

# Hepato Pancreatic Protective Potentials of Iraqi Aqueous *Allium cepa* Extract against Low Double Doses of Alloxan Induced Oxidoreductive Stress Mediated Diabetes mellitus in Rats

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## ABSTRACT

Biochemical and histopathological assessment of using aqueous *Allium cepa* extract against oxidoreductive stress and hepato pancreatic cytoarchitectural changes induced by two low double doses of alloxan (75mg/kg body weight/dose) was studied. Forty eight male rats were divided into 4 main groups (n=12), viz; Group 1 (CN – Gr) which was treated as the negative control and was allowed for the free access to the rodents chow and the tap water for the whole study period, Group 2 or the (AACE – Gr) which was assigned as the herb control received a daily oral dose of 1 ml of aqueous *Allium cepa* extract/100g body weight that was equivalent to (0.45 g/100 g body weight), Group 3 or (ALX-Gr), the alloxan-injected group which was given alloxan at (75 mg/kg body weight) on the first day of the first and 5<sup>th</sup> week of the experimental period and Group 4 (ALX+AACE-Gr) which was injected by double doses of alloxan as G3 and received a daily oral dose of 1 ml of aqueous *Allium cepa* extract/100 g body weight that was equivalent to (0.45 g/100 g body weight). Each of the mentioned groups was subdivided into two halves (n=6) that one was exsanguinated on day 28 (subgroup A) while the rest were kept till the end of the study period (56 days) (Subgroup B). Serum malondialdehyde concentration, total oxidative stress and oxidative stress index as oxidoreductive stress biomarker, activities of certain anti-oxidoreductive stress enzymes (glutathione peroxidase, super

oxide dismutase and catalase) and concentration of reduced form glutathione with total antioxidative stress capacity and lipid profile were estimated. In Addition, liver and pancreas histopathological findings were evaluated.

In this study, the continuous daily administration of aqueous *Allium cepa* extract significantly protect against deleterious impact of free radicals action and thus preserving hepatic and pancreatic cellular restoration and architecture. Taken together, the biochemical and histopathological findings of the current study indicate a hepato-pancreatic protective effects and antidiabetic efficacy of aqueous *Allium cepa* extract against time and dose dependent manner of oxidoreductive stress mediated alloxan diabetogenicity in rats

**Keywords:** Alloxan, oxidoreductive stress, Diabetes mellitus, *Allium cepa*, Histopathology.

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## INTRODUCTION

Diabetes mellitus is a heterogeