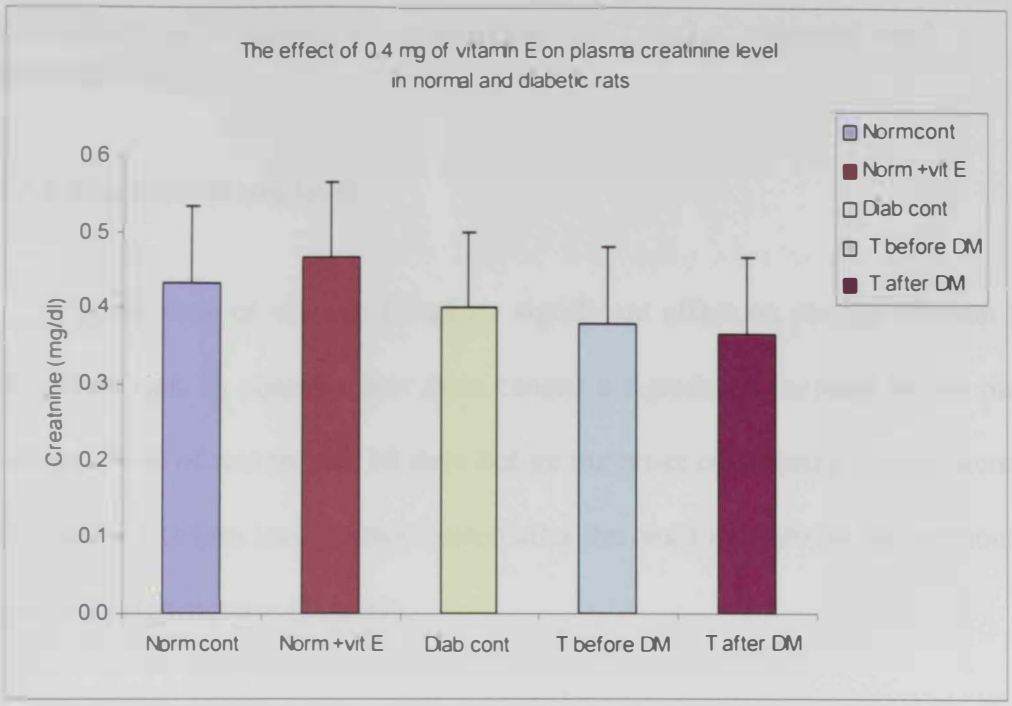


Figure 31: Histograms showing the effect of 0.4 mg of vitamin E on plasma creatinine level of normal and diabetic rats. (Data are mean ± SD, n=6) DM= diabetes mellitus.

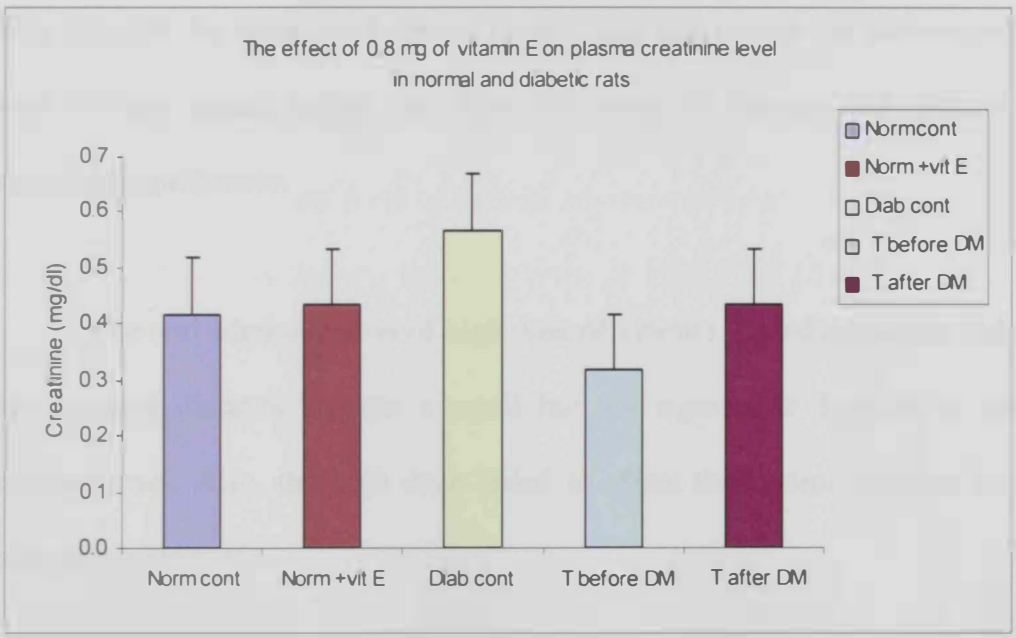


Figure 32: Histograms showing the effect of 0.8 mg of vitamin E on plasma creatinine level of normal and diabetic rats. (Data are mean ± SD, n=6) DM= diabetes mellitus

2.3 Effect of Vitamin E on electrolytes level of normal and diabetic rats

2.3.1 Plasma calcium level

Low dose of vitamin E had no significant effect on plasma calcium level of normal rats. In contrast, low dose caused a significant increase in the plasma calcium level of rats treated 10 days before the onset of diabetes. It also increased the plasma calcium level of rats treated after the onset of diabetes rats without any statistical significance (Fig. 33).

The moderate dose of vitamin E induced significant increases ($p<0.002$) in plasma calcium level of the normal rats when compared to normal untreated rats (Fig. 34). On the other hand, the moderate dose can reduce the plasma calcium level of rats treated before and after the onset of diabetes but without any statistical significance.

The oral administration of high dose of vitamin E 10 days before and after the onset of diabetes brought a small but not significant decrease in plasma calcium level. Also, the high dose failed to affect the plasma calcium level in normal rats (Fig. 35).

2.3.2 Plasma phosphorus level

Figure 36 shows the effect of low dose of vitamin E on plasma phosphorus level of normal rats. The result indicates that low dose of vitamin E failed to change the plasma phosphorus level in normal and diabetic rats.

The moderate dose of vitamin E did not have any effect on the plasma phosphours level of normal and rats treated 10 days before the onset of diabetes. however, the moderate dose caused a significant decrease in the plasma phosphorus level of rats treated after the onset of diabetes compared to diabetic control (Fig. 37).

The oral administration of high dose of vitamin E did not have any effect on the plasma phosphours level of normal rats and rats treated 10 days before the onset of diabetes. However, the high dose of vitamin E caused a significant increase in the plasma phosphorus level of rats treated after the onset of diabetes compared to diabetic control (Fig. 38).

2.3.3 Plasma sodium level

Low dose of vitamin E affected the plasma sodium level in normal rats. However, in diabetic rats, the low dose of vitamin E caused significant increases in the plasma sodium level in rats treated before ($p<0.02$) and after ($p<0.005$) the onset of diabetes (Fig. 39). The moderate dose of vitamin E also caused

significant ($p< 0.003$) reduction in the plasma sodium level of rats treated 10 days after the onset of diabetes. There was an increase in the plasma sodium level of the normal rats without any statistical significance (Fig. 40). In rats treated before and after the onset of diabetes the level of sodium was slightly increased when compared to control (Fig. 41).

2.3.4 Plasma potassium level

Figure 42 shows the effect of low dose of vitamin E on plasma potassium level of normal and diabetic rats. The result shows that low dose of vitamin E has no effect on the plasma potassium level of normal rats and rats treated after the onset of diabetes. However, the low dose of vitamin E increased plasma potassium level of rats treated before the onset of diabetes. However, this increase did not reach statistical significance.

The oral administration of moderate dose of vitamin E increased the plasma potassium level of normal rats and rats treated before the onset of diabetes. While the plasma potassium level of rats treated after the onset of diabetes was reduced (Fig. 43).

The oral administration of high dose of vitamin E significantly increased the level of potassium in rats treated normal rats ($p<0.003$) and in rats treated before the onset of diabetes ($p<0.032$). It also increased the plasma potassium level of rats treated before the onset of the diabetes without any statistical significance (Fig. 44).

2.3.5 Plasma magnesium level

Figure 45 shows the effect of low dose of vitamin E on plasma magnesium level of normal and diabetic rats. The result indicates that low dose of vitamin E had no effect on plasma magnesium level of normal and diabetic rats. Moreover, the oral administration of moderate dose of vitamin E also has no significant effect on the plasma magnesium level of normal and diabetics rats (Fig. 46).The oral administration of high dose of vitamin E had no effect on the plasma magnesium level of both normal and diabetic treated rats (Fig. 47).

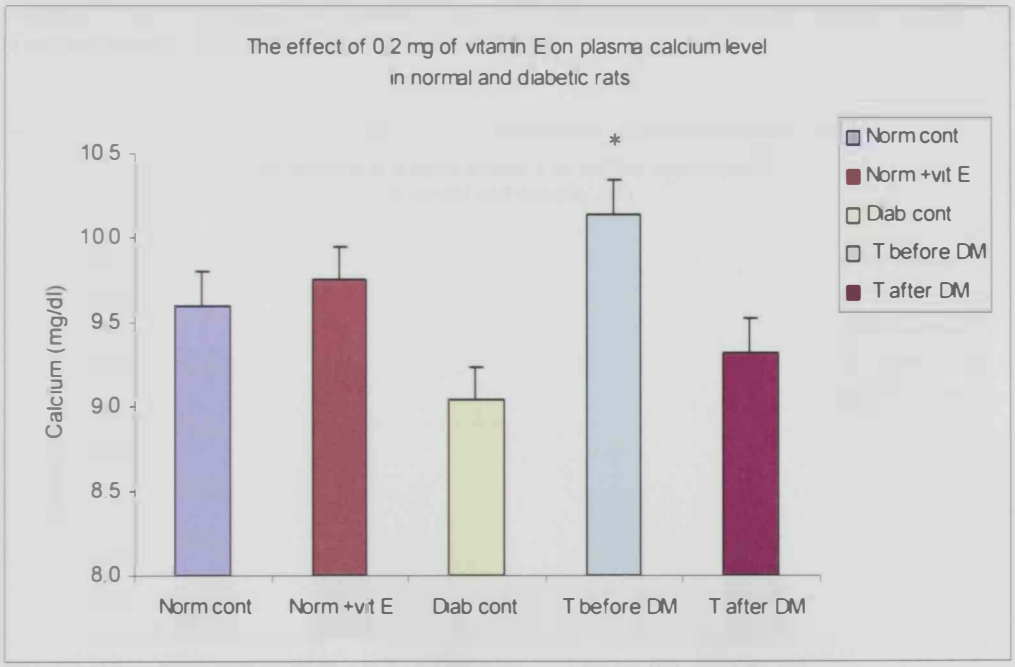


Figure 33: Histograms showing the effect of 0.2 mg of vitamin E on plasma calcium level of normal and diabetic rats. 0.2 mg of vitamin E induced a significant increase on the plasma calcium level in rats treated before the onset of DM *$p<0.05$ (diab cont versus treated before DM). (Data are mean ± SD, n=6) DM= diabetes mellitus.

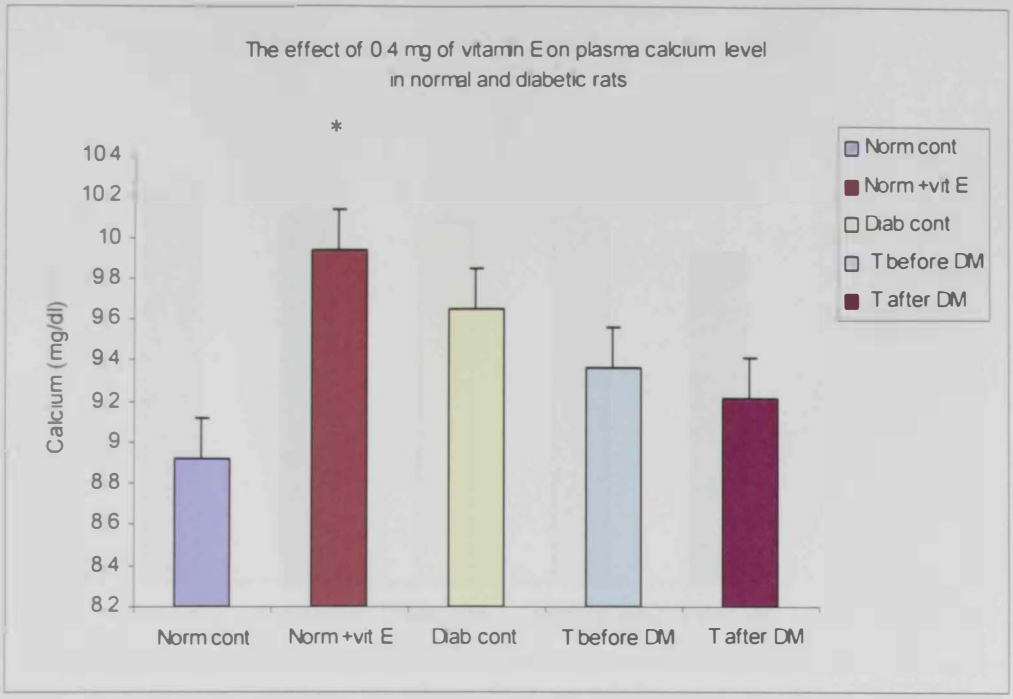


Figure 34: Histograms showing the effect of 0.4 mg of vitamin E on plasma calcium level of normal and diabetic rats. 0.4 mg of vitamin E induced a significant increase in the plasma calcium level of normal non diabetic rats *p<0.05 (norm cont versus norm+ vitamin E). (Data are mean ± SD, n=6) DM= diabetes mellitus.

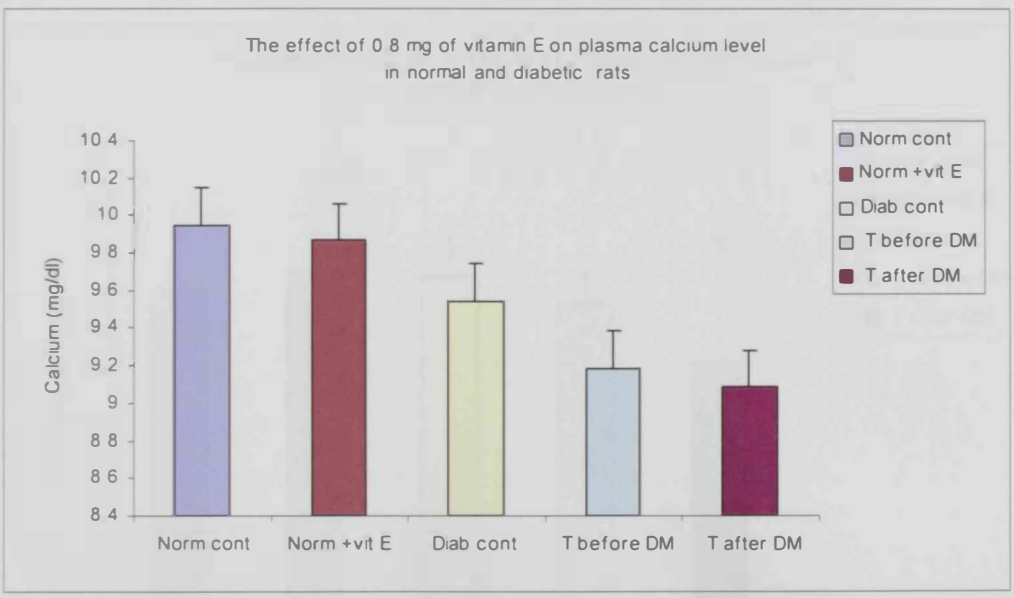


Figure 35: Histograms showing the effect of 0.8 mg of vitamin E on plasma calcium level of normal and diabetic rats. (Data are mean ± SD, n=6) DM= diabetes mellitus.

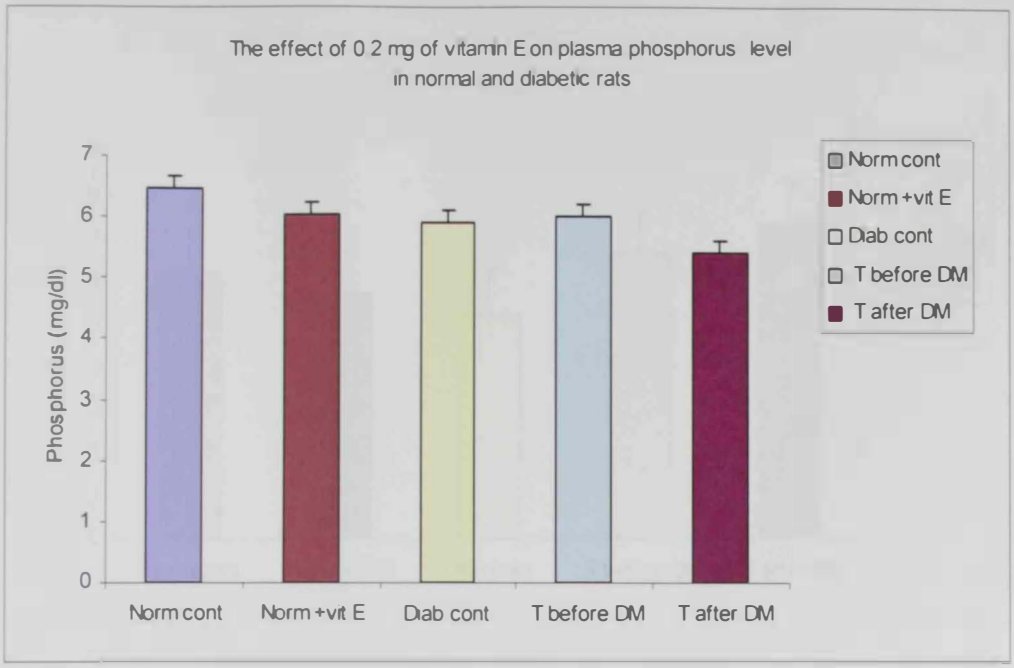


Figure 36: Histograms showing the effect of 0.2 mg of vitamin E on plasma phosphorus level of normal and diabetic rats. (Data are mean ± SD, n=6) DM= diabetes mellitus.

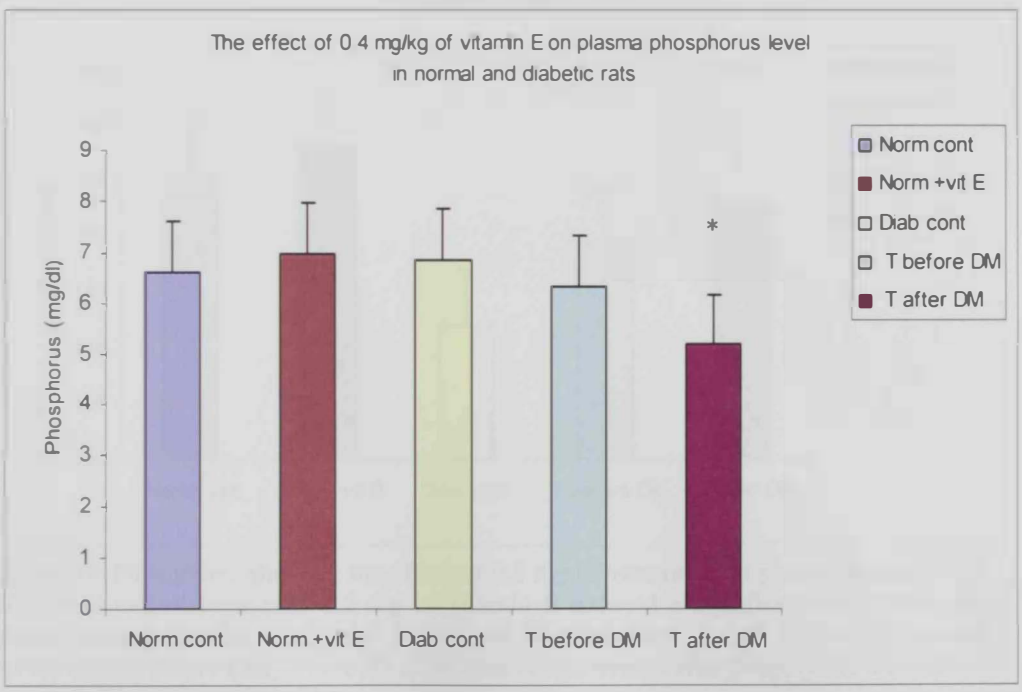


Figure 37: Histograms showing the effect of 0.4 mg of vitamin E on plasma phosphorus level of normal and diabetic rats. 0.4 mg of vitamin E induced a significant increase in the plasma phosphorus level in rats treated after the onset of DM *$p<0.05$ (diab cont versus treated after DM). (Data are mean ± SD, n=6) DM= diabetes mellitus.

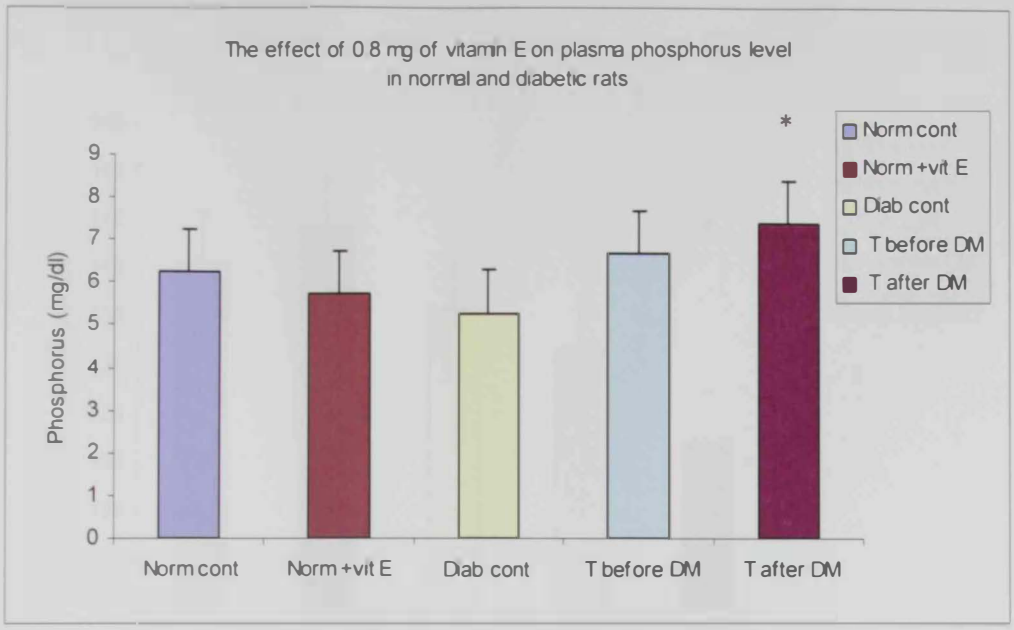


Figure 38: Histograms showing the effect of 0.8 mg of vitamin E on plasma phosphorus level of normal and diabetic rats. 0.8 mg of vitamin E induced a significant increase in the plasma phosphorus level in rats treated after the onset of DM *p<0.05 (diab cont versus treated after DM). (Data are mean ± SD, n=6) DM= diabetes mellitus.

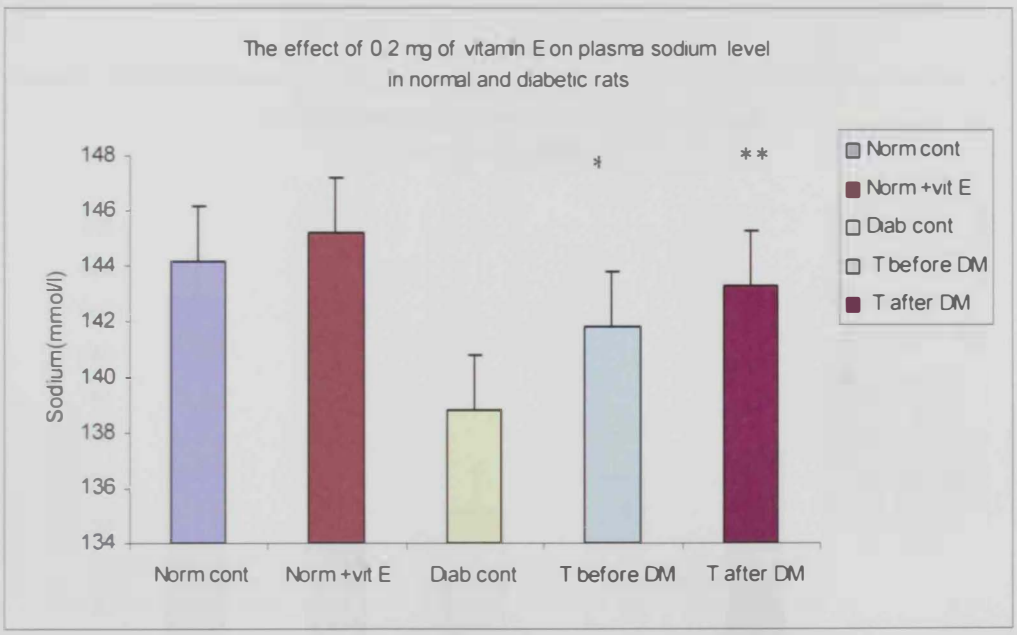


Figure 39: Histograms showing the effect of 0.2 mg of vitamin E on plasma sodium level of normal and diabetic rats. 0.2 mg of vitamin E induced a significant increase in the plasma sodium level in rats treated before and after the onset of DM *p<0.05 (diab cont versus treated before DM) **p<0.05 (diab cont versus treated after DM). (Data are mean ± SD, n=6) DM= diabetes mellitus.

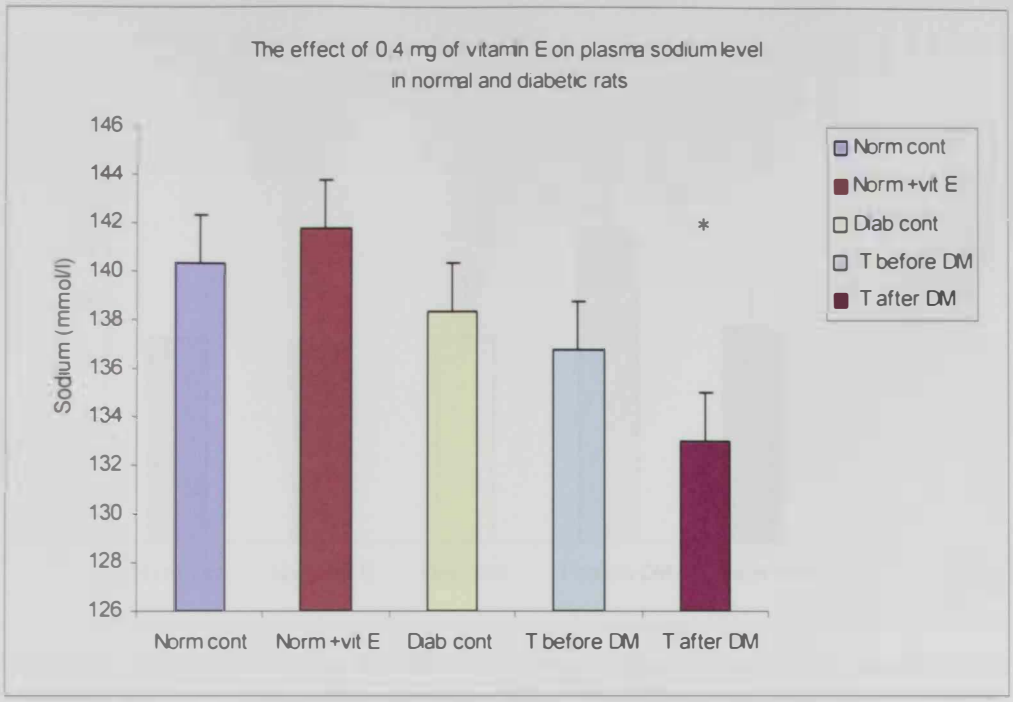


Figure 40: Histograms showing the effect of 0.4 mg of vitamin E on plasma sodium level of normal and diabetic rats. 0.4 mg of vitamin E induced a significant decrease in the plasma sodium level in rats treated after the onset of DM *$p<0.05$ (diab cont versus treated after DM). (Data are mean ± SD, n=6) DM= diabetes.

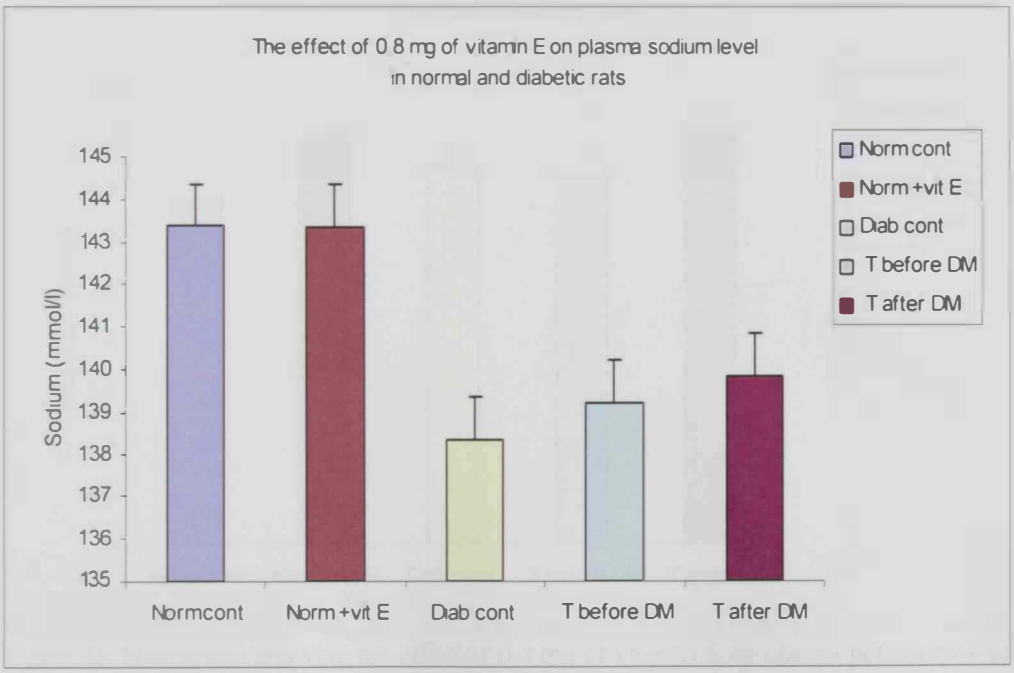


Figure 41: Histograms showing the effect of 0.8 mg of vitamin E on plasma sodium level of normal and diabetic rats. (Data are mean ± SD, n=6) DM= diabetes mellitus.

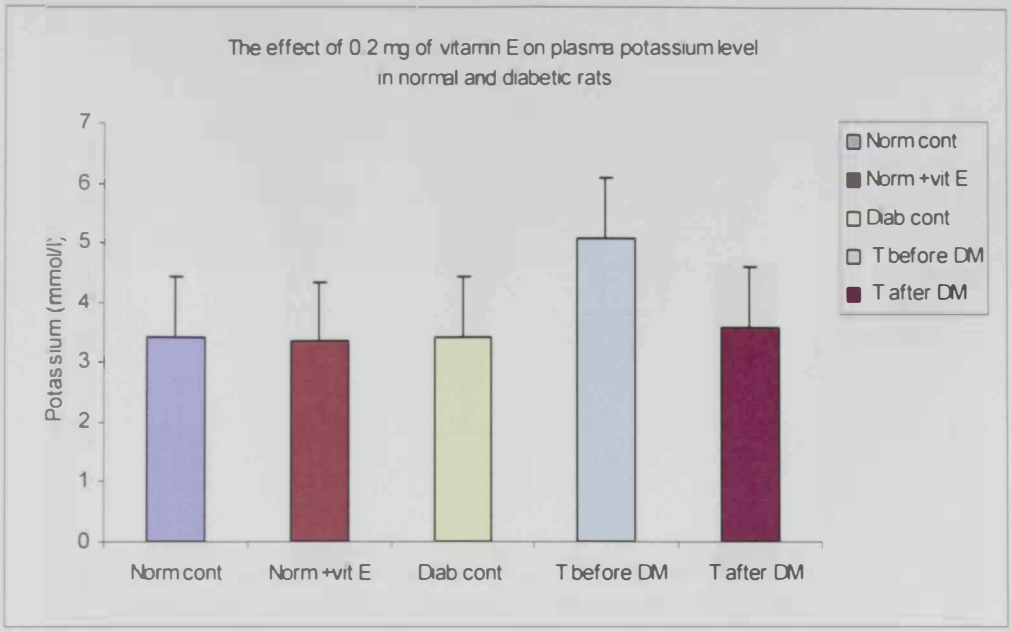


Figure 42: Histograms showing the effect of 0.2 mg of vitamin E on plasma potassium level of normal and diabetic rats. (Data are mean ± SD, n=6) DM= diabetes mellitus.

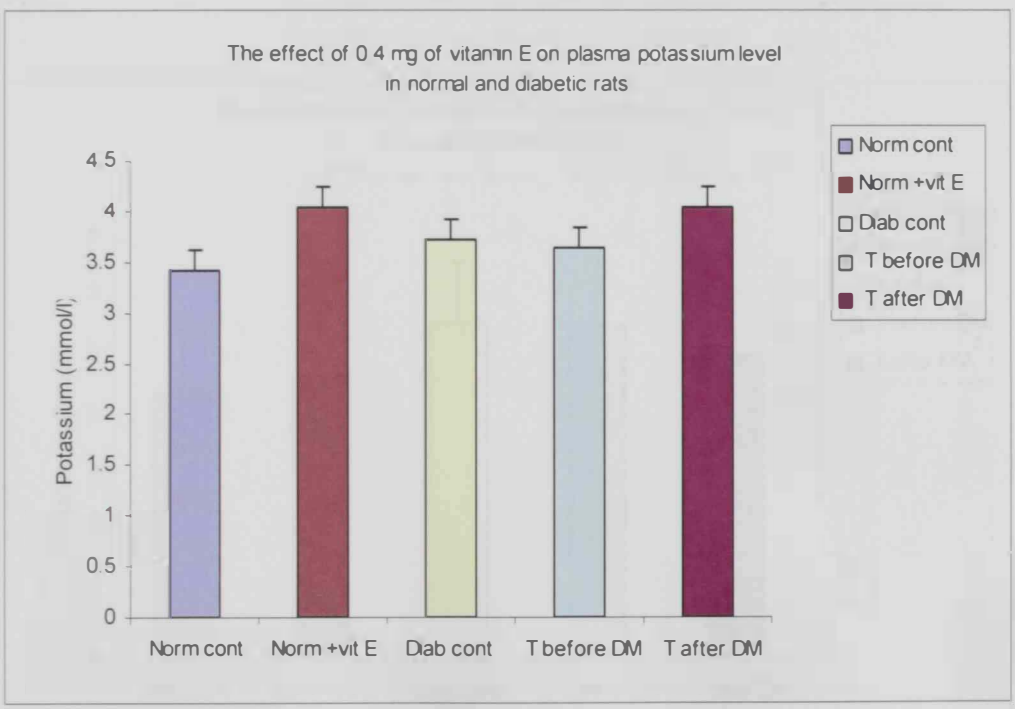


Figure 43: Histograms showing the effect of 0.4 mg of vitamin E on plasma potassium level of normal and diabetic rats. (Data are mean ± SD, n=6) DM= diabetes mellitus.

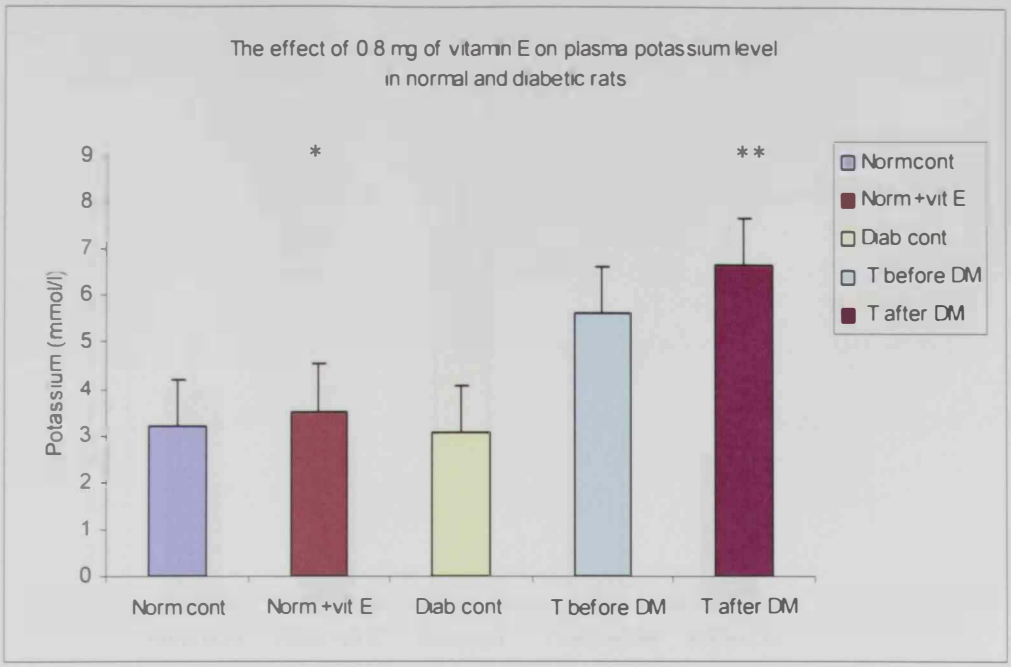


Figure 44: Histograms showing the effect of 0.8 mg of vitamin E on plasma potassium level of normal and diabetic rats. 0.8 mg of vitamin E induced a significant increase in the plasma potassium level in normal non diabetic rats and in rats treated after the onset of DM *p<0.05 (norm cont versus norm +vitamin E) **p<0.05 (diab cont versus treated after DM). (Data are mean ± SD, n=6) DM= diabetes mellitus.

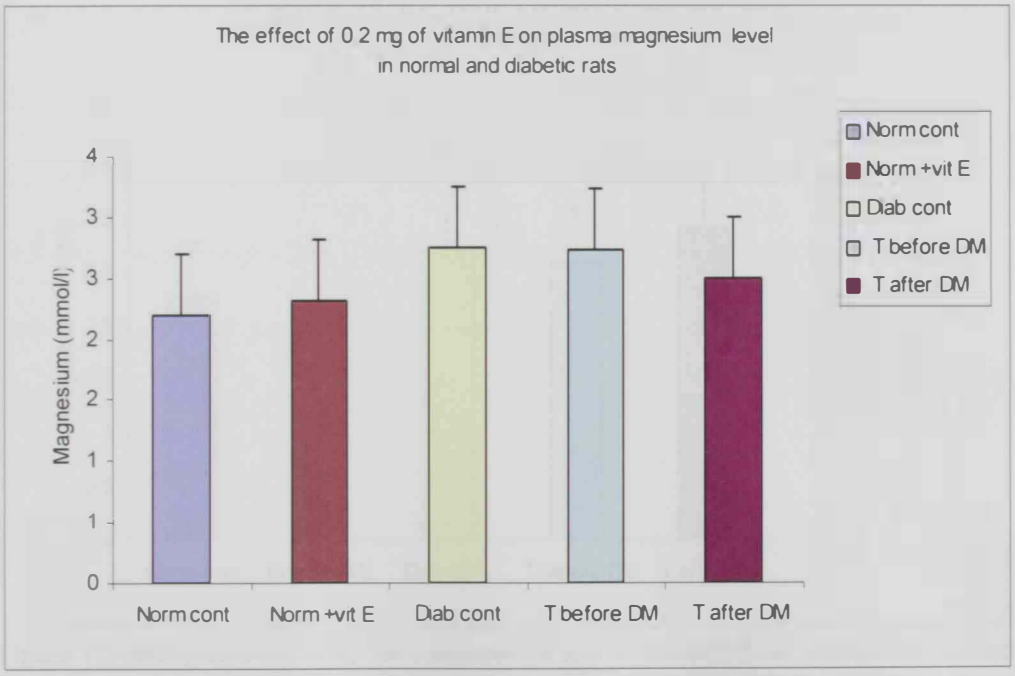


Figure 45: Histograms showing the effect of 0.2 mg of vitamin E on plasma magnesium level of normal and diabetic rats. (Data are mean ± SD, n=6) DM= diabetes mellitus.

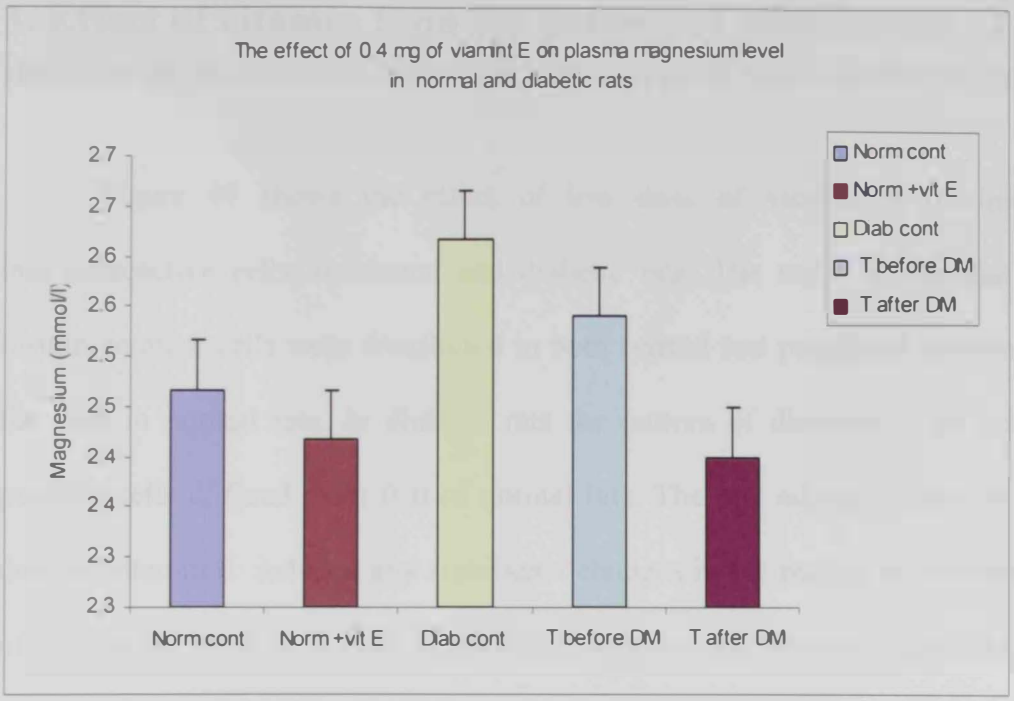


Figure 46: Histograms showing the effect of 0.4 mg of vitamin E on plasma magnesium level of normal and diabetic rats. (Data are mean ± SD, n=6) DM= diabetes mellitus.

