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ANTIOXIDANT EFFECT OF HUMAN URINE IN ALLOXAN-INDUCED DIABETIC RATS

[1]Ogugua, Victor N.; [1,2]*Okeke, Emmanuel S.; [1]Anaduaka, Emeka G.; [1]Ugwu, Obumneke J.; [1,2]Nwankwo, Nicodemus E.; [1]Okenwa, Ezinne J.; [1]Ihim, Chiamaka A. and [1]Enwerem, Uchechukwu

[1]Department of Biochemistry, University of Nigeria, Nsukka.
[2]Natural Science Unit, School of General Studies, University of Nigeria, Nsukka.

emmanuel.okeke@unn.edu.ng

Abstract

This work evaluated the effects of different doses of the human urine on normal and alloxan-induced diabetic rats. The experimental design comprises of five (5) groups of four (4) animals each. At the end of the treatment period, blood glucose levels, protein, MDA, catalase, Glutathione and vitamin C concentrations were determined in both the normal and diabetic rats. Oral administration of low and higher doses (5ml/kg and 10 ml/kg) of human urine for 7 days resulted in significant ($p < 0.05$) decrease in blood glucose and protein concentrations of the diabetic rats compared to the untreated diabetic rats. However, the catalase concentration of the rats treated with (5ml/kg and 10 ml/kg) of human urine showed a significant ($p < 0.05$) increase compared to the untreated diabetic rats. This results show that human urine has great potentials of increasing the ascorbic acid concentration of diabetic rats treated with standard drug, 5ml/kg and 10ml/kg of urine when compared to diabetic untreated group and with a concomitant antioxidant effect on alloxan-induced lipid peroxidation in rats. However, it was observed that there is a significant ($p < 0.05$) increase in the mean concentration of catalase of the groups treated with standard drug, 5ml/kg and 10ml/kg of urine when compared to diabetic untreated group. Furthermore, there was an observed significant ($p < 0.05$) increase in the mean concentration of glutathione and SOD of the groups treated with standard drug, 5ml/kg and 10ml/kg of urine when compared to diabetic untreated group which proves human urine to have an antioxidant property.

Keywords: Diabetes; Urine; Alloxan; Glutathione; Lipid peroxidation; catalase; SOD.

Introduction

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia and insufficiency of secretion or action of endogenous insulin [1]. It is a metabolic disorders with micro-and macrovascular complications that results in significant morbidity and mortality. It is considered as one of the five leading causes of death in the world [2, 3]. In modern medicine no satisfactory effective therapy is still available to cure diabetes mellitus [4]. Although the etiology of this disease is not well defined, viral infection, autoimmune disease and environmental factors have been implicated. Studies demonstrated that oxidative stress and free radical are the lead or supporting actor in pathogenesis of diabetic complications.[5, 6]. Diabetes is usually accompanied by increased production of free radicals or impaired antioxidant defenses.[5-7]. There is increasing demand by patients to use natural products with antidiabetic activity due to side effects associated with the use of insulin and oral hypoglycemic agents [8-10]. From these facts, it is needed to find nutrients and food with effective antioxidant activity against oxidative damage to prevent various diseases. There are therefore many natural integrative therapies available to reduce casualties from communicative and non-communicative diseases [11]. A perfect example of such substance is human urine[12-14]. Along the history, people have been toying with their own urine to find the solution for various diseases. Martha [12] stated that there is an extraordinary natural healing substance produced by our own bodies that modern medicine science has proven to be one of the most powerful natural medicines known to man. Human urine contains among other substances a hormone called dehydroepiandrosterone (DHEA). Dehydroepiandrosterone (DHEA), a natural hormone in the body, helped rebalance the set of chemical reactions that falls out of balance due to chronic high blood sugar in type 2 diabetes. This work was carried out to scientifically ascertain the traditional use of human urine in treatment of many ailments and diseases.

Materials and Methods

Chemicals

Chemicals and reagents: all chemicals and reagents were obtained from Sigma- Aldrich Chemical Co. (St Louis, MO, USA). All the chemicals used including the solvents, were of analytical grade.

Treatment Material

About 20 ml of early morning urine of a healthy individual free from drug of any type was freshly collected and the pH 6.2 was determined daily, and administered to the experimental animals based on their body weight.

