

Protective Effect of L-ascorbic Acid in Alloxan Diabetic Rats

M. A. A. Metwally, E.A.Abulghasem and K. M. Naffati

Central Laboratory for Aquaculture Research, Agriculture Research Center, Egypt

Abstract: The present study was designated to investigate the effect of ascorbic acid on toxicity induced by Alloxan through biochemical changes as the levels of some liver enzymes and blood constituents in alloxan-induced diabetic Wister rats. Diabetes was induced in animals by intraperitoneal injection of Alloxan (100 mg/kg). Alloxan induce a significant ($p < 0.05$) rise in aspartate aminotransferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), total bilirubin, direct bilirubin, albumin and total protein. The results of this study strongly indicate that vitamin C has protective effect against toxicity induced by Alloxan.

Key words: Alloxan • Rats • Diabetes Mellitus • Enzymes • Vitamin C

INTRODUCTION

Vitamin C was discovered by Albert Imre Szent-Gyorgyi (1893), a Hungarian-born biochemist, was the first to isolate vitamin C [1]. Ascorbic acid (vitamin C) is an important water soluble vitamin. Most plants and animals synthesize ascorbic acid for their own requirements. However, human cannot synthesize ascorbic acid due to lack of the enzyme gulonolactone oxidase. Hence, ascorbic acid has to be supplemented mainly through fruits, vegetables and tablets. Many health benefits have been attributed to ascorbic acid such as being an antioxidant, anti-atherogenic, immune modulator and prevent cold etc. [2]. L-Ascorbic acid is the enol form of 2-oxo-L-gulofuranolactone. It is a white, odorless, crystalline powder. It is stable in dry form, but is easily oxidized as solution in the presence of air. Oxidation is accelerated by heat, light, alkalis and traces of copper and iron. Ascorbic acid is a molecule composed of six carbon atoms, six oxygen atoms and eight hydrogen atoms, all linked together by chemical bonds [3]. Human and other primates as well as guinea pigs and some bats are the only mammals known to be unable to synthesized ascorbic acid from glucose because they lack the enzyme gulonolactone oxidase [4, 5].

It is found intra- and extracellularly as ascorbate and is well absorbed from the gastrointestinal tract [6]. Vitamin C is a natural antioxidant that prevents the increase production of free radicals induced by oxidative damage to lipids and lipoproteins in various cellular compartments

and tissues [7]. It has been shown to react directly with superoxide hydroxyl radicals and singlet oxygen [8]. The antioxidant function of vitamin C is related to its reversible oxidation and reduction characteristics. Thus, vitamin C may particularly prevent certain types of hepatic cellular damage [3].

Alloxan is one of the usual substances used for the induction of diabetes mellitus and it has a destructive effect on the beta cells of the pancreas [9]. Alloxan, a β -cytotoxic agent, causes β -Cell death and apoptosis by generation of ROS, superoxide radicals and hydrogen peroxide [10]. β Cell death causes hyperglycemia due to insulin deficiency which further aggravates the oxidative stress induced by Alloxan [11]. Alloxan is a toxic glucose analogue, which selectively destroys insulin-producing cells in the pancreas when administered to rodents and many other animal species. This causes an insulin-dependent diabetes mellitus (called "Alloxan Diabetes") in these animals, with characteristics similar to type I diabetes in humans [12].

The aim of the present study was to investigate the biochemical effects of vitamin C in Alloxan-induced diabetic Wister rats.

MATERIALS AND METHODS

Alloxan (Alloxan crystalline form) was obtained from Algamhoria Company, Egypt. Vitamin C manufactured by Bayer Company, Germany.

Corresponding Author: Mahmoud. A.A. Metwally, Chemistry Department, Faculty of Science El-Dawadmi, Shqra Univ., KSA. Central Laboratory for Aquaculture Research, Agriculture Research Center, Egypt.
E-mail: metwallymahmoud2002@yahoo.com.

Experimental Animals: Thirty six male albino rats weighting 210-240g were obtained from the experimental Animals House at Twaisha Biotechnology Research Centre, Tripoli Libya. The animal was housed in metal cages under normal temperature, pressure, humidity, good ventilation and day and night illumination cycle. Four similar cages were used as ordinary living cages, each cage contains 9 animals. The animals were fed with commercial pelleted diet and water was ad libitum. All Thirty six male albino rats, weighting 210-240g, were divided equally into four groups, each comprising nine animals (n=9). Rats were allowed to acclimatize for two weeks prior to experimentation.