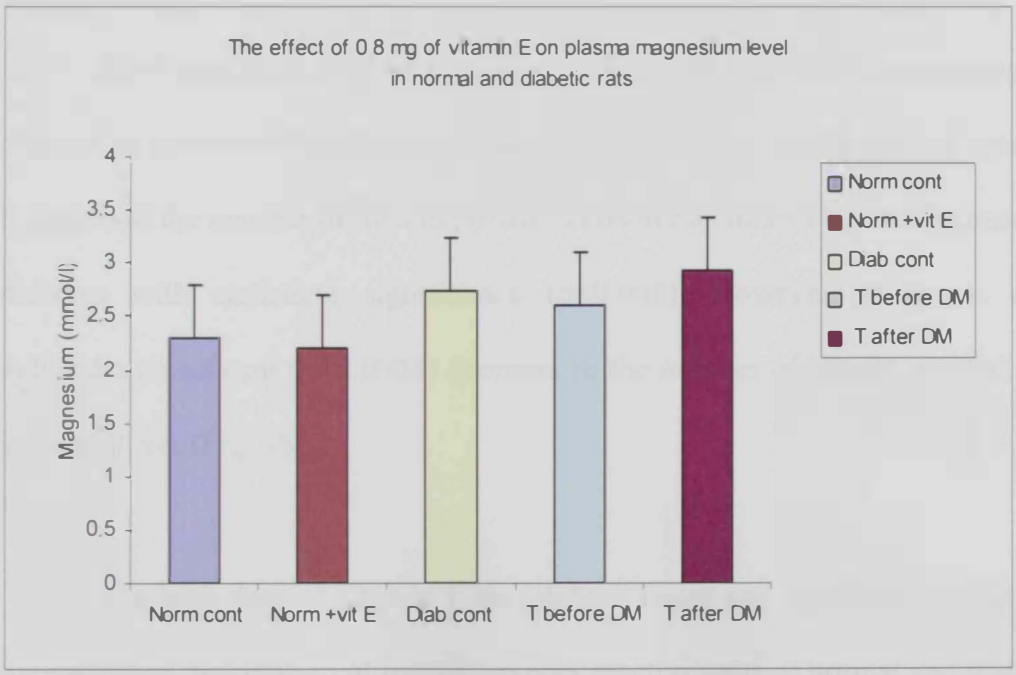


Figure 47: Histograms showing the effect of 0.8 mg of vitamin E on plasma magnesium level of normal and diabetic rats. (Data are mean ± SD, n=6) DM= diabetes mellitus.

3. Effect of vitamin E on the pattern of distribution of insulin immunoreactive cells in normal and diabetic rats.

Figure 49 shows the effect of low dose of vitamin E on insulin immunoreactive cells in normal and diabetic rats. The result shows that the insulin positive cells were distributed in both central and peripheral portions of the islet in normal rats. In diabetic rats the pattern of distribution of insulin positive cells differed from that of normal rats. The oral administration of low dose of vitamin E induced any significant changes in the pattern of distribution of the insulin positive cells in normal and diabetic rats. Moreover, low dose of vitamin E induced a significant ($p<0.002$) decrease in the number of positive insulin immunoreactive cells of normal rats (Fig. 48).

Also, moderate dose of vitamin E did caused significant morphological changes to pancreatic islets of diabetic rats (Fig. 50). Moderate dose of vitamin E increased the number of insulin positive cells in rats treated before the onset of diabetes with statistical significance ($p<0.008$). However, moderate dose induced a significant ($p<0.0008$) decrease in the number of insulin positive cell in normal rats (Fig. 48).

The high dose of vitamin E also did not cause any significant changes in the pattern of distribution of insulin immunoreactive cells of normal and diabetic rats (Fig. 51). Moreover, the high dose of vitamin E induced a significant increase in the number of insulin positive cells in rats treated before ($p<0.0027$) and after ($p<0.0023$) the onset of diabetes (Fig. 48).

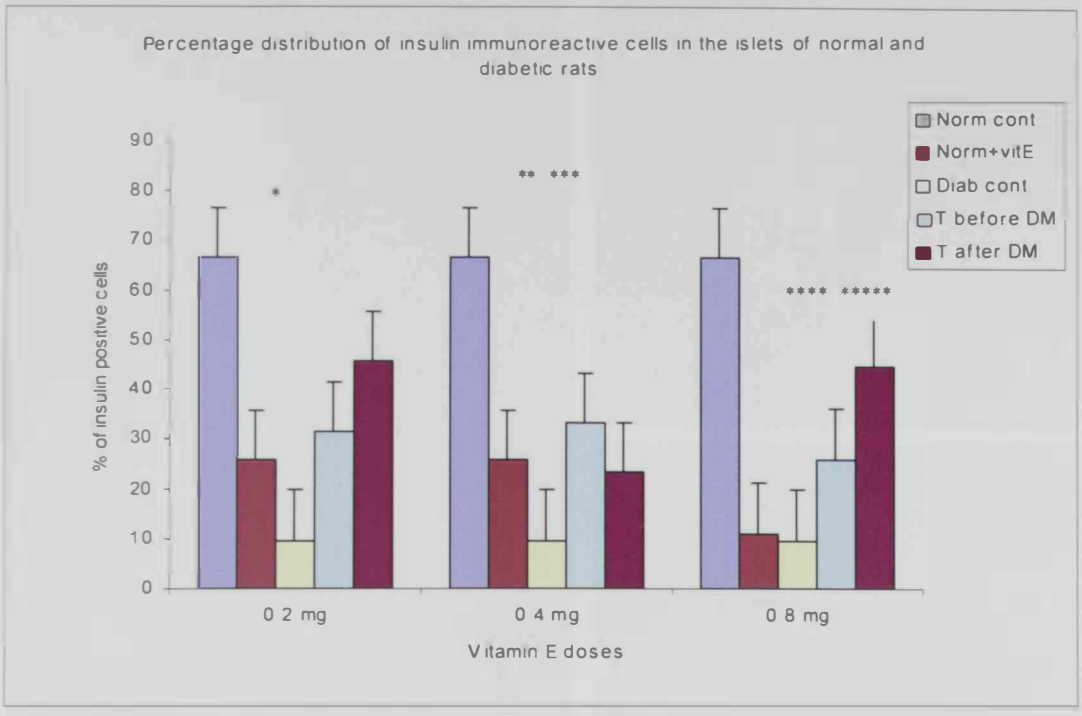


Figure 48: Histogram showing the effect of different doses of vitamin E on percentage distribution of insulin immunoreactive cells of normal and diabetic rats. Different doses vitamin E induced significant *p<0.002 (norm cont versus norm+ 0.2 mg of vitamin E), **p<0.0005 (norm cont versus norm+ 0.4 mg of vitamin E) decrease in the insulin positive cells of normal pancreas, and induced a significant ***p<0.008 (diab cont versus treated with 0.4 mg of vitamin E before DM) and ****p<0.027 (diab cont versus with 0.8 mg of vitamin E before DM), *****p<0.023 (diab cont versus with 0.8 mg of vitamin E after DM) increase in the percentage of insulin immunoreactive cells in diabetic rats when compared to control. (Data are mean ± SD, n=10). DM= diabetes mellitus.

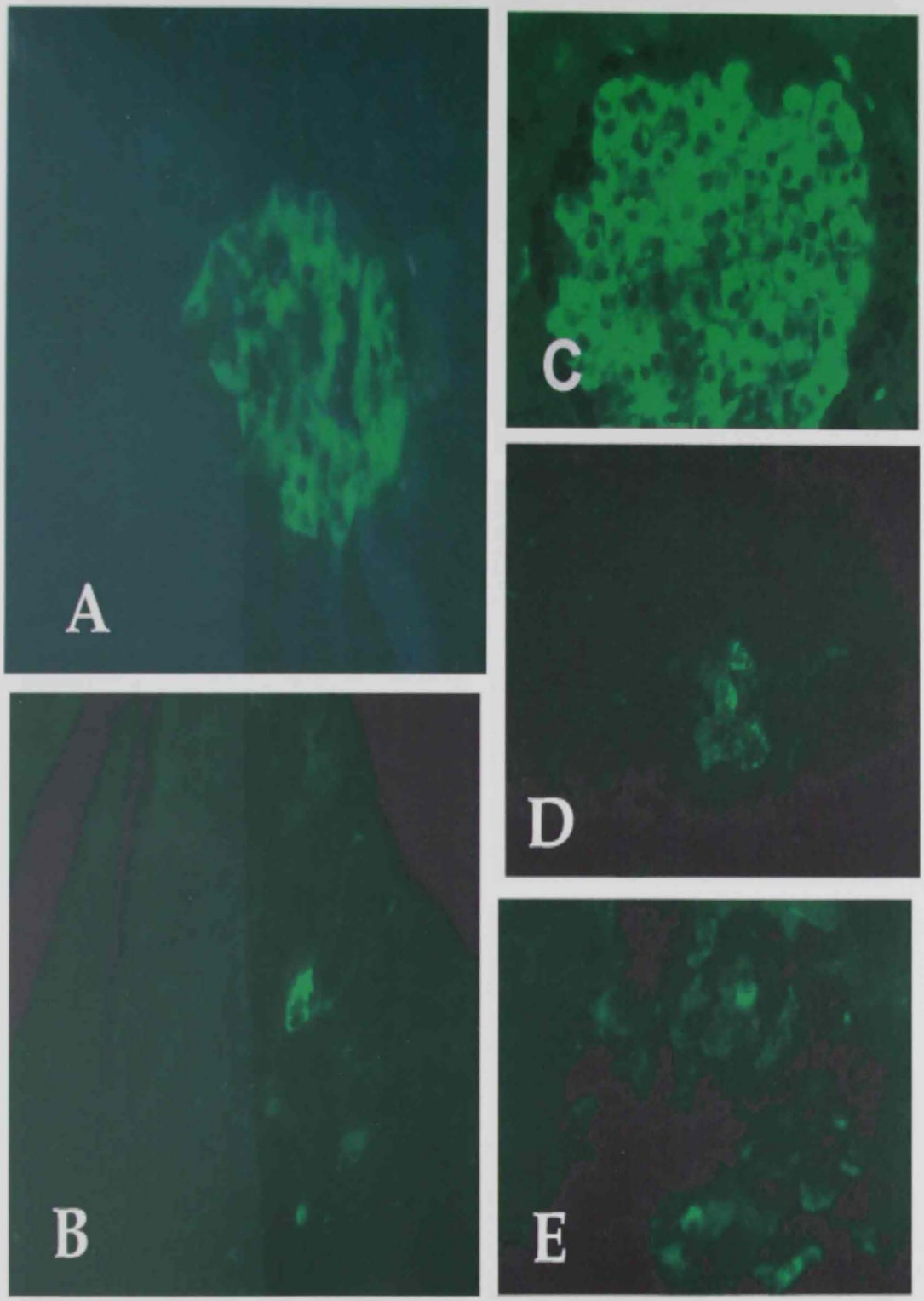


Figure 49: Representative micrographs showing insulin-positive cells in the islet of Langerhans of normal and diabetic rats. A = normal rat; B = untreated diabetic rat; C = normal rats treated with 0.2 mg of vitamin E; D = rats treated with 0.2 mg of vitamin E before the onset of diabetes and E = rats treated with 0.2 mg of vitamin E after the onset of diabetes. Magnification: X 400.

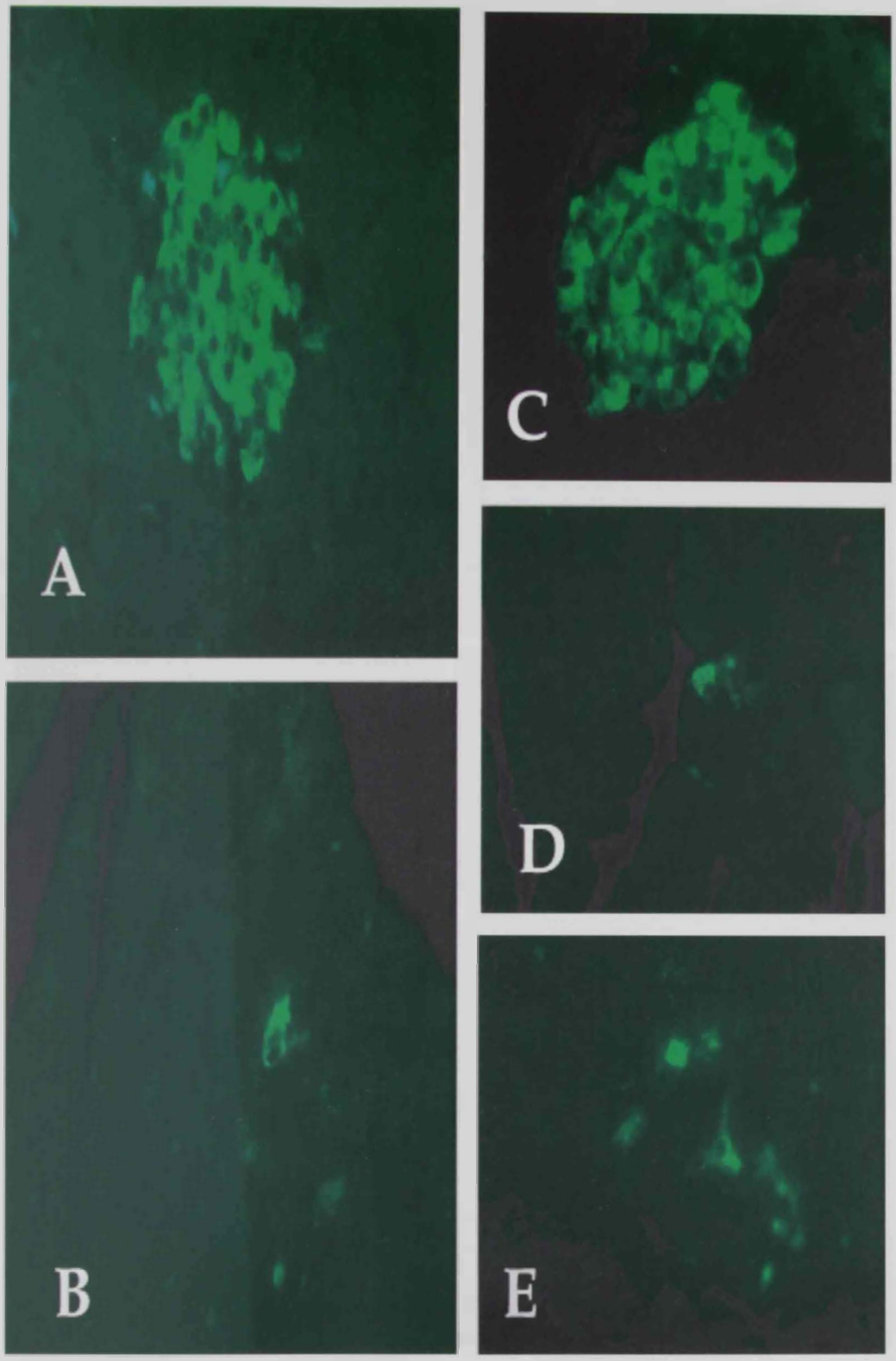


Figure 50: Representative micrographs showing insulin-positive cells in the islet of Langerhans of normal and diabetic rats. A = normal rat; B = untreated diabetic rat; C = normal rats treated with 0.4 mg of vitamin E; D = rats treated with 0.4 mg of vitamin E before the onset of diabetes and E = rats treated with 0.4 mg of vitamin E after the onset of diabetes. Magnification: X 400.

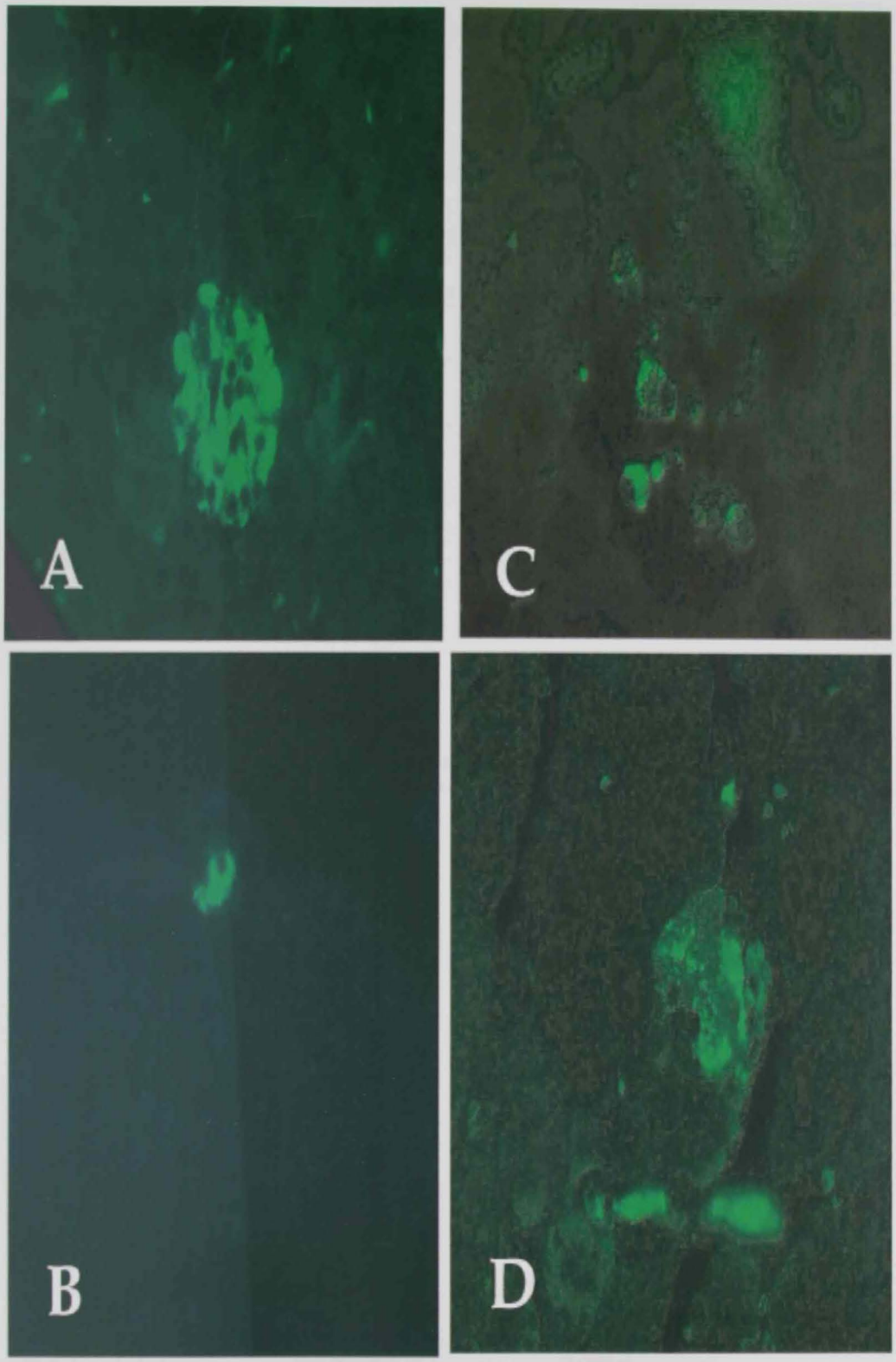


Figure 51: Representative micrographs showing insulin-positive cells in the islet of Langerhans of normal and diabetic rats. A = normal rat; B = untreated diabetic rat; C rats treated with 0.8 mg of vitamin E before the onset of diabetes and D= rats treated with 0.8 mg of vitamin E after the onset of diabetes. Magnification: X 400.

3.1 Effect of vitamin E on plasma insulin level of normal and diabetic rats

Figure 52 shows the effect of low dose (0.2 mg) of vitamin E on plasma insulin level in normal and diabetic rats. No change was observed in plasma insulin level of normal rats compared with normal untreated rats. Similarly, there was no significant difference in insulin level of rats given 0.2 mg of vitamin E before the onset of diabetes. The level of insulin was reduced in diabetic rats treated after the onset of diabetes when compared with untreated diabetes rats.

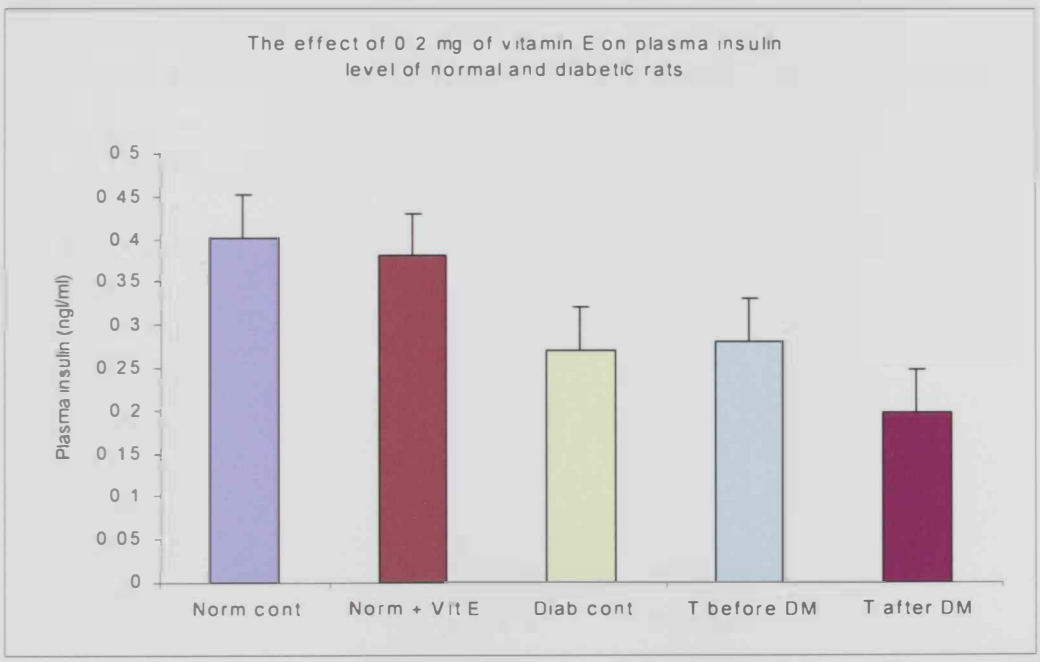


Figure 52: Histograms showing the effect of 0.2 mg of vitamin E on plasma insulin level of normal and diabetic rats. (Data are mean ± SD, n=6) DM= diabetes mellitus.

As shown in figure 53, the plasma insulin level was highly increased in normal rats treated with moderate dose (0.4 mg) of vitamin E when compared to normal control. However, the increase in the plasma insulin level was not statistically significant. The moderate dose did not induce any significant increase in plasma insulin level.

Also, the high dose (0.8 mg) of vitamin E caused an increase in the plasma insulin level in normal rats. However, this increase was not statistically different compared to control (Fig. 54). In diabetic rats, the high dose induced a significant decrease in the plasma insulin level in rats treated before the onset of diabetes. However, the high dose had no significant effect on plasma insulin level in rats treated after the onset of diabetes when compared to diabetic controls.

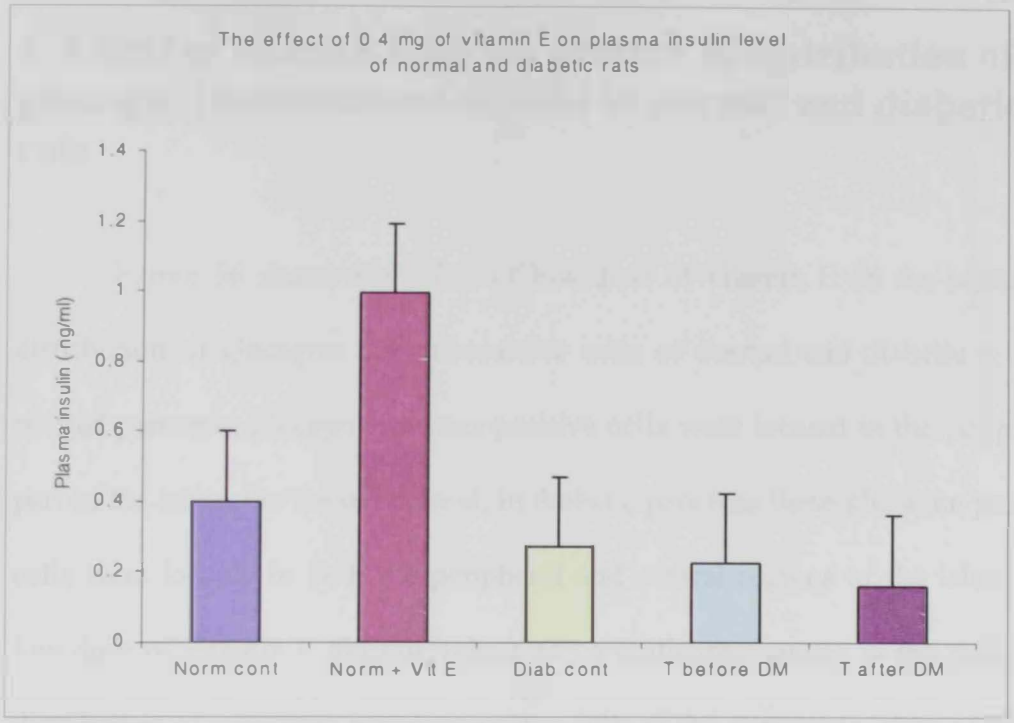


Figure 53: Histograms showing the effect of 0.4 mg of vitamin E on plasma insulin level of normal and diabetic rats. (Data are mean ± SD, n=6) DM= diabetes mellitus.

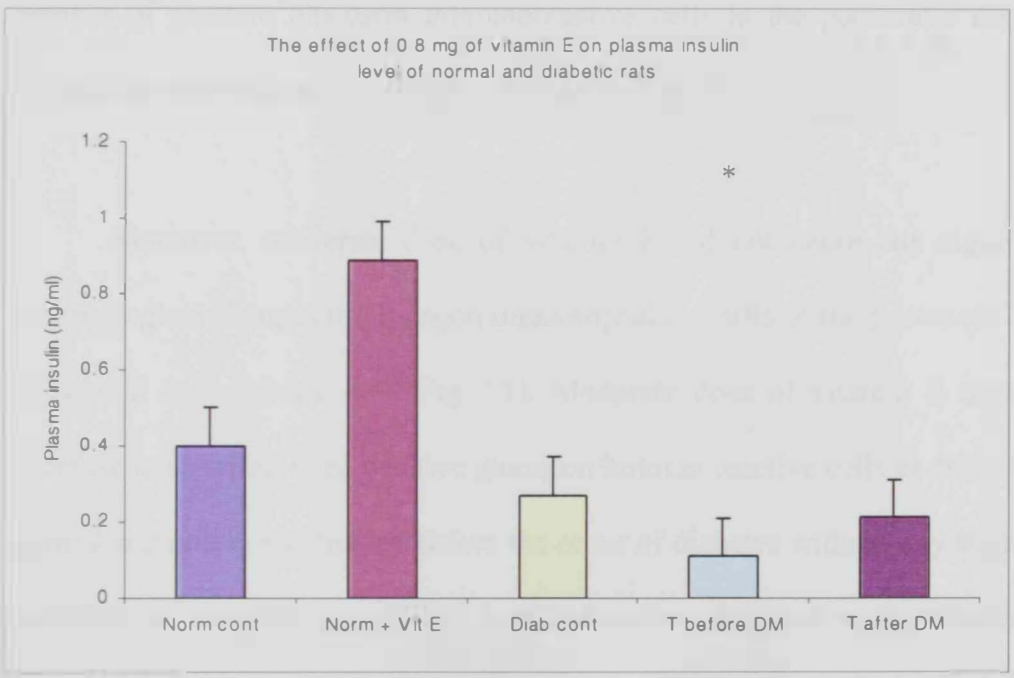


Figure 54: Histograms showing the effect of 0.8 mg of vitamin E on plasma insulin level of normal and diabetic rats. 0.8 mg of vitamin E induced a significant decrease in the plasma insulin level in rats treated 10 days before the onset of DM *$p<0.05$ (diab cont versus treated before DM). (Data are mean ± SD, n=6) DM= diabetes mellitus.

4. Effect of vitamin E on the pattern of distribution of glucagon immunoreactive cells of normal and diabetic rats

Figure 56 shows the effect of low dose of vitamin E on the pattern of distribution of glucagon immunoreactive cells of normal and diabetic rats. In normal pancreas, glucagon immunopositive cells were located in the peripheral part of the islets. On the other hand, in diabetic pancreas these glucagon positive cells were located in both the peripheral and central regions of the islets. The low dose of vitamin E did not induce any significant changes in the pattern of distribution of glucagon immunopositive cells of the pancreatic islets of normal and diabetic rats. Moreover, low dose of vitamin E induced an increase in the number of positive glucagon immunoreactive cells in the pancreatic islets of normal rats but with no statistical significance (Fig. 55).

Moreover, moderate dose of vitamin E did not cause any significant morphological changes to glucagon immunopositive cells of the pancreatic islets of normal and diabetic rats (Fig. 57). Moderate dose of vitamin E caused a decrease in the number of positive glucagon immunoreactive cells in the islets of normal rats and in rats treated before the onset of diabetes without any statistical significance. However, moderate dose induced an increase in the number of glucagon positive cells in the islets of rats treated 10 days after the onset of diabetes (Fig. 55).

The high dose of vitamin E also did not cause any significant changes in the pattern of distribution of glucagon immunoreactive cells of normal and diabetic rats (Fig. 58). Moreover, the high dose of vitamin E induced an increase in the number of glucagon positive cells in the islets of normal rats and a decrease in the number of glucagon immunopositive cells in the islets of rats treated after the onset of diabetes when compared to control (Fig. 55).

Figure 55: Histogram showing the effect of different doses of vitamin E on percentage distribution of glucagon immunoreactive cells of normal and diabetic rats. (Data are mean ± SD, n=10). DM= diabetes mellitus. Vitamin E induced no significant change in the insulin positive cells in the islets of normal and diabetic rats

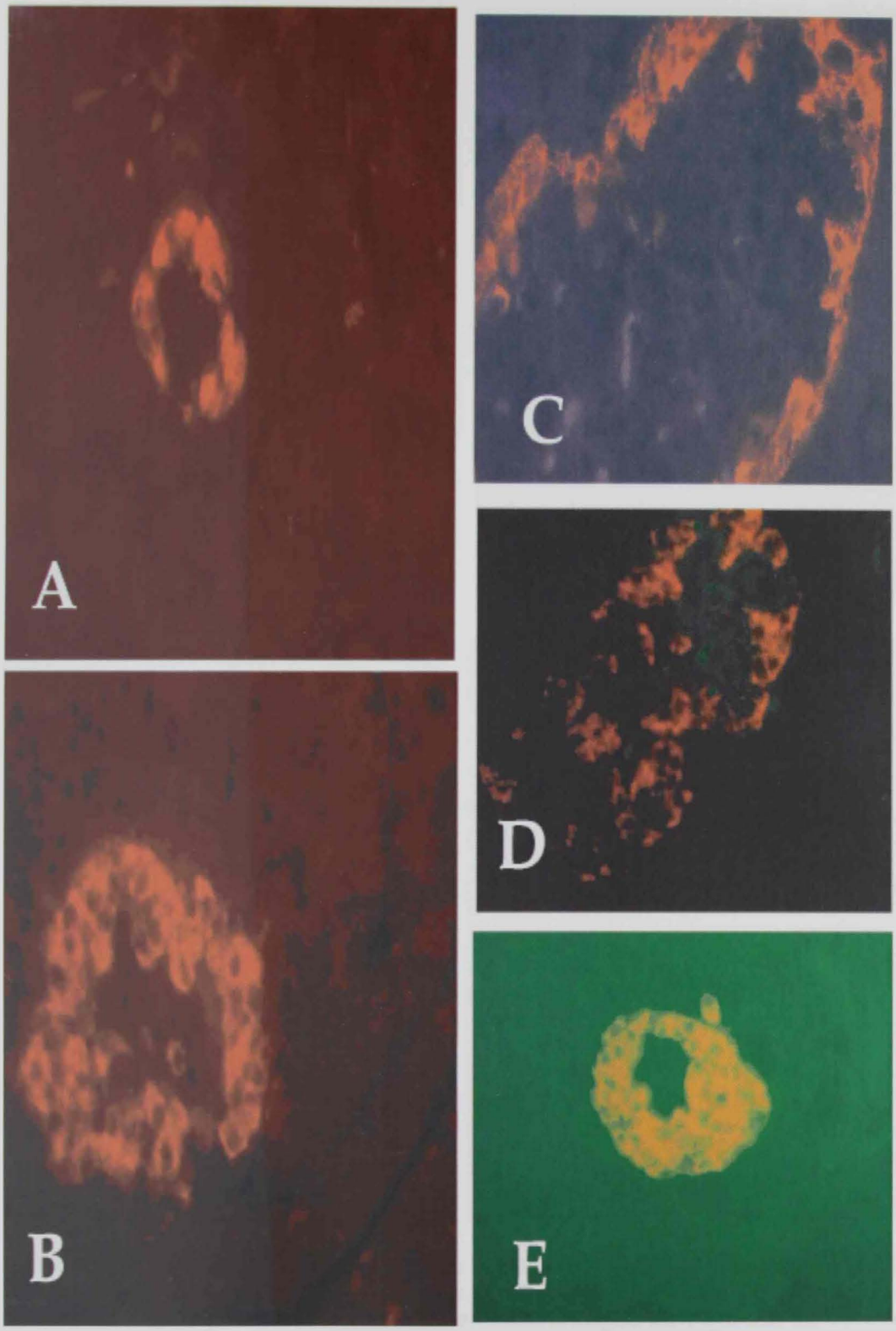


Figure 56: Representative micrographs showing glucagon-positive cells in the islet of Langerhans of normal and diabetic rats. A = normal rat; B = untreated diabetic rat; C = normal rats treated with 0.2 mg of vitamin E; D = rats treated with 0.2 mg of vitamin E before the onset of diabetes and E = rats treated with 0.2 mg of vitamin E after the onset of diabetes. Magnification: X 400.

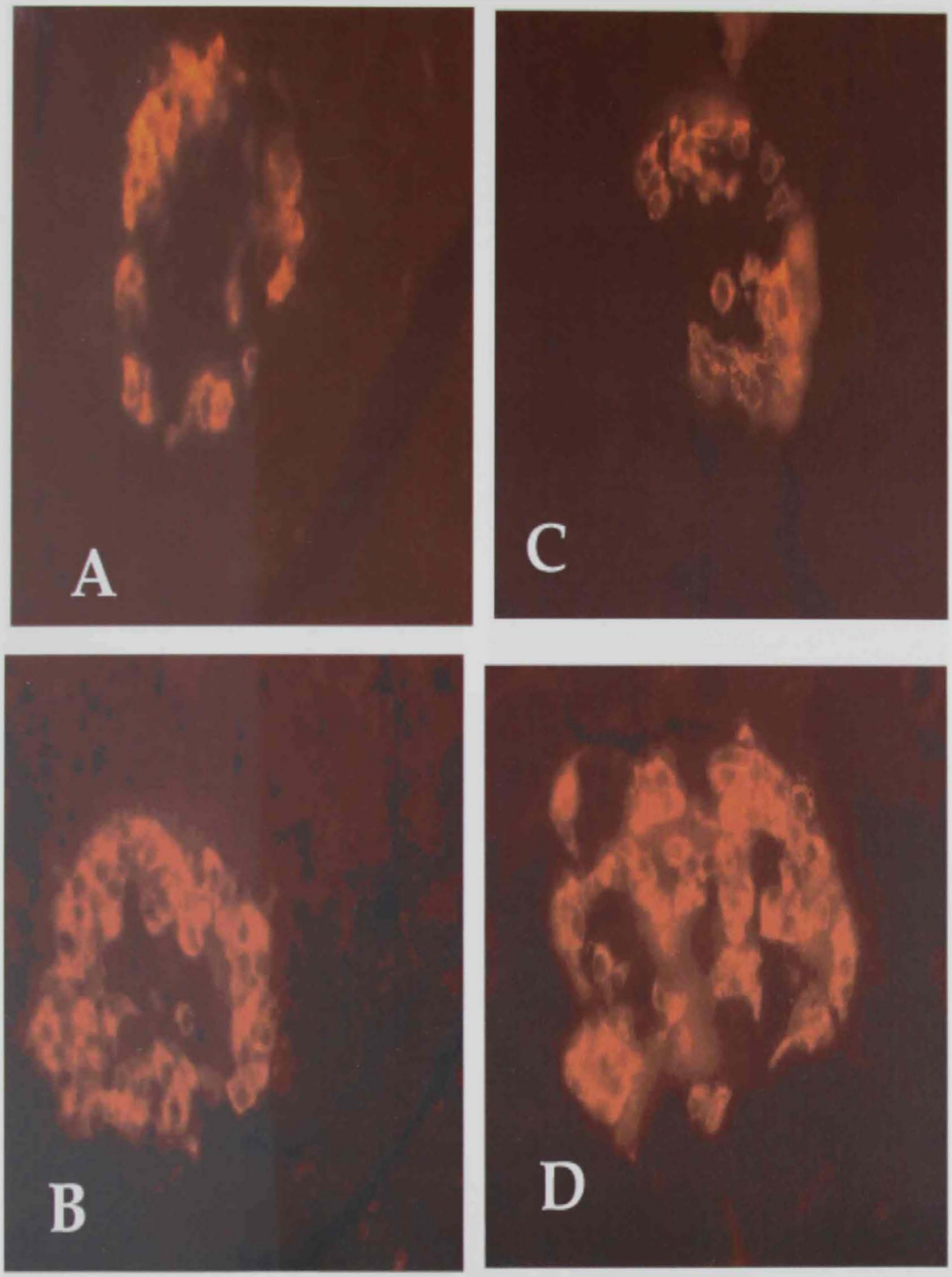


Figure 57: Representative micrographs showing glucagon-positive cells in the islet of Langerhans of normal and diabetic rats. A = normal rat; B = untreated diabetic rat; C = normal rats treated with 0.4 mg of vitamin E; D = rats treated with 0.4 mg of vitamin E after the onset of diabetes. Magnification: X 400.

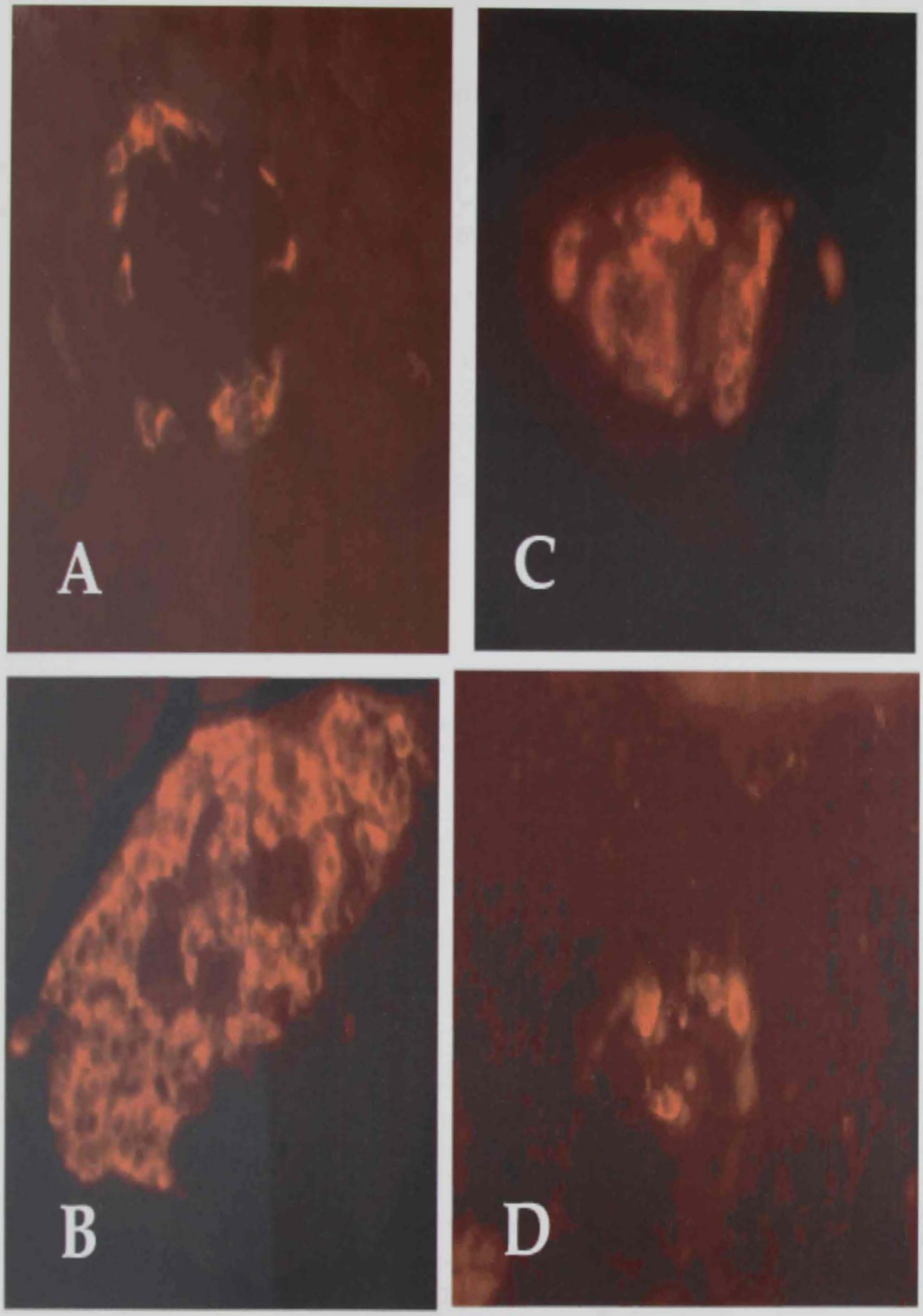


Figure 58: Representative micrographs showing glucagon-positive cells in the islet of Langerhans of normal and diabetic rats. A = normal rat; B = untreated diabetic rat; C = rats treated with 0.8 mg of vitamin E before the onset of diabetes and D = rats treated with 0.8 mg of vitamin E after the onset of diabetes. Magnification: X 400.

4.1 Effect of vitamin E on plasma glucagon level of normal and diabetic rats.

Figure 59 shows the effect of low dose (0.2 mg) of vitamin E on plasma glucagon level of normal and diabetic rats. In normal rats, low dose of vitamin E had no significant effect compared with normal untreated rats. Low dose of vitamin E reduced the plasma glucagon level in rats treated before and after the onset of diabetes without any statistical significance when compared to untreated diabetic rats.

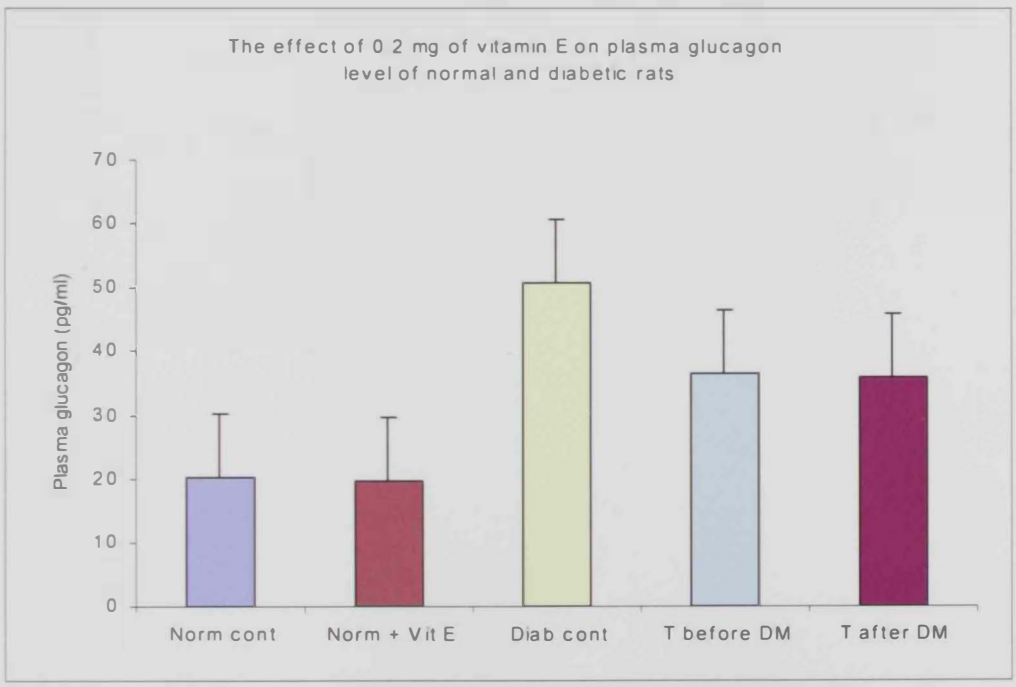


Figure 59: Histograms showing the effect of 0.2 mg of vitamin E on plasma glucagon level of normal and diabetic rats. (Data are mean ± SD, n=6) DM= diabetes mellitus.

As shown in figure 60, the moderate dose (0.4 mg) of vitamin E increased the plasma glucagon level in normal rats without any statistical significance. On the other hand, the moderate dose significantly ($p<0.0073$) reduced the plasma glucagon level in rats treated before the onset of diabetes.

Furthermore, the level of glucagon was increased in normal rats given a high dose (0.8mg) of vitamin E compared to untreated normal rats (Fig. 61). On the other hand, the level of plasma glucagon level was significantly decreased ($p<0.02$) in rats treated before the onset of diabetes while the plasma glucagon level decreased in rats treated after the onset of diabetes without any statistical significance.

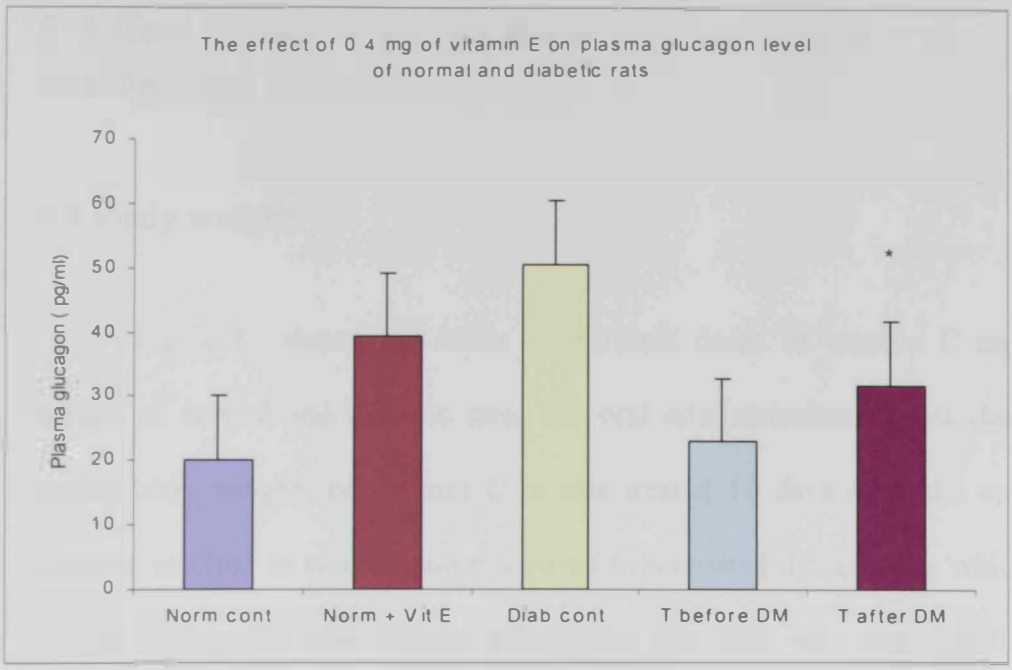


Figure 60: Histograms showing the effect of 0.4 mg of vitamin E on plasma glucagon level of normal and diabetic rats. 0.4 mg of vitamin E induced a significant decrease in the plasma glucagon level in rats treated after the onset of DM *p<0.05 (diab cont versus treated after DM). (Data are mean ± SD, n=6) DM= diabetes mellitus.

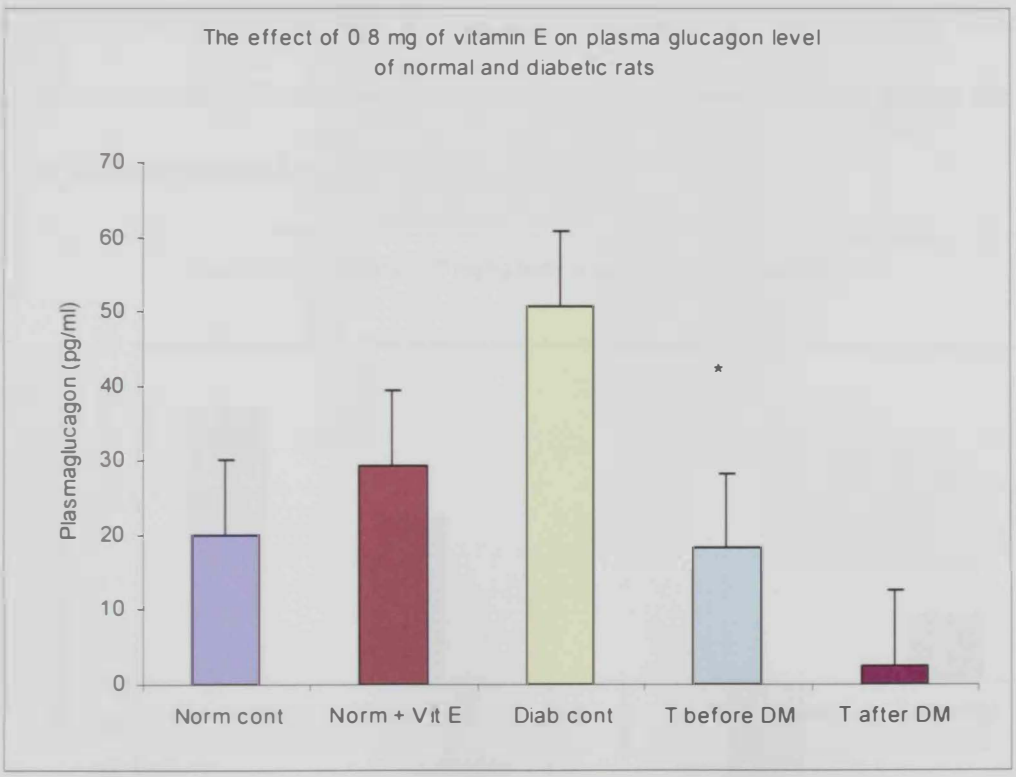


Figure 61: Histograms showing the effect of 0.8 mg of vitamin E on plasma glucagon level of normal and diabetic rats. 0.8 mg of vitamin E induced a significant decrease in the plasma sodium level in treated before the onset of DM *p<0.05 (diab cont versus treated before DM). (Data are mean ± SD, n=6) DM= diabetes mellitus.

5. Effect of vitamin C on metabolic parameters of normal and diabetic rats

5.1 Body weight

Figure 62 shows the effect of different doses of vitamin C on body weight of normal and diabetic rats. The oral administration of low dose (10 mg/kg body weight) of vitamin C to rats treated 10 days after the onset of diabetes resulted in weight gain compared to untreated diabetic rats which lost weight during the experimental period but this gain was still significantly smaller compared to that of normal untreated and normal treated rats. Also, the weight gained by normal treated rats with low dose of vitamin C was still smaller compared to that of untreated normal rats. On the other hand, the administration of low dose of vitamin C to rats treated 10 days before the onset of diabetes resulted in high weight loss.

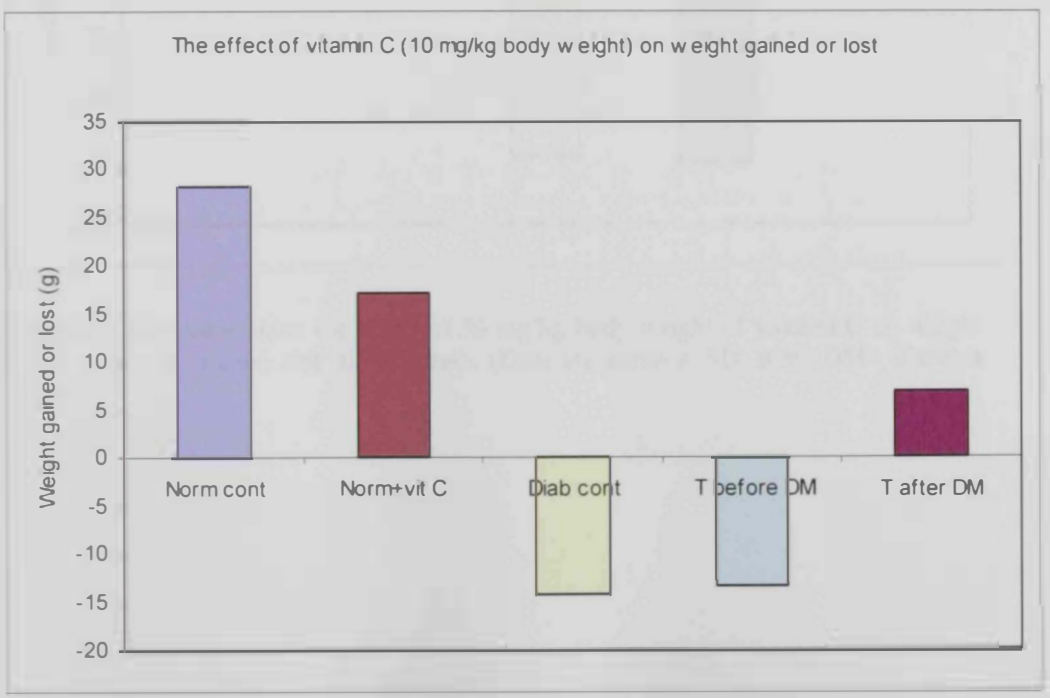


Figure 62: Histograms showing the effect of 10 mg/kg body weight of vitamin C on weight gained or lost of normal and diabetic rats. (Data are mean ± SD, n=6) DM= diabetes mellitus.

Rat receiving a moderate dose (50 mg/kg body weight) of vitamin C 10 days after the onset of diabetes achieved lower weight gain compared to normal control. However, oral administration of moderate dose (50 mg/kg body weight) of vitamin C failed to increase the weight of normal rats compared to control Fig. 63.

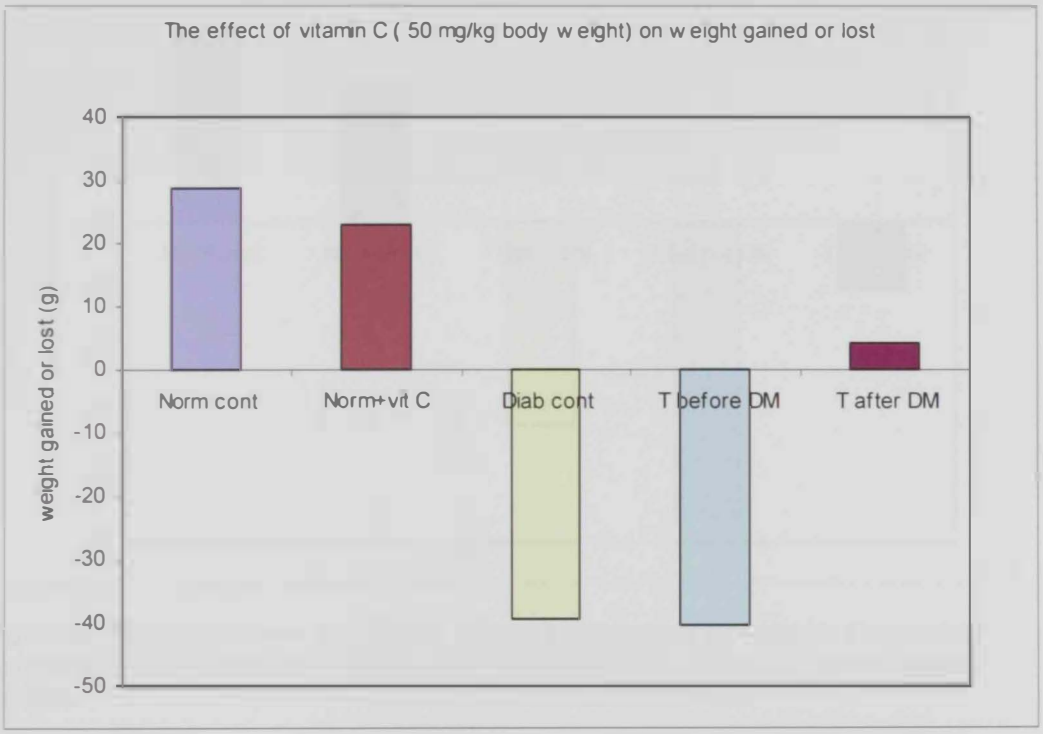


Figure 63: Histograms show the effect of 50 mg/kg body weight of vitamin C on weight gained or lost in normal and diabetic rats. (Data are mean ± SD, n=6) DM= diabetes mellitus.

The high dose (100mg/kg body weight) of vitamin C given to diabetic rats also resulted in weight loss in both rats treated before and after the onset of diabetes. Normal rats, which received high dose of vitamin C failed to gain weight in the same rate as untreated normal rats (Fig. 64).

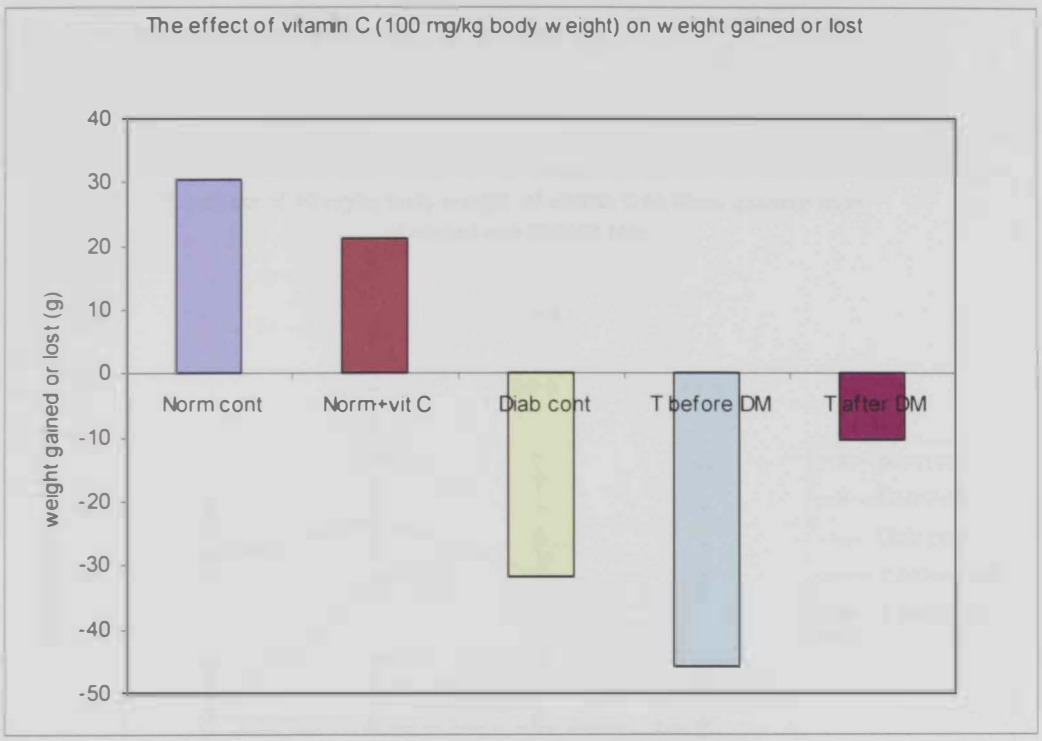


Figure 64: Histograms show the effect of 100 mg/kg body weight of vitamin C on weight gained or lost in normal and diabetic rats. (Data are mean ± SD, n=6) DM= diabetes mellitus.

5.2 Blood glucose level

Figure 65 shows that the oral administration of low dose (10 mg/kg body weight) of vitamin C reduced the level of blood glucose in rats treated before and after the onset of diabetes. Moreover, normal rats treated with low dose (10 mg/kg body weight) of vitamin C had a significant decrease in blood glucose level when compared to normal untreated rats.

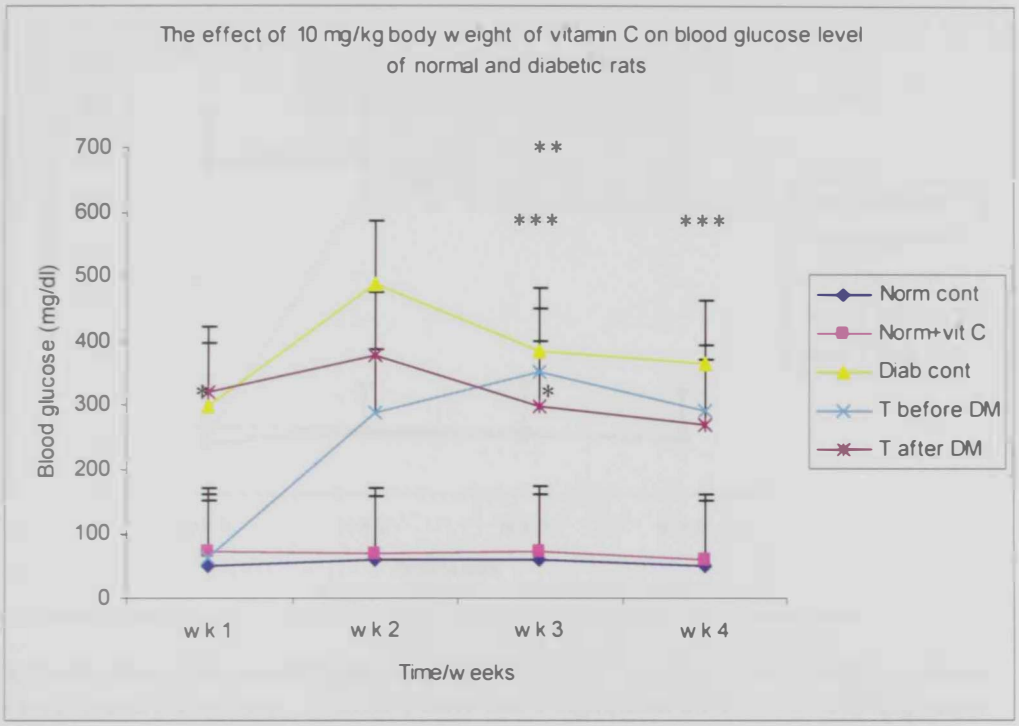


Figure 65: Histograms showing the effect of 10 mg/kg of vitamin C on blood glucose level of normal and diabetic rats. In weeks 3 and 4 there was a significant difference in blood glucose level in normal non diabetic rats *$p<0.05$ (norm cont versus norm + vitamin C). Also, there was a significant difference in blood glucose level in rats treated before the onset of DM rats in week 3 **$p<0.05$ (diab cont versus treated before DM) and in rats reated after the onset of DM in weeks 3 and 4 ***$p<0.05$ (diab cont versus treated after DM) (Data are mean ± SD, n=6) DM= diabetes mellitus.

The blood glucose level was slightly unchanged in diabetic rats treated with moderate dose (50mg/kg body weight) of vitamin C after the onset of diabetes compared with untreated diabetic rats. On the other hand, the level of blood glucose was significantly reduced compared with untreated normal rats (Fig. 66).

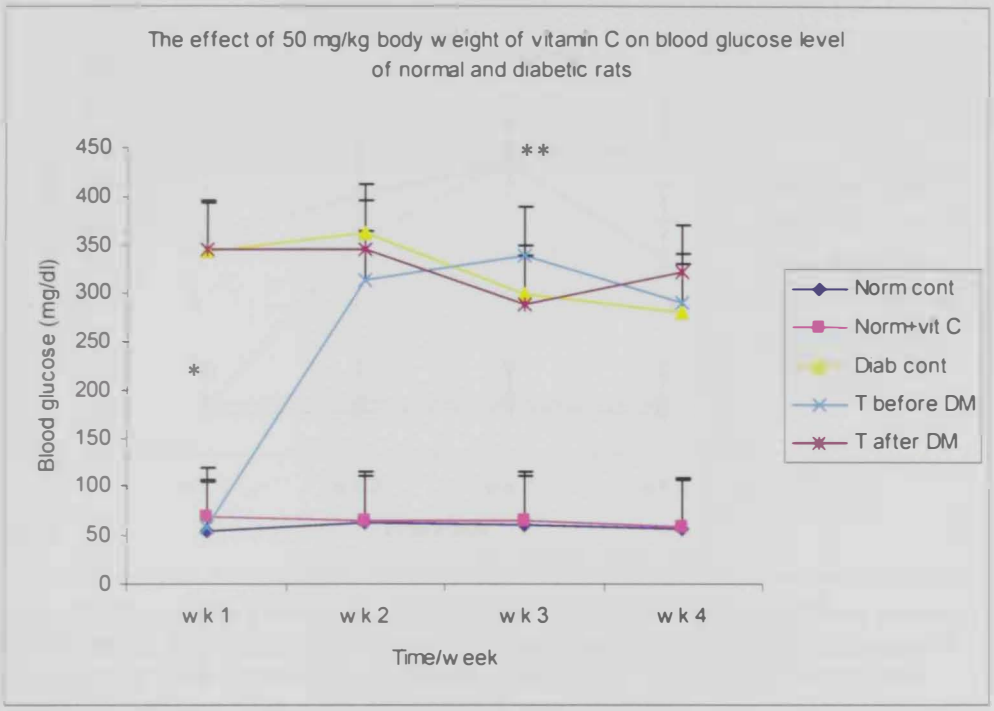


Figure 66: Histograms showing the effect of 50 mg/kg of vitamin C on blood glucose level of normal and diabetic rats. In week 1 there was a significant difference in blood glucose level in normal non diabetic rats *$p<0.05$ (norm cont versus norm + vitamin C). Also, there was a significant difference in blood glucose level in rats treated after the onset of DM rats in week 3 **$p<0.05$ (diab cont versus treated after DM) (Data are mean ± SD, n=6) DM= diabetes mellitus

Figure 67 shows that the oral administration of high dose (100 mg/kg body weight) of vitamin C failed to reduce blood glucose level in rats treated before and after the onset the onset of diabetes.

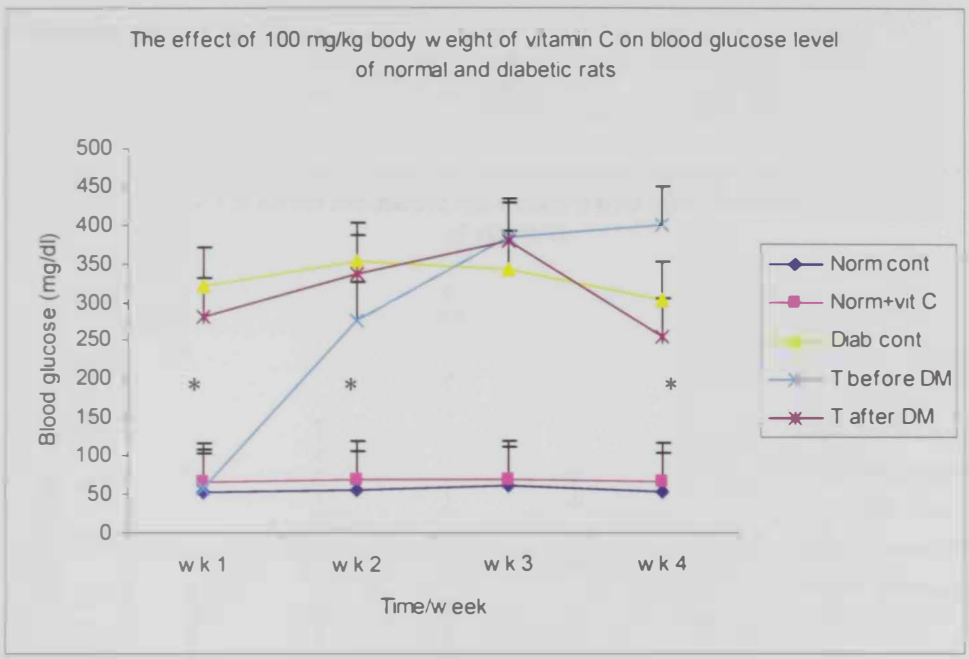


Figure 67: Histograms showing the effect of 100 mg/kg of vitamin C on blood glucose level of normal and diabetic rats. In week 1, 2 and 4 there was a significant difference in blood glucose level in normal non diabetic rats *$p<0.05$ (norm cont versus norm + vitamin C). (Data are mean ± SD, n=6) DM= diabetes mellitus.

5.3 Oral glucose tolerance test (OGTT)

The OGTT of rats that received vitamin C (10 mg/kg body weight) after the onset of diabetes was better than that of untreated diabetic rats. However, rats that received low dose of vitamin C before and after the onset of diabetes had a better OGTT at 60 min after the glucose load compared to untreated diabetic rats (Fig. 68).

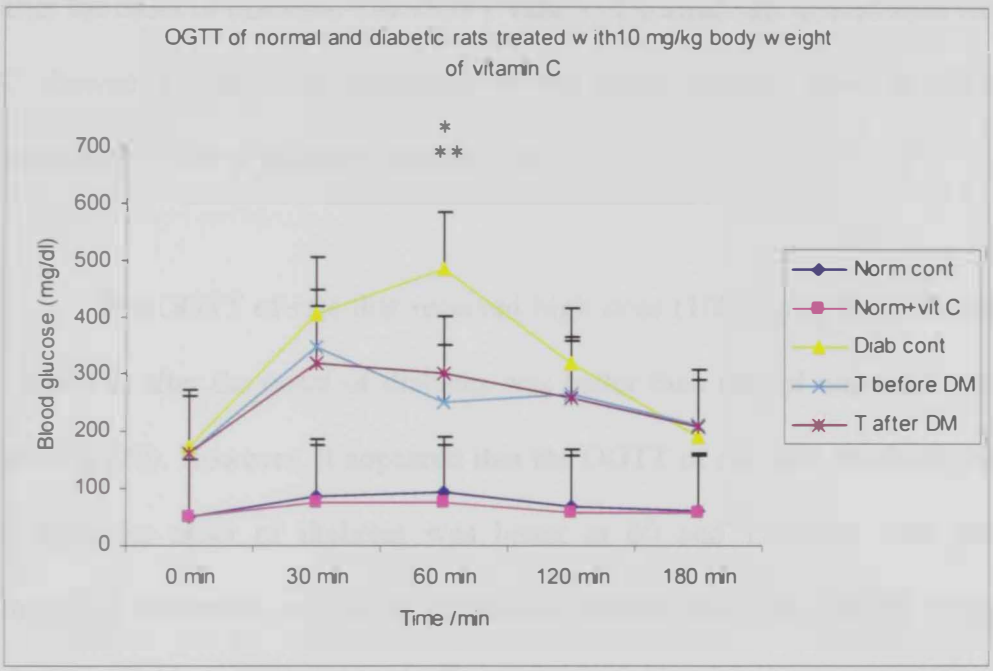


Figure 68: Histograms showing the effect of 10 mg/kg of vitamin C on OGTT of normal and diabetic rats. At 60 min there was a significant difference in blood glucose level in rats treated after the onset of DM *p<0.05 (diab cont versus treated after DM) and in rats treated before the onset of DM **p<0.05 (diab cont versus treated before DM). (Data are mean ± SD, n=6) DM= diabetes mellitus.

The OGTT of rats that received vitamin C (50 mg/kg body weight) before the onset of diabetes was better than that of untreated diabetic rats (Fig. 69). Moreover, the OGTT of rats that received vitamin C before the onset of diabetes showed a significant reduction in the blood glucose level at 120 min after glucose challenge compared to untreated diabetic rats. However, it appeared that the OGTT of rats that received vitamin C (50 mg/kg body weight) before the onset of diabetes was better than that of rats that received vitamin C after the onset of diabetes. The OGTT values of normal rats treated with vitamin C showed a significant reduction in the blood glucose level at 120 min compared to that of untreated diabetic rats.

The OGTT of rats that received high dose (100 mg/kg body weight) of vitamin C after the onset of diabetes was better than that of untreated diabetic rats Fig (70). However, it appeared that the OGTT of rats that received vitamin C after the onset of diabetes was better at 60 and 120 min after glucose challenge compared to that of untreated diabetic rats. The OGTT values of normal rats treated with vitamin C was comparable to that of untreated normal rats.

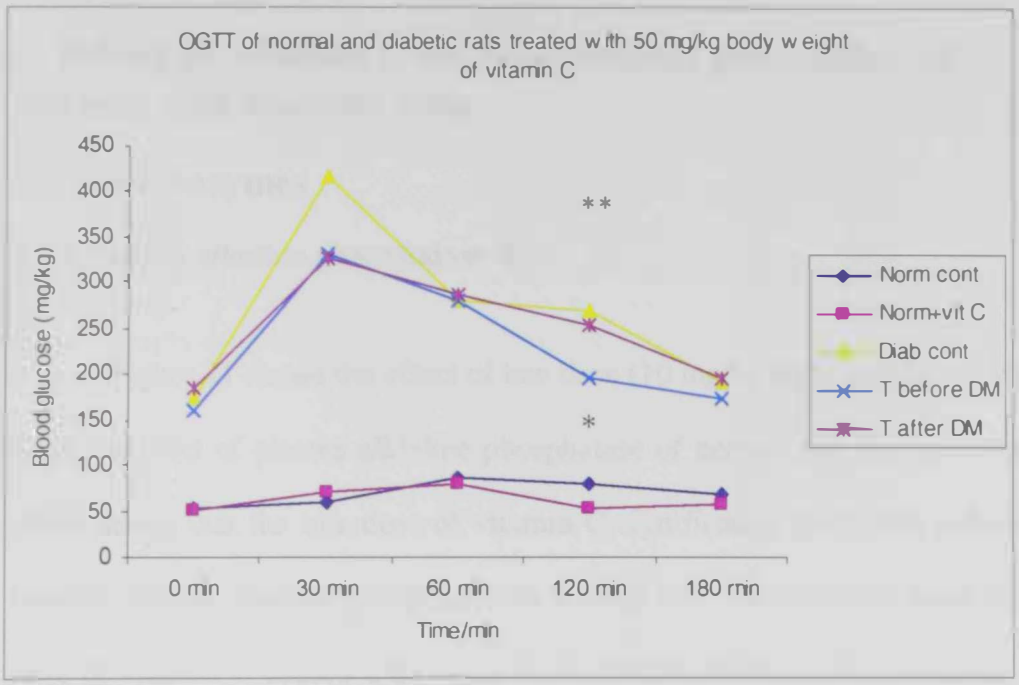


Figure 69. Histograms showing the effect of 50 mg/kg of vitamin C on OGTT of normal and diabetic rats. At 120 min there was a significant difference in blood glucose level in rats treated before the onset of DM rats *p<0.05 (diab cont versus treated before DM) and in normal non diabetic rats **p<0.05 (norm cont versus norm+ vitamin C). (Data are mean ± SD, n=6) DM= diabetes mellitus.

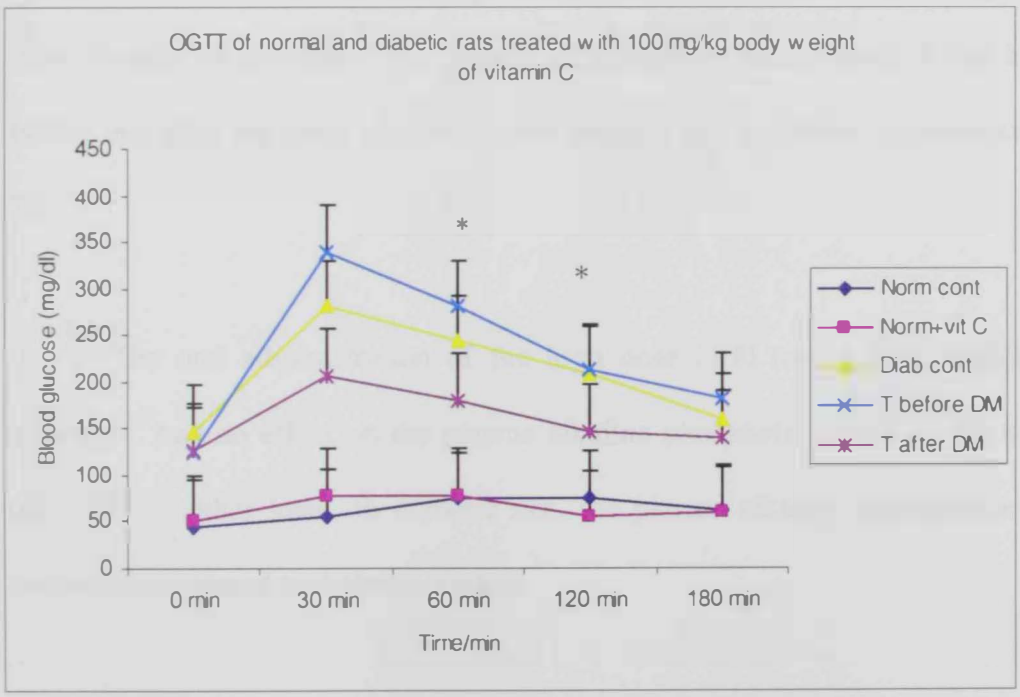


Figure 70: Histograms showing the effect of 100 mg/kg of vitamin C on OGTT of normal and diabetic rats. At 60 and 120 min there was a significant difference in blood glucose level in normal non diabetic rats *p<0.05 (norm cont versus norm+ vitamin C). (Data are mean ± SD, n=6) DM= diabetes mellitus.

6. Effect of vitamin C on biochemical parameter of normal and diabetic rats

6.1 Liver enzymes

6.1.1 Plasma alkaline phosphatase level

Figure 71 shows the effect of low dose (10 mg/kg body weight) of vitamin C on the level of plasma alklaline phosphatase of normal and diabetic rats. The result shows that the low dose of vitamin C significantly ($p<0.004$) reduced the level of plasma alkaline phosphatase in normal rats. On the other hand, the low dose of vitamin C caused a decrease in the plasma alkaline phosphatase level of rats treated before the onset of diabetes.

The moderate dose (50mg/kg body weight) of vitamin C had no effect on the plasma alkaline phosphatase level of normal rats. Moreover, the moderate dose brought an increase in the plasma alkaline phosphatase level of rats treated before and after the onset of diabetes but without any statistical significance (Fig 72).

The oral administration of the high dose (100 mg/kg body weight) of vitamin C had no effect on the plasma alkaline phosphatase level of the normal rats. On the other hand, in diabetic rats, the plasma alkaline phosphatase level increased compared to diabetic control.

The oral administration of the high dose of vitamin C can elicit a significant ($p<0.005$) increase in the plasma alkaline phosphatase level in rats treated before the onset of diabetes. While after administration of high dose, the plasma alkaline phosphatase level increased in rats treated after the onset of diabetes compared to diabetic control (Fig. 73).

6.1.2 Plasma alanine aminotransferase level

Figure 74 shows that the oral administration of low dose (10 mg/kg body weight) of vitamin C had no effect on the plasma alanine aminotransferase level of the normal rats. The low dose of vitamin C reduced the plasma alanine aminotransferase level in rats treated 10 days before the onset of diabetes without any statistical significance. In contrast, after the oral administration of a low dose of vitamin C, the plasma alanine aminotransferase level in rats treated 10 days after the onset of diabetes increased without any statistical significance.

On the other hand, moderate dose (50 mg/kg body weight) of vitamin C increased the plasma alanine aminotransferase level in rats treated 10 days before and after the onset of the diabetes without any statistical significance (Fig. 75). The plasma alanine aminotransferase level of normal rats did not change after the oral administration of moderate dose of vitamin C.

After the oral administration of high dose (100 mg/kg body weight) of vitamin C no significant change was observed in the alanine aminotransferase level in normal rats and in rats treated 10 days before the onset of diabetes.

However, there was a high increase in the alanine aminotransferase level in rats treated 10 days after the onset diabetes without any statistical significance (Fig. 76).

6.1.3 Plasma aspartate aminotransferase level

Figure 77 shows that the oral administration of low dose of vitamin C had no effect on the level of plasma asparatate aminotransferase in normal rats. There was a large but not significant reduction in the plasma asparatate aminotransferase level in rats treated before and after the onset of diabetes.

After the oral administration of moderate dose (50mg/kg body weight) of vitamin C the plasma asparatate aminotransferase level in rats treated 10 days before the onset of diabetes increased with no statistical significance (Fig. 78). However, there was a significant reduction in the plasma asparatate aminotransfrase level of normal rats after treatment with moderate dose of vitamin C.

On the other hand, the high dose (100 mg/kg body weight) of vitamin C significantly increased the level of plasma asparatate aminotransfrase in normal rats. The high dose of vitamin C increased the plasma asparatate aminotransferase level in rats treated before and after the onset of diabetes (Fig. 79).

6.1.4 Plasma lactic dehydrogenase level

The effect of low dose (10 mg/kg body weight) of vitamin C on plasma lactic dehydrogenase level in normal and diabetic rats is shown in figure 80. The result shows that low dose of vitamin C had no significant effect on the plasma lactic dehydrogenase level of normal rats and in rats treated before the onset of diabetes. Moreover, the plasma lactic dehydrogenase level of rats treated 10 days after the onset of diabetes increased without any statistical significance after low dose treatement of vitamin C.

The oral administration of moderate dose (50 mg/kg body weight) of vitamin C significantly ($p<0.00005$) increased the plasma lactic dehydrogenase level of normal rats. However, the moderate dose of vitamin C decreased the plasma lactic dehydrogenase level in rats treated 10 days before and after the onset of diabetes without any statistical significance (Fig. 81).

The high dose (100 mg/kg body weight) of vitamin C significantly ($p<0.0001$) reduced the plasma lactic dehydrogenase level in normal rats. On the other hand, the high dose of vitamin C elicited an increase in the plasma lactic dehydrogenase level after the onset of diabetes. Vitamin C had no significant effect on plasma lactic dehydrogenase level in diabetic rats (Fig. 82).

6.1.5 Plasma gamma-glutamyl transferase

The oral administration of low dose (10 mg/kg body weight) of vitamin C failed to induce significant increase in plasma gamma-glutamyl tansferase level in normal rats. However, there was a decrease in the plasma gamma-glutamyl tansferase level of rats treated 10 days after and before the onset of diabetes compared to untreated diabetic rats (Fig. 83).

The treatment with moderate dose (50 mg/kg body weight) of vitamin C significantly decreased the plasma gamma-glutamyl tansferase level of rats treated 10 days before ($p< 0.018$) and after ($p<0.0008$) the onset of diabetes and it had no effect on normal rats (Fig. 84).

The oral administration of the high dose (100 mg/kg body weight) of vitamin C was capable of reducing the plasma gamma-glutamyl tansferase level in rats treated before and after the onset of diabetes without any statistical significance. Also, the high doses of vitamin C had no effect on the plasma gamma-glutamyl tansferase level of normal rats compared to normal control (Fig. 85).

6.2 Effect of vitamin C on kidney parameters of normal and diabetic rats

6.2.1 Blood urea nitrogen level

Figure 86 shows that low dose (10mg/kg body weight) of vitamin C had no effect on plasma urea nitrogen level in both normal and diabetic rats compared to control rats. The administration of moderate dose (50 mg/kg body weight) of vitamin C significantly increased the plasma urea nitrogen level in normal rats. On the other hand, moderate dose of vitamin C caused a decrease in the level of plasma urea nitrogen in rats treated 10 days before the onset of diabetes without any statistical significance and in rats treated 10 days after the onset of diabetes with statistical significance ($p<0.012$) (Fig. 87).

The level of plasma urea nitrogen in rats treated 10 days before the onset of diabetes significantly ($p<0.011$) increased after oral administration of high dose (100mg/kg body weight) of vitamin C (Fig. 88) while no change was observed in the blood urea nitrogen of rats treated 10 days after the onset of diabetes. Moreover, the high dose of vitamin C caused a significant increase in the blood urea nitrogen level of normal treated rats compared to normal control.

6.2.2 Plasma creatinine level

Figure 89 shows the effect of low dose (10 mg/kg body weight) of vitamin C on plasma creatinine level of normal and diabetic rats. The level of plasma creatinine of non diabetic rats was not affected by the treatment with low dose of vitamin C. Moreover, the plasma creatinine level of rats treated 10 days before and after the onset of diabetes decreased but without any statistical significance.

The moderate dose of vitamin C had no effect on the plasma creatinine level of normal rats. However, moderate dose reduced the plasma creatinine level of rats treated 10 days before and after the onset of diabetes but with no statistical significance (Fig. 90).

After the oral administration of high dose (100 mg/kg body weight) of vitamin C, the plasma creatinine level of normal and in rats treated 10 days before the onset of diabetes did not change significantly. However, high dose induced a significant ($p<0.019$) increase in the plasma creatinine level in rats treated 10 days after the onset of diabetes when compared to diabetic control (Fig. 91).

6.3 Effect of vitamin C on electrolytes level in the normal and diabetic rats

6.3.1 Plasma calcium level

The oral administration of low dose (10mg/kg body weight) of vitamin C had no significant effect on the plasma calcium level of normal rats. On the other hand, the low dose caused a significant increase in rats treated 10 days before ($p< 0.02$) and after ($p< 0.008$) the onset of diabetes compared to diabetic controls (Fig. 92).

The moderate dose (50 mg/kg body weight) of vitamin C increased the plasma calcium level of normal rats but without any statistical significance. However, moderate administration of vitamin C caused significant increases in the plasma calcium level in rats treated 10 days after the onset of the diabetes (Fig. 93).

After the oral administration of high (100 mg/kg body weight) of vitamin C, a decrease in the plasma calcium level was observed in normal rats when compared to control. However, this decrease did not reach statistical significance. No significant change was observed on the plasma calcium level in rats treated 10 days before and after the onset of the diabetes (Fig. 94).

6.3.2 Plasma phosphorus level

The results presented in figure 95 demonstrated the effect of low dose (50 mg/kg body weight) of vitamin C on plasma phosphorus level in normal and diabetes rats. The result shows that low dose of vitamin C caused no significant change in the plasma phosphorus level of normal treated rats compared to normal controls. In diabetic rats, low dose of vitamin C caused a slight but not significant increase in plasma phosphorus level when compared to diabetic controls.

The oral administration of moderate dose (50 mg/kg body weight) of vitamin C induced a significant ($p<0.016$) increase in the level of plasma phosphorus level in rats treated 10 days before the onset of the diabetes (Fig. 96). The moderate dose had no effect on the plasma phosphorus level of treated normal rats.

Figure 97 shows the effect of high dose (100 mg/kg body weight) of vitamin C on the plasma phosphorus level of normal and diabetic rats. The result indicated that high dose of vitamin C had no significant effect on the plasma phosphorus level of normal and diabetic rats.

6.3.3 Plasma sodium level

The oral administration of low dose vitamin C (10 mg/kg body weight) caused significant ($p<0.00001$) increase in plasma sodium level of normal rats. Also, in diabetic rats it induced significant increases in the level of plasma sodium in rats treated 10 days before and after the onset of the diabetes compared to diabetic controls (Fig. 98).

The moderate dose (50 mg/kg body weight) of vitamin C caused increase in plasma sodium level without any statistical significance in normal rats when compared to normal control. In contrast, moderate dose increased the plasma sodium level without any statistical significance in rats treated 10 days before the onset of diabetes. However, moderate dose of vitamin C induced significant ($p<0.033$) increase in plasma sodium level of rats treated 10 days after the onset of the diabetes when compared to control (Fig. 99).

The oral administration of high dose (100 mg/kg body weight) of vitamin C increased the plasma sodium level significantly in normal rats and in rats treated 10 days before and after the onset of the diabetes (Fig. 100).

6.3.4 Plasma potassium level

Figure 101 shows that the oral treatment with low dose of vitamin C had no significant effect on the plasma potassium level in normal rats when compared to normal control. The level of potassium was slightly increased in rats treated 10 days before and after the onset of the diabetes when compared with untreated diabetic control.

The moderate dose (50 mg/kg body weight) of vitamin C given to normal and diabetic rats had no significant effect on the plasma potassium level (Fig. 102).

The oral administration of high dose (100 mg/kg body weight) of vitamin C given to normal and diabetic rats had no significant effect on the plasma potassium level (Fig. 103).

6.3.4 Plasma magnesium level

Figure 104 shows the effect of low dose (10mg/kg body weight) of vitamin C on plasma magnesium level in normal and diabetic rats. These results show that normal rats treated with low dose of vitamin C had a significant ($p<0.020$) decrease in plasma magnesium level when compared to normal control. In contrast, there was no significant difference in plasma magnesium level of rats treated before and after the onset of the diabetes when compared to diabetic control.

The moderate dose of vitamin C increased the level of plasma magnesium but without any statistical significance and there was no effect on normal and diabetic rats either before or after the onset of diabetes when compared to control significantly (Fig. 105).

The effect of high dose of vitamin C on plasma magnesium level is shown in Fig 106. The result showed that high dose of vitamin C significantly ($p<0.004$) increased the plasma magnesium level of normal rats when compared to normal control. There was a significant ($p<0.016$) reduction in the plasma magnesium level in rats given high dose of vitamin C treated 10 days after the onset of diabetes when compared to diabetic control.

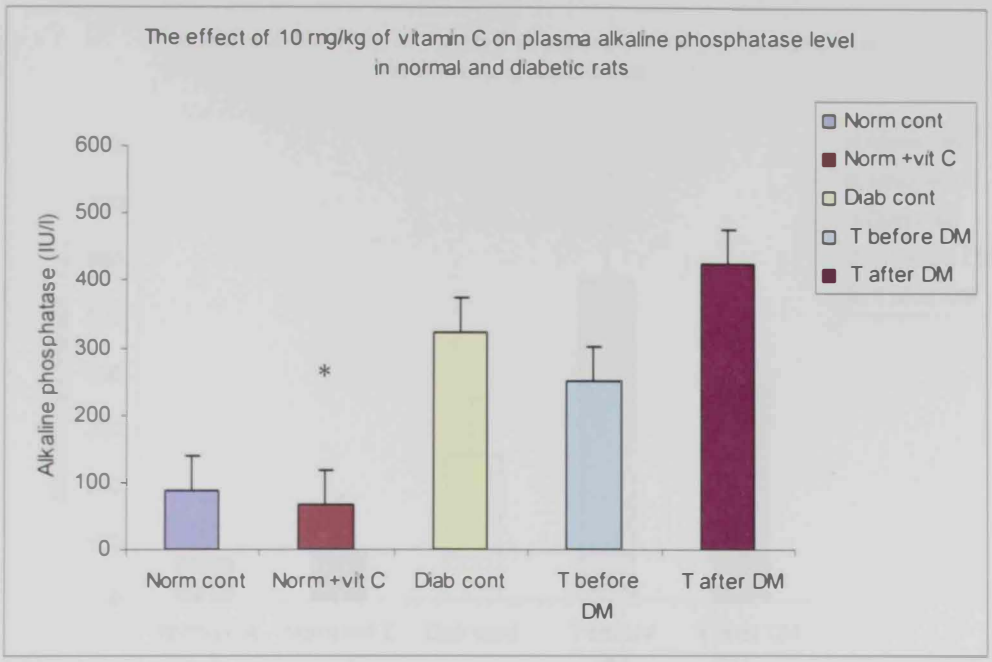


Figure 71: Histograms showing the effect of 10 mg/kg of vitamin C on plasma alkaline phosphatase level of normal and diabetic rats. 10 mg/kg of vitamin C induced a significant decrease in the plasma alkaline phosphatase level in normal non diabetic rats *p< 0.05 (norm cont versus norm+ vitamin C). (Data are mean ± SD, n=6). DM= diabetes mellitus.

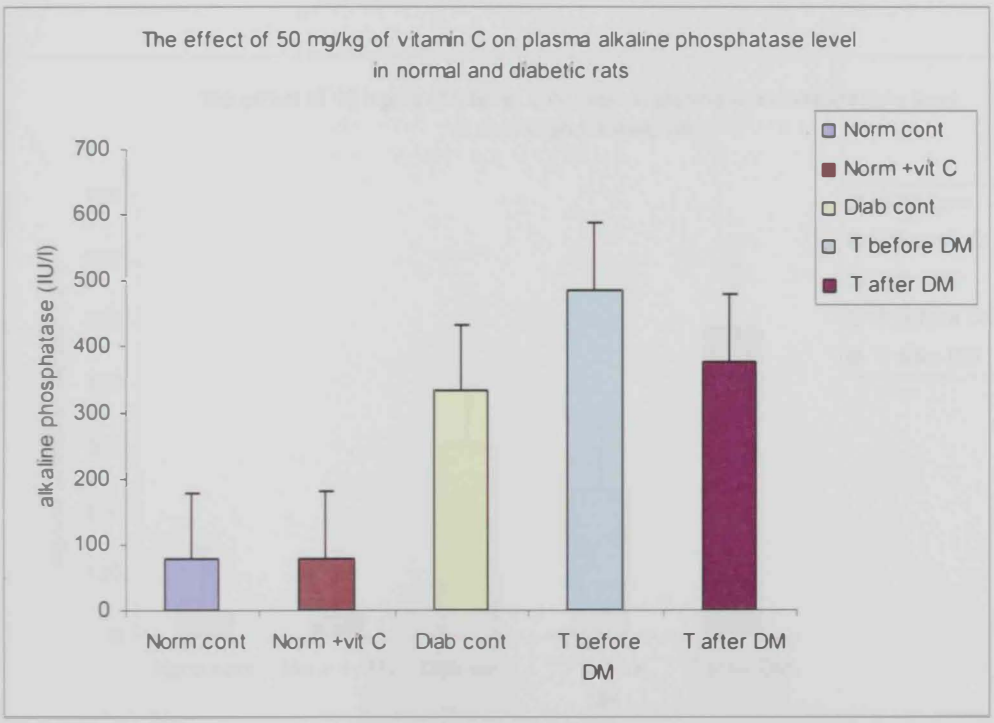


Figure 72: Histograms showing the effect of 50 mg/kg of vitamin C on plasma alkaline phosphatase level of normal and diabetic rats. (Data are mean ± SD, n=6). DM= diabetes mellitus.

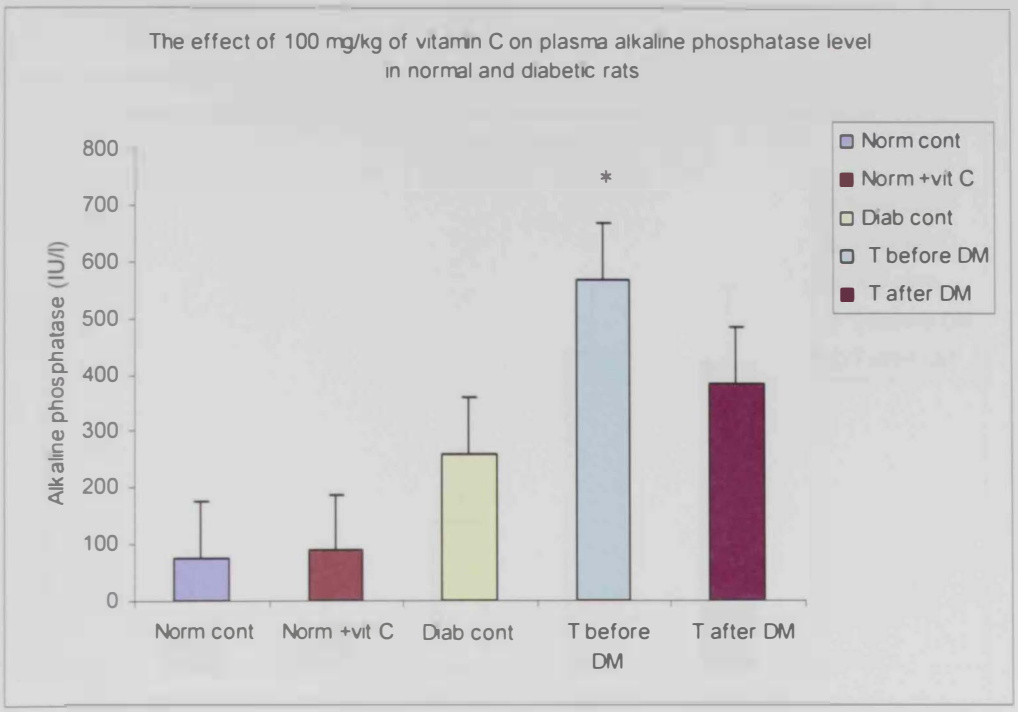


Figure 73: Histograms showing the effect of 100 mg/kg of vitamin C on plasma alkaline phosphatase level of normal and diabetic rats. 100 mg/kg of vitamin C induced a significant increase in the plasma alkaline phosphatase level in rats treated before onset of the DM *p< 0.05 (diab cont versus treated before DM). (Data are mean ± SD, n=6). DM= diabetes mellitus.

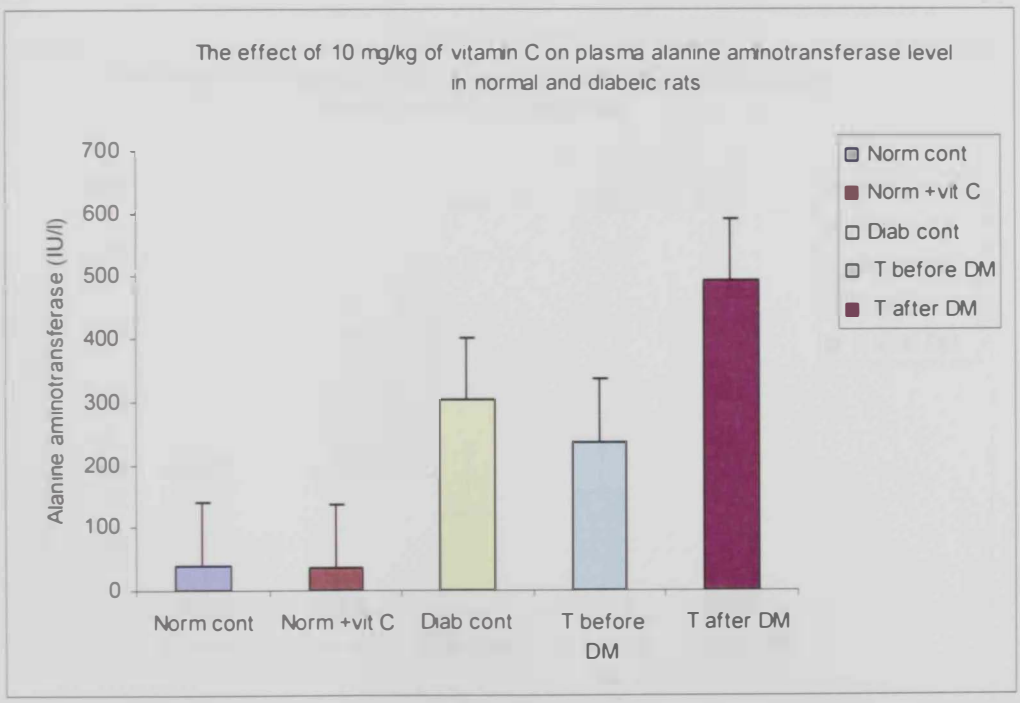


Figure 74: Histograms showing the effect of 10 mg/kg of vitamin C on plasma alanine aminotransferase level of normal and diabetic rats. (Data are mean ± SD, n=6). DM= diabetes mellitus.

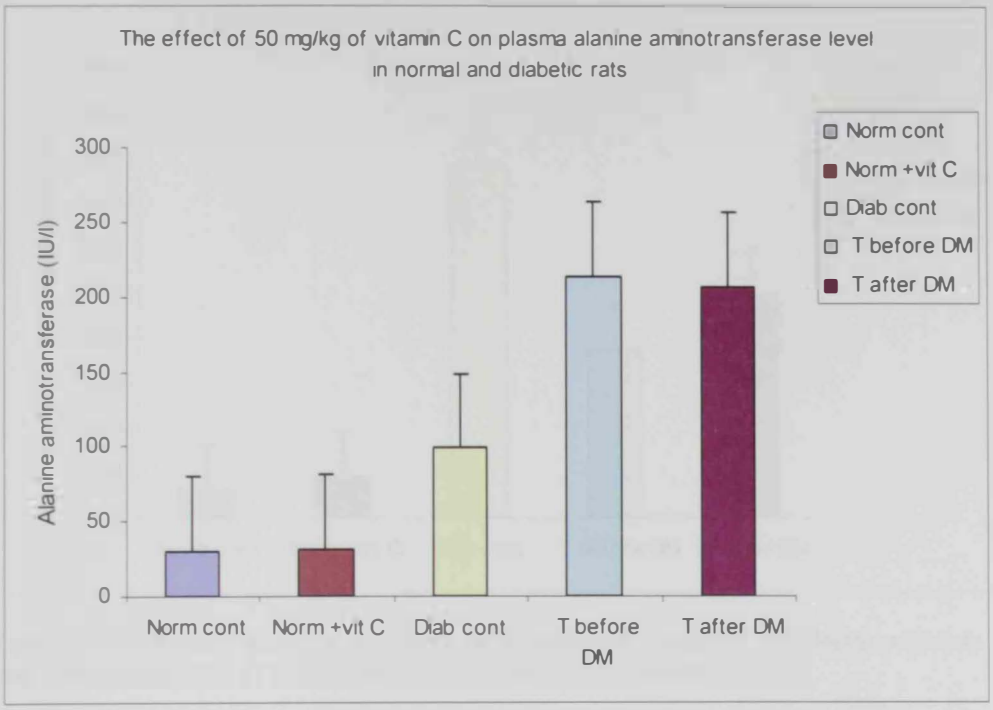


Figure 75: Histograms showing the effect of 50 mg/kg of vitamin C on plasma alanine aminotransferase level of normal and diabetic rats. (Data are mean ± SD, n=6). DM= diabetes mellitus.

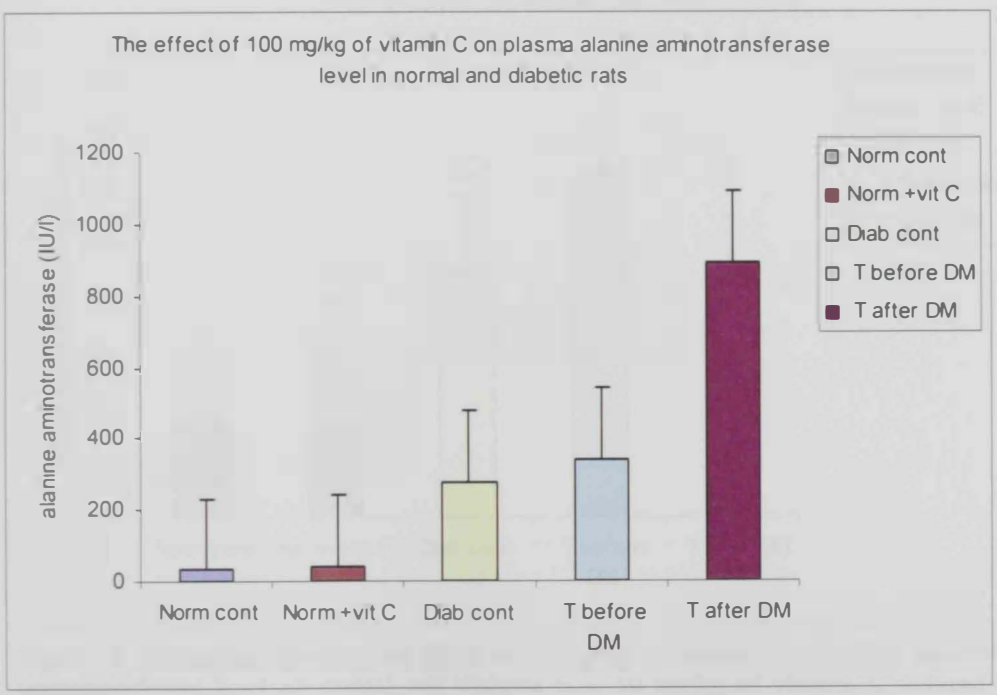


Figure 76: Histograms showing the effect of 100 mg/kg of vitamin C on plasma alanine aminotransferase level of normal and diabetic rats. (Data are mean ± SD, n=6). DM= diabetes mellitus

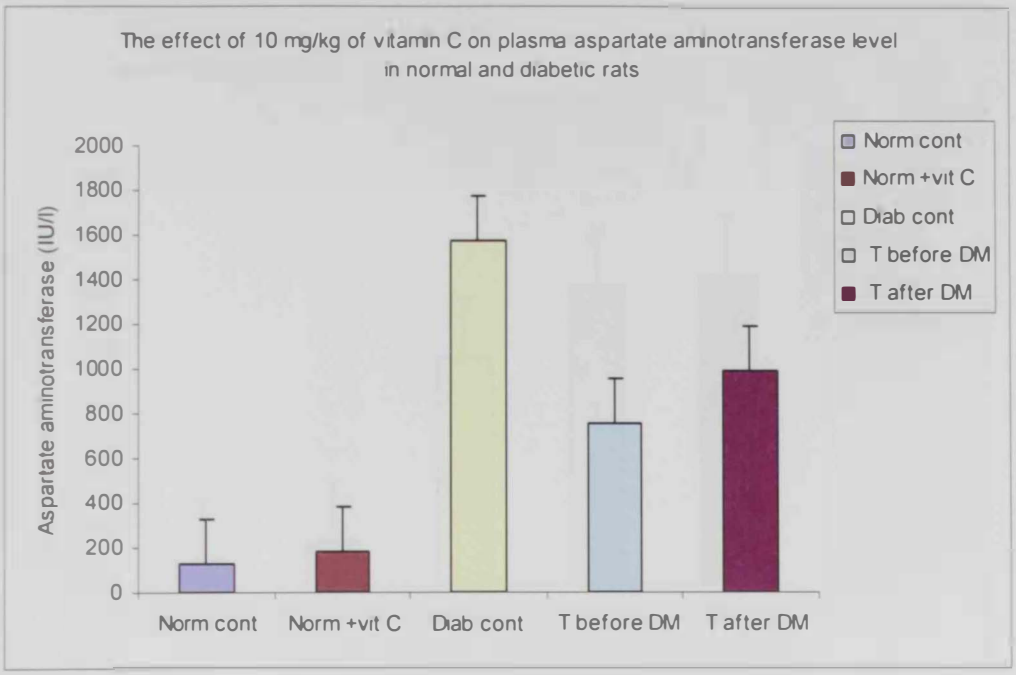


Figure 77: Histograms showing the effect of 10 mg/kg of vitamin C on plasma aspartate aminotransferase level of normal and diabetic rats. DM= diabetes mellitus.

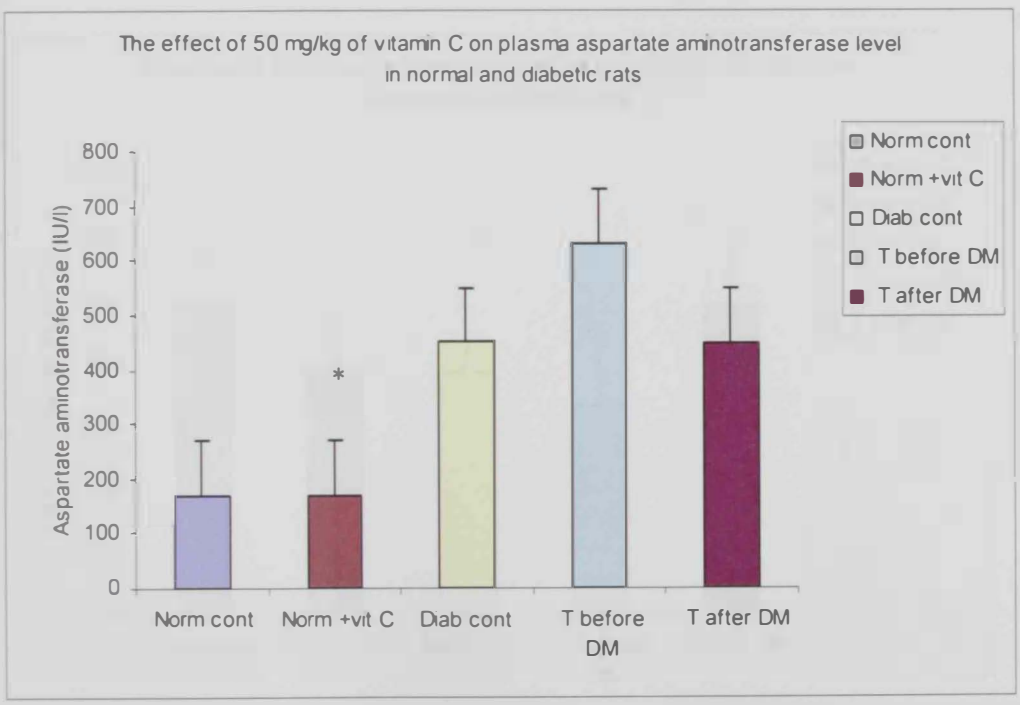


Figure 78: Histograms showing the effect of 50 mg/kg of vitamin C on plasma aspartate aminotransferase level of normal and diabetic rats. 50 mg/kg of vitamin C induced a significant increase in the plasma aspartate aminotransferase level in normal non diabetic rats *p< 0.05 (norm cont versus norm +vitamin C). (Data are mean ± SD, n=6). DM= diabetes mellitus.

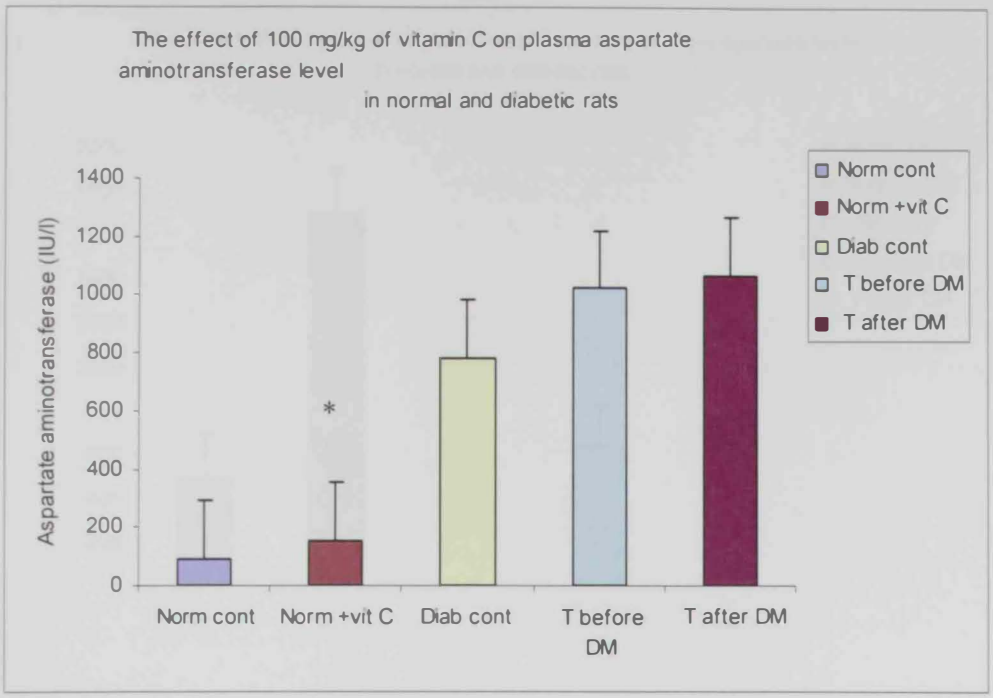


Figure 79: Histograms showing the effect of 100 mg/kg of vitamin C on plasma aspartate aminotransferase level of normal and diabetic rats. 100 mg/kg of vitamin C induced a significant increase in the plasma aspartate aminotransferase level in normal non diabetic rats *p< 0.05 (norm cont versus norm +vitamin C). (Data are mean ± SD, n=6). DM= diabetes mellitus.

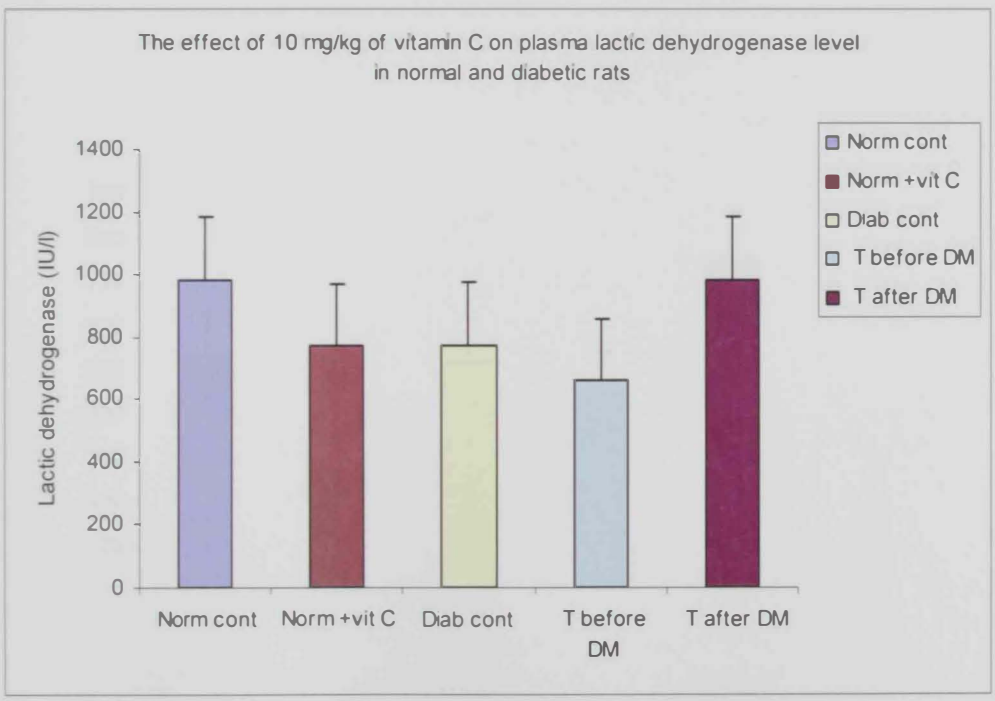


Figure 80: Histograms showing the effect of 10 mg/kg of vitamin C on plasma lactic dehydrogenase level of normal and diabetic rats. 50 mg/kg of vitamin C had no significant effect on the plasma lactic dehydrogenase level of the normal and diabetic rats. (Data are mean ± SD, n=6). DM= diabetes mellitus.

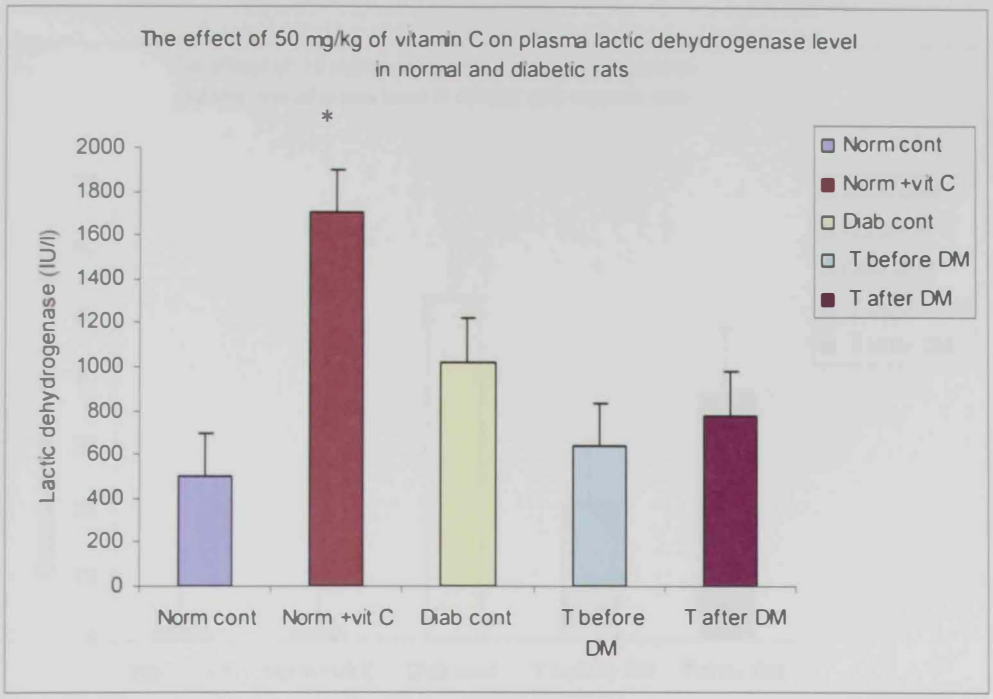


Figure 81: Histograms showing the effect of 50 mg/kg of vitamin C on plasma lactic dehydrogenase level of normal and diabetic rats. 50 mg/kg of vitamin C induced a significant increase in the plasma lactic dehydrogenase level in normal non diabetic rats *p< 0.05 (norm cont versus norm +vitamin C). (Data are mean ± SD, n=6). DM= diabetes mellitus.

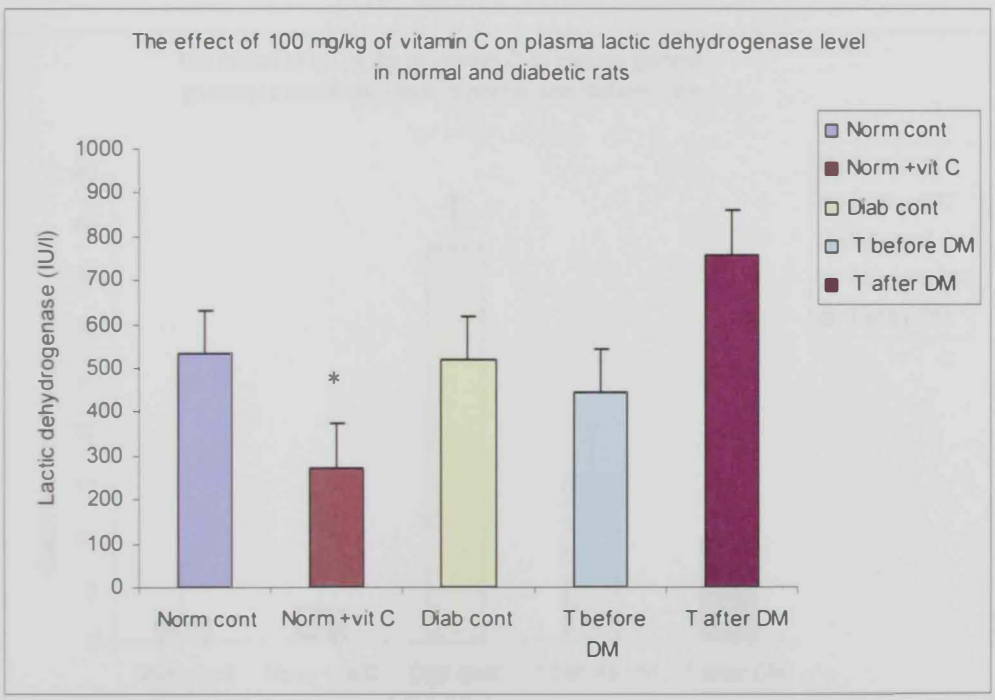


Figure 82: Histograms showing the effect of 100 mg/kg of vitamin C on plasma lactic dehydrogenase level of normal and diabetic rats. 100 mg/kg of vitamin C induced a significant decrease in the plasma lactic dehydrogenase level of normal non diabetic rats *p< 0.05 (norm cont versus norm +vitamin C). (Data are mean ± SD, n=6). DM= diabetes mellitus.

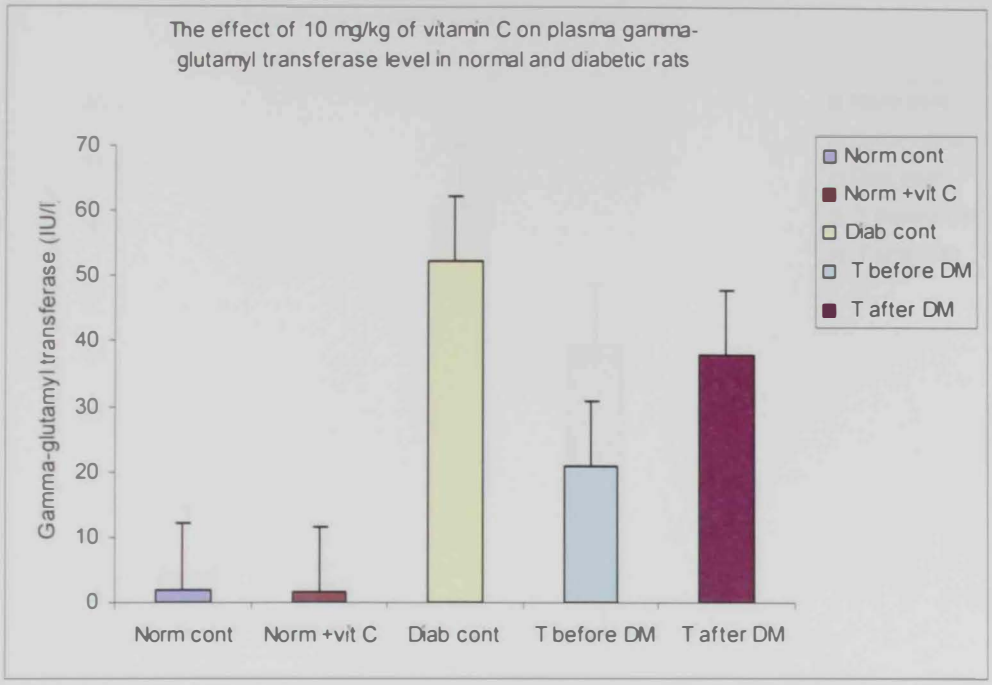


Figure 83: Histograms showing the effect of 10 mg/kg of vitamin C on plasma gamma-glutamyl transferase level of normal and diabetic rats. 10 mg/kg of vitamin C had no significant effect on the plasma gamma-glutamyl transferase level of normal and diabetic rats. (Data are mean ± SD, n=6). DM= diabetes mellitus.

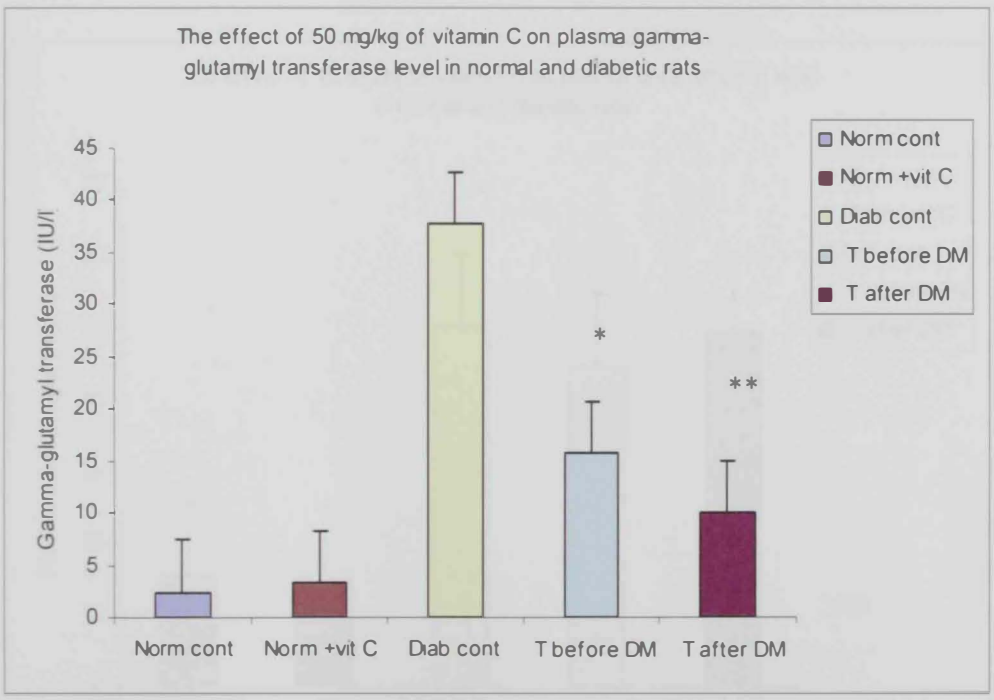


Figure 84: Histograms showing the effect of 50 mg/kg of vitamin C on plasma gamma-glutamyl transferase level of normal and diabetic rats. 50 mg/kg of vitamin C induced a significant decrease in the plasma gamma-glutamyl transferase level in rats treated before and after the onset of DM *$p<0.05$ (diab cont versus treated before DM) **$p<0.05$ (diab cont versus treated after DM). (Data are mean ± SD, n=6) DM= diabetes

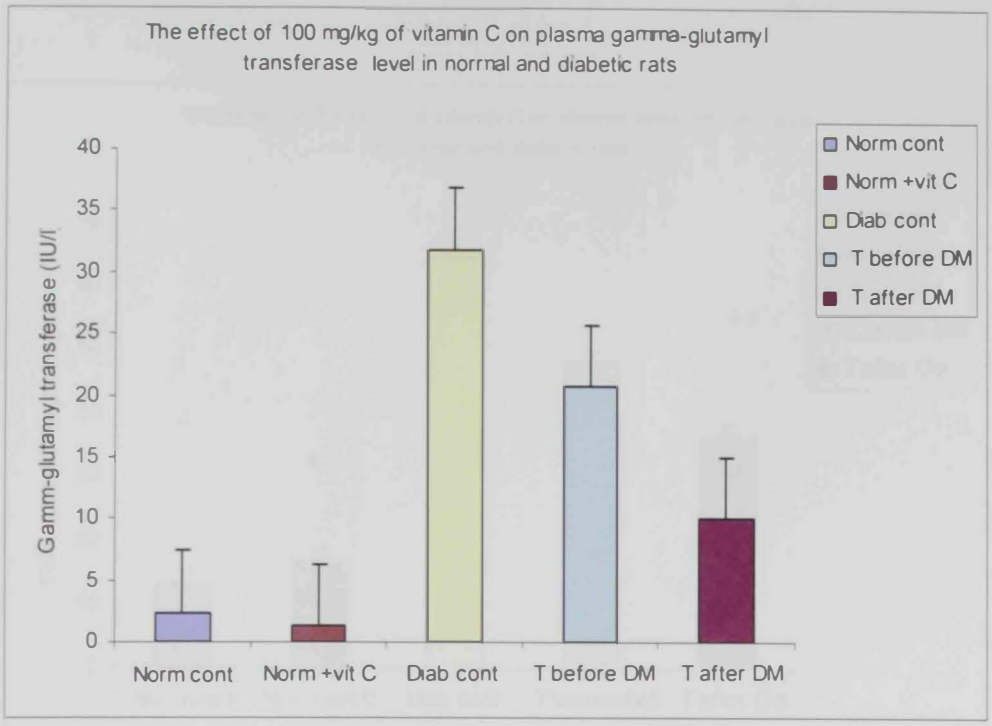


Figure 85: Histograms showing the effect of 100 mg/kg of vitamin C on plasma gamma-glutamyl transferase level of normal and diabetic rats. 100 mg/kg of vitamin C had no significant effect on the plasma gamm-glutamyl transferase level of the normal and diabetic rats. (Data are mean ± SD, n=6). DM= diabetes mellitus.

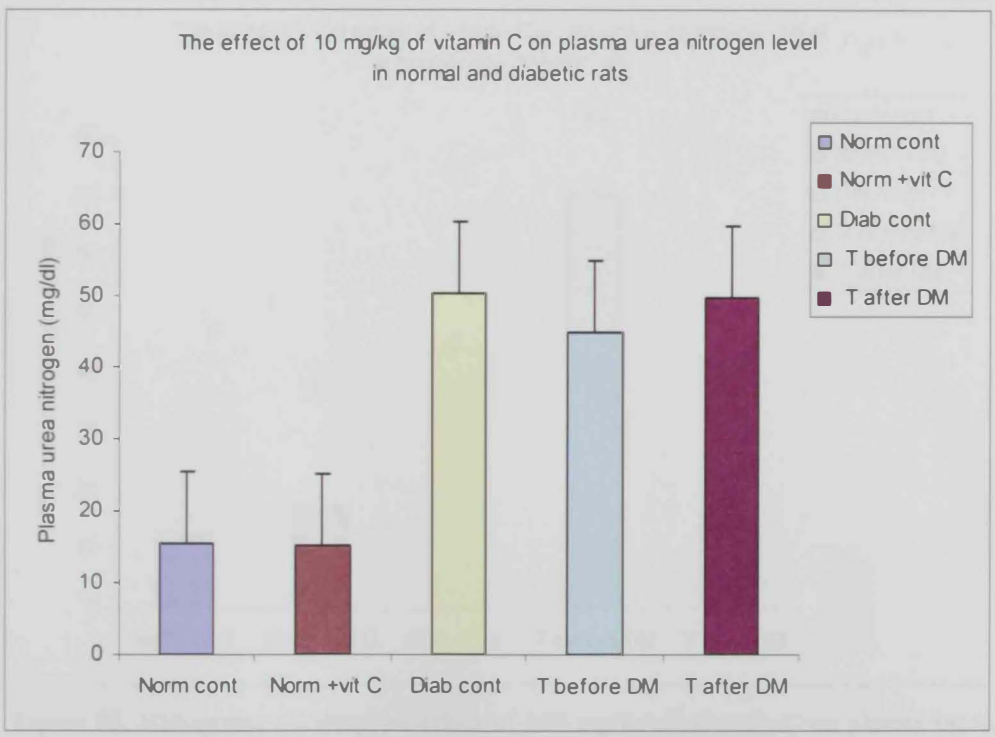


Figure 86: Histograms showing the effect of 10 mg/kg of vitamin C on plasma urea nitrogen level of normal and diabetic rats. 10 mg/kg of vitamin C had no significant effect on the plasma urea nitrogen level of the normal and diabetic rats. (Data are mean ± SD, n=6). DM= diabetes mellitus.

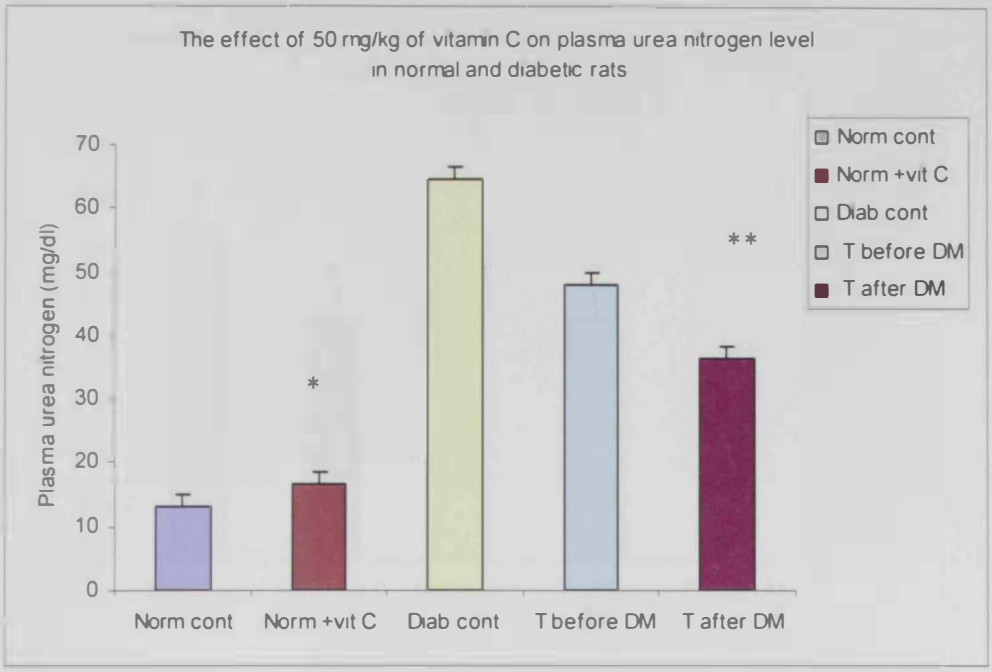


Figure 87: Histograms showing the effect of 50 mg/kg of vitamin C on plasma urea nitrogen level of normal and diabetic rats. 50 mg/kg of vitamin C induced a significant increase in the plasma urea nitrogen level of normal non diabetic rats *p<0.05 (norm cont versus norm+ vitamin C). Also, there was a significant decrease in plasma urea nitrogen in rats treated after the onset of DM **p<0.05 (diab cont versus treated after DM)). (Data are mean ± SD, n=6) DM= diabetes

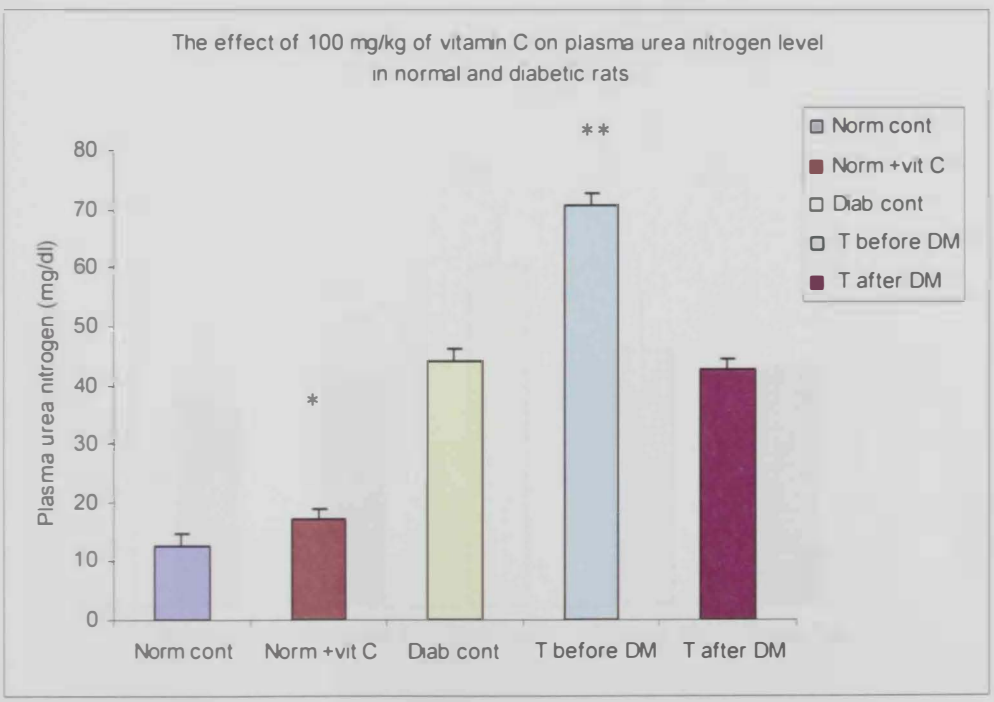


Figure 88: Histograms showing the effect of 100 mg/kg of vitamin C on plasma urea nitrogen level of normal and diabetic rats. 100 mg/kg of vitamin C had a significant increase in the plasma urea nitrogen level of normal non diabetic rats *p<0.05 (norm cont versus norm+ vitamin C) and in rats treated before the onset of DM **p<0.05 (diab cont versus before DM)). (Data are mean ± SD, n=6) DM= diabetes mellitus.

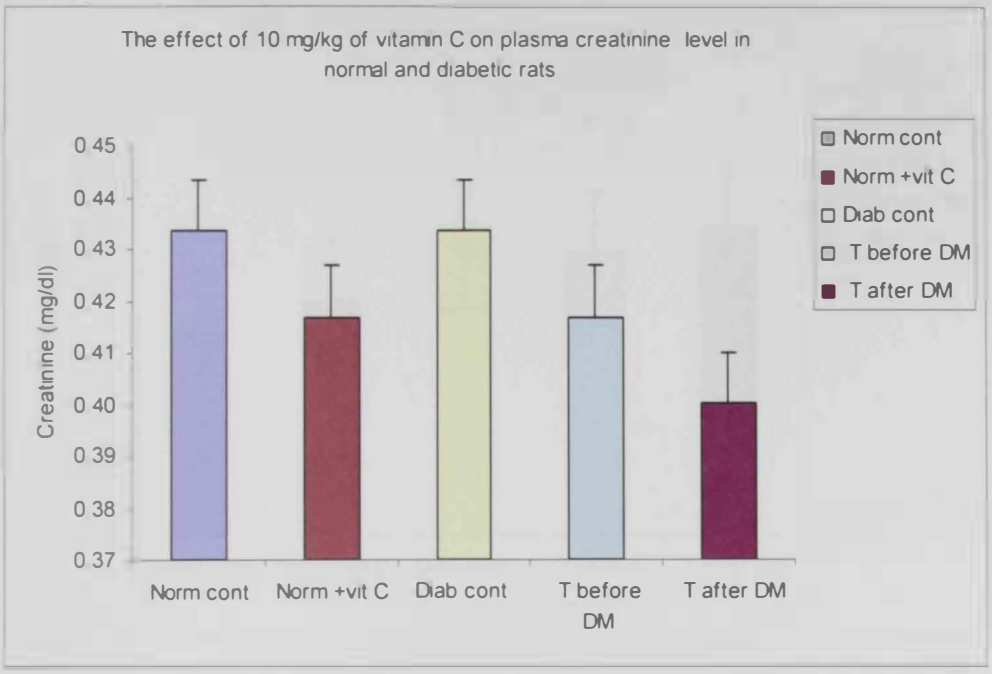


Figure 89: Histograms showing the effect of 10 mg/kg of vitamin C on plasma creatinine level of normal and diabetic rats. 10 mg/kg of vitamin C had no significant effect on the plasma creatinine level of the normal and diabetic rats. (Data are mean ± SD, n=6). DM= diabetes mellitus.

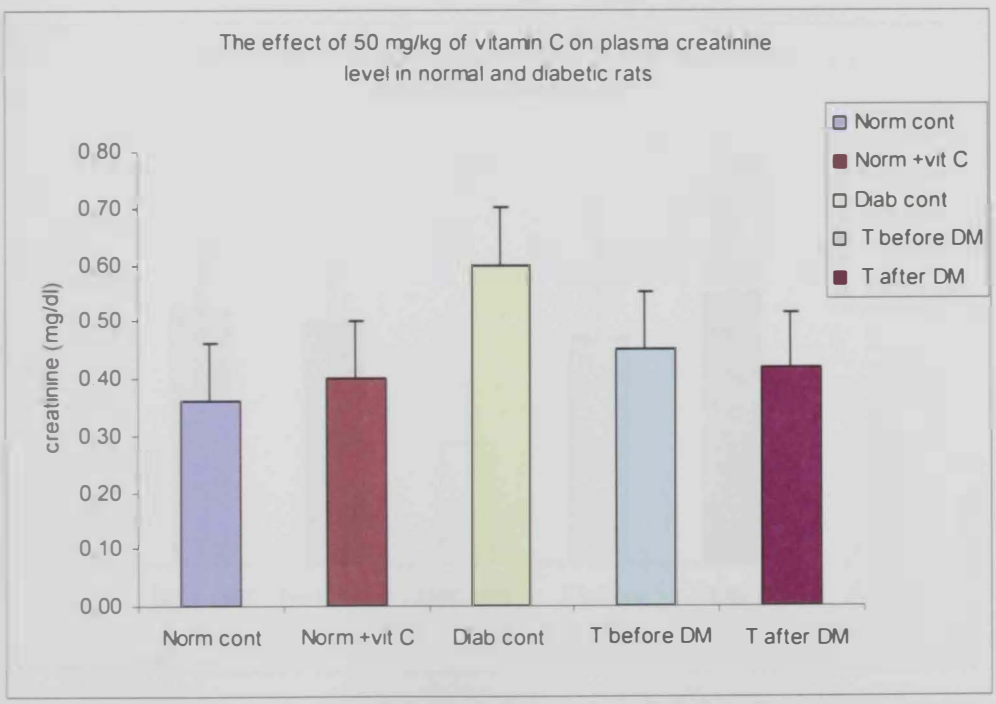


Figure 90. Histograms showing the effect of 50 mg/kg of vitamin C on plasma creatinine level of normal and diabetic rats. 50 mg/kg of vitamin C had no significant effect on the plasma creatinine level of the normal and diabetic rats. (Data are mean ± SD, n=6). DM= diabetes mellitus.

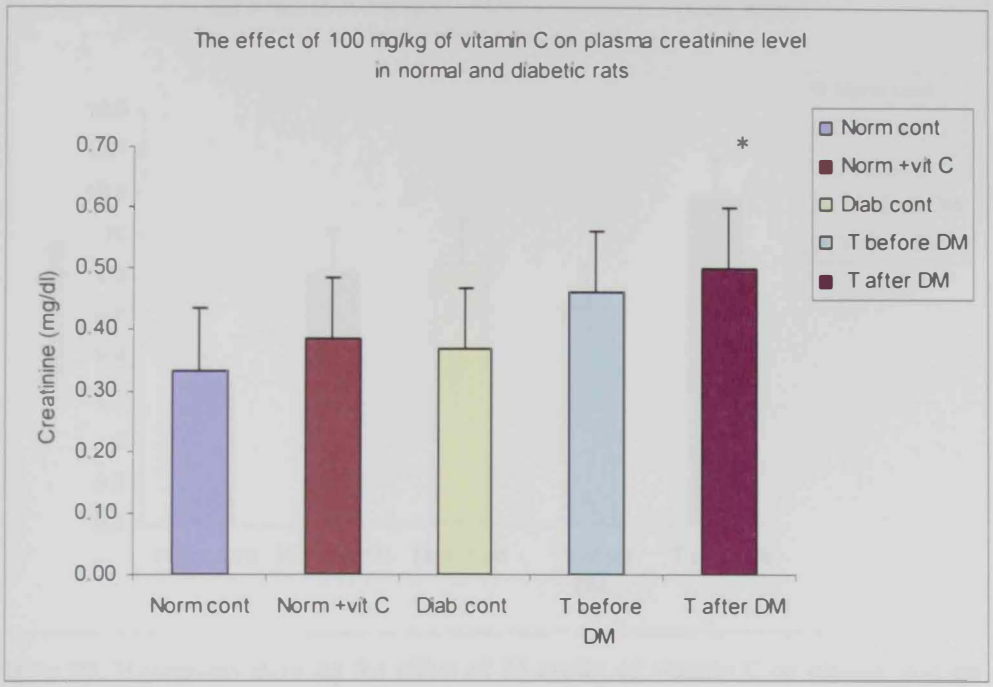


Figure 91: Histograms showing the effect of 100 mg/kg of vitamin C on plasma creatinine level of normal and diabetic rats. 100 mg/kg of vitamin C induced a significant increase in the plasma creatinine level in rats treated after the onset of DM *p<0.05 (diab cont versus after DM)). (Data are mean ± SD, n=6) DM= diabetes mellitus.

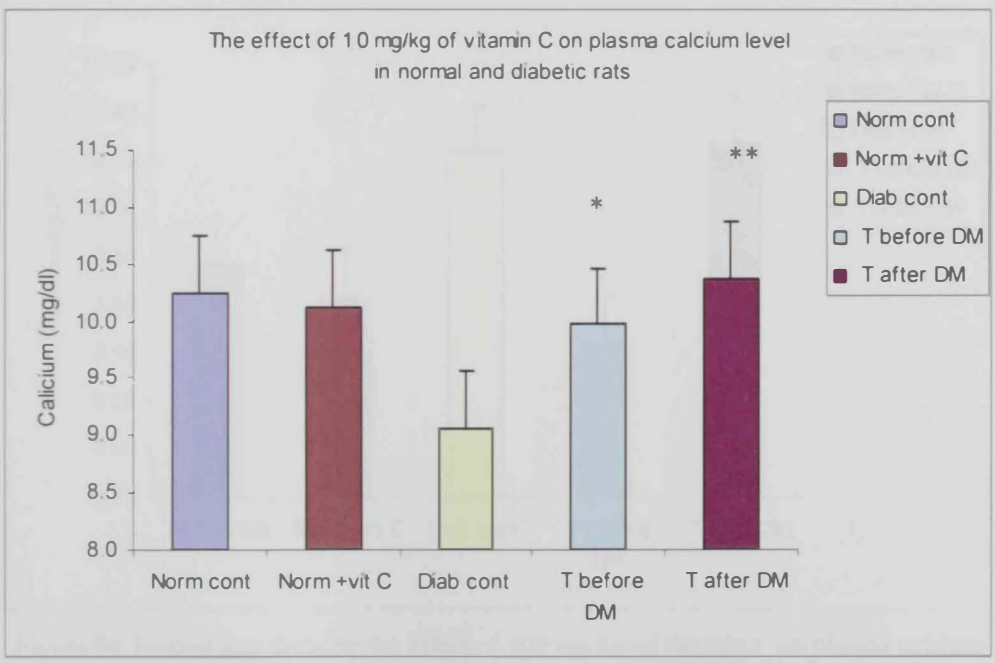


Figure 92: Histograms showing the effect of 10 mg/kg of vitamin C on plasma calcium level of normal and diabetic rats. 10 mg/kg of vitamin C induced a significant decrease in the plasma calcium level in rats treated before and after the onset of DM *p<0.05 (diab cont versus treated before DM) **p<0.05 (diab cont versus treated after DM). (Data are mean ± SD, n=6) DM= diabetes mellitus.

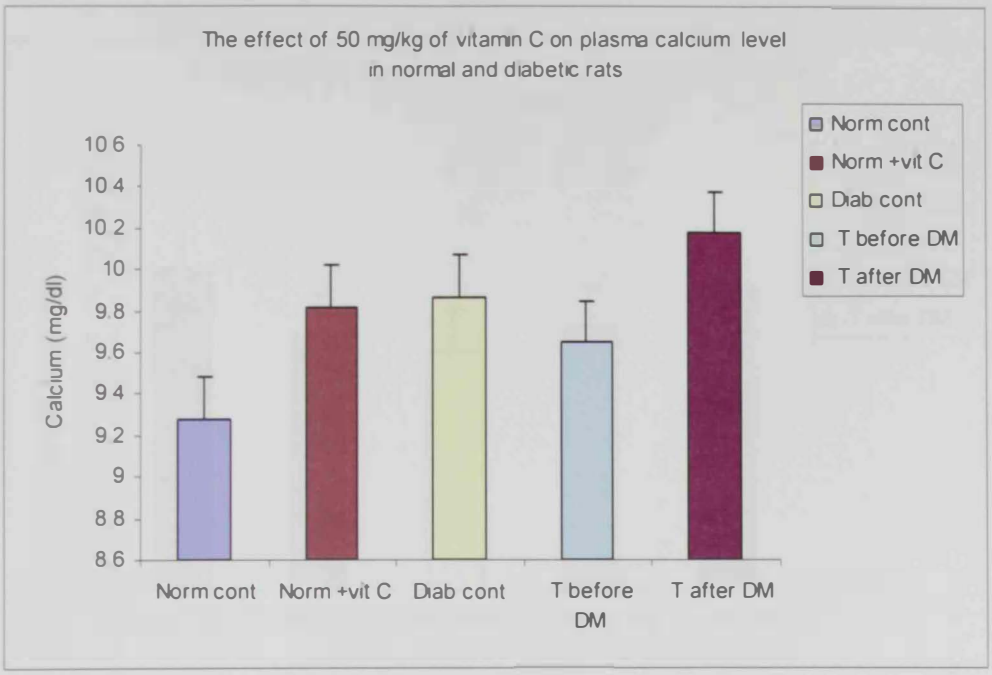


Figure 93: Histograms showing the effect of 50 mg/kg of vitamin C on plasma calcium level of normal and diabetic rats. 50 mg/kg of vitamin C had no significant effect on the plasma calcium level of normal and diabetic rats. (Data are mean ± SD, n=6). DM= diabetes mellitus.

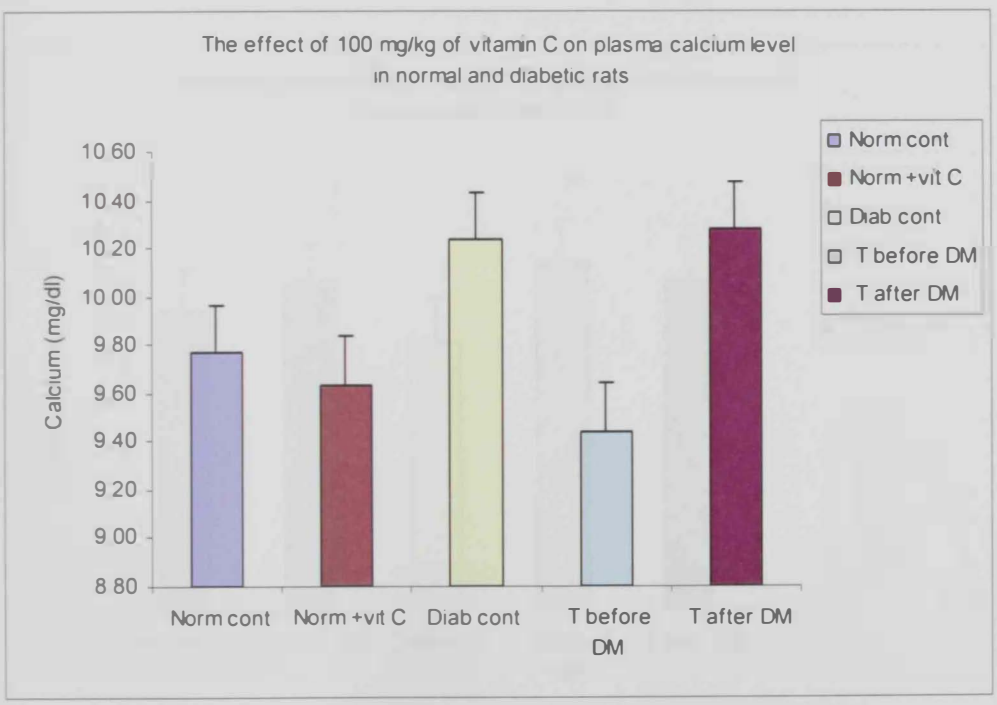


Figure 94: Histograms showing the effect of 100 mg/kg of vitamin C on plasma calcium level of normal and diabetic rats. 100 mg/kg of vitamin C had no significant effect on the plasma calcium level of normal and diabetic rats. (Data are mean ± SD, n=6). DM= diabetes mellitus.

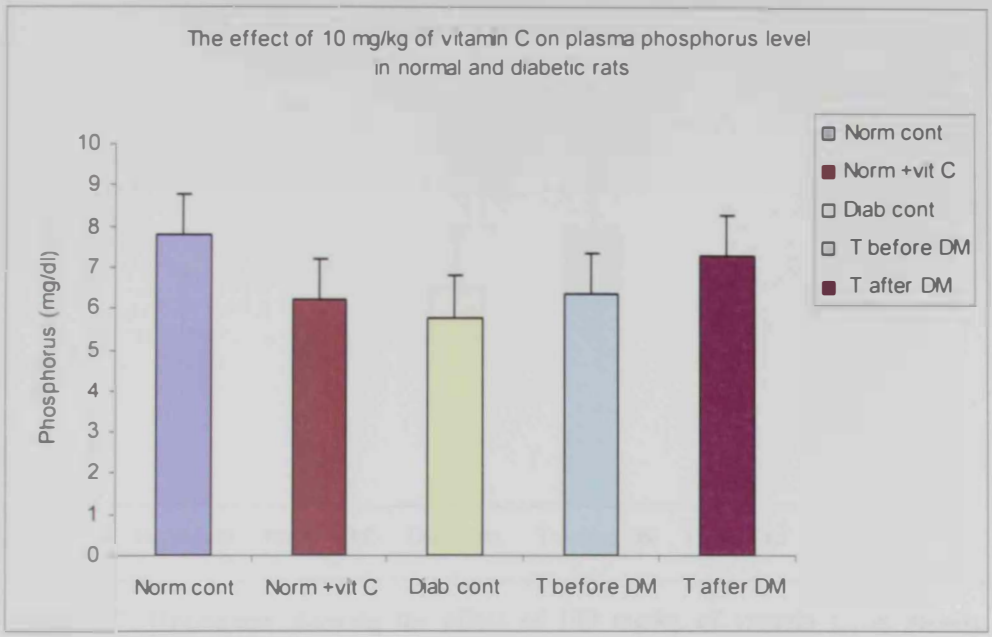


Figure 95: Histograms showing the effect of 10 mg/kg of vitamin C on plasma phosphorus level of normal and diabetic rats. 10 mg/kg of vitamin C had no significant effect on the plasma phosphorus level of normal and diabetic rats. (Data are mean ± SD, n=6). DM= diabetes mellitus.

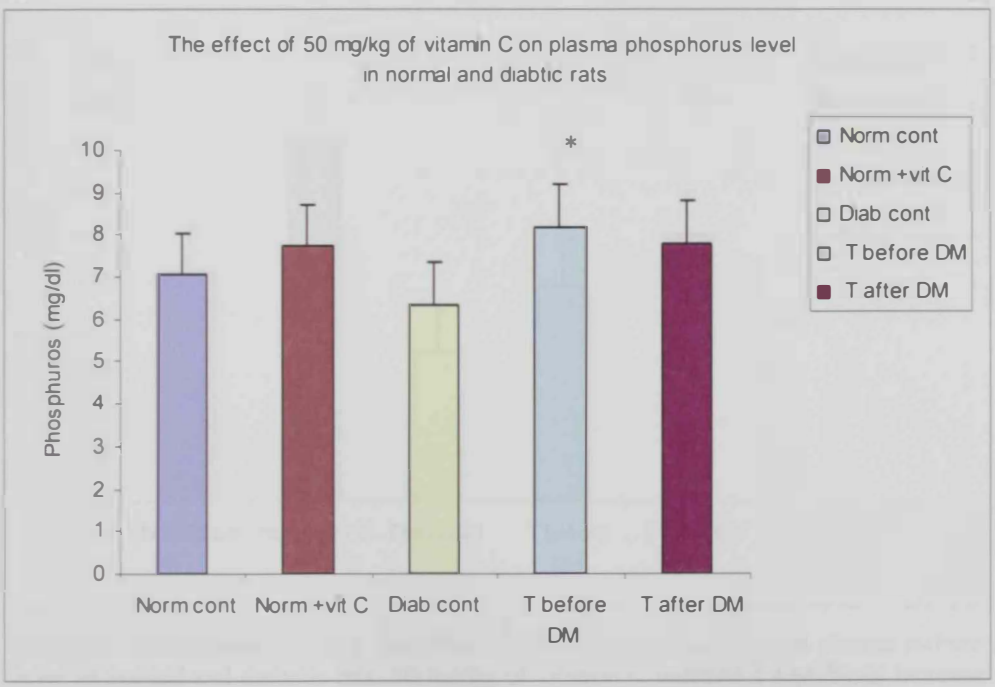


Figure 96: Histograms showing the effect of 50 mg/kg of vitamin C on plasma phosphorus level of normal and diabetic rats. 50 mg/kg of vitamin C induced a significant decrease in the plasma phosphorus level in rats treated before the onset of DM *p<0.05 (diab cont versus treated before DM). (Data are mean ± SD, n=6) DM= diabetes mellitus.

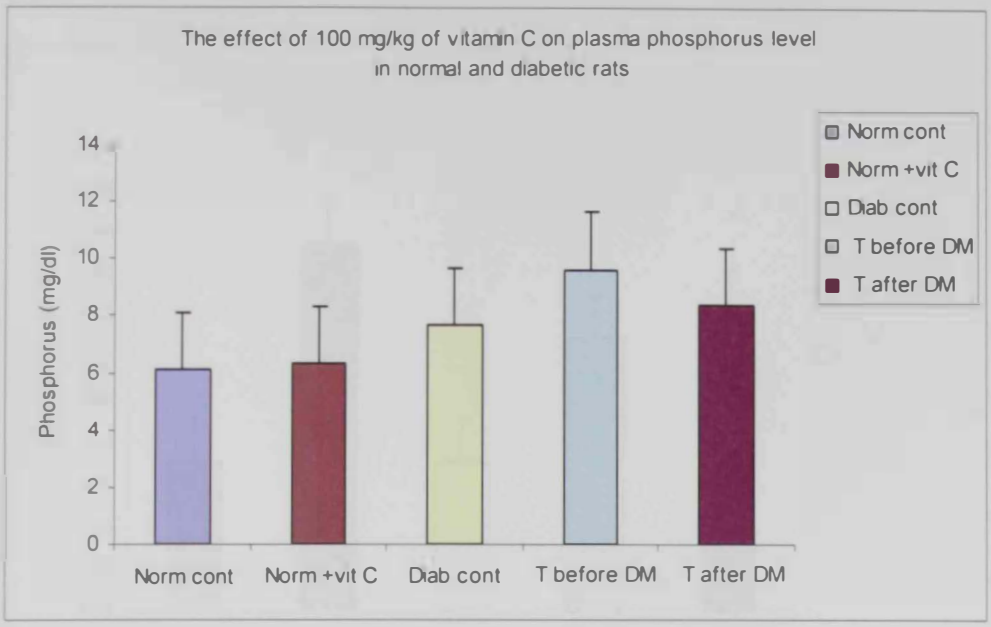


Figure 97: Histograms showing the effect of 100 mg/kg of vitamin C on plasma phosphorus level of normal and diabetic rats. 100 mg/kg of vitamin C had no significant effect on the plasma phosphorus level of normal and diabetic rats. (Data are mean ± SD, n=6). DM= diabetes mellitus.

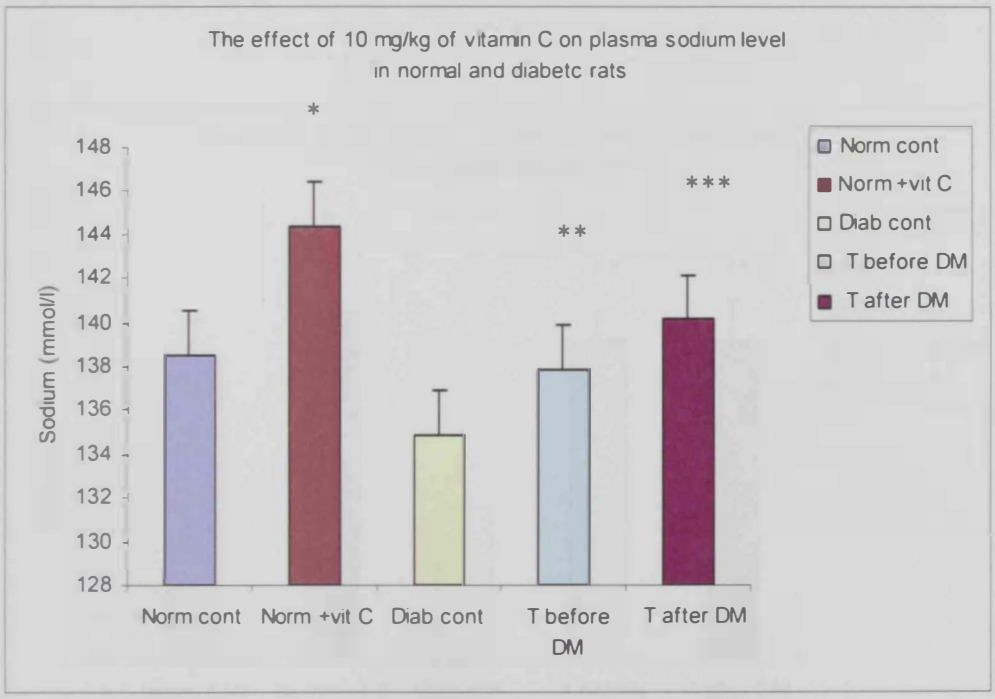


Figure 98: Histograms showing the effect of 10 mg/kg of vitamin C on plasma sodium level of normal and diabetic rats. 10 mg/kg of vitamin C induced a significant increase in the plasma sodium level of normal non diabetic rats *p<0.05 (norm cont versus norm+ vitamin C). Also, there was a significant increase in the sodium level in rats treated before **p<0.05 (diab cont versus before DM) and after ***p<0.05 (diab cont versus after DM) the onset of DM. (Data are mean ± SD, n=6) DM= diabetes mellitus.

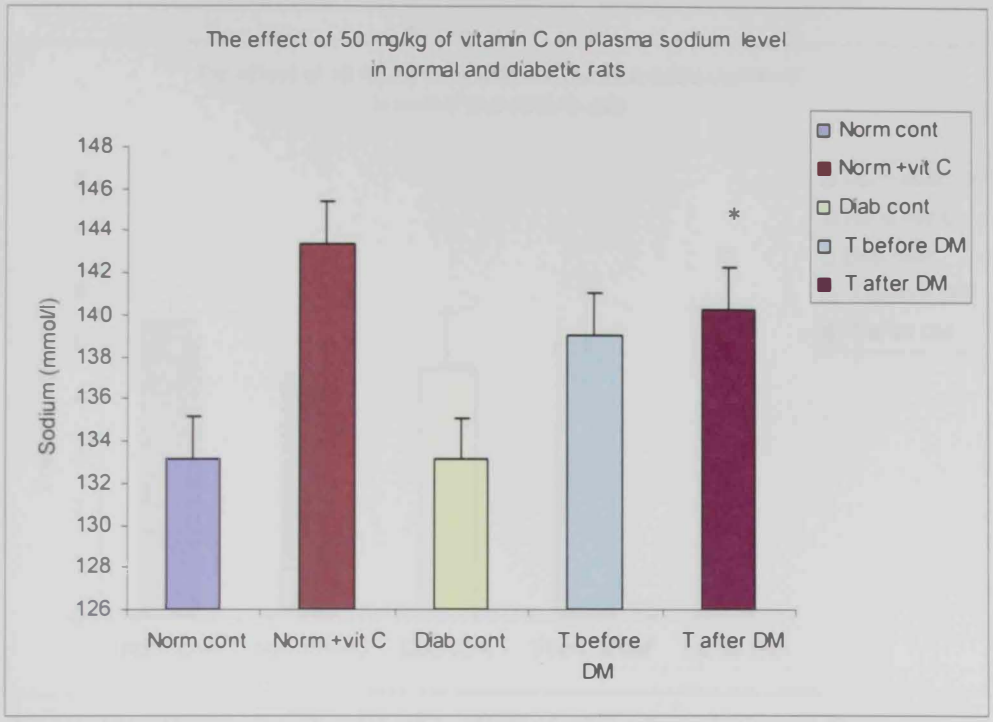


Figure 99: Histograms showing the effect of 50 mg/kg of vitamin C on plasma sodium level of normal and diabetic rats. 50 mg/kg of vitamin C induced a significant increase in the plasma sodium level in rats after the onset of DM *p<0.05 (diab cont versus after DM). (Data are mean ± SD, n=6) DM= diabetes mellitus.

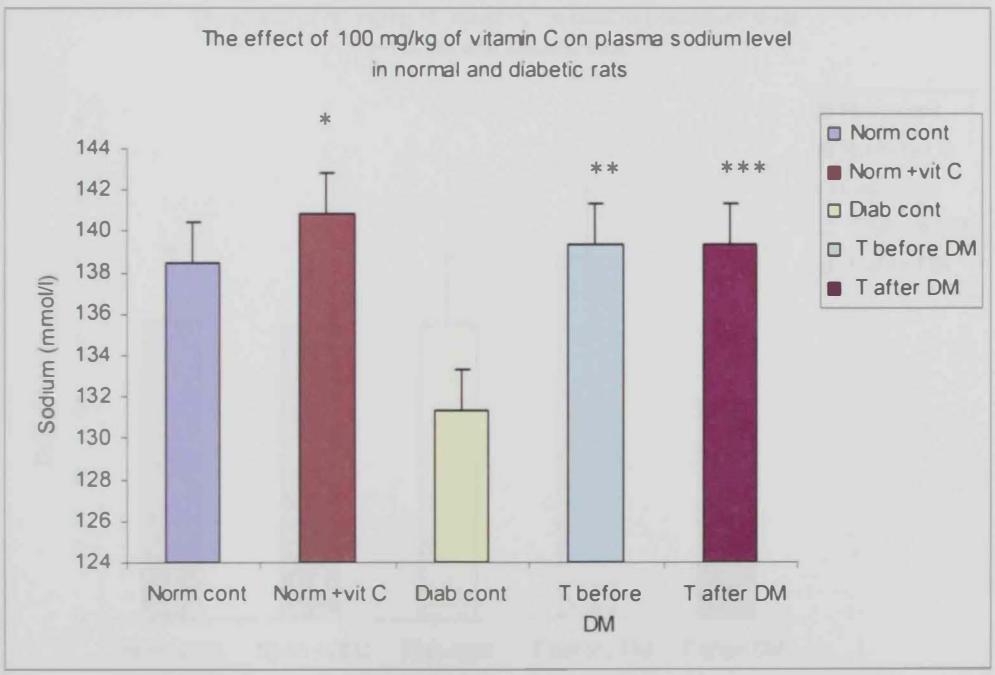


Figure 100: Histograms showing the effect of 100 mg/kg of vitamin C on plasma sodium level of normal and diabetic rats. 100 mg/kg of vitamin C induced a significant increase in the plasma sodium level of normal non diabetic rats *p<0.05 (norm cont versus norm+ vitamin C). Also, there was a significant increase in the sodium level in rats treated before **p<0.05 (diab cont versus before DM) and after ***p<0.05 (diab cont versus after DM) the onset of DM. (Data are mean ± SD, n=6) DM= diabetes mellitus.

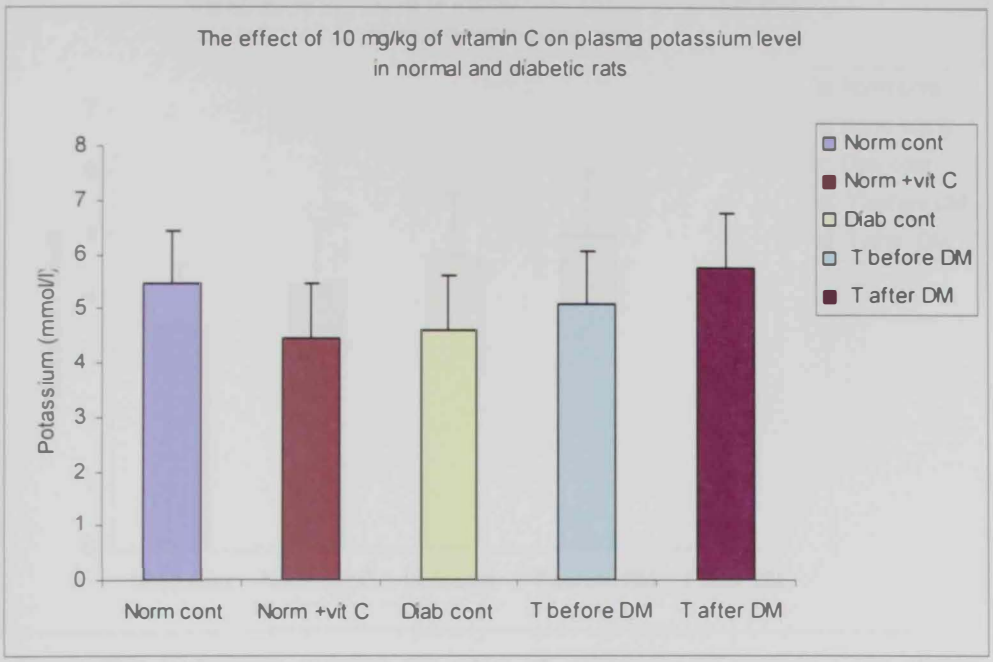


Figure 101: Histograms showing the effect of 10 mg/kg of vitamin C on plasma potassium level of normal and diabetic rats. 10 mg/kg of vitamin C had no significant effect on the plasma potassium level of normal and diabetic rats. (Data are mean ± SD, n=6). DM= diabetes mellitus.

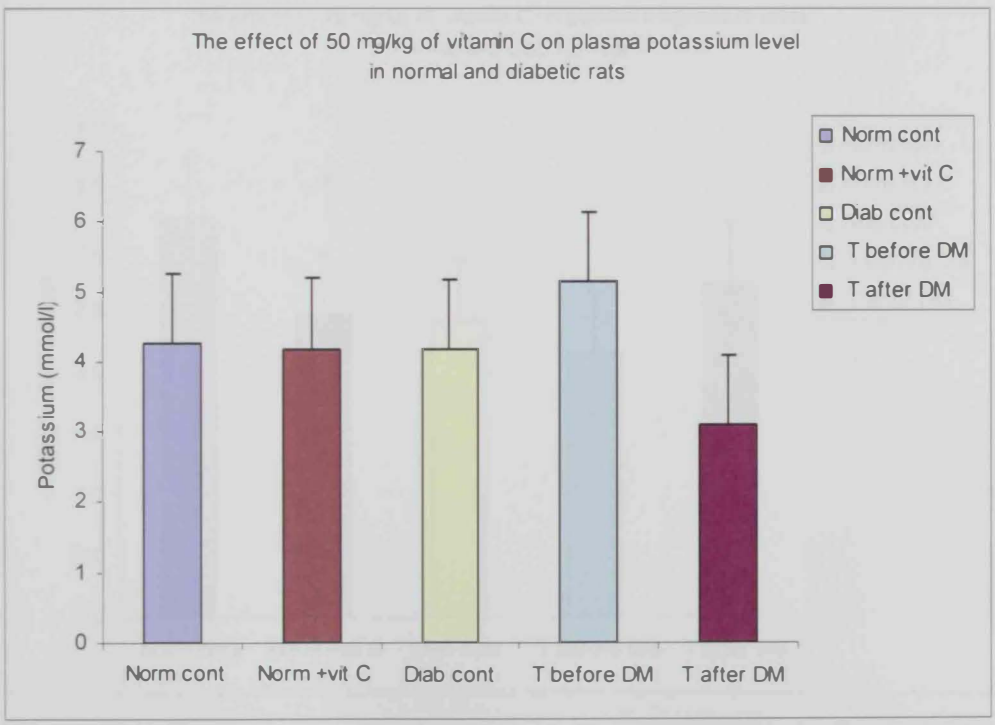


Figure 102: Histograms showing the effect of 50 mg/kg of vitamin C on plasma potassium level of normal and diabetic rats. 50 mg/kg of vitamin C had no significant effect on the plasma potassium level of normal and diabetic rats. (Data are mean ± SD, n=6). DM= diabetes mellitus.

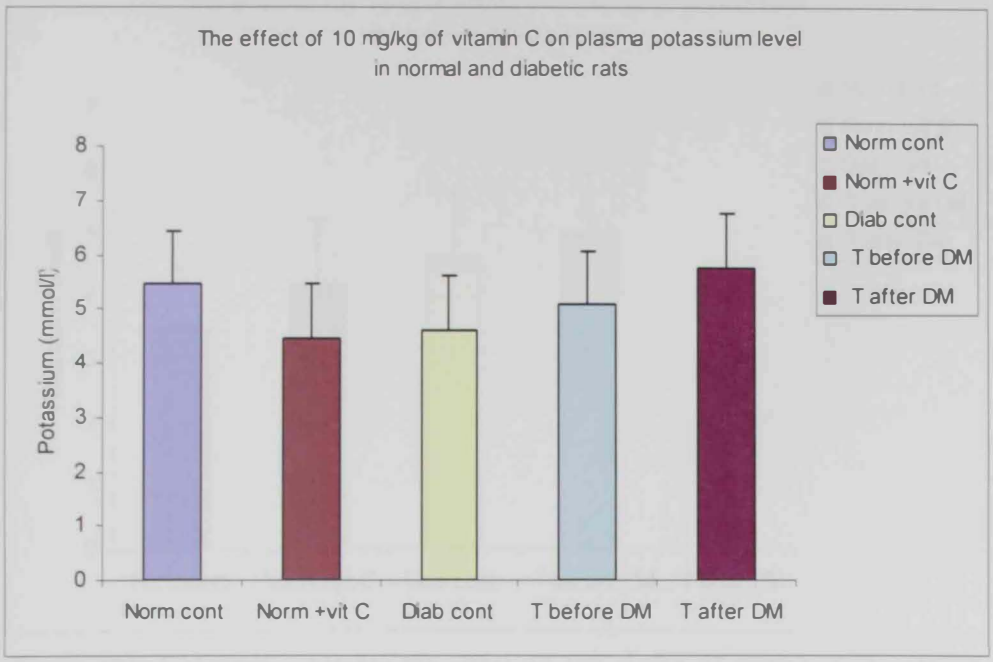


Figure 101: Histograms showing the effect of 10 mg/kg of vitamin C on plasma potassium level of normal and diabetic rats. 10 mg/kg of vitamin C had no significant effect on the plasma potassium level of normal and diabetic rats. (Data are mean ± SD, n=6). DM= diabetes mellitus.

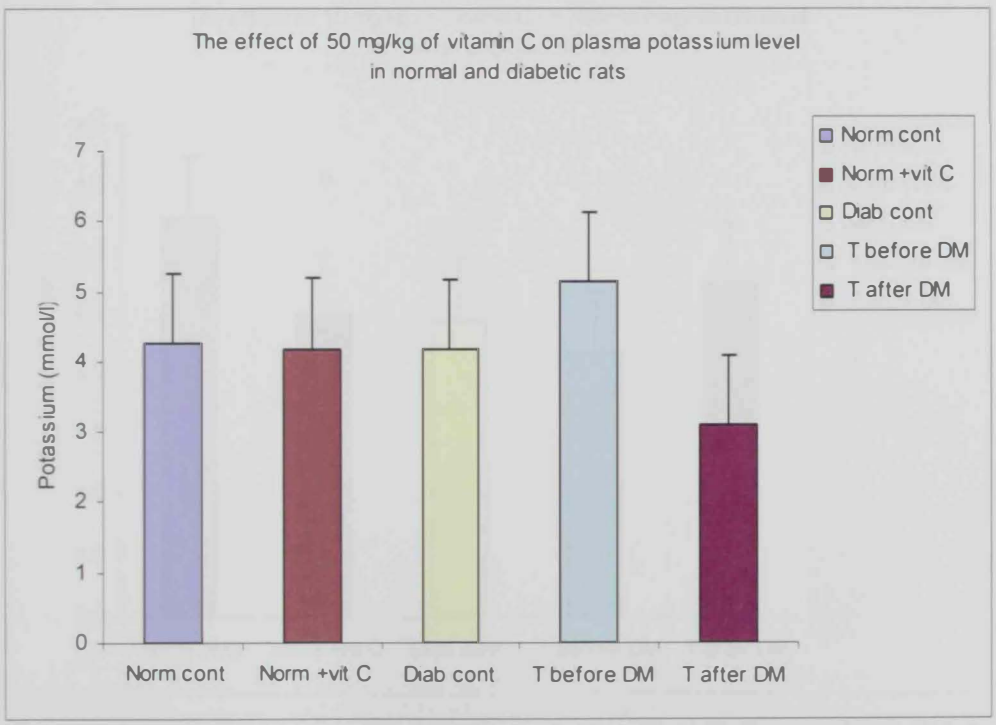


Figure 102: Histograms showing the effect of 50 mg/kg of vitamin C on plasma potassium level of normal and diabetic rats. 50 mg/kg of vitamin C had no significant effect on the plasma potassium level of normal and diabetic rats. (Data are mean ± SD, n=6). DM= diabetes mellitus.

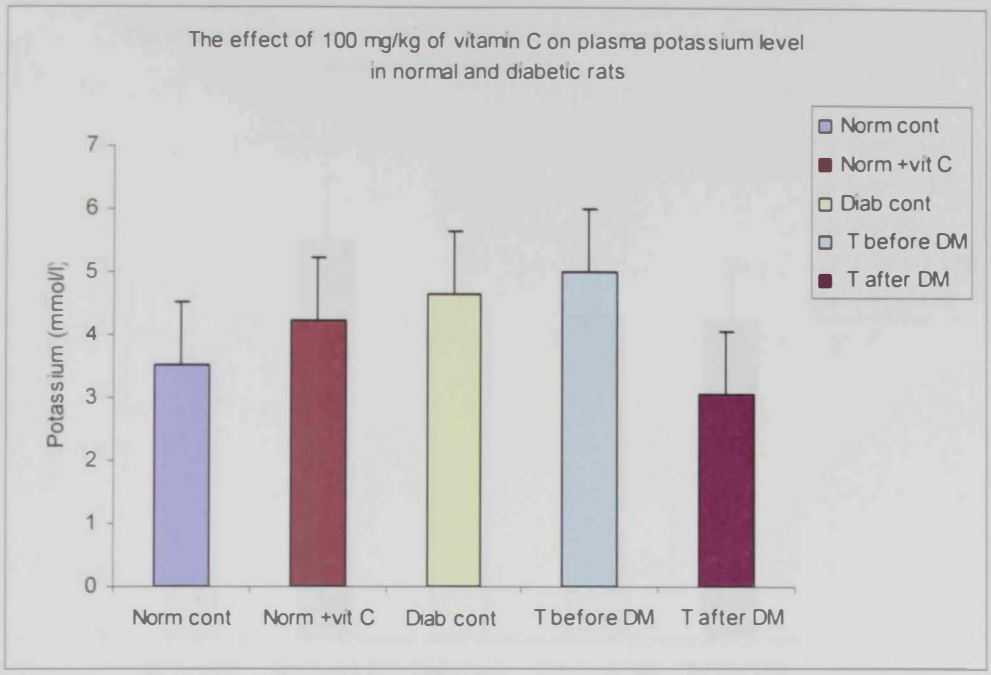


Figure 103: Histograms showing the effect of 100 mg/kg of vitamin C on plasma potassium level of normal and diabetic rats. 100 mg/kg of vitamin C had no significant effect on the plasma potassium level of normal and diabetic rats. (Data are mean ± SD, n=6). DM= diabetes mellitus.

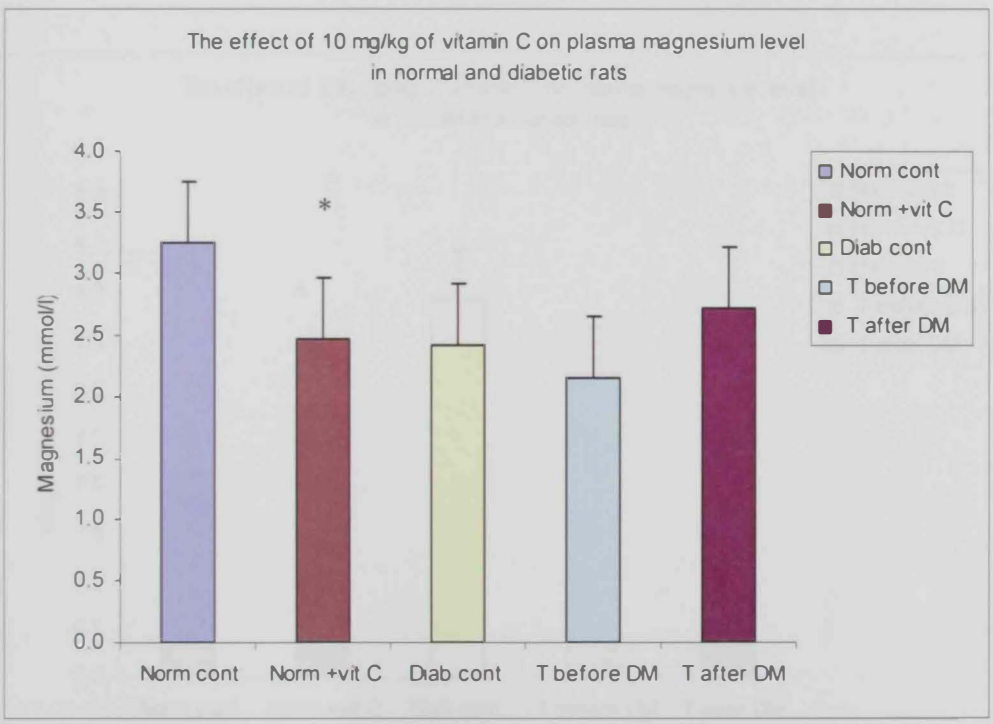


Figure 104: Histograms showing the effect of 10 mg/kg of vitamin C on plasma magnesium level of normal and diabetic rats. 10 mg/kg of vitamin C induced a significant decrease in the plasma magnesium level in normal non diabetic rats (norm cont versus norm+ vitamin C). (Data are mean ± SD, n=6) DM= diabetes mellitus.

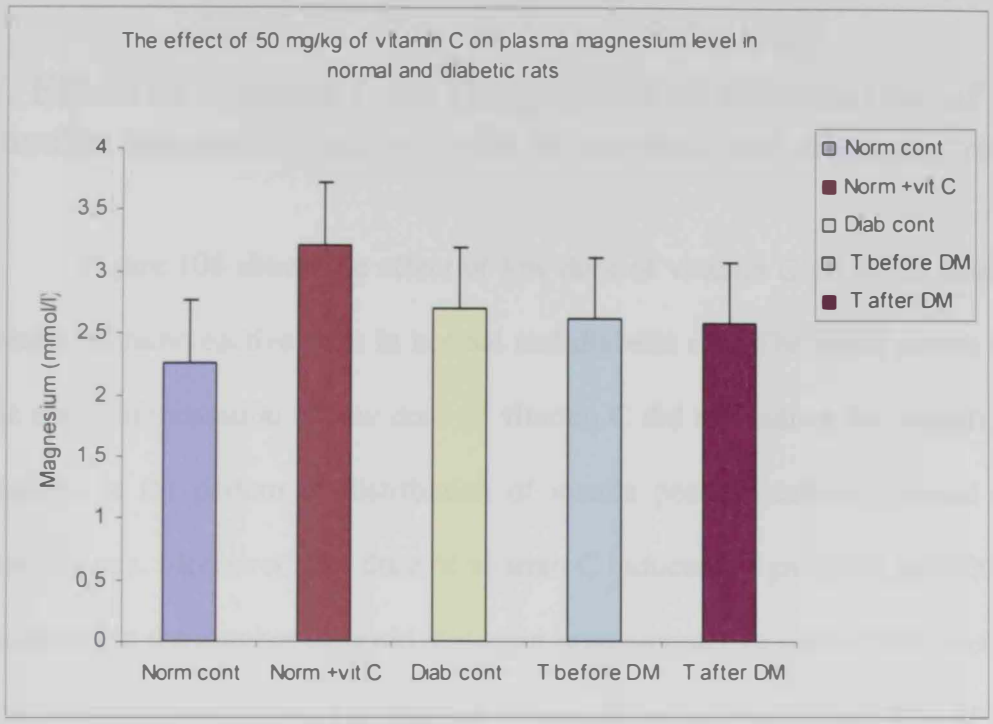


Figure 105: Histograms showing the effect of 50 mg/kg of vitamin C on plasma magnesium level of normal and diabetic rats. 50 mg/kg of vitamin C had no significant effect on the plasma magnesium level of the normal and diabetic rats. (Data are mean ± SD, n=6). DM= diabetes mellitus.

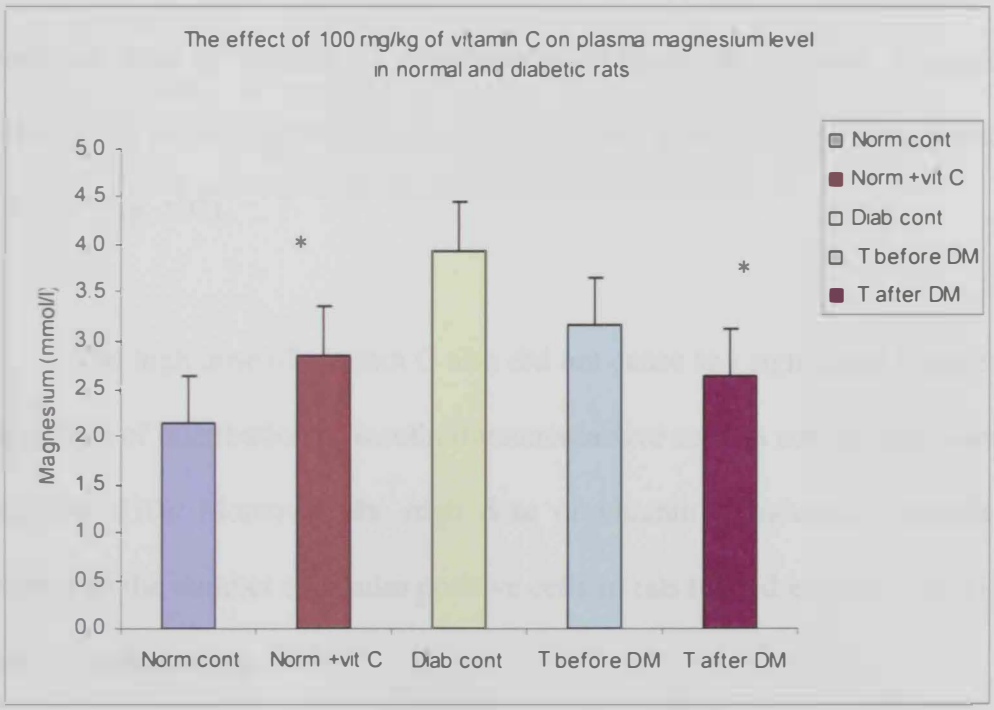


Figure 106: Histograms showing the effect of 100 mg/kg of vitamin C on plasma magnesium level of normal and diabetic rats. 100 mg/kg of vitamin C induced a significant increase in the plasma magnesium level of normal non diabetic rats *$p<0.05$ (norm cont versus norm+ vitamin C) and in rats treated after the onset of DM **$p<0.05$ (diab cont versus after DM). (Data are mean ± SD, n=6) DM= diabetes mellitus.

7. Effect of vitamin C on the pattern of distribution of insulin immunoreactive cells in normal and diabetic rats

Figure 108 shows the effect of low dose of vitamin C on the number of insulin immunoreactive cells in normal and diabetic rats. The result shows that the oral administration of low dose of vitamin C did not induce any significant changes in the pattern of distribution of insulin positive cells in normal and diabetic rats. Moreover, low dose of vitamin C induced a significant ($p<0.0003$) increased in the number of positive insulin immunoreactive cells of the islets of rats treated before the onset of diabetes compared to diabetic control (Fig. 107).

Also, moderate dose of vitamin C did not cause any significant morphological changes to pancreatic islets of normal and diabetic rats (Fig.109). Moderate dose of vitamin C significantly increased the number of positive insulin cells in rats treated before ($p<0.013$) and after ($p<0.001$) the onset of diabetes (Fig. 107).

The high dose of vitamin C also did not cause any significant changes in the pattern of distribution of insulin immunoreactive cells in normal and diabetic rats (Fig. 110). Moreover, the high dose of vitamin C induced a significant increase in the number of insulin positive cells in rats treated after ($p<0.031$) the onset of diabetes (Fig. 107).

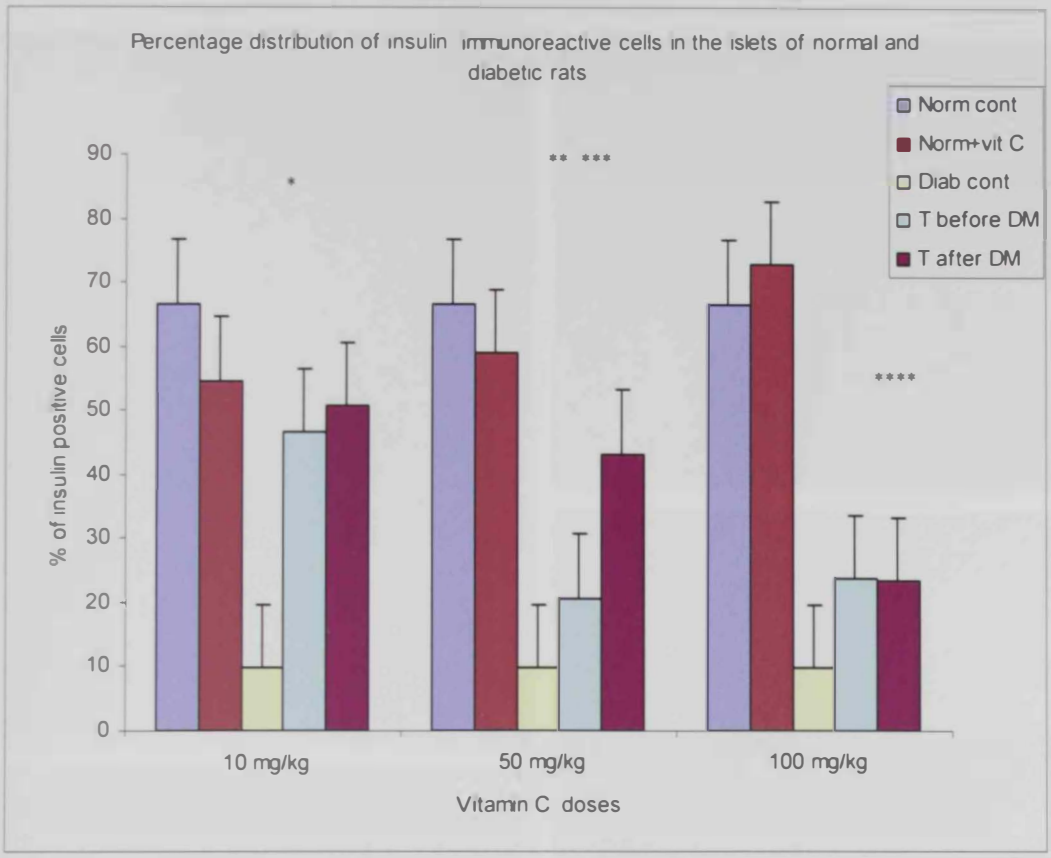


Figure 107: Histogram showing the effect of different doses of vitamin C on percentage distribution of insulin immunoreactive cells of normal and diabetic rats. Different doses of vitamin C induced significant *p<0.0003 (diab cont versus treated with 10 mg/kg of vitamin C before DM), **p<0.013(diab cont versus treated with 50 mg/kg of vitamin C before DM), ***p<0.001(diab cont versus treated with 50 mg/kg of vitamin C before DM after DM) and ****p<0.031(diab cont versus treated with 100 mg/kg of vitamin C DM after DM) increase in the percentage of insulin immunoreactive cells in islets of diabetic rats when compared to control. (Data are mean ± SD, n=10). DM= diabetes mellitus.

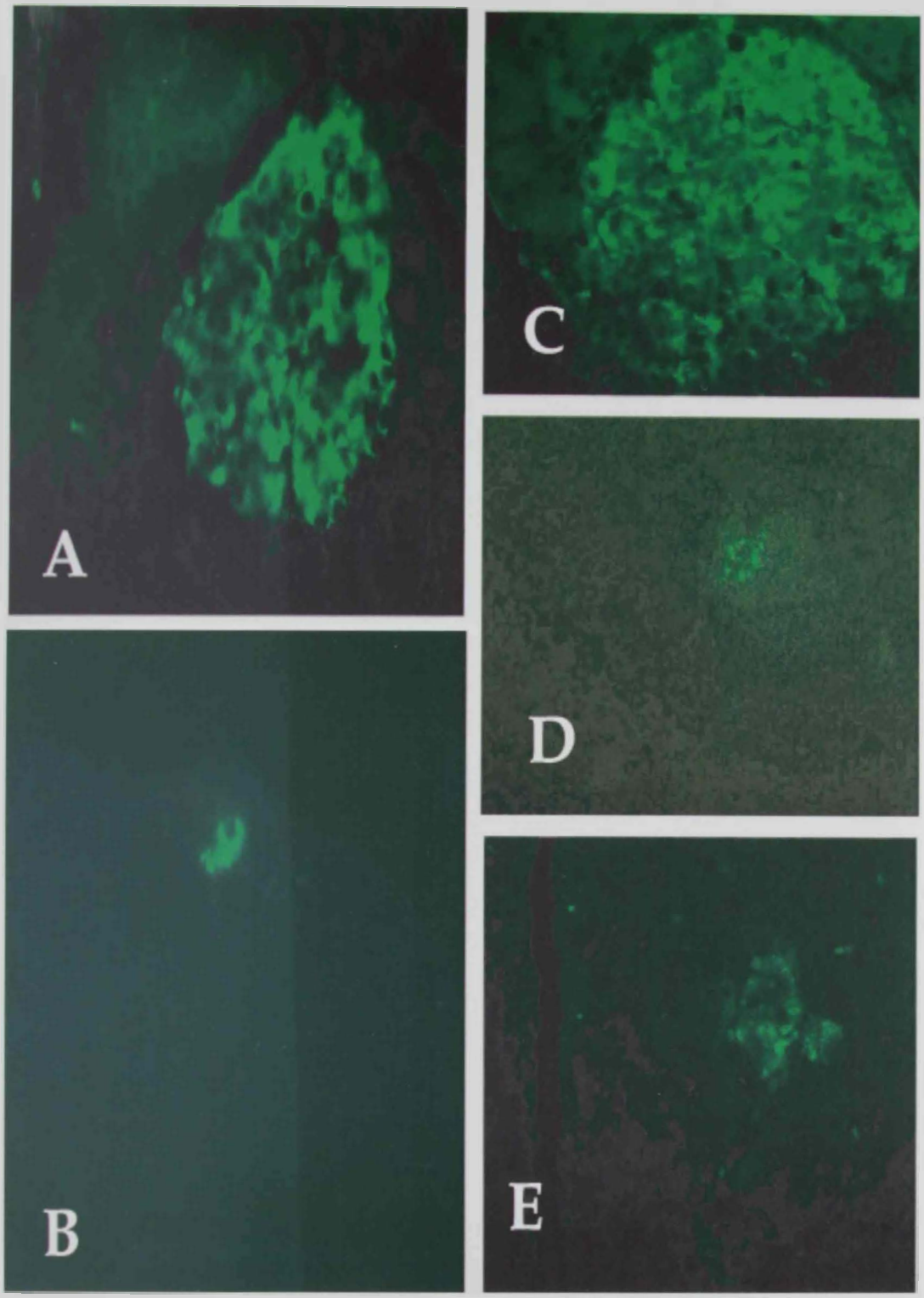


Figure 108: Representative micrographs showing insulin-positive cells in the islet of Langerhans of normal and diabetic rats. A = normal rat; B = untreated diabetic rat; C = normal rats treated with 10 mg/kg of vitamin E; D = rats treated with 10 mg/kg of vitamin C before the onset of diabetes and E = rats treated with 10 mg/kg of vitamin C after the onset of diabetes. Magnification: X 400.

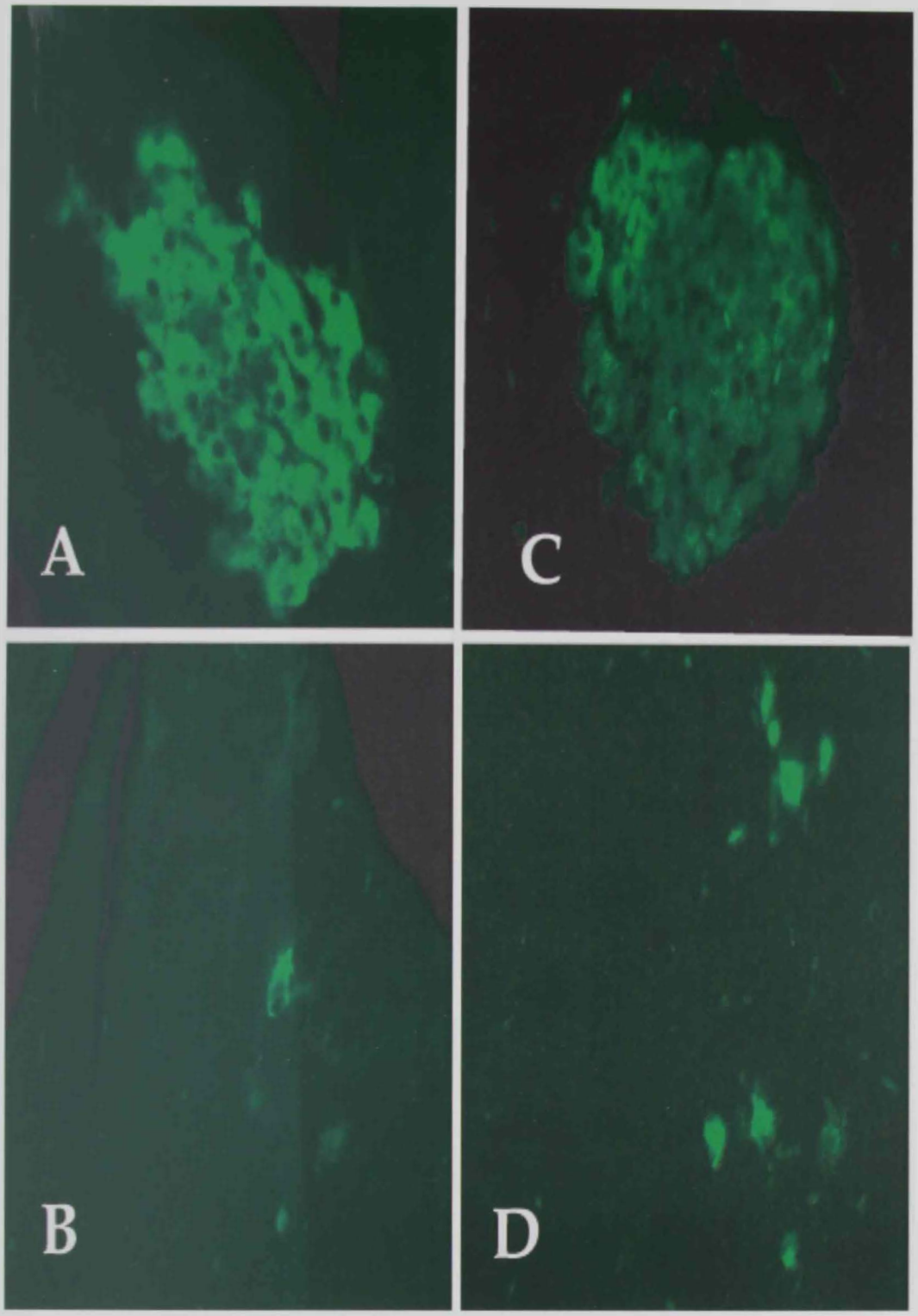


Figure 109: Representative micrographs showing insulin-positive cells in the islet of Langerhans of normal and diabetic rats. A = normal rat; B = untreated diabetic rat; C = normal rats treated with 50 mg/kg of vitamin C and D = rats treated with 50 mg/kg of vitamin C before the onset of diabetes. Magnification: X 400.

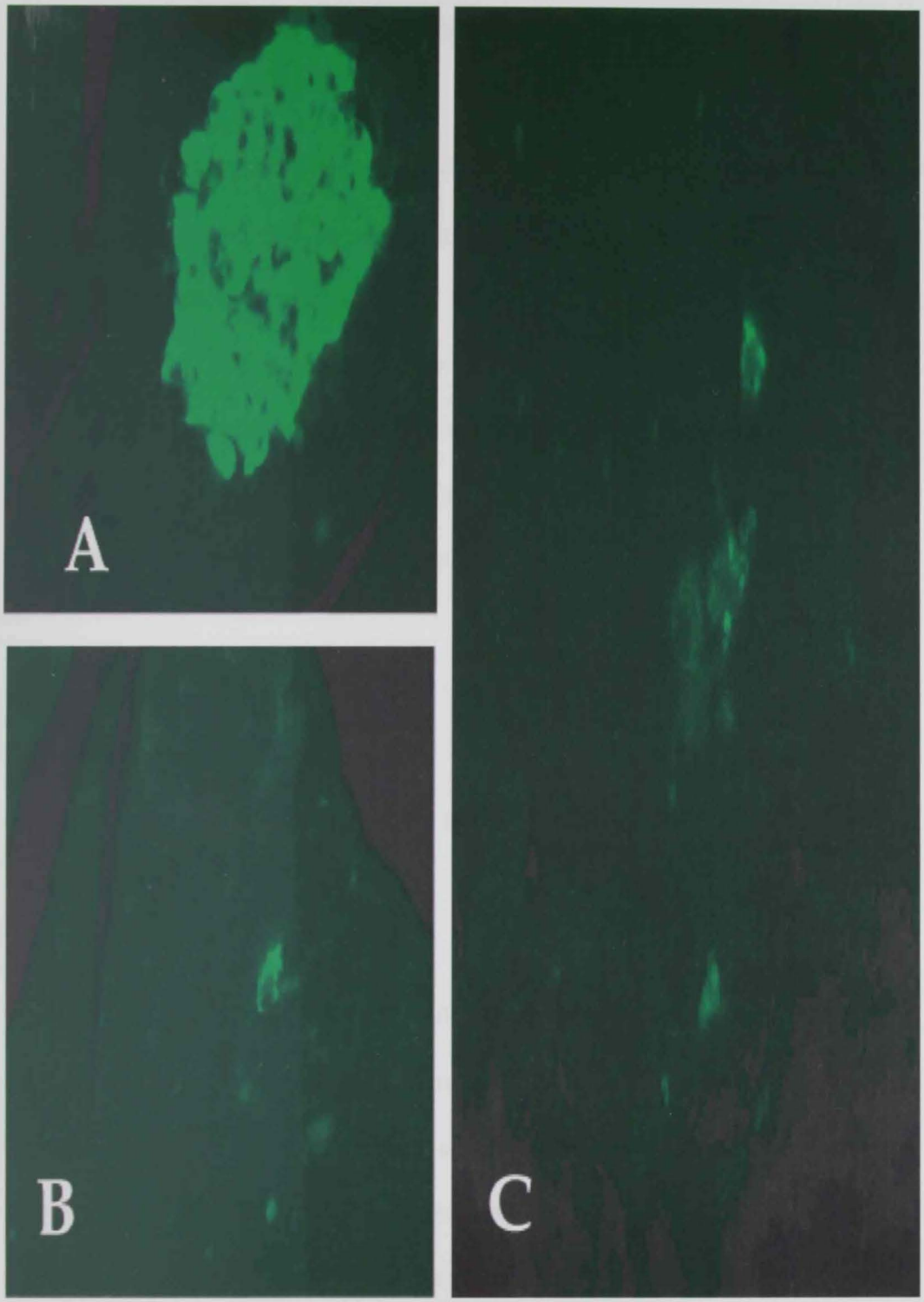


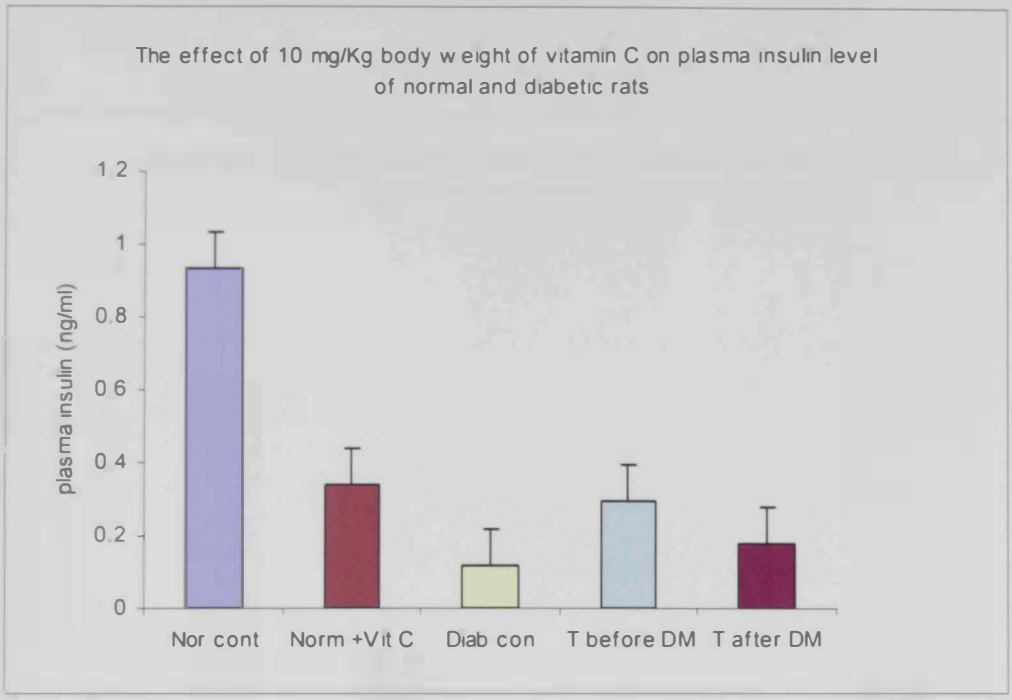
Figure 110: Representative micrographs showing insulin-positive cells in the islet of Langerhans of normal and diabetic rats. A = normal rat; B = untreated diabetic rat; C = rats treated with 100 mg/kg of vitamin C after the onset of diabetes. Magnification: X 400.

7.1 Effect of vitamin C on plasma insulin level of normal and diabetic rats.

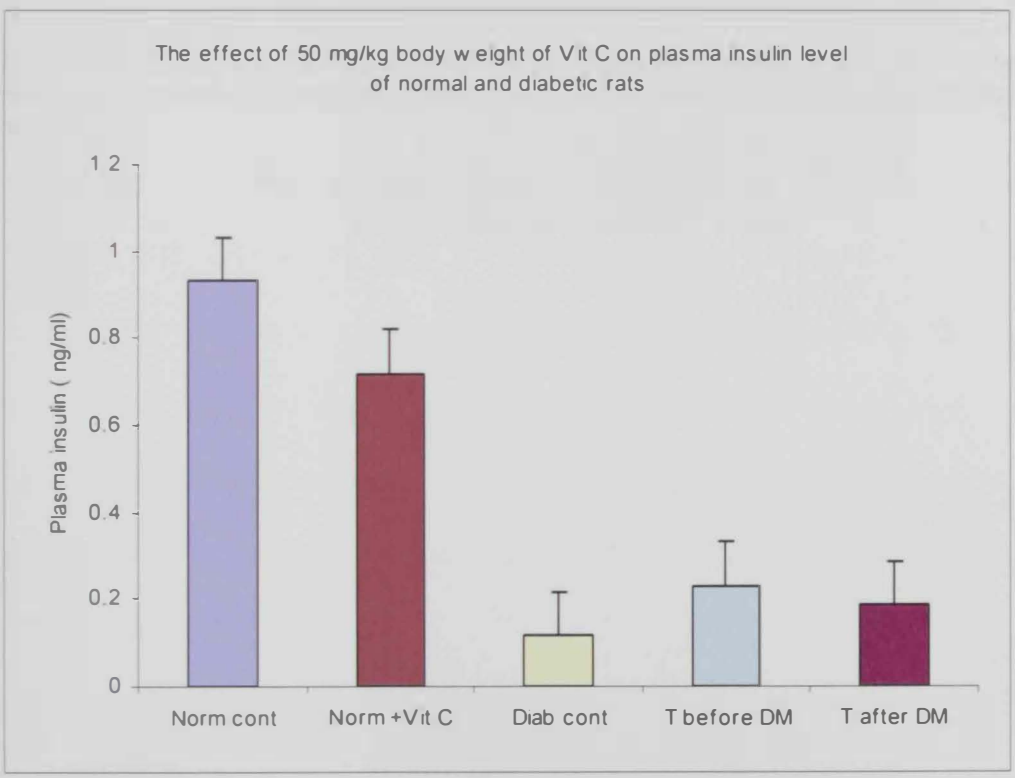
Figure 111 shows the effect of low dose of (10 mg/kg body weight) of vitamin C on insulin level in normal and diabetic rats. The insulin plasma level was slightly increased in rats treated 10 days with low dose of vitamin C (10 mg/kg body weight) before and after the onset of the diabetes compared to diabetic controls compared to diabetic controls. The oral administration of the low dose of vitamin C decreased the plasma insulin level of the normal treated rats compared to untreated normal controls.

As shown in figure 112, the plasma insulin level of normal rats treated with moderate dose (50mg/kg body weight) of vitamin C decreased when compared to normal control. Moderate dose did not induce any significant change in insulin level.

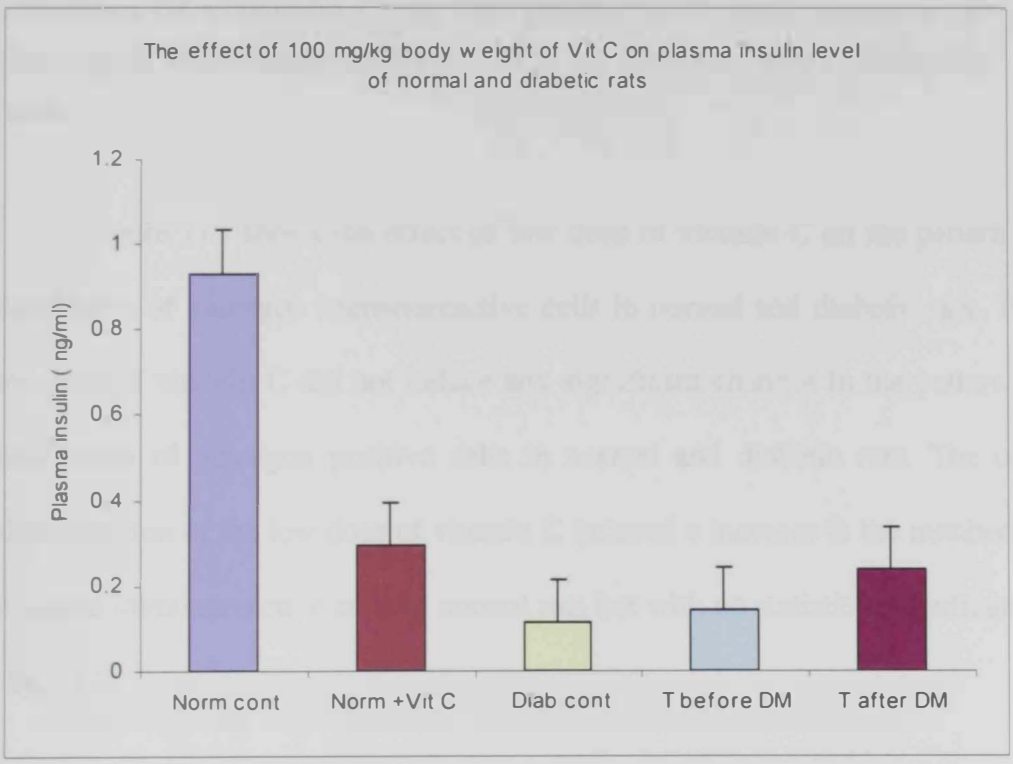
Moreover, high dose (100mg/kg body weight) of vitamin C failed to increase the plasma insulin level in rats treated with high dose of vitamin C before and after the onset of diabetes and the level was slightly increased when compared diabetic control but without any statistical significance. Also, the plasma insulin level of normal rats decreased after treatment of the high dose of vitamin C when compared to untreated normal controls (Fig. 113)



Figures 111: Histograms showing the effect of 10 mg/kg body weight of vitamin C on the plasma insulin level of normal and diabetic rats. (Data are mean ± SD, n=6) DM= diabetes mellitus.



Figures 112: Histograms showing the effect of 50 mg/kg body weight of vitamin C on the plasma insulin level of normal and diabetic rats. (Data are mean ± SD, n=6) DM= diabetes mellitus.



Figures 113: Histograms showing the effect of 100 mg/kg body weight of vitamin C on the plasma insulin level of normal and diabetic rats. (Data are mean ± SD, n=6) DM= diabetes mellitus.

8. Effect of vitamin C on the pattern of distribution of glucagon immunoreactive cells in normal and diabetic rats.

Figure 115 shows the effect of low dose of vitamin C on the pattern of distribution of glucagon immunoreactive cells in normal and diabetic rats. The low dose of vitamin C did not induce any significant changes in the pattern of distribution of glucagon positive cells in normal and diabetic rats. The oral administration of the low dose of vitamin C induced a increase in the number of glucagon immunoreactive cells in normal rats but with no statistical significance (Fig. 114).

Moreover, moderate dose of vitamin C did not cause any significant morphological changes to glucagon immunopositive cells of the pancreatic islets of normal and diabetic rats (Fig. 116). Moderate dose of vitamin C slightly increased the number of glucagon positive cells in the islets of normal rats compared to untreated normal controls. However, there was a significant ($p<0.019$) decrease in the number of glucagon positive cells in rats treated with moderate dose of vitamin C 10 days after the onset of diabetes compared to diabetic controls (Fig. 114).

The high dose of vitamin C also did not cause any significant changes in the pattern of distribution of glucagon immunoreactive cells in normal and diabetic rats (Fig. 117). Moreover, the high dose of vitamin C induced a decrease in the

number of glucagon positive cells in the islets of normal rats and in rats treated after the onset of diabetes without any statistical significance (Fig. 114).

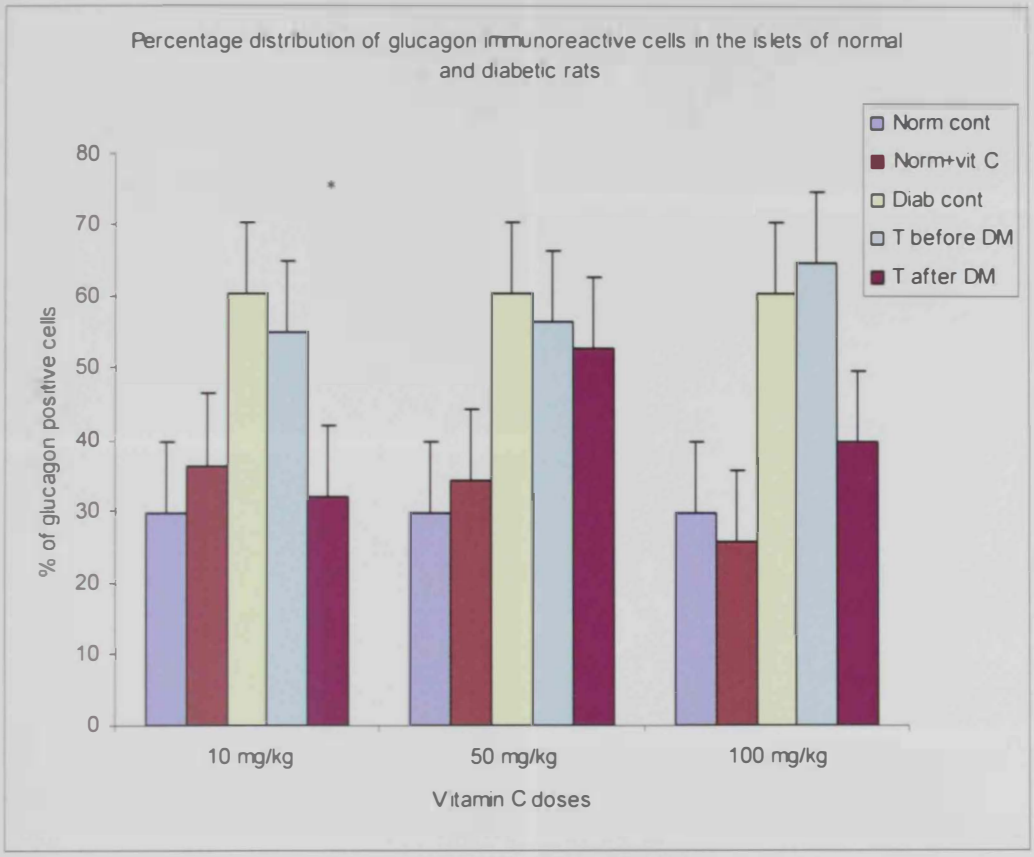


Figure 114: Histogram showing the effect of different doses of vitamin C on percentage distribution of glucagon immunoreactive cells of normal and diabetic rats. 10 mg/kg of vitamin C induced a significant *p< 0.019 (diab cont versus treated after DM) decrease in the percentage distribution of glucagons immunoreactive cells in the islets of rats treated 10 days after the onset of diabetes. (Data are mean ± SD, n=10). DM= diabetes mellitus.

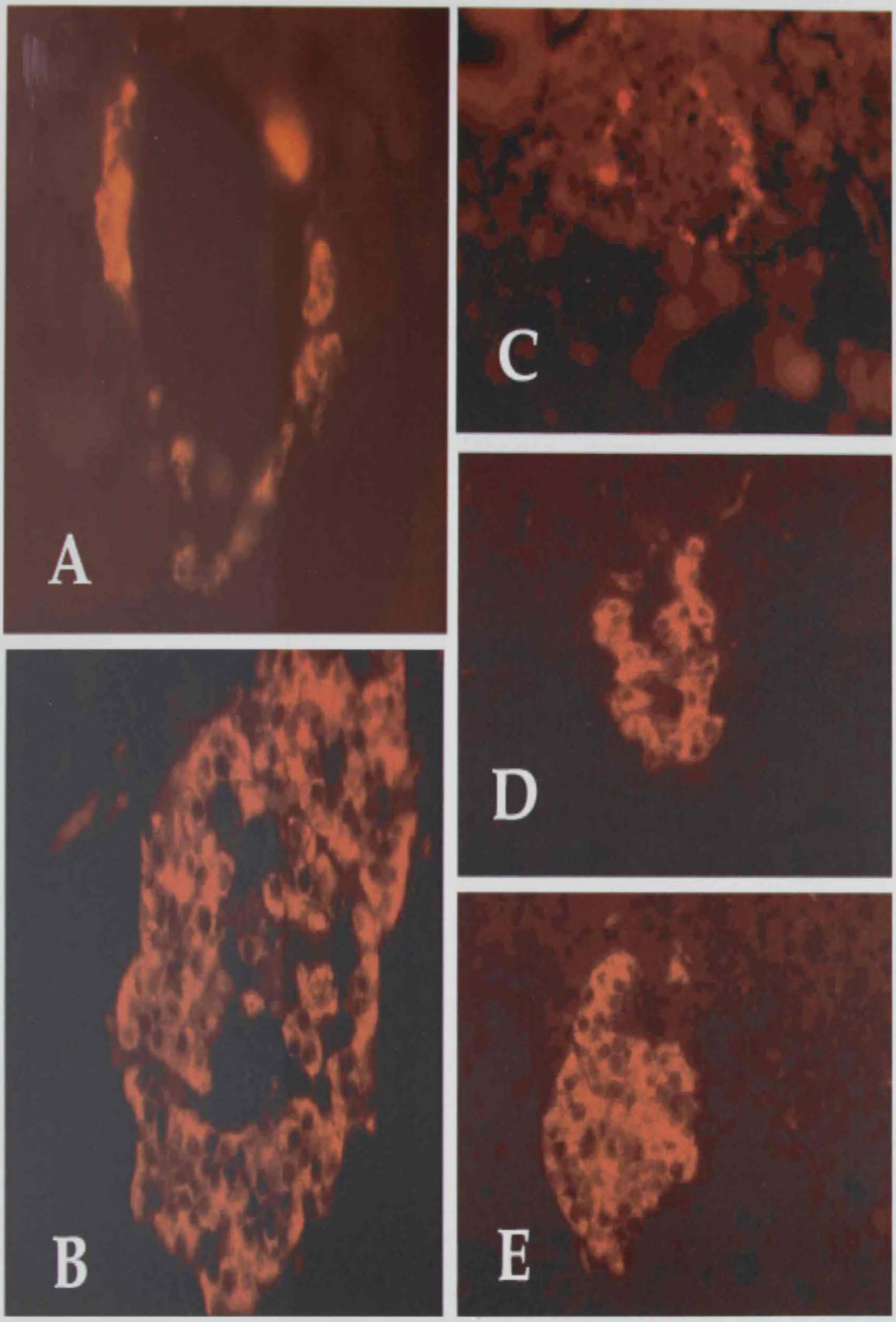


Figure 115: Representative micrographs showing glucagon-positive cells in the islet of Langerhans of normal and diabetic rats. A = normal rat; B = untreated diabetic rat; C = normal rats treated with 10 mg/kg of vitamin E; D = rats treated with 10 mg/kg of vitamin C before the onset of diabetes and E = rats treated with 10 mg/kg of vitamin C after the onset of diabetes. Magnification: X 400.

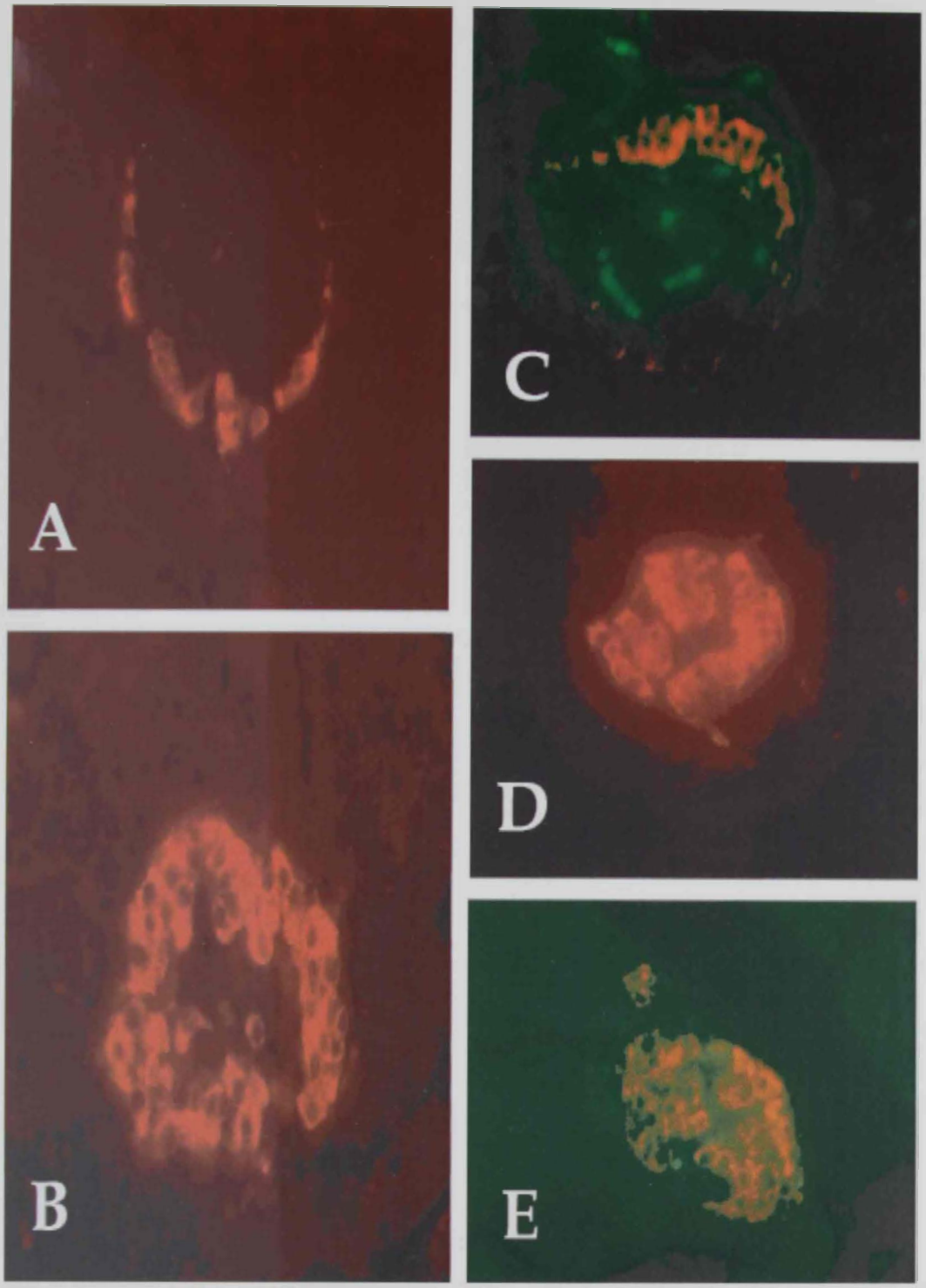


Figure 116: Representative micrographs showing glucagon-positive cells in the islet of Langerhans of normal and diabetic rats. A = normal rat; B = untreated diabetic rat; C = normal rats treated with 50 mg/kg of vitamin E; D = rats treated with 50 mg/kg of vitamin C before the onset of diabetes and E = rats treated with 50 mg/kg of vitamin C after the onset of diabetes. Magnification: X 400.

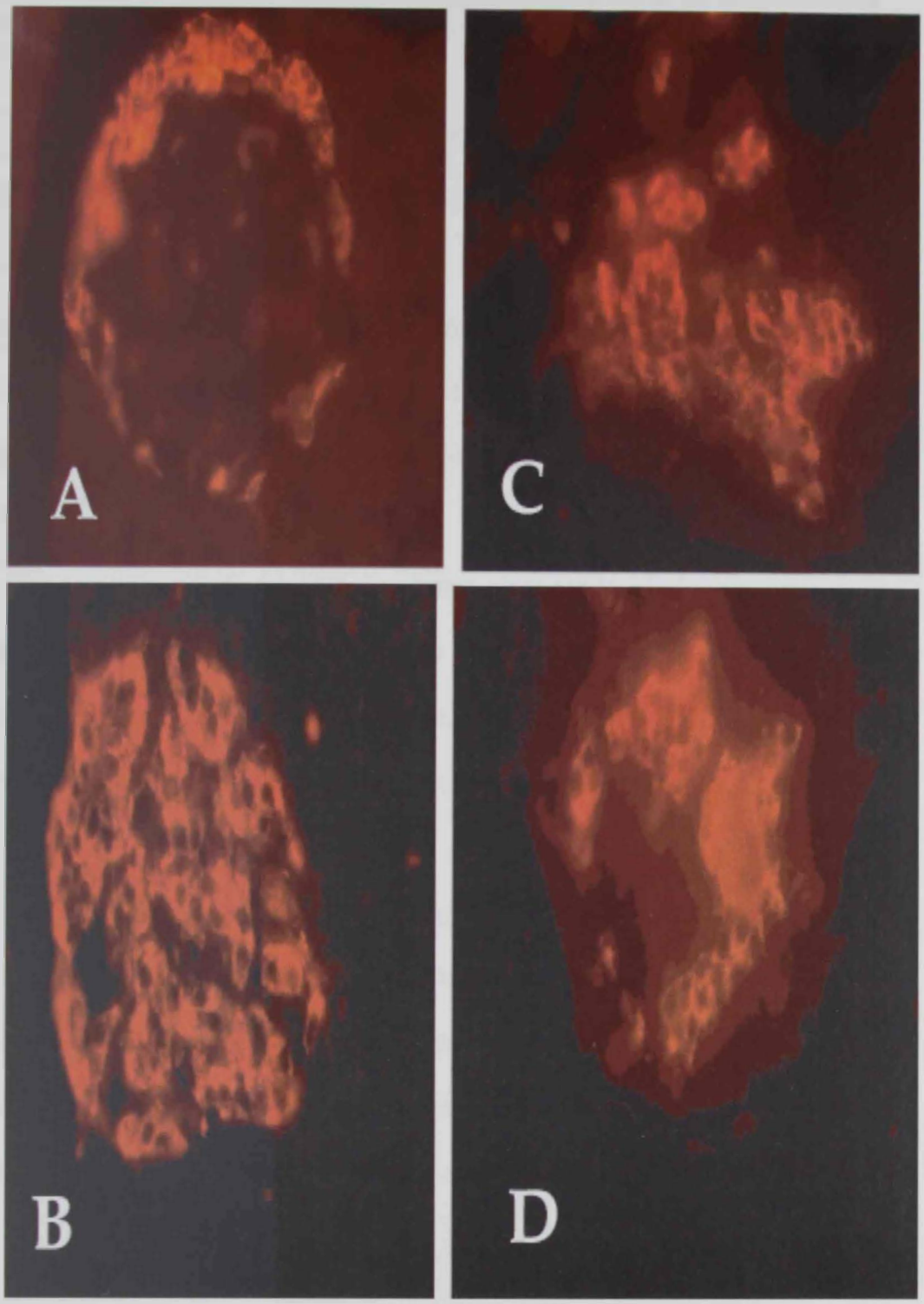


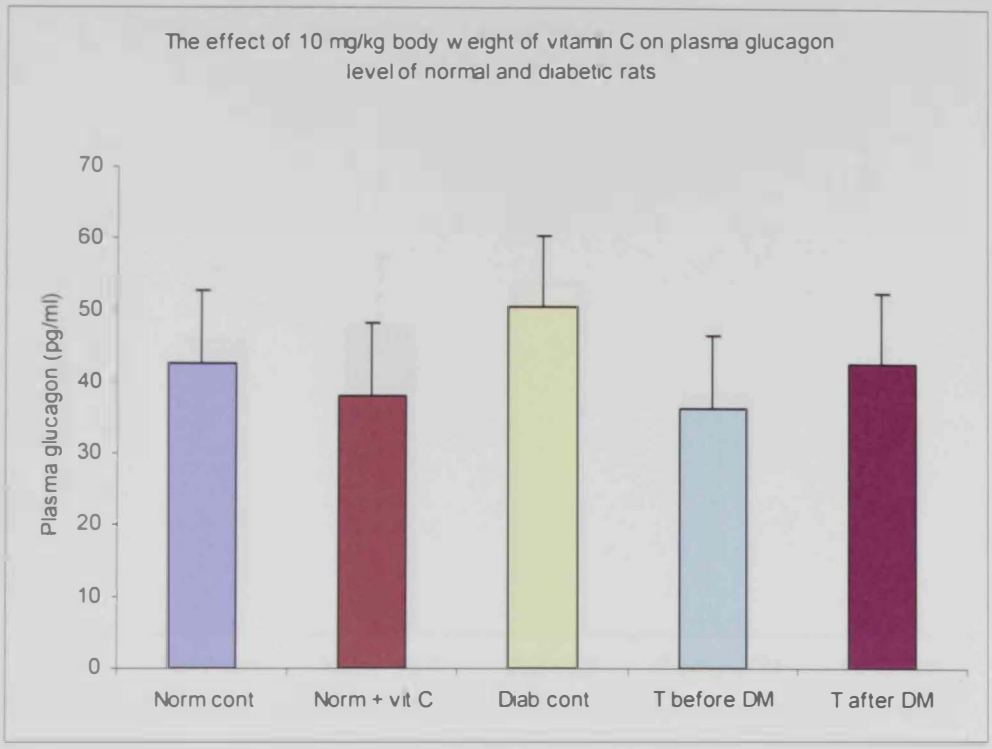
Figure 117: Representative micrographs showing glucagon-positive cells in the islet of Langerhans of normal and diabetic rats. A = normal rat; B = untreated diabetic rat; C = normal rats treated with 100 mg/kg of vitamin E and D = rats treated with 100 mg/kg of vitamin C after the onset of diabetes. Magnification: X 400.

8.1 Effect of vitamin C on plasma glucagon level of normal and diabetic rats.

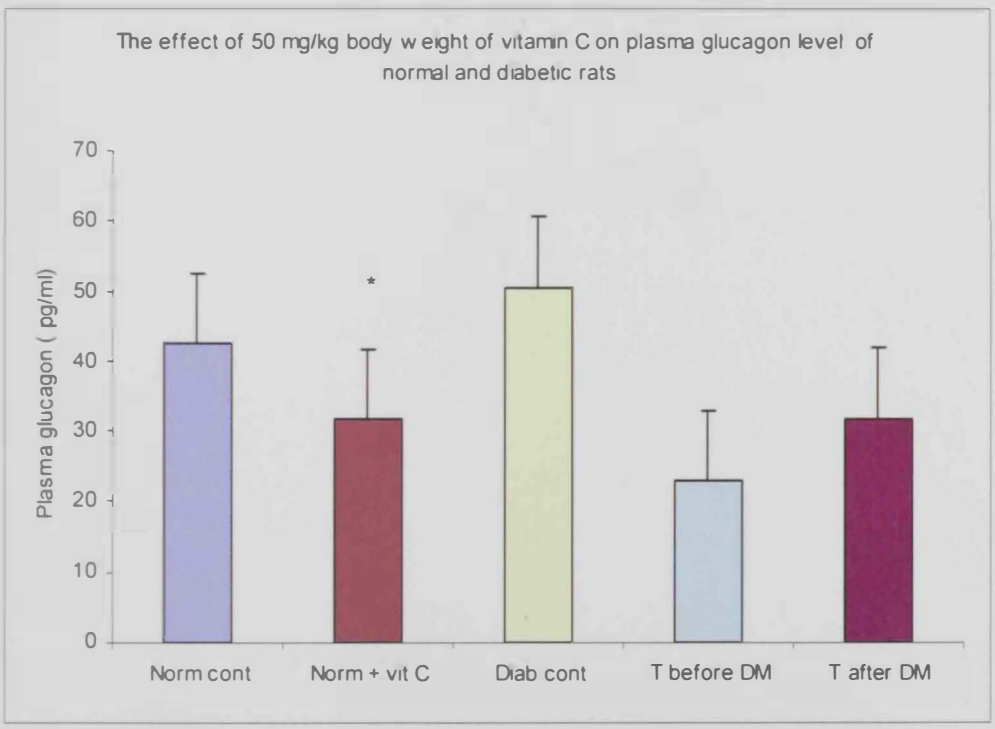
Figure 118 shows the effect of low dose of (10 mg/kg body weight) of vitamin C on plasma glucagon level in normal and diabetic rats. The glucagon plasma level was slightly decreased in rats treated 10 days with low dose of vitamin C (10 mg/kg body weight) before and after the onset of the diabetes without any statistical significance. Moreover, the plasma glucagons level decreased in normal rats after administration low dose of vitamin C.

As shown in figure 119, the plasma glucagon level of normal rats treated with moderate dose (50mg/kg body weight) of vitamin C significantly decreased when compared to normal controls. Moderate dose did not induce any significant change in plasma glucagon level in diabetic rats.

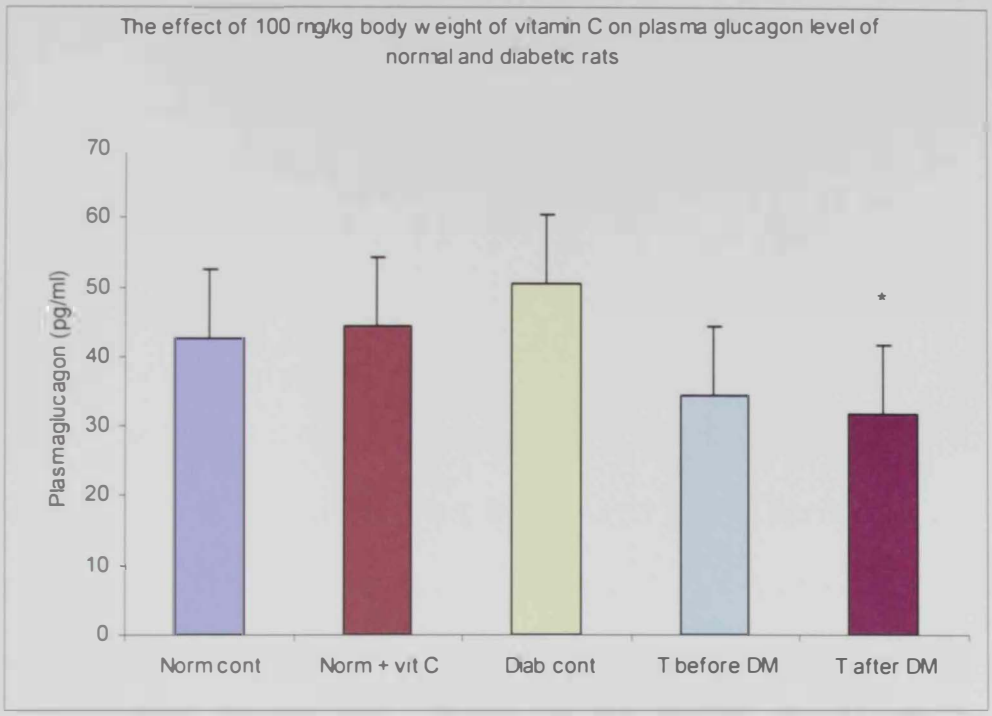
High dose (100mg/kg body weight) of vitamin C decreased the plasma glucagon level in rats treated 10 days after the onset of diabetes with a statistical significance. Moreover, high dose of vitamin C decreased the plasma glucagon level in rats treated 10 days before the onset of diabetes when compared to diabetic control but without any statistical significance (Fig. 120).



Figures 118 Histograms showing the effect of 10 mg/kg body weight of vitamin C on the plasma glucagon level of normal and diabetic rats. (Data are mean ± SD, n=6) DM= diabetes mellitus



Figures 119: Histograms showing the effect of 50 mg/kg body weight of vitamin C on the plasma glucagon level of normal and diabetic rats. 50 mg/kg of vitamin C induced a significant decrease in plasma glucagons level in normal rats *$p<0.05$ (norm cont versus norm+ vitamin C) in normal rats. (Data are mean ± SD, n=6) DM= diabetes mellitus.



Figures 120: Histograms show the effect of 100 mg/kg of body weight of vitamin C on the plasma glucagon level of normal and diabetic rats. 100 mg/kg of vitamin C induced a significant decrease in plasma glucagons level in rats treated 10 days after the onset of DM *$p<0.05$ (diab cont versus T after DM). (Data are mean ± SD, n=6) DM= diabetes mellitus.

DISSCUSSION

Effect of vitamin E on metabolic parameters of normal and diabetic rats

1. Body weight

The finding of this study have demonstrated that oral administration of different doses of vitamin E reduced body weight gain in normal and diabetic rats. Vitamin E induced weight loss is associated with a decrease in the blood glucose level. The mechanism by which vitamin E reduced rats body weight is unknown. On the other hand, vitamin E supplementation did not affect body weight gain of diabetic Wistar male rats (75). Moreover, the administration of vitamin E has no effect on body weight gain in Goto Kakizaki (GK) (76).The supplementation with 60, 200 and 800 IU of vitamin E for 30 days did not affect the body weight of healthy persons (77). There was a negative relationship between body mass index and serum Tocopherols level in Costa Rican adolescents (78). This reduction in body weight after oral administration of vitamin E may be due to the presence of tocotrienol in Tocopherols. There was a suppression of body weight gain of Fisher male rats after oral administration of 3% of tocotrienol, which was purified from vitamin E (79).

2- Plasma glucose level

Significant increase in blood glucose level was observed in diabetic rats and this was maintained at high level throughout the experimental period. Interestingly, the blood glucose level of untreated diabetic rats was slightly lower compared to the untreated group. However, this decrease in the blood glucose level did not reach statistical significance. This observation may be due to the relatively small number of samples. The present study revealed that the oral administration of vitamin E reduced blood glucose level. Other studies have shown that vitamin E supplementation significantly improves glucose level in Goto Kakizaki (GK) rats model and this improvement may result from minimizing free radical damage to pancreatic cells. Also there was an improvement in glucose metabolism and insulin action in obese Zucker rats and this may be due to the reduction in oxidative stress. Some investigators have reported that glucose stimulated hyperinsulinemia and lipid peroxidation in obese Zucker rats could be significantly reduced after vitamin E supplementation (80). However, the diabetes state induced by STZ associated with increase in fasting blood glucose level and administration of vitamin E could not normalize the hyperglycemia but it prevent lipid peroxidation induced by hyperglycemia in eyes and aorta of diabetic rats (76). The supplementation (800 IU/1 day) of vitamin E significantly reduced fasting glucose level in NIDDM patients (81).

3. Glucose tolerance test (OGTT)

The present study also showed that oral administration of vitamin E can improve glucose tolerance. The moderate and high dose of this vitamin were effective in improving glucose tolerance in rats treated 10 days before the onset of diabetes mellitus compared to rats treated 10 days after the onset of diabetes mellitus especially at 30, 60,120, and 180 min after glucose challenge. The baseline blood glucose levels were slightly lower compared to severe diabetes. This may be due to the vitamin E treatment and even a possible recovery of pancreatic β-cells.

Vitamin E thus has a beneficial effect in the improvement of glucose tolerance. The blood glucose levels were decreased significantly at 30 min and 120 min in tocopherol supplemented Goto Kakizaki (GK) rats. The reduction of oxidative stress by tocopherol decreased glucose level due to accumulation of tocopherol in pancreatic islets and improvement of insulin secretion (76).

4. Plasma insulin level

The present study demonstrated that insulin resistance may be accompanied by intracellular production of free radicals. Ceriello (81) noticed that insulin increases the production of hydrogen peroxide which has been shown to mimic the action of insulin and form this vicious circle between hyperinsulinemia and free radical could be operating. Insulin resistance might cause elevated plasma free radical concentrations which may contribute to the

deterioration of insulin action. He found that vitamin E improves insulin action in healthy, elderly and noninsulin dependent diabetes (82).

3. Effect of vitamin E on biochemical parameters of normal and diabetic rats

3.1 Effect of vitamin E on liver enzymes

Liver enzymes including ALT, AST, ALP and GGT reflect different functions of the liver such as excretion of anions, hepatocellular integration, formation and subsequent free flow of bile and protein synthesis. The aminotransferase ALT and AST are important indicators of hepatocellular damage. They also play an important role in gluconeogenesis (83).

AST is found in several organs like liver, brain, kidney, lungs and pancreas and it is present in cytosolic and mitochondrial enzymes. In the present study the plasma level of AST increased after the onset of diabetes mellitus. Only treatment with low dose of vitamin E significantly reduced the AST level in rats treated 10 days after the onset of diabetes. The reason for the inability of other doses to significantly increase or decrease the level of AST in normal and diabetic rats is not clear.

ALT is more specific to liver and it is present in cytosolic enzyme. It is found in large quantity in liver. The plasma level of ALT increased after the onset of diabetes mellitus. In contrasts, the oral administration of low dose of

vitamin E significantly decreased ALT in the plasma in rats treated 10 days before and after the onset of diabetes.

The hepatocellular injury is the trigger for the release of these enzymes into the circulation. The increase in the plasma level of these liver enzymes shows that STZ had a toxic effect on the liver. Treatment with low dose of vitamin E reduced this toxic effect on the liver of diabetic rats. Also, this result indicated that low dose of vitamin E is more effective than moderate and high does on plasma AST and ALT and this may be due to what was reported previously, that the use of high single doses of micronutrient antioxidant supplements such as vitamin E poses potential risks because it could pertub the antioxidant peroxidant balance. It has been recommended that high doses of micronutrient antioxidants vitamins should be administered in combination rather than in single supplements (84).

The plasma ALP level also increased after the onset of diabetes. The ALP originates mainly form two sources, the liver and bone and it may be present in many other tissues such as the intestine, kidney, placenta and blood. Hepatic ALP is present in the canalicular and luminal domain of bile duct epithelium and its level rise as a result of increased synthesis and before being release into circulation. The level of ALP may not rise until two days after biliary obstruction (85). The oral administration of vitamin E failed to decrease the ALP level in rats treated 10 days before and after the onset of diabetes. The plasma level of ALP increased significant after administration of low dose of vitamin E in normal non diabetic rats.

The data obtained from this study show that low vitamin E had a significant effect on LDH level in untreated normal rats. On the other hand, the LDH level in rats treated 10 days after the onset of DM is slightly similar to the level in normal rats. The high oral administration of vitamin E significantly increased LDH level.

GGT is found in hepatocytes and biliary epithelial cells. Its level is raised in pancreatic disease, renal failure and diabetes (85). There was a significant increase in the GGT level in diabetic rats. After the oral administration of vitamin E there was no significant change in GGT level in rats treated either before and after of the onset of diabetes. This means that treatment with vitamin E did not have any beneficial effect on decreasing GGT level in diabetic rats.

3-2 Effect of vitamin E on kidney parameters of normal and diabetic rats

In the present study, only administration of low dose of vitamin E significantly increased blood creatinine level while all other two doses of vitamin E had no effect on creatinine the in the normal rats and this suggest that low dose of vitamin E may have adverse effect on kidney function. Diabetes elevated the plasma creatinine level in rats and vitamin E treatment failed to reduce its level. Other study indicated that the disturbance of renal function which appeared in high level of plasma creatinine and they found that a decrease

in creatinine level of diabetic rats correlated significantly with vitamin E treatment and this effect may be due to the influence of vitamin E on the disturbance of redox balance. The high dose of vitamin E significantly increased blood urea nitrogen level (BUN) of treated 10 days after the onset of diabetes. It does not appear that the higher the vitamin E dose the higher the level of BUN.

4. Effect of vitamin E on the pattern distribution of insulin immunoreactive cells in the pancreas of normal and diabetic rats

There were significant changes in the pattern of distribution of insulin positive cells in the pancreas of diabetic rats when compared to normal control. Insulin positive cells were located on the central and peripheral parts of the islets in normal pancreas. This observation on the pattern distribution of insulin producing cells is similar to other reports (87). Moreover, the oral administration of different doses of vitamin E did not change the pattern of distribution of insulin immunoreactive cells in the islets of normal and diabetic rats. The number of insulin positive cells was significantly higher in normal pancreas compared to STZ-induced diabetic rats. The STZ does not destroy all of the ß-cells in the diabetic pancreatic islets and this result corroborates with previous report (88). The moderate and high doses of vitamin E have beneficial effect by increasing the number of insulin positive cells in diabetic rats. The mechanism by which vitamin E causes the increase in the number of insulin positive cells is unknown. However, this increase in the number of insulin positive cells may be due the ability of vitamin E to reduce the toxic

effect of STZ on ß-cell and it may help in the regeneration of pancreatic ß-cells which are damaged by STZ. The low and moderate doses of vitamin E induced a significant decrease in the number of insulin positive cells in normal rats. The ability of vitamin E to induce a decrease in the number of pancreatic β-cell is unknown. This may indicate that vitamin E is useful in tissue subjected to oxidative stress such as STZ-induced diabetes.

5. Effect of vitamin E on the pattern of distribution of glucagon immunoreactive cells in the pancreas of normal and diabetic rats

There were significant changes in the pattern of distribution of glucagon positive cells in the pancreas of diabetic rats when compared to normal control. In normal pancreas, glucagon positive cells were located mainly on the peripheral parts of the islets and this is similar to other report (89). After the onset of diabetes, the number and pattern of distribution of glucagon positive cells changed significantly when compared to normal control. Glucagon positive cells were located in both peripheral and central regions of the islets of diabetic rats. Moreover, the oral administration of different doses of vitamin E did not induce any statistical significance on the pattern of distribution and number of glucagon immunoreactive cells in the islets of normal and diabetic rats. The reason for the increase in the number of glucagon positive cells is unknown but this could be due to the absence of the inhibitory effect of insulin on glucagon.

Effect of vitamin C on metabolic parameters of normal and diabetic rats

1- Body weight

The present study showed that the administration of vitamin C has completely inverse correlation with body weight gain in rats treated 10 days before the onset of diabetes. Low dose of vitamin C can elevate body weight gain in rats treated 10 days after the onset of diabetes. Moderate dose also increase body weight gain in a rate smaller than that of low dose. However, high dose decreased body weight gain in treated 10 days after onset of DM. This observation suggested that only the low dose of vitamin C has a beneficial effect on diabetes and the moderate and high doses had an adverse effect on body weight gain especially after the onset of diabetes.

Also, the administration of vitamin C on rats before the onset of diabetes did not reduce the effect of diabetes on body weight. Further studies are required to establish the mechanism of action of vitamin C on body weight. The mechanism by which high dose of vitamin C reduces body weight is not clear.

2. Blood glucose level

The present study showed that vitamin C can improve plasma glucose level. The administration of low dose of vitamin C was more effective than moderate and high doses. It significantly reduced the plasma glucose level in rats treated 10 days before and after the onset of diabetes. Also, vitamin C supplementation significantly elevated glucose level in non diabetic rats.

The data from this study showed that low dose of vitamin C significantly improved the glucose tolerance in rats treated 10 days before and after the onset of diabetes especially at 60 min. On the other hand, administration of high dose of vitamin C significantly reduced glucose level at 60 and 120 min only in rats treated 10 days after the onset of diabetes. The beneficial effect of ascorbic acid noted in the present study can be attributed to the antioxidant effects of vitamin C. vitamin C is a scavenger of oxygen free radicals which are toxic by-products of many metabolic process and in STZ-induced diabetes.

Effect of vitamin C on biochemical parameters of normal and diabetic rats

1. Liver enzyme

As mentioned before, liver enzymes are used as markers of hepatotoxicity especially ALT, which is a more specific indicator for liver damage. From the demonstrated data, the plasma level of alanine aminotransferase increased after diabetes and vitamin C administration had no beneficial effect in reducing ALT level also it can't reduce the level of plasma AST.

Serum alkaline phosphatase level also increased in diabetes mellitus. The low dose of vitamin C significantly reduced the ALP level. However, the administration of high dose of vitamin C had a negative effect on ALP level because the level of ALP is increased. There was no significant effect on plasma level of lactic dehydrogenase in diabetic group after treatment with vitamin C but low dose significantly increase LDH level and high dose can also significantly reduce its level in normal non diabetic rats .

The plasma level of GGT was significantly increased in diabetic rats. Low dose of vitamin C did not reduce GGT plasma level in rats treated 10 days before and after the onset of diabetes. There was no significant effect of vitamin C on normal non diabetic rats. This may be due to the dosage of vitamin C

applied. A much lower or higher dose of vitamin C may be required for an effective reduction in the plasma GGT level of diabetic rats.

2. Effect of vitamin C on kidney parameters of normal and diabetic rats.

In the present study, all the three doses of vitamin C had no effect on creatinine level in the normal rats. The administration of high dose of vitamin C significantly increased blood creatinine level in rats treated 10 days after the onset of diabetes and this may be because that high dose of vitamin C had adverse effect on kidney function. The high dose of vitamin C significantly increased blood urea nitrogen level in both treated 10 days before and after onset of diabetes.

Effect of vitamin C on the pattern of distribution of insulin immunoreactive cells in the pancreas of normal and diabetic rats

The oral administration of different doses of vitamin C did not induce any changes in the pattern of distribution of insulin immunoreactive cells in islets of normal and diabetic rats. However, the oral administration of vitamin C has beneficial effect by the increasing the number of positive insulin cells in diabetic rats. The mechanism by which vitamin C causes an increase in the number of insulin positive cells is unknown. This increase in the number of insulin positive cells may be due the ability of vitamin C to prevent the death of β-cells or permitting the recovery of the destroyed β-cell by STZ. The STZ-

induced a cascade of event during which cytokines will be able to destroy pancreatic β-cell islets (88) and the effects of these cytokines may be neutralized by vitamin C, and thus increase the number of insulin positive cells.

Effect of vitamin C on the pattern of distribution of glucagon immunoreactive cells in the pancreas of normal and diabetic rats

The oral administration of the low dose of vitamin C is more effective in reducing the number of glucagon positive cells in the islets of rats treated 10 days after the onset of diabetes. The other two doses of vitamin C failed to induce any statistical significance on the pattern of distribution and number of glucagon immunoreactive cells in the islets of normal and diabetic rats. As mention earlier the reason for the increase in the number of glucagon positive cells is unknown but this could be due to the absence of the inhibitory effect of insulin on glucagon.

CONCULSION

Conclusion

It can be concluded from this study that the administration of vitamins E and C to normal and STZ-induced diabetic rats significantly decreased body weight gain in a dose-correlated manner. The oral administration of vitamin E and C also, reduced blood glucose level and improved glucose tolerance. Both vitamin E and C significantly affected the biochemical parameters of both normal and diabetic rats. The investigation on liver enzymes, kidney parameters and electrolytes showed that the levels of these parameters are altered during STZ-induced diabetes. The administration of vitamin E and C affect the level of these biochemical parameters. The insulin immunoreactive cells were significantly decreased in STZ-induced diabetic rats and the administration of vitamin E and C significantly increased insulin positive cells. However, glucagon positive cells increased in STZ-induced diabetic rats and the administration of vitamin E and C failed to decreased glucagon positive cells. The result of obtained from this study have provided insight into the hypoglycemic effect of vitamin E and C and they may be a useful therapies in the management of diabetes mellitus.

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ARABIC ABSTRACT

الملخص العربي

يعرف داء السكري بأنه واحد من الأسباب الرئيسية المؤدية للمرض والموت في العالم و يتزايد عدد الأشخاص المصابين بمرض السكري يتسارع حيث يتراوح عدد المصابين بهذا المرض ما بين 5- 6 % من سكان العالم.

وهناك ارتباط وثيق بين مرض السكري و حالات الإجهاد الناجمة عن التأكسد . ويعتبر ارتفاع نسبة السكر في الدم ، من احد أهم المؤشرات على الإجهاد الناجم عن التأكسد . ومن الطبيعي بأن الآليات الذاتية لمضادات الأكسدة لها القدرة على تحطيم المجموعات النشطة ، وإيجاد التوازن ما بينها وبين هذه المجموعات النشطة. وعند الإصابة بالسكري فان تأثير التأكسد يزداد بسبب النقص في نظام دفاع مضادات الأكسدة والتي بدورها يمكن أن تقلل من تأثير المجموعات النشطة ، ويساعد في استعادة نظام دفاع مضادات الأكسدة .

إن الهدف من هذه الدراسة هو دراسة مدى تأثير وفعالية جرعات مختلفة من كل من فيتامين ج و وفيتامين سي في علاج مرض السكري. فقد تم دراسة تأثير هذه الفيتامينات على العمليات الايضية والبيوكيميائية لفئران طبيعية وأخرى مصابة بمرض السكري. وقد توصل هذا البحث إلى أن فيتامين ج وفيتامين سي قد اخفضا وبشكل ملحوظ ومعنوي الزيادة في وزن الجسم.

وعلاوة على ذلك فان كل من فيتامين سي , ج أدى إلى تقليل وخفض مستوى السكر في الدم في فئران المصابة بمرض السكري. وبالإضافة لذلك فأن هذه الفيتامينات أيضا لها القدرة على زيادة عدد الخلايا المفروزة الأنسولين. وأيضا إن كل من فيتامين ج وفيتامين سي له أثر ملحوظ على المحددات البيوكيماوية. فإن كلا من فيتامين ج وفيتامين سي له أثر على عمل الأنزيمات التي يفرزها الكبد وعلى عمل الكلى وعلى مستوى العناصر المتحللة كهر بائيا في البلازما مثل الكالسيوم و الصوديوم. ومنه فان مضادات الأكسدة كفيتامين ج وفيتامين س ويمكنها تحسين المحددات الايضية و البيوكيميائية للفئران المصابة بالسكري .

PUBLICATIONS

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Beneficial effect of vitamin E on the metabolic parameters of diabetic rats

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Abstract

The role of vitamin E in the pathogenesis of diabetes mellitus is unknown. The purpose of this study was to examine the effect of oral administration of vitamin E on some of the metabolic parameters of experimental diabetic rats. Diabetes was induced by intraperitoneal injection of streptozotocin (60 mg/kg body weight at 12 weeks of age). Vitamin E (0.2, 0.4, 0.8 mg/kg body weight) was administered orally for a period of 3 weeks to normal and diabetic Wistar rats. In some experiments, Vitamin E was given either before or after the induction of diabetes mellitus. Blood glucose level and weight were recorded for each rat in different groups on a weekly basis. Oral glucose tolerance test (OGTT) was performed on fasted normal, diabetic and vitamin E treated rats at the end of the experiment. Vitamin E significantly ($p < 0.01$) reduced blood glucose levels in experimental diabetes mellitus at all doses as compared to untreated rats. Vitamin E induced weight loss in normal as well as in diabetic rats. The beneficial effect of vitamin E on the hyperglycaemia of diabetic rats was dose-dependent. Moreover, vitamin E also improved OGTT in diabetic rats compared to untreated diabetics. In conclusion, vitamin E may play a role in glucose metabolism and may be a useful adjuvant therapy in type I diabetes. (Mol Cell Biochem **261**: 35–42, 2004)

Key words: vitamin E, diabetes mellitus, anti-oxidants, glucose, glucose tolerance test, weight

Introduction

There is little doubt that we are in the midst of a worldwide epidemic of diabetes. There are an estimated 143 million people worldwide with the disease, almost five times more than estimates of 10 years ago. This number will probably double by 2030 [1]. Although diabetes is more prevalent in developed countries, it is likely that the developing world will bear the brunt of the epidemic in the future. In diabetics, the risks of heart disease and stroke are escalating. Moreover, diabetes is the leading cause of end-stage renal disease, blindness, and non-traumatic limb amputation. It is rather difficult to calculate the human and economic costs of diabetes worldwide. However, the total medical costs incurred annually in the United States alone are close to $100 billion [2].

Diabetes mellitus is a metabolic disorder caused by an absolute or relative lack of insulin. As a result, the metabolism of fuels including carbohydrates, fats, and proteins is altered. This results in elevated fasting and post-prandial serum glucose (hyperglycaemia) that leads to complications if left untreated.

Hyperglycaemia induces increases in glucose autoxidation and protein glycation, and the subsequent oxidative degradation of glycated proteins leads to enhanced production of reactive oxygen species (ROS) [4]. ROS are thought to play a major role in a variety of physiologic and pathophysiological processes in which increased oxidative stress may play an important role in disease mechanisms; however, increased oxidative stress may be a result of the pathologic process [5]. In diabetes, persistent hyperglycaemia may cause high production of free radicals generated in direct autoxidation processes of glucose [6], the non-enzymatic and progressive glycation of proteins with the consequent increased formation of glucose-derived advanced glycosylation end products [7], activation of NAD(P)H oxidases [8], nitric oxide synthase [9], and a specific enzyme activity, xanthine oxidase, which produces oxidant species and subsequent oxidative stress [10]. There is also evidence that hyperglycaemia may

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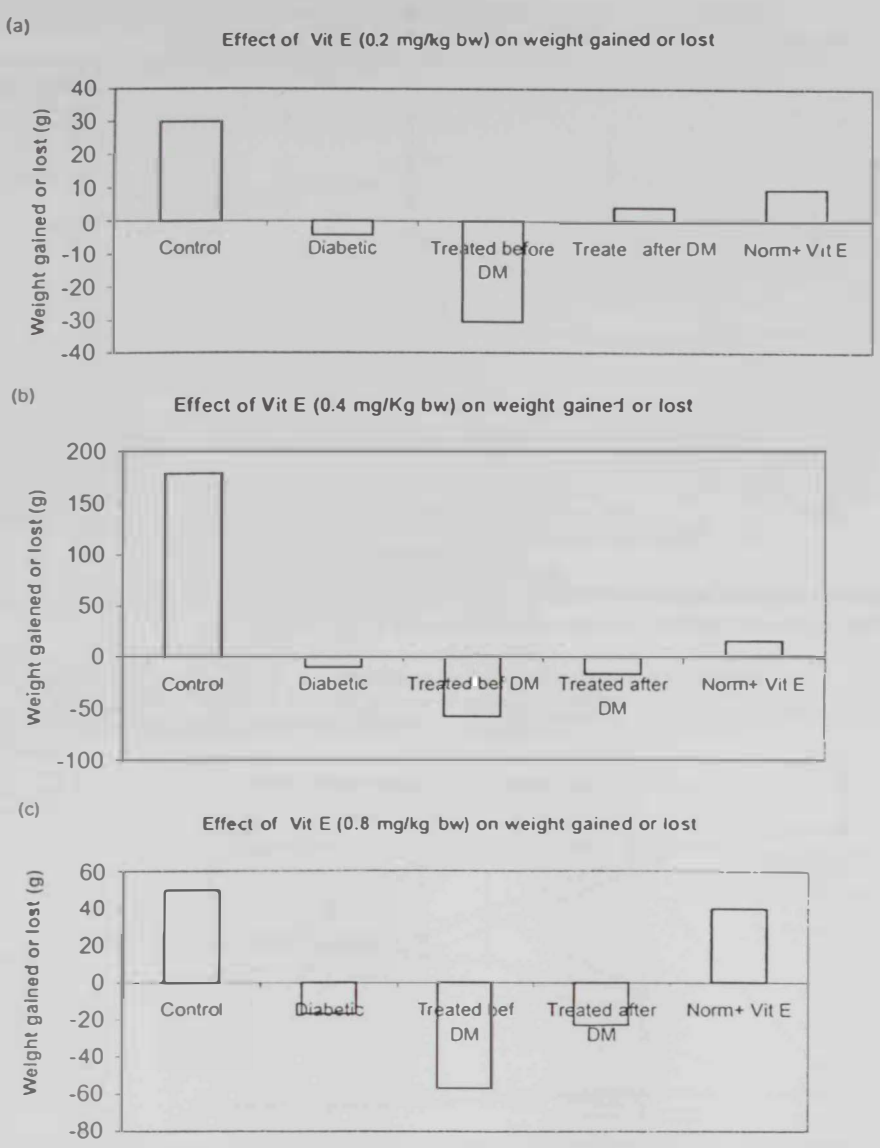

. Histograms showing the effect of different doses (a: 0.2, b: 0.4; c: 0.8 mg/kg body weight) of vitamin E on body weight of normal and diabetic rats. Diabetes mellitus. $N = 10$ rats.

at of untreated normal control. There was a significant ht loss in rats that received vitamin E before the onset abetes compared to that in untreated diabetic rats.

ie administration of a medium dose of 0.4 mg of vitamin rats after the onset of diabetes resulted in weight loss 1b). This weight loss was more pronounced compared to f untreated diabetic rats. The weight loss observed in rats d with 0.4 mg vitamin E before the onset of diabetes was ficantly higher than that of untreated diabetics. Normal hat received vitamin E failed to gain weight in the same on as untreated control rats.

e oral administration of 0.8 mg of vitamin E to diabetic esulted in weight loss (Fig. 1c). The weight loss ob- d in rats treated with 0.8 mg vitamin E after the onset of diabetes was comparable to that untreated diabetics. However, the treatment of rats with vitamin E before the onset of diabetes inflicted severe weight loss compared to untreated diabetic rats. Normal rats that received vitamin E, did not gain as much weight as untreated control rats.

Effect of vitamin E on blood glucose level

Figure 2 shows the fasting blood glucose (FBG) level of normal and diabetic rats after treatment with vitamin E. Oral administration of 0.2 mg/kg body weight of vitamin E to rats that were already diabetic caused a significant ($p < 0.05$) reduction in FBG level compared to untreated diabetic rats. In contrast, there was no significant difference in the FBG

disrupt natural antioxidant defence mechanisms [11, 12]. Such metabolic perturbations elicit alterations in tissues that undergo insulin-independent glucose uptake, thereby provoking early tissue damage in target organs of diabetic complications such as ocular lens, retina, peripheral nerve and renal glomerulus [13–15].

There are two common mechanisms that lead to increased oxidative stress in diabetes: one of them is increased free radical production during elevated autoxidation of glucose and the other is the hyperglycaemia-induced reduction in the levels of protective endogenous antioxidants such as vitamins A, E, C, glutathione, etc., and decrease in the activities of antioxidant enzymes such as glutathione peroxidase. Reduction of the antioxidant capacity generates more H_2O_2 and other reactive intermediates such as hydroxyl radicals. Therefore, lipids but also proteins, carbohydrates and nucleic acids are affected by alteration of the oxidant and antioxidant systems. Increased free radical production and reduced activity of antioxidant defence systems in diabetes and hence tissue damage are facilitated [16, 17]. Lower endogenous antioxidants and elevated lipid peroxidation levels are risk factors for the development of diabetic complications such as atherosclerosis [18, 19].

Studies in animal models revealed that some of the diabetes-related functional and morphological abnormalities could be prevented by antioxidants [20]. However, in general, these findings are inconclusive because several reports indicated the absence of improvement [21] and even worsening [22] of diabetic nephropathy with antioxidant treatment. The latter could result from insufficient understanding of the mechanisms underlying renal oxidative damage and, in particular, very contradictory information on the effects of diabetes on major components of free radical production and anti-oxidative defence, which hampers the selection of specific targets for antioxidant therapy as well as proper antioxidants and antioxidant combinations [23].

Vitamin E is a lipid-soluble, chain-breaking antioxidant, which protects especially biological membranes from lipid peroxidation [24]. It was recently reported that vitamin E prevents elevated lipid peroxidation in rats [25]. Other vitamins such as vitamin C are also involved in antioxidative defence [26].

The purpose of this study was to characterize some STZ-induced metabolic activities as well as to examine the antioxidant effect of vitamin E on diabetic rats.

Materials and methods

Animals and operation procedures

Seven- to eight-week-old male Wistar rats (200–300 g) were used in this study. All rats were housed in temperature- and humidity-controlled rooms, and allowed free access to tap water and standard rat chow *ad libitum*. Diabetes was induced by a single intraperitoneal injection of STZ (Sigma, St. Louis, MO, USA) at a dose of 60 mg/kg body weight [27]. STZ was dissolved immediately before use in 0.05 mol/l sodium citrate (pH 4.5) [28]. Blood was drawn from the tail vein, and blood glucose was measured weekly using a glucometer (One Touch II Glucometer, LifeScan, Milpitas, CA, USA). Rats were considered diabetic if their blood glucose levels exceeded 250 mg/dl [29]. Rat body weights were also recorded on a weekly basis.

Rats were assigned to three groups according to the dose of vitamin E they received (low, 0.2; moderate, 0.4; and high, 0.8 mg/kg body weight). Each group was then divided into five subgroups: (a) untreated non-diabetic (control), where rats have not been treated with either STZ or vitamin E; (b) untreated diabetic, STZ-induced diabetic rats that were not treated with vitamin E; (c) treated diabetic I, where rats were treated with vitamin E for 10 days prior to the STZ-induction of diabetes; (d) treated diabetic II, where rats were treated with vitamin E 10 days after the onset of diabetes; (e) treated non-diabetic, in this subgroup, rats were treated with vitamin E only.

Oral glucose tolerance test (OGTT)

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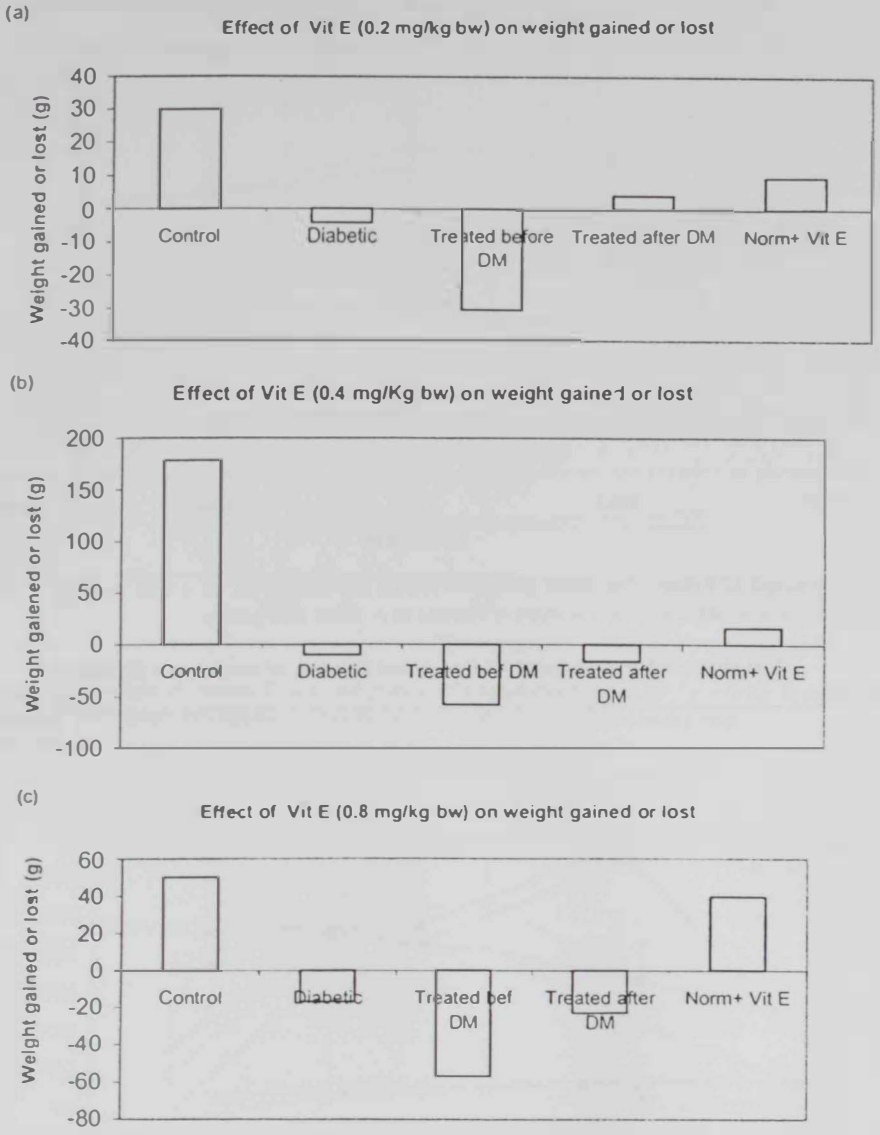

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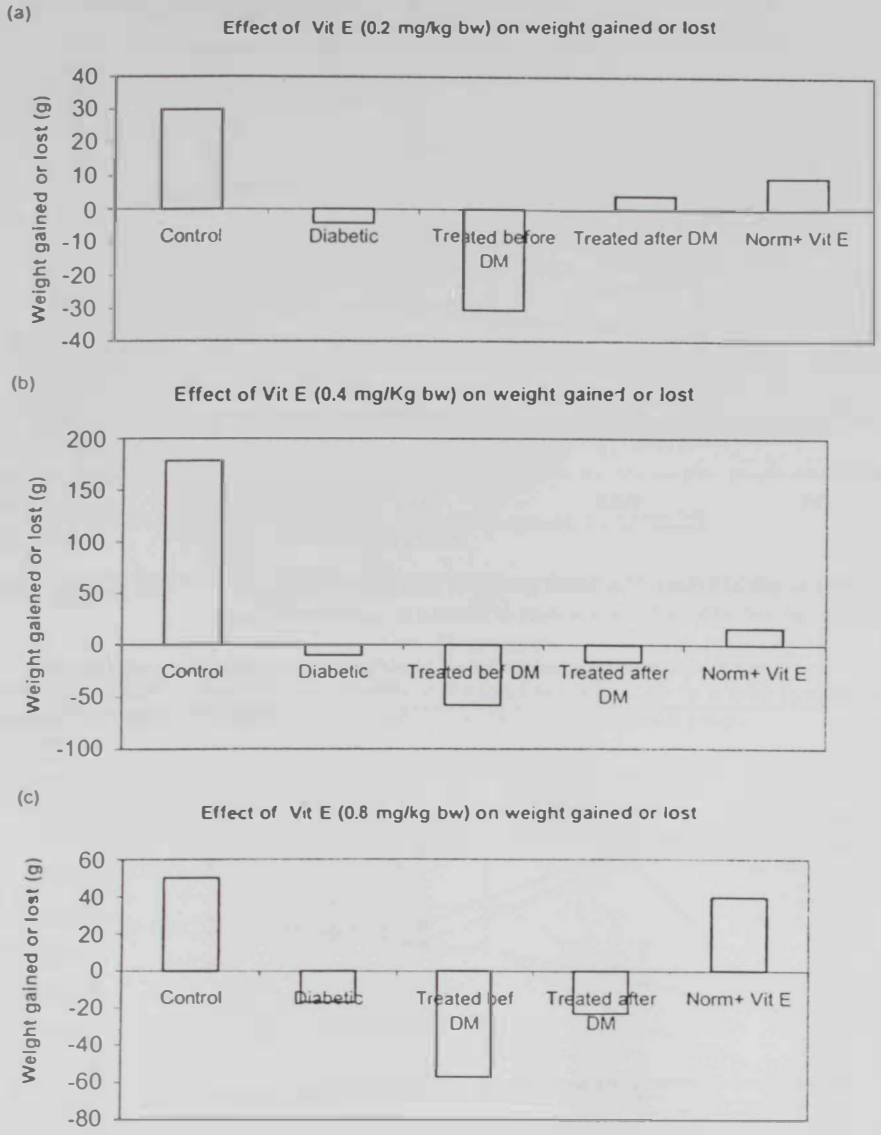

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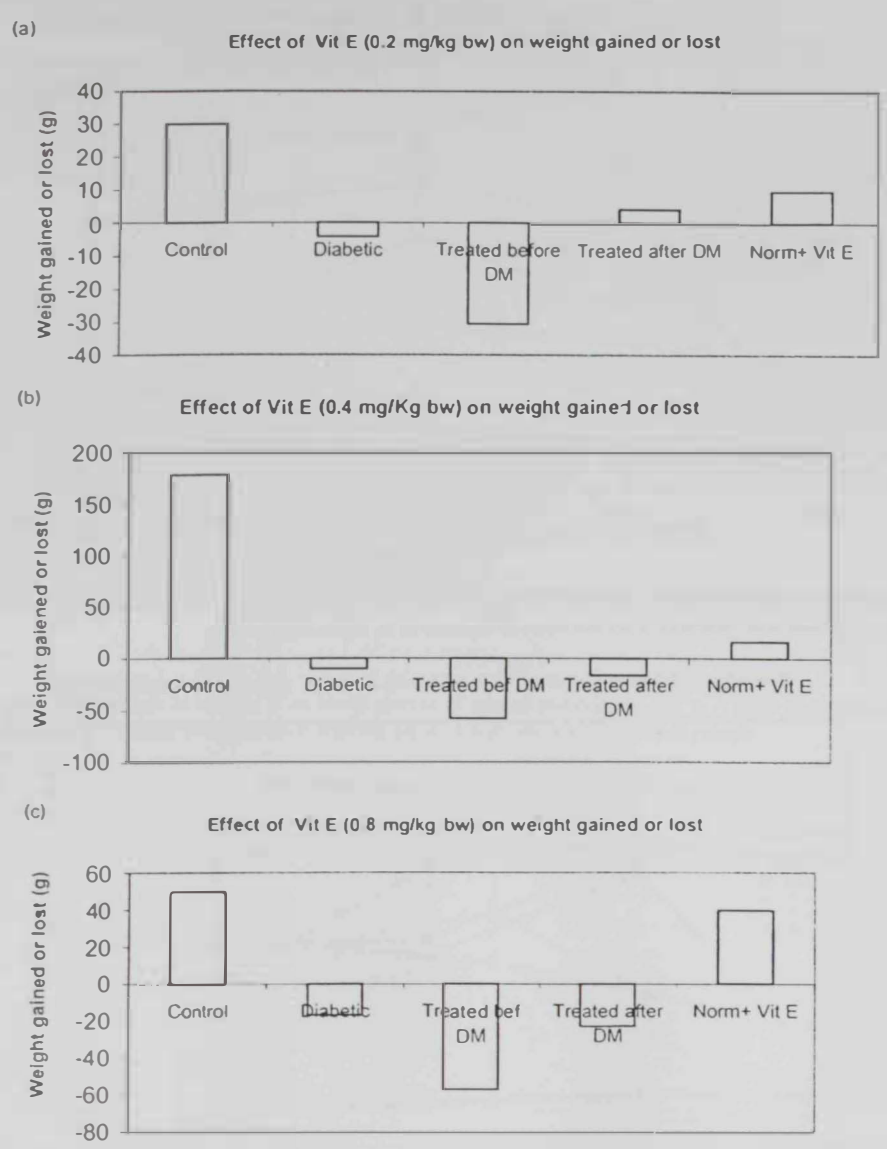

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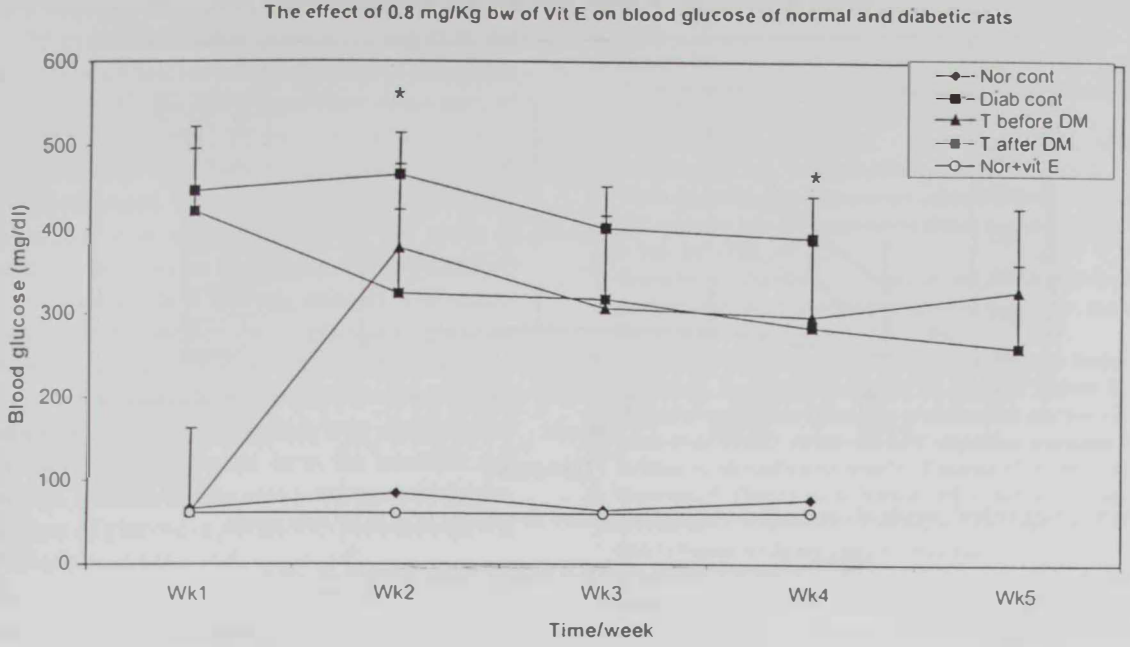
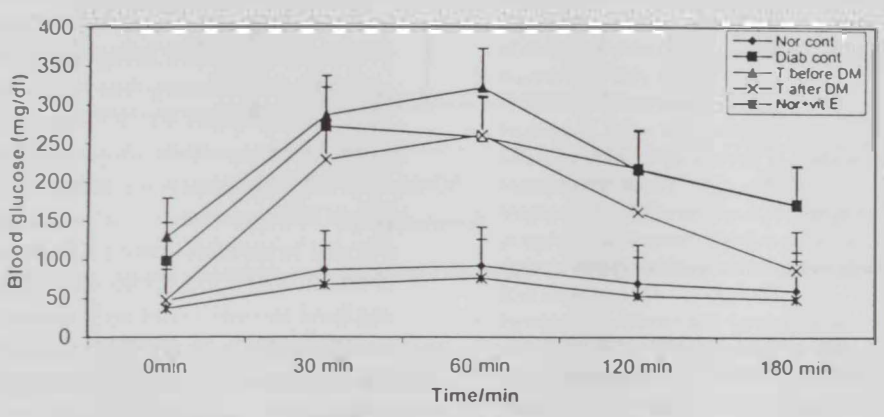


4. The effect of 0.8 mg/kg body weight of vitamin E on blood glucose of normal and diabetic rat $^{*}p < 0.05$: In weeks 2 and 4, there are a significant erent in blood glucose levels of rats treated with vitamin E after the onset of diabetes ($n = 10$ for each group).



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ct of vitamin E on OGTT

OGTT of rats that received vitamin E (0.28 mg/kg body ght) after the onset of diabetes was better than that of un- ted diabetic rats (Fig. 5). However, rats that were given min E before the onset of diabetes had a comparable TT value 120 min after the glucose load as compared ntreated diabetic rats. Although the OGTT values of nor- rats treated with vitamin E was better than that of the eated normal rats, it was nonetheless comparable.

he OGTT of normal and vitamin E (0.4 mg/kg body ght) treated rats is shown in Fig. 6. Rats treated with vitamin E before and after the onset of diabetes had a better OGTT value compared to untreated diabetic rats. However, it appeared that the OGTT of rats that received vitamin E before the onset of diabetes was better than that of rats treated with vitamin E after the onset of diabetes. The OGTT values of normal rats treated with vitamin E was comparable to those of untreated normal rats.

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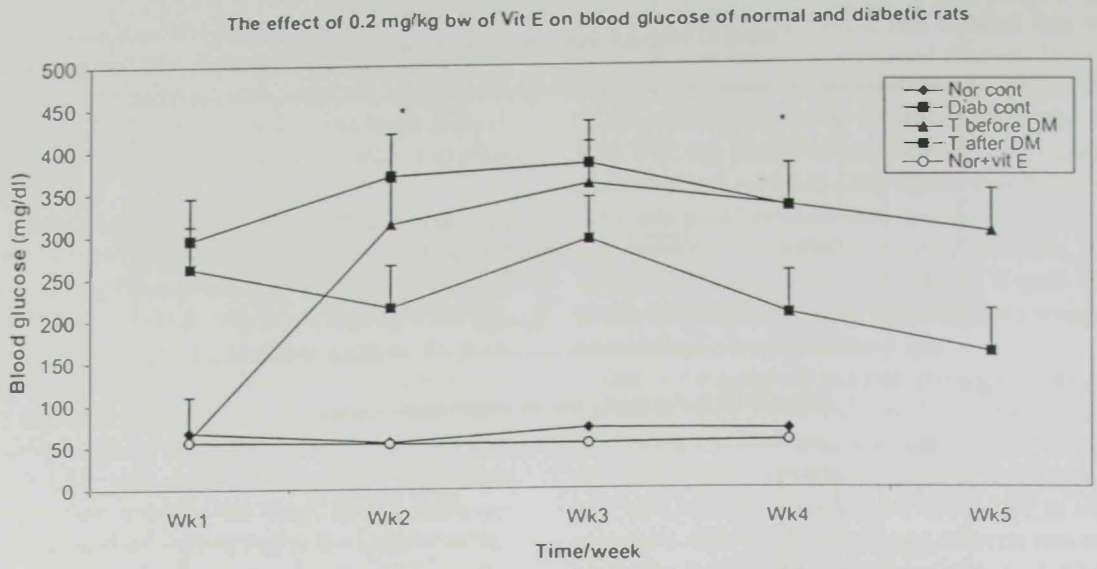

Fig. 2. The effect of 0.2 mg/kg body weight of vitamin E on blood glucose of normal and diabetic rat. $^{*}p < 0.05$ in weeks 2 and 4 there was a significant difference in blood glucose of rats treated with vitamin E after the onset of diabetes ($n = 10$ for each group).

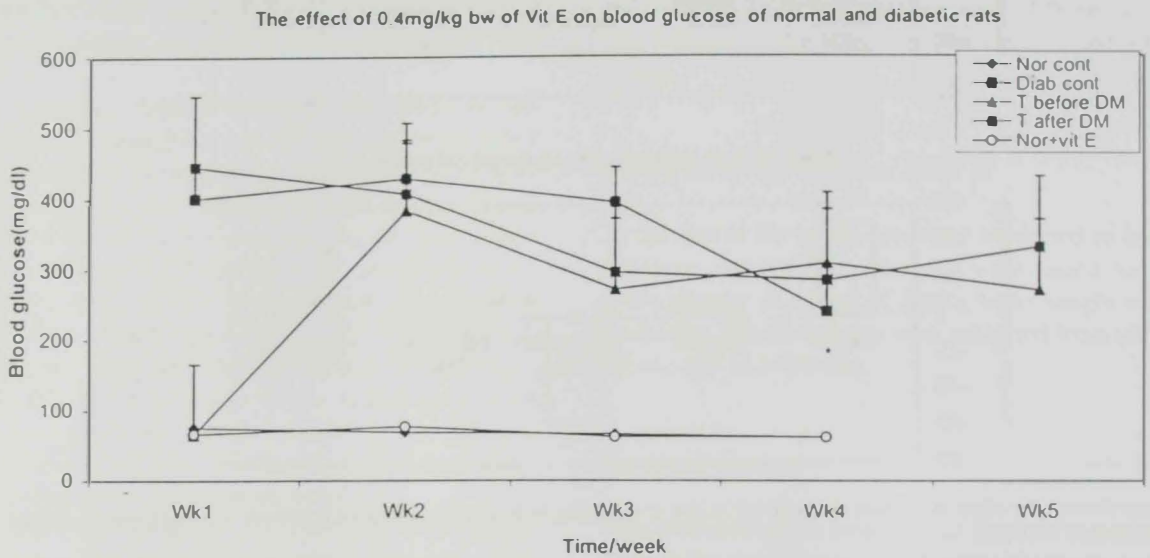

Fig. 3. The effect of 0.4 mg/kg body weight of vitamin E on blood glucose of normal and diabetic rat. $^{*}p < 0.05$ in week 4 there is a significant difference in blood glucose levels of rats treated with vitamin E before the onset of diabetes ($n = 10$ for each group).

level of rats given 0.2 mg/kg body weight before the onset of diabetes when compared to untreated diabetic rats. Normal rats treated with vitamin E had a small but not significant decrease in FBG level compared to untreated normal rats.

The FBG level was slightly reduced in diabetic rats treated with a higher dose of vitamin E (0.4 mg/kg body weight) either before or after the onset of diabetes compared to untreated diabetics. However, the reduction in FBG level was not statistically significant (Fig. 3). The FBG level of normal rats treated with vitamin E was comparable with that of untreated normal rats.

The oral administration of 0.8 mg/Kg body weight of vitamin E to rats after the onset of diabetes significantly ($p < 0.05$) reduced the FBG level when compared to untreated diabetic rats. The reduction in the level of FBG was consistent throughout the experimental period. Similarly, there was a significant ($p < 0.05$) reduction in the FBG level when rats were given higher doses of vitamin E (0.8 mg/kg body weight) before the onset of diabetes. Vitamin E (0.8 mg/kg body weight) also decreased the FBG level of normal rats compared to untreated normal rats (Fig. 4).

ls in Goto Kakizaki (GK) rats fed with vitamin E (20 and mg/kg) at 30 and 120 min after glucose loading [33]. An peritoneal tolerance test revealed a significant increment sulin secretion at 30 min and a significant decrement of d glucose levels at 30 and 120 min after glucose chal- e in the GK rats fed with high α-tocopherol diet [33]. levels of glycosylated haemoglobin A1c, an indicator ycaemic control, were also reduced [33]. The result of study and those of previous studies show that vitamin E ces blood glucose levels in diabetic animals. The mech- n by which vitamin E reduces blood glucose levels is far clear. However, vitamin E as an antioxidant may help e clearance of free radicals responsible for the compli- ns of diabetes mellitus. In addition, it may also promote bsorption or uptake of glucose from the intestine and respectively. Vitamin E may also participate in the in- ed catabolism of glucose, a possible explanation for the ction in weight in rats fed with vitamin E.

hat are the effects of vitamin E at the tissue level? High of vitamin E caused interstitial inflammation and ade- tous hyperplasia of the lung [31]. Moreover, high doses mg/kg) of vitamin E have been associated with haem- gic diathesis in both males and females and increased llary erythropoiesis in the spleen of one male [31]. How- it has been reported that vitamin E supplementation sig- ntly increased glutathione *S*-transferase (GST) levels in nal plasma of rabbits as compared with the controls [34]. min E is thus a stimulant of endogenous antioxidant en- s such as GST. An increase in the tissue level of GST n turn help organisms destroy the ROS, reactive nitrogen s and other free radicals that are released into the cyto- and interstitia of diabetic rat cells as a result of hyper- emia. Hyperglycaemia is a known inductor of reactive en and nitrogen species [35] in diabetics. In addition to eneficial effect of vitamin E on blood glucose level and T in diabetic rats, it has been shown that vitamin E can ficantly reduce total plasma cholesterol, triglyceride and lensity lipoprotein levels in diabetic rats [19]. In conclu- vitamin E decreases body weight and has a beneficial on the metabolic parameters of STZ-induced diabetic

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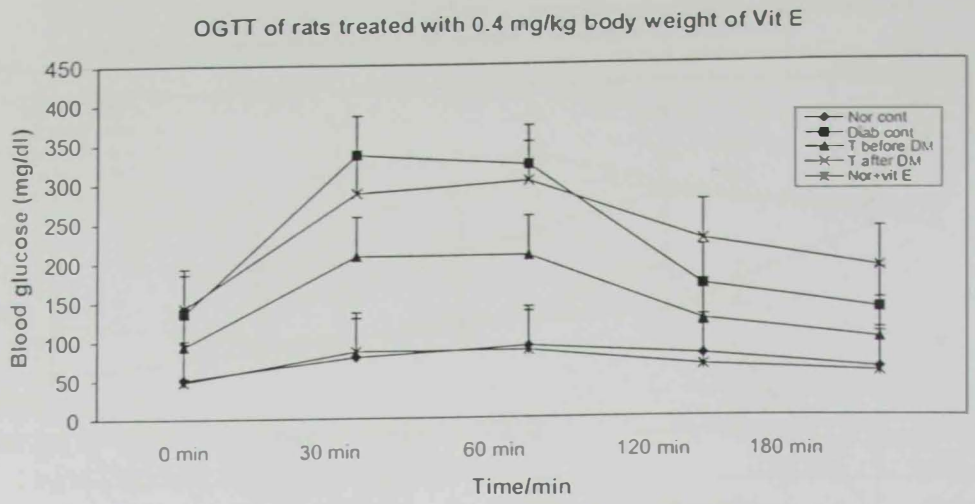

Fig. 6. The effect of 0.4 mg/kg body weight of vitamin E on OGTT of normal and diabetic rats ($n = 10$ for each group).

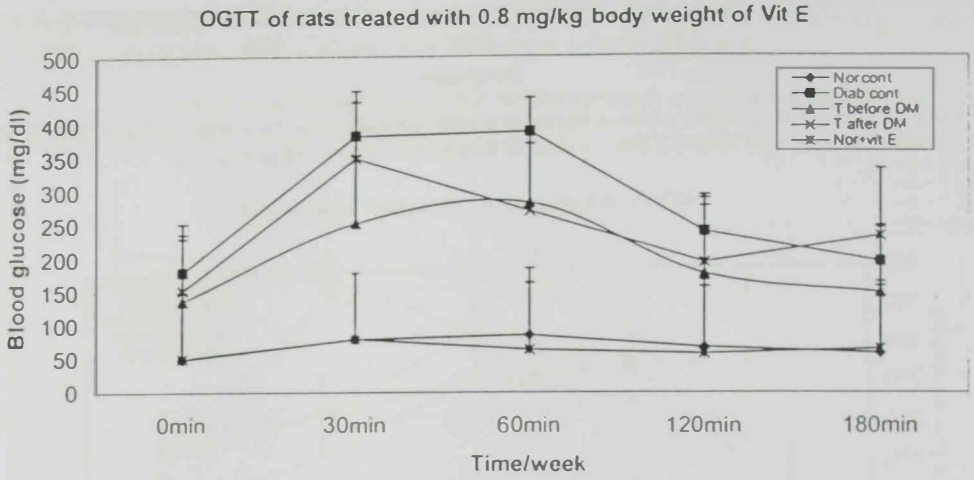

Fig. 7. The effect of 0.8 mg/kg body weight of vitamin E on OGTT of normal and diabetic rats ($n = 10$ for each group).

onset of diabetes was better than that of rats that received vitamin E after the onset of diabetes. The OGTT of normal rats treated with vitamin E was comparable to that of untreated normal rats.

Discussion

Effect of vitamin E on body weight

The result of this study shows that vitamin E can reduce body weight in both normal and diabetic rats. The mechanism by which vitamin E reduces weight is unknown. Reports performed in rabbits showed that dietary vitamin E supplementation does not affect their growth or the lipid concentration in the plasma [30, 31]. A reduction in the weight of these animals was not reported. A possible reason for this discrepancy is that different doses were used in the experiments. Studies performed by Abdo *et al.* [31] used high doses (125–200 mg/kg) of vitamin E. Many of the animals died especially when the higher doses were used. In a more recent study, Sharma *et al.* [32] showed that vitamin E did not have any effect on the weight of diabetic rats after treatment with oral 650 mg/kg body weight of α-tocopherol.

The relatively smaller doses of vitamin E employed in this study decreased body weight in both normal and diabetic rats. It is not known how vitamin E contributes to weight loss in this group of animals. The weight loss may, however, play a role in the lowering of blood glucose in diabetic rats treated with vitamin E.

Effect of vitamin E on blood glucose and OGTT

The result of this study showed that vitamin E can reduce blood glucose levels and improve the values of OGTT. Similar studies reported significant decrease in blood glucose

Abstracts

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Na^+/Ca^{2+} exchange current ($I_{Na/Ca}$) and sarcoplasmic reticulum (SR) Ca^{2+} release in catecholamine-induced Cardiac hypertrophy

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We investigated the effects of cardiac hypertrophy, produced by catecholamine administration, on $I_{Na/Ca}$ and SR Ca^{2+} release in isolated rat left ventricular myocytes. Steady state $I_{Na/Ca}$ density, measured using descending (+80 to −10 mV) voltage ramps in the whole cell configuration, was increased in hypertrophied myocytes ($P<0.05$). Ca^{2+} release from the SR was also increased, whereas resting $[Ca^{2+}]_i$ and the rate of decline of $[Ca^{2+}]_i$ to control levels were unchanged. SR Ca^{2+} content, estimated by using 10.0 mmol/L caffeine was also significantly increased in hypertrophied myocytes, but only when myocytes were held and stimulated from their normal resting potential (−80 mV) but not from −40 mV. However, the rate of decline of caffeine-induced Ca^{2+} transients or $I_{Na/Ca}$ was not significantly different between control and hypertrophied myocytes. Ca^{2+}-dependence of $I_{Na/Ca}$, examined by comparing the slope of the descending phase of the hysteresis plots of $I_{Na/Ca}$ vs. $[Ca^{2+}]_i$, was also similar in the two groups of cells. The observation that increased SR function occurred only when myocytes were stimulated from −80 mV suggests that Na^+ influx may play a role in altering Ca^{2+} homeostasis in hypertrophied cardiac muscle, possibly through increased reverse Na^+/Ca^{2+} exchange.

Effect of streptozotocin on insulin and glucagon secretion from the isolated rat pancreas.

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Streptozotocin (STZ) is one of the most commonly used agents in studies on experimental diabetes. It was the aim of this study to examine the effect of STZ on insulin and glucagon secretion from the isolated rat pancreas. Pancreatic tissue fragments were incubated with different concentrations of STZ for 1 h and the supernatant radioimmunoassayed for insulin and glucagon. Stimulation of the isolated pancreas with STZ (10^{-8} – 10^{-4} M) resulted in a dose-dependent increase in insulin secretion. Insulin secreted into supernatant (mean ± standard deviation) was 30.31± 2.05, 37.28 ± 7.99 and 41.94 ± 11.42 µIU L^{-1} when pancreatic tissue fragments were incubated with 10^{-8} M, 10^{-6} M and 10^{-4} M of STZ respectively. The level of insulin secretion obtained with STZ stimulation at 10^{-6} and 10^{-4} M were significantly ($P < 0.02$) higher than that of basal (19.83± 5.10 µIU L^{-1}). STZ elicited a dose-dependent increase in glucagons secretion from the pancreas. Glucagon secretion expressed as mean ± standard deviation was 184.75 ± 44.44, 205.83 ± 44.30 and 248.01 ± 98.20 pg mol^{-1}, after stimulation with 10^{-8} M, 10^{-6} M and 10^{-4} M of STZ, respectively. Glucagon secretion at 10^{-4} M of STZ was almost a two-fold of that obtained in the basal (156.27 ± 9.52 pg mol^{-1}). In summary, STZ can elicit significant dose-dependent increases in insulin and glucagons secretion from the isolated pancreas. STZ can thus be regarded as a secretagogue of pancreatic hormones.

An update on the aetiology and epidemiology of diabetes mellitus

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Diabetes mellitus is one of the most common endocrine disorders affecting almost 6 percent of the world's population. The number of diabetic patients will reach 300 million in 2025 (International Diabetes Federation, 2001). More than 97 % of these patients will have type II diabetes. The projected increase in the number of diabetic patients will strain the capabilities of health care providers the world over. Thus it is of paramount importance to revisit the causes and epidemiology of diabetes mellitus. Diabetes mellitus is caused by both environmental and genetic factors. The environmental factors that may lead to the development of diabetes mellitus include physical inactivity, drugs and toxic agents, obesity, viral infection and location. While Type I diabetes is not a genetically predestined disease, an increased susceptibility can be inherited. Genetic susceptibility plays a crucial role in the aetiology and manifestation of Type II diabetes with concordance in monozygotic twins approaching 100%. Genetic factors may have to be modified by environmental factors for diabetes mellitus to become overt. An individual with a susceptible gene may become diabetic if environmental factors modify the expression of these genes. Since there is an increase in the trend at which diabetes

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body wt) and cardiac function and protein phosphatase activities examined after 1, 2, 3, 4 and 8 weeks. One group of 4-week diabetic animals received subcutaneous injection of insulin (3U/day) for a further period of 4 weeks. Cardiac dysfunction was apparent early on (after 2 weeks of induction of diabetes) and deteriorated with time. A significant increase in protein phosphatase activity appeared very early (after one week) and persisted until 8 weeks. Increased protein phosphatase activity was consistent with a corresponding increase in the protein contents of protein phosphatase 1 and protein phosphatase 2A. Treatment with insulin partly reversed the abnormalities observed in diabetic animals. The results of this study suggest that increased protein phosphatase activities and therefore enhanced protein dephosphoryation may play a role in diabetes induced cardiac dysfunction.

Fructose transport and metabolism in adipose tissue of Zucker rats: diminished GLUT5 activity during obesity and insulin resistance

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Fructose is a major dietary sugar which is elevated in the serum of diabetic humans, and is associated with metabolic syndromes important in the pathogenesis of diabetic complications. The facilitative fructose transporter, GLUT5, is expressed in insulin sensitive tissues of humans and rodents, where it mediates the uptake of substantial quantities of dietary fructose, although little is known about its regulation. Here, we present evidence that GLUT5 expression and activity are compromised severely during obesity and insulin resistance in Zucker (*fa/fa*) rat adipocytes. Adipocytes from young (*fa/fa*), highly insulin-responsive Zucker rats present considerably more surface GLUT5 than those from their lean counterparts (1.8-fold per microgram membrane protein), and exhibit higher fructose transport (4-fold) and metabolism (3-fold) rates, largely directed toward lactate production. As rats age and become more obese and insulin-resistant, adipocyte GLUT5 density (12-fold) and fructose transport (10-fold) and utilisation rates (3-fold) fall markedly. The GLUT5 loss is more dramatic in adipocytes from obese animals, which develop a more marked insulin resistance than lean counterparts. The decline of GLUT5 density in adipocytes from older, obese animals does not appear to be a generalised effect, being neither shared by the Na^+/K^+ ATPase, a plasma membrane marker, nor observed in kidney. Our findings suggest that GLUT5 expression and thus fructose utilisation rates in adipocytes are dependent upon cellular insulin sensitivity, inferring a significant role for GLUT5 in the elevated circulating fructose observed during diabetes, and the likely pathological consequences.

The effects of Type 1 diabetes mellitus on the morphology, cytosolic calcium ($[Ca^{2+}]_i$) signals and fatty acid lipid profiles in the isolated rat parotid gland.

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Diabetes mellitus (DM) is associated with numerous conditions including hypo-secretion of digestive enzymes. This study investigates the morphology, $[Ca^{2+}]_i$ signals and fatty acid lipid profiles in isolated parotid glands of diabetic and age-matched control rats in order to understand the cellular mechanism of hypo-secretion. The techniques employed included light microscopy, fluorimetric and gas chromatography analysis (GC), respectively. DM was induced in adult male Wistar rats by a single (IP) injection of streptozotocin (STZ) (60 mg kg/ body weight). Control animals were injected with a similar volume of citrate buffer. The animals were tested for DM 4 days after STZ injection and 2 months later when they were humanely killed for the experiment. The morphological results showed diabetic parotid glands to be extensively infiltrated with lipid droplets of various magnitudes, whereas glands from control animals, display normal structure with the absence of lipid droplets. DM produced no significant change in either basal or Ach-evoked initial peak $[Ca^{2+}]_i$ signals when compared to age-matched control cells. In contrast, DM induced a significant ($P<0.01$) reduction in the plateau phase of the $[Ca^{2+}]_i$ signal compared to control cells. Levels of fatty acids (16:0, 16:1 and 18:1) in diabetic parotid glands were significantly ($P<0.01$) reduced compared to control glands. The results indicate DM can elicit changes in the morphology, $[Ca^{2+}]_i$ signals and in fatty acid lipid profiles of the isolated rat parotid gland, compared to glands from healthy age-matched control rats.

The effect of Vitamin E on metabolic parameters of Experimental Diabetes Mellitus

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The role of vitamin E on Diabetes Mellitus is unknown. The purpose of this study was to examine the effect of oral administration of vitamin E on some of the metabolic effects of experimental diabetic rats. Diabetes was induced by intraperitoneal injection of STZ (60 mg/kg body weight at 12 weeks of age). Vitamin E (0.2, 0.4, 0.8 mg/Kg body weight) was administered orally for a period of three to normal and diabetic Wistar rats. In some experiments Vitamin E was given either before or after the induction of diabetes mellitus. Oral glucose tolerance test (OGTT) were performed on fasted normal, diabetic and vitamin E-treated rats at the end of the experimental period. Blood sugar level and weight were also recorded on a weekly basis for each

rat in different groups. Vitamin E significantly ($p < 0.01$) reduced blood sugar level and improved weight gain in experimental diabetes mellitus at all doses when compared to untreated rats. This beneficial effect of vitamin E on the hyperglycaemia of diabetic rats was dose-dependent. Moreover, vitamin E also improved OGTT in diabetic rats compared to untreated diabetic rats. In conclusion, Vitamin E may play a role in insulin metabolism and thus be a useful adjuvant therapy in type I diabetes mellitus.

The time course of changes in amine concentrations in the rat tail artery following induction of diabetes with streptozotocin.

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Introduction: We have examined the changes in concentrations of noradrenaline, dopamine and serotonin in the proximal, middle and distal segments of the rat tail artery following the induction of diabetes by injection of STZ (60 mgs/kg ip at 10 weeks of age); the present abstract is concerned with the time course of the changes from 17.5 weeks of age to 52 weeks of age. Tissues were taken under pentobarbitone anaesthesia, weighed and treated with 10% sodium metabisulphite. Amines were extracted and analysed using HPLC. Results: The control blood glucose concentrations were in the range 57 to 68 mgs/dl and the diabetic levels were 227 to 380 mgs/dl. In the control animals there was a significant increase in noradrenaline, dopamine and serotonin concentrations with age in the proximal portion of the tail artery, whereas this was not seen in the middle (except in the case of serotonin) and distal segments. Between 20 and 30 weeks of age, noradrenaline was the most abundant amine, followed by serotonin, dopamine and adrenaline. STZ diabetes resulted in major increases in the concentrations of noradrenaline, dopamine and serotonin (4-11fold) that generally occurred about 16 weeks following the injection of STZ. The maximum increases in amine concentrations occurred in the distal segments of the tail artery.

Conclusion: STZ-diabetes is associated with an increase in the concentrations of noradrenaline, dopamine and serotonin in all segments of the tail artery of the rat. These peak between 10 and 20 weeks following the initial treatment.

Biochemical effects of *Citrullus colocynthis* in non-diabetic and diabetic rats.

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Diabetes mellitus is one of the most common endocrine diseases in the world that affects almost 6% of the world population (1). In UAE many traditional plants are used as anti-diabetic remedies, such as the *Citrullus colocynthis* (Handal). The aim of this study was examine the effect of the oral administration of aqueous extract of *Citrullus colocynthis* seeds on biochemical and metabolic parameters of normal and streptozotocin (STZ)-induced diabetes rats. Diabetes mellitus (DM) was induced by a single intraperitoneal (60 mg Kg body wt $^{-1}$) injection of streptozotocin. Normal and diabetic rats were fed with the plant extract daily by oral intubations for two weeks. Blood sample collected at the beginning and at the end of the experiment for measurement of biochemical parameters. The plasma level of ALT increased significantly after the onset of experimental diabetes. In contrast, the plasma level of ALT decreased significantly after the administration of the plant extract. All of these results raised and confirm a number of interesting issues. Firstly, that STZ has a hepatotoxic effect by increasing the plasma level of ALT and secondly, the aqueous extract of the *C. colocynthis* can ameliorate the toxic effect of STZ in the liver. Moreover, this study suggests that *C. colocynthis* is not toxic at least when given in the higher doses in this study. In diabetic rats there was a significant increase in plasma GGT level. This elevation is an indicator of hepatotoxicity caused by STZ. There were no significant changes in the level of GGT even after treating diabetic rats with low and moderate doses of the aqueous extract of the plant. There was no significant change in the blood level of creatinine, calcium, sodium, phosphorus. It is not known why there were no changes in these biochemical parameters after treatment with the plant extract. It is possible that the concentration of the extract may be too weak to have a significant effect or the tissue or organ damage may be too severe to show any detectable improvement. In conclusion, the results of this study revealed that oral administration of the aqueous extract of the *Citrullus colocynthis* can ameliorate the toxic effect of STZ.

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Increased Incidence of Diabetic Neuropathy on Smokers: 9 year Follow Up Result of Sheffield Prospective Diabetes Study

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Introduction: A number of macrovascular risk factors have been associated with the development of diabetic neuropathy (DN), however the relation of smoking to DN has not been clearly established in a prospective study. **Aims:** The aim of Sheffield Prospective Diabetes study to identify the early abnormalities of clinical, biochemical, neurophysiological and haemorrheological functions for the development of complications of type 1 diabetes. **Materials and Methods:** 66 newly diagnosed diabetes subjects (mean age 31 ± 9 (SD) duration (3 years ± 2) were identified and followed up for 9 years. They had detailed smoking history

جامعة الإمارات العربية المتحدة
عمادة الدراسات العليا

تأثير مضادات الأكسدة على الغدة الصماء والمقاييس الأيضية في الفئران المصابة بمرض السكري

رسالة مقدمة من الطالبة

مريم سرور مشعان سليم الشامسي

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علوم البيئة

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