Animals

Adult *Wistar* rats of between 12 to 14 weeks with average weight of 180 ± 13 g were obtained from the Department of Zoology and housed in the animal House of the Department of Biological Sciences, both in University of Nigeria, Nsukka. The animals were acclimatized for 7 days under standard environmental conditions, with a 12 hour light/dark cycle maintained on a regular feed (Top feed; grower mash) and water *ad libitum.*

Methods

Experimental Protocol

A total of twenty (20) *Wistar* rats were divided into 5 groups of four (4) animals each. The animals were grouped as follows;

Group 1: Normal rats (Normal control)
Group 2: Diabetic rats untreated (Positive control)
Group 3: Diabetic rats treated with 2.5 mg/kg body weight of standard drug
Group 4: Diabetic rats treated with 5 ml/kg body weight of human urine
Group 5: Diabetic rats treated with 10 ml/kg body weight of human urine

Induction of experimental diabetes

Diabetes was induced by intraperitoneal injection of 1% Alloxan (150 mg/kg b/w) in overnight – fasted animals after acclimatization. Diabetes was confirmed three (3) days later in the alloxan – treated animal showing blood glucose of 250 mg/dL or higher were reflected to be diabetic and were encompassed in our study [15]. This day was considered as the 1st day of our experiment. Further, rats were divided into five groups (*n* = 4 per

group). Group 1 received normal saline (NS) and served as control; group 2 served as diabetic untreated group; group 3 diabetic rats treated with 2.5 mg/kg body weight of standard drug serving as positive control; group 4 are diabetic rats treated with 5 ml/kg body weight of human urine; group 5 are diabetic rats treated with 10ml/kg body weight of human urine. Blood sample was collected through ocular puncture for further biochemical analysis. The glucose level was determined on the first day, the third day and on the seventh day. After treatment, the animals were sacrificed by decapitation and the blood was collected in tubes containing EDTA. The plasma was immediately separated by low-speed centrifugation (for 1500 g for 15 min, 4°C).

Biochemical Analysis

Glucose concentration: was measured by an oxygen rate method employing a Beckman Coulter Oxygen electrode.

Determination of Proteins Concentrations: Protein content in tissue homogenate was measured by the method of Lowry [16].

Oxidative Stress Assessment in Hepatic Tissue. Oxidative stress markers were determined in the liver homogenate using commercial kits for reduced glutathione (GSH), and malondialdehyde (MDA) according to the manufacturer's instructions.

Antioxidant Activity Assessment in Hepatic Tissue: Antioxidant activity in hepatic tissue was assessed in the liverhomogenate using commercial kits for the determination of catalase (CAT), and super oxide dismutase (SOD) activitiesaccording to the manufacturer's instructions.

Determination Vitamin C Concentrations : A quantity, 1.0 g of sample was macerated with 20 ml of 0.4% oxalic acid and filtered. About 9 ml of indophenols reagent was added to 1 ml of the filtrate. The standard solution of vitamin C was prepared similarly and the absorbances of the standard solution and the sample were read at 520 nm. The concentration of vitamin C was extrapolated from the standard curve of vitamin C.

Statistical Analysis

Data were reported as means ± SEM, where appropriate. Both one- and two- way analysis of variance (ANOVA) were used to analyze the experimental data and Duncan multiple test range was used to compare the group means obtained after each treatment with control measurements. Differences were considered significant when $p < 0.05$.

Results

Figure 1. below shows the mean value of glucose concentration for the normal control after seven days of treatment is 103mg/dl that of diabetic untreated is 342.5mg/dl. The mean glucose levels for groups III (Glibenclamide), IV (5ml/kg), V (10ml/kg) are 179.5ml/dl, 113.5ml/dl and 146.25ml/dl respectively. There was a significant decrease ($p < 0.05$) in the glucose concentration of the groups treated with 5ml/kg and 10ml/kg of urine when compared to the diabetic untreated group. The result also shows a significant decrease ($P <0.05$) in glucose concentration from day 3 to day 7 in group III, IV, and V. There was also more decrease in mean glucose concentration of the group treated with 5ml/kg than those treated with 10ml/kg.

Fig. 2 below shows the mean concentrations of protein for the normal group is 4.60mg/dL, diabetic untreated/ is 5.40mg/dL, group treated with glibenclamide is 5.43mg/dL, those treated with 5ml/kg of urine is 5.10mg/dL, while that of those treated with 10ml/kg of urine is 4.866mg/dL. There was no significant difference ($p > 0.05$) in the mean concentration of protein in the group treated with 5ml/kg of urine when compared to those of the normal group. There was also no significant difference ($p > 0.05$) in the mean concentration of protein in the group treated with 10ml/kg of urine when compared to those of the normal group.

Figure 3 reveals the mean concentrations of MDA in normal group, Diabetic untreated, group treated with Glibenclamide, group treated with 5ml/kg of urine, and that of those treated with 10 ml/kg of urine are 58.7650 mg/dl, 67.8667 mg/dl, 38.8600 mg/dl, 44.0767 mg/dl, and 43.5467 mg/dl respectively. The result shows that there was a significant increase ($p < 0.05$) in mean concentration

of MDA in diabetic untreated group than in the group treated with urine.