Animal Grouping: Group I (Normal control rats): The animals of this group were given (1ml/100g body weight) of normal saline for 10 days by oral gavages. Group II (Alloxan group): This group was treated with (100mg/kg body weight) of Alloxan monohydrate for 3 days. Group III (vitamin C group): This group was orally treated with vitamin C (150mg/kg body weight) [13]. Group IV: This group was treated with Alloxan which injected intraperitoneum (100mg/kg body weight) for 3 days and in 4th day vitamin C was administrated orally (150 mg/kg body weight) for three weeks.

Sample Collection: At the end of experimental period (3 weeks), animals in all groups were fasted for 12 hours and blood samples were taken for biochemical and hematological parameters to assess the effect of vitamin C treatment in Alloxan induced diabetes, animals were sacrificed by cervical dislocation. Blood samples were collected from each rat into Falcon tube (without anticoagulant) and centrifuged (1500 rpm, 10 min). Serum was separated and stored at -20 °C until analyzed.

Blood Serum Enzymes Analysis: Quantitative determination of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity were performed according to the method described by Reitman and Frankel [14]. Determination of alkaline phosphatase (ALP) activity was performed according to the method described by Babson *et al.*, [15]. Determination of albumin was performed according to the method described by Webster [16]. Total protein concentration was estimated in serum by using the Biuret method Plummer [17]. While the serum bilirubin concentration was done using the method described by Malloy and Evelyn [18].

Statistical Analysis: Data analysis was performed with computer software (SPSS, version 15.0, SPSS Inc., Chicago, IL). Data was expressed as among different groups were analyzed using one way analysis of variance (ANOVA)[19]. Significance were considered significant if $p < 0.01$.

RESULTS

Aspartate Aminotransferases AST: The results of AST activity in blood serum of control rats were 152.5 ± 4.6 whereas in group II (Alloxan) treated rats were 241.1 ± 3.6 showed highly significant increase compared to control groups. The activity of AST in blood serum of vitamin C treated group III was 136.8 ± 3.5 showed significant decreases compared to control group I 152.5 ± 4.6 , whereas AST activity in group IV (Alloxan and vit. C) treated rats were 166.4 ± 16.9 . These groups showed significant decreased compared to Alloxan treated groups as shown in Table 1.

Alanine Aminotransferase (ALT): Serum ALT activity of control rats were 44.4 ± 9.8 whereas in group II were 165.3 ± 5.6 , showed significant increase compared to control group. The activity of ALT in blood serum of vitamin C treated group III was 37.1 ± 9.7 showed significant decrease compared to control group 44.4 ± 9.8 . Whereas ALT activity in group of (Alloxan and vit C) treated rats were 134.6 ± 7.9 , showed significant increase compared to control. But these groups showed significant decrease compared to Alloxan treated group as shown in table 1.

Alkaline Phosphatase (ALP): Serum ALP activity of control rats were 143.3 ± 5.1 , whereas in Alloxan treated rats were 192.6 ± 4 , showed significant increase compared to control group. The activity of ALP in blood serum of vitamin C treated group was 90.6 ± 8.2 showed significant decreased compared to control group 143.3 ± 5.1 , whereas ALP activity in group of (Alloxan and vit °C) treated rats.

Table 1: Aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase activity (U/l) in serum of normal and treated rats (Mean \pm SD).

groups	AST(U/l)	ALT(U/l)	ALP(U/l)
Control	152.5 ± 4.6^b	44.4 ± 9.8^c	143.3 ± 5.1^b
Alloxan	241.1 ± 3.6^a	165.3 ± 5.6^a	192.6 ± 4.2^a
Vit °C	136.8 ± 3.5^c	37.1 ± 9.7^c	90.6 ± 8.2^d
Alloxan and vit °C	166.4 ± 16.9^b	134.6 ± 7.9^b	121.6 ± 7.7^c

Means with the different letters for each parameter is significantly different at $P < 0.01$

Table 2: Bilirubin, albumin and total protein in serum of normal and treated (Alloxan, Vit. C) rats (Mean \pm SD).

groups	Total bilirubin (mg/dl)	Direct Bilirubin (mg/dl)	Albumin (g/dl)	Total protein (g/dl)
Control	0.33 \pm 0.09 ^b	0.027 \pm 0.009 ^b	3.92 \pm 0.26 ^a	7.4 \pm 0.1 ^a
Alloxan	0.65 \pm 0.11 ^a	0.072 \pm 0.009 ^a	2.66 \pm 0.10 ^c	5.2 \pm 0.3 ^c
Vit °C	0.20 \pm 0.05 ^c	0.028 \pm 0.010 ^b	3.95 \pm 0.16 ^a	7.0 \pm 0.4 ^a
Alloxan and vit °C	0.36 \pm 0.06 ^b	0.027 \pm 0.009 ^b	3.58 \pm 0.23 ^b	6.5 \pm 0.1 ^b

Means with the different letters for each parameter is significantly different at $P < 0.01$

were 121.6 \pm 7.7, showed significant decrease compared to control. But this group showed highly significant decreased compared to Alloxan treated group as shown in table 1.

Total Bilirubin: levated levels of total bilirubin was found in Alloxan treated rats 0.65 \pm 0.11 when compared to the normal 0.33 \pm 0.09, while the oral administration of vit °C to the (vit °C group) caused significant decrease 0.20 \pm 0.05 when compared to the control rats. Whereas significantly decrease in total bilirubin level was showed in groups of (Alloxan and vit °C) 0.36 \pm 0.06 when compared with Alloxan group as shown in table 2.

Direct Bilirubin: irect bilirubin in blood serum of the control group was 0.027 \pm 0.009 whereas in Alloxan group was 0.072 \pm 0.009, showed significant increase compared to control. But in vitamin C group the amount of direct bilirubin was significantly increased 0.028 \pm 0.010 when compared with control. The activity of direct bilirubin in group of (Alloxan and vit °C) treated rats were 0.027 \pm 0.009 showed highly significantly decreased when compared with Alloxan treated rats as shown in table 2.

Albumin: Albumin concentration in blood serum of control group was 3.92 \pm 0.26 whereas in Alloxan group was 2.66 \pm 0.10 showed significant decrease compared with control one. But in vitamin C group the amount of albumin was not affected when compared to the normal group. The albumin concentration in groups of (Alloxan and vit °C) treated rats were 3.58 \pm 0.23 showed significantly increased when compared with Alloxan treated rats as shown in table 2.

Total Protein: A significant decrease in the total protein level was observed in Alloxan treated rats 5.2 \pm 0.3 treated rats when compared with normal control rats 7.4 \pm 0.1. There was a significant decrease in protein level in the rats treated with vit °C only 7.0 \pm 0.4 when compared with control rats. On the other hand significant increase was observed in rats treated with (Alloxan and vit °C) 6.5 \pm 0.1 when compared with Alloxan treated rats as shown in table 2.

DISCUSSION

The results of the present study showed that serum aspartate aminotransaminase (AST), alanine aminotransaminase (ALT) and alkaline phosphatase (ALP) activity in diabetic animal groups (Alloxan treated rats) showed significant increase compared to control group. Elevated level of the activity of AST, ALT and ALP in blood serum is a strong indicator of hepatic and cardiac damage. These enzymes are usually liver makers whose plasma concentration above homeostatic limit could be associated with various forms of disorders which affect the functional integrity of the liver tissue [20, 21]. In addition the measurement of enzymatic activities of phosphatase such as acid phosphatase (ACP) and alkaline phosphatase (ALP) during diabetes is of clinical and toxicological importance as changes in their activities are indicative of tissue damage by toxicants [20]. This results are in agreement with [22] and [23]. The AST, ALT and ALP activity in blood serum in group (vit.C) and group (Alloxan and vit C) treated rats showed significant decrease compared to Alloxan group. In this investigation vitamin C significantly reduced elevated levels of AST, ALT and ALP. Vitamin C has a role to improve the liver function under the effects of Alloxan [24, 25].

The results of bilirubin in group (Alloxan) treated rats showed significant increased compared to control group these may occur due to peroxidation reactions, arising from Alloxan biotransformation during diabetes and these reactions may inflict oxidative injury to cellular components of liver and may cause cirrhosis. The same results were noticed by vitamin °C lowered the serum bilirubin in group (Alloxan and vit C)[26,27].

A significant decrease in total protein and albumin levels were observed in serum of Alloxan treated rats compared with control rats. The administration of vit. C restored the protein and albumin levels to near normal. Insulin deficiency leads to various metabolic aberrations in animals, such as decreased protein content. Insulin deficiency causes excessive catabolism of protein and the amino acids released are used for gluconeogenesis.

Similar results showed that total protein and albumin concentrations increased with dietary vitamin C and folic acid supplementation [28].

CONCLUSION

The administration of vitamin C in these models might be beneficial for the restoration of biochemical and hematological parameters, in the present study have revealed that oral administration of vitamin C (150 mg/kg b w) reduced blood nonfunctional plasma enzymes and liver function parameters in Alloxan induced hyperglycemia in Wister rats. On the other hand Alloxan-induced diabetes could increase the liver enzyme levels. The increase in these enzymes may occur due to peroxidation reactions, arising from Alloxan biotransformation during diabetes and these reactions may inflict oxidative injury to cellular components. Vitamin C prevents the hepatopancreatic cellular injury produced by Alloxan.

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