Fig 4. below shows that the mean value for the vitamin C concentration of the normal group was found to be 0.8 mg/dL, diabetic untreated is 0.567 mg/dL, group treated with the standard drug is 0.667 mg/dL, those treated with 5 ml/kg of urine is 0.733 mg/dL, while that of those treated with 10 ml/kg of urine revealed the result to be 0.667 mg/dL. There was a significant increase ($p < 0.05$) in the mean concentration of the group treated with 5 ml/kg of urine when compared to those of diabetic untreated group. There was no significant difference ($p > 0.05$) in the mean concentartiobn of the group treated with 10ml/kg body weight of urine when compared to normal group or diabetic untreated group.

From fig 5 below, the mean concentration of catalase for normal group is 7.154 IU/L, diabetic untreated is 7.668 IU/L, for those treated with glibenclamide is 8.259 IU/L, those treated with 5 ml/kg is 8.298 IU/L, and those treated with 10 ml/kg is 8.09967 IU/L. There was no a significant difference ($p > 0.05$) in the mean concentration of the diabetic untreated group when compared to the group treated with standard drug, 5 ml/kg and 10 ml/kg of urine.

From the result shown in fig 6 below, the mean concentration of SOD for the normal group is30.105IU/L, that of diabetic untreated is 27.483IU/L, for those with gliberclamide is 35.060IU/L, for those treated with 5ml/kg of urine is 40.510IU/L, while those treated with 10ml/kg of urine is 34.603IU/L. There is a significant increase($p<0.05$) in the mean concentration of the group treated with 5ml/kg body weight of urine when compared to the diabetic untreated group. There was also a significant increase ($p<0.05$) in the mean concentration of the group treated with 10ml/kg body weight of urine when compared to the normal group.

Figure 7 shows the mean concentration of GSH for the normal group is 8.23 mg/dL, that of diabetic untreated is 5.28 mg/dL, for those treated with gliberclamide is 6.77 mg/dL, for those treated with 5 ml/kg of urine is 6.51 mg/dL, while those treated with 10 ml/kg of urine is 7.35 mg/dL. There was a significant decrease ($p < 0.05$) in the mean concentration of the group treated with 5 ml/kg body weight of urine when compared to the diabetic untreated group or the normal group. There was also a significant decrease ($p < 0.05$) in the mean concentration of the group treated with 5 ml/kg body weight of urine when compared to the diabetic untreated group or the normal group.

Discussion

Diabetes mellitus is a metabolic disorder that is considered a major health problem and affects millions of people worldwide. The adjunctive use of standardized pharmaceutical-grade nutrients, known as nutraceuticals, has recently gained the increased interest of many research groups [17] and many nutraceuticals are now being used for treating several diseases. Human urine has been shown to have some beneficial health imlplications.This research investigated the positive antioxidant effects of human urine on diabetes with the view of proposing a better management scheme for diabetic patients. Alloxan has been shown to produce hyperglycemia which may be due to partial or complete destruction of the beta cells. Insulin deficiency leads to various metabolic alterations in the animals viz increased blood glucose levels [18]Increasing evidence in both experimental and clinical studies suggests that oxidative stress plays a major role in the pathogenesis of both types of diabetes mellitus. Free radicals are formed disproportionately in diabetes by glucose oxidation, nonenzymatic glycation of proteins, and the subsequent oxidative degradation of glycated proteins. Abnormally high levels of free radicals and the simultaneous decline of antioxidant defense mechanisms can lead to damage of cellular organelles and enzymes, increased lipid peroxidation, and development of insulin resistance. These consequences of oxidative stress can promote the development of complications of diabetes mellitus. Changes in oxidative stress biomarkers, including superoxide dismutase, catalase, glutathione levels, vitamins C, lipid peroxidation (MDA) are surveyed in this research work.

The mechanisms of antidiabetic effect of human urineinvolve multiple factors. It may be resulting

from the influence of bioactive constituents such as DHEA.Recent studies have yielded very encouraging results supporting dehydroepiandrosterone (DHEA) supplementation in diabetics. DHEA has been shown to improve insulin sensitivity and obesity in human and animal models [19]. Although its mechanism of action is poorly understood, it is thought that DHEA improves glucose metabolism in the liver [19]. Animal studies have also demonstrated that DHEA increases beta cells on the pancreas, which are responsible for producing insulin [20]. In humans, DHEA levels are sensitive to elevated glucose: higher glucose levels tend to be associated with decreased DHEA levels[21]. One proposed mechanism of action in humans is linked to DHEA's metabolism into testosterone. DHEA is an adrenal hormone that can be converted into either testosterone or estrogen. Studies have shown that testosterone improves insulin sensitivity in men, suggesting that DHEA's conversion into testosterone may be responsible for its beneficial effects in improving insulin sensitivity [22].

During diabetes, the excess glucose present in the blood reacts with hemoglobin to form glycosylated hemoglobin. It has been reported that various proteins, including hemoglobin, albumin, collagen, LDL, or crystalline proteins undergo non-enzymatic glycation in diabetes [23]. The result from the study showed a reduction in blood glucose and protein after treatment with human urine which is an indication that human urine could be responsible for the possess anti-diabetic properties. From the results obtained for blood glucose determination, human urine significantly reduced($p < 0.05$) the glucose concentration at both test doses compared to the untreated diabetic group. This result suggests that human urine is a potential anti-diabetic drug and can be developed further possibly in synergy with other agents to help fight against diabetes.

The decrease in protein concentration of the groups treated with varying doses of human urine when compared to the diabetic untreated group is in synergy with the work performed by Ronald [24], that there is an increase in the degradation of cellular proteins as a result of increase in free radicals. This again shows that human urine may have contributed to this effect.

Lipid peroxidation is one of the characteristic features of chronic diabetes. The increased free radicals produced may react with polyunsaturated fatty acids in cell membranes leading to lipid peroxidation. Lipid peroxidation will in turn result in the elevated production of free radicals [25]. Lipid peroxide-mediated damage has been observed in the development of type I and type II diabetes mellitus. Insulin secretion is also closely associated with lipoxygenase-derived peroxides [26]. Low levels of lipoxygenase peroxides stimulate the secretion of insulin, but when the concentration of endogenous peroxides increases, it may initiate uncontrolled lipid peroxidation leading to cellular infiltration and islet cell damage in type I diabetes [27]. The most commonly used indicator of lipid peroxidation is MDA. The increased lipid peroxidation in the tissues of diabetic animals may be due to the observed remarkable increase in the concentration of MDA in tissues of diabetic rats [28]. In diabetes, changes in the antioxidant parameters status have been reported in various tissues [29]. Mechanisms that contribute to increase **oxidative stress** in diabetes included non-enzymatic glycosylation, autooxidative glycosylation and metabolic stress [30, 31]. T1he increase of **lipid peroxidation** products, in tissue of alloxan-induced **diabetic rats** has been previously reported and it is well known that hyperglycaemia increases **lipid peroxidation,** which may contribute to long-term tissue damage [29]. In our investigation, treatment with human urine reduces significantly the MDA concentrations in the tissue of diabetic rats.

The concentration of vitamin C reduces during diabetic conditions [32] and this can be likened to the significant decrease ($p < 0.05$) in the mean concentration of vitamin C in the diabetic untreated when compared to the diabetic group treated with different doses of human urine, this also suggests the antioxidant effect of human urine.

SOD scavenges the superoxide radical by converting it to H_2O_2 and molecular oxygen [33]. The activity of SOD was found to be lower in diabetic control rats. The observed decrease in SOD activity could result from inactivation by H_2O_2 or by glycation of the enzyme, which have been reported to occur in diabetes [34]. CAT is a hemeprotein, which catalyzes the reduction of hydrogen

peroxides and protects the tissues from highly reactive hydroxyl radicals [35]. This decrease in CAT activity could result from inactivation by glycation of the enzyme [36]. Further, an increase in the SOD activity may protect CAT against enzyme inactivation by superoxide radical as these radicals have been shown to inactivate CAT [37]. Thus, the increase in SOD activity may indirectly play an important protective role in preserving the activity of CAT. The reduced activities of SOD and CAT in the liver and kidney have been observed during diabetes and this may result in a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxides. Our study revealed and increased SOD and Catalase activities after treatment with human urine. In this context, other researchers also reported a decrease in the activity of these antioxidant enzymes (SOD, CAT) in the liver and kidney of diabetic rats [38, 28]. In diabetic rats treated with human urine, a significant increase in activity of these enzymes was observed. This might reflect the antioxidant potency of the human urine, which by reducing blood glucose levels prevented glycation and inactivation of enzymes. Similar kinds of effect, i.e. prevention of potential glycation of antioxidant enzymes and the ensuing decrease in activity, have been reported with other medicinal plants, such as *Gongronemalatifolium* and *Eugenia jambolana* well known for their antidiabetic activity [39, 40].

Cooperative defense systems that protect the body from free radical damage include the antioxidant nutrients and enzymes. Oxidative stress in diabetes coexists with a reduction in the antioxidant capacity, which can increase the deleterious effects of free radicals. Glutathione is a tripeptide normally present at high concentrations intracellularly, and constitutes the major reducing capacity of the cytoplasm [41]. Glutathione is known to protect the cellular system against toxic effects of lipid peroxidation [42]. Decreased level of GSH in the liver and kidney during diabetes represents its increased utilization due to oxidative stress [38]. In the present study, a significant elevation of GSH level was observed in the urine and standard drug-treated diabetic rats. This indicates that the extract can either increase the biosynthesis of GSH or reduce the oxidative stress leading to less degradation of GSH, or have both effects.

Conclusion

In conclusion, the effect of human urine on tissue antioxidants is due to reduction in blood glucose (hypoglycemic) level in diabetic rats, which prevents excessive formation of free radicals through various biochemical pathways such as reduction of lipid peroxidation also reduction in cellular protein degradation. Further study will investigate the mechanism of actions and identify the required biological active ingredients.

Conflicts of interest

The authors declare no conflicts of interest.

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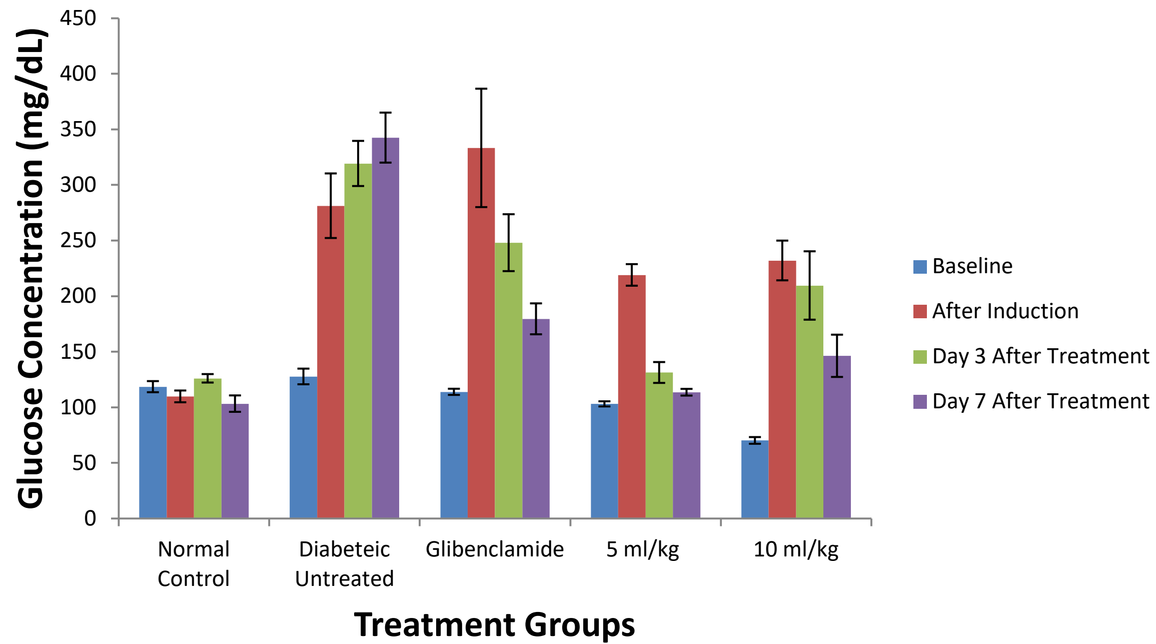

Fig 1. *Effect of human urine on glucose concentration in alloxan-induced diabetic rats.*

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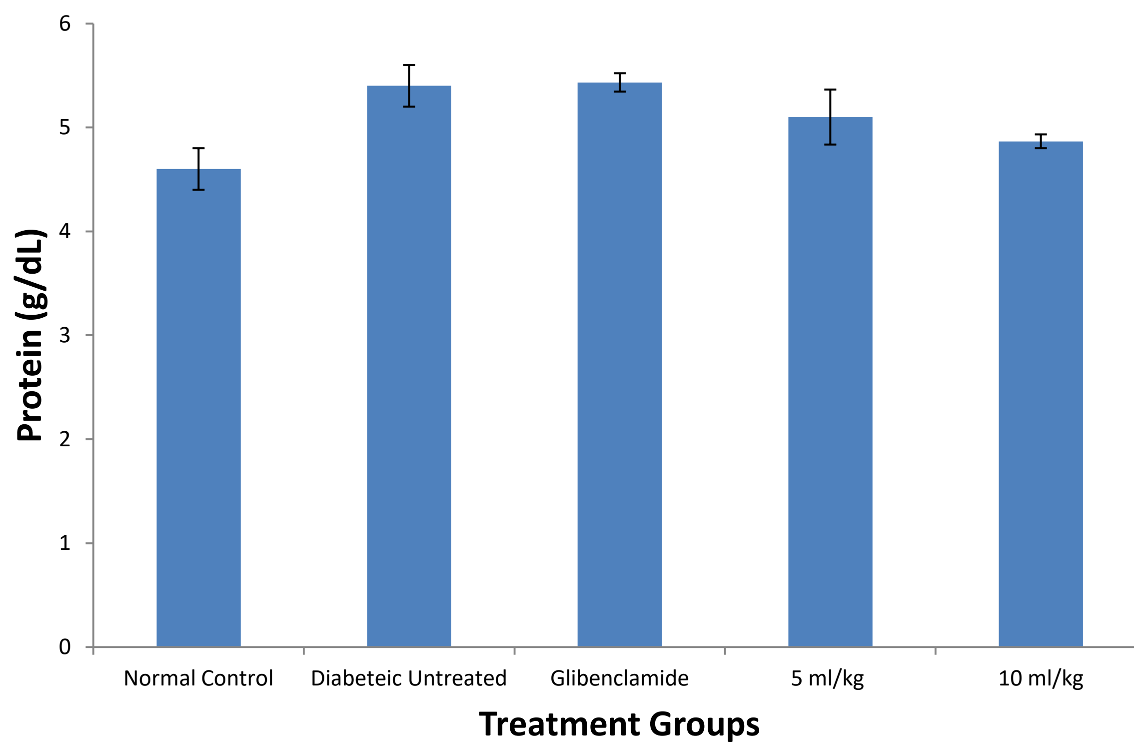

Fig. 2 Effect of human urine on protein concentration in alloxan-induced diabetic rats.

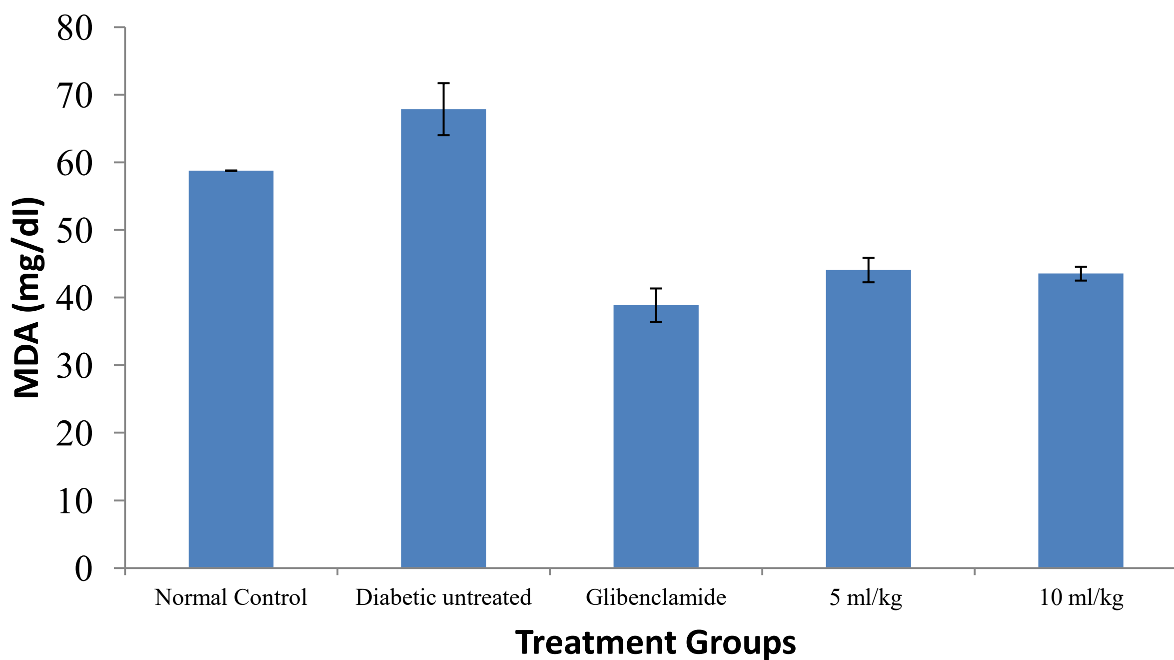

Fig. 3 Effect of human urine on MDA levels in alloxan-induced diabetic rats

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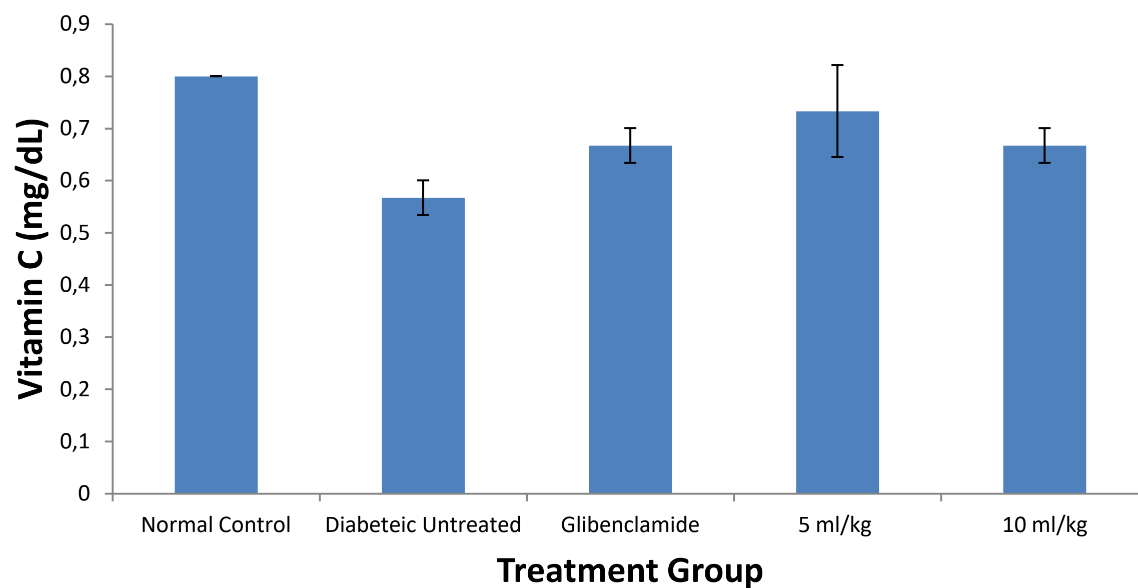

Fig. 4 Effect of human urine on the vitamin C concentration in alloxan induced diabetic rats.

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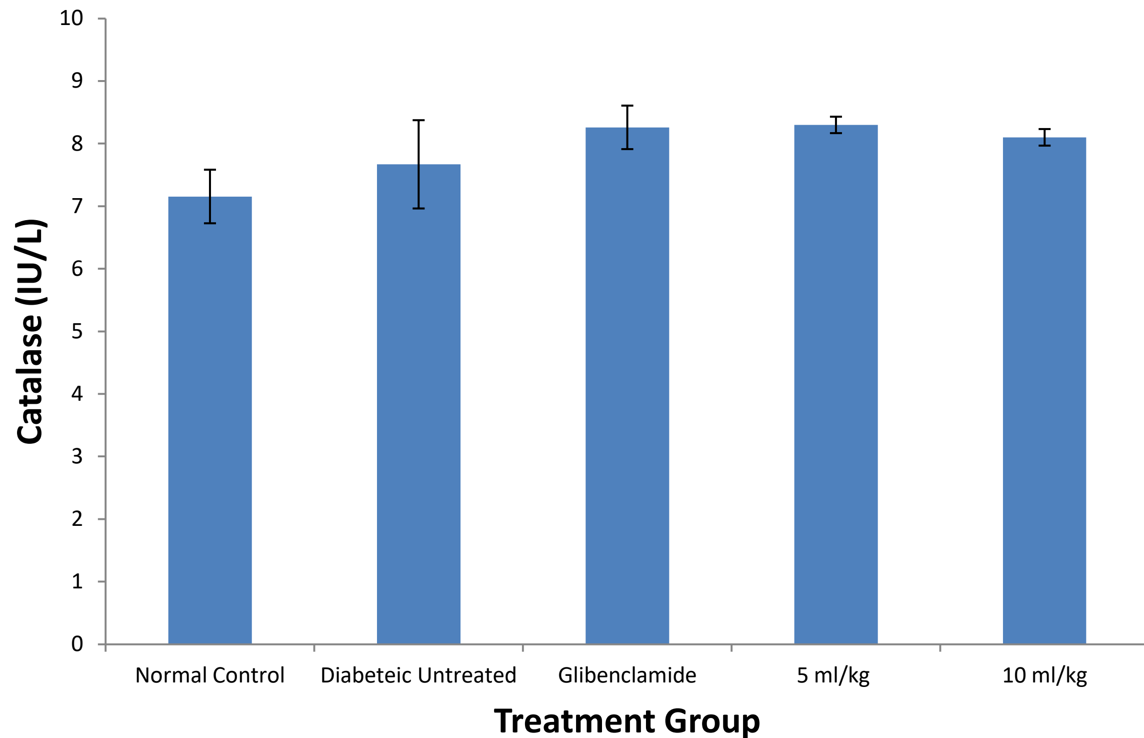

Fig. 5 Effect of human urine on catalase concentration in alloxan-induced diabetic rats.

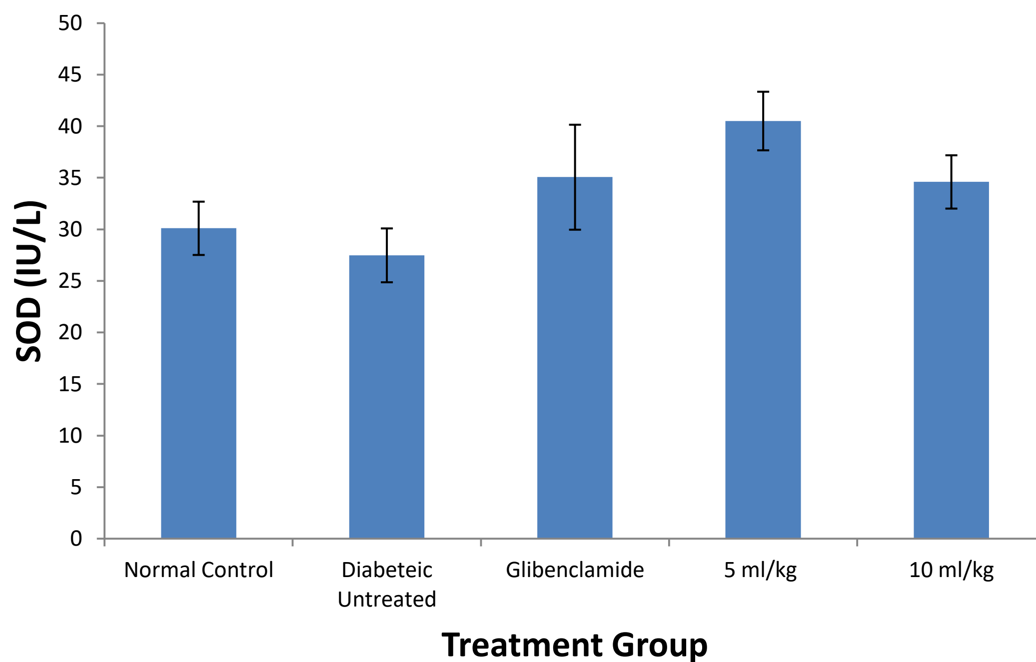

Fig. 6 Effect of human urine on SOD concentration in alloxan-induced diabetic rats.

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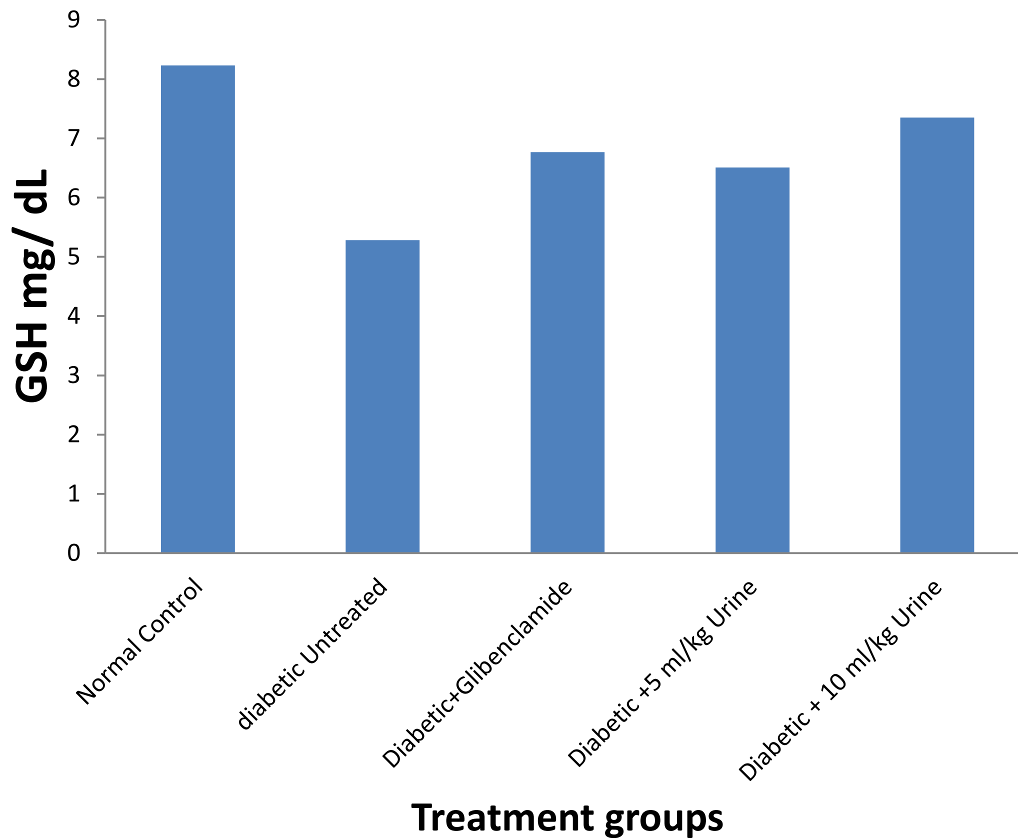

Fig 7: Effect of human urine on GSH concentration in the different groups of rats.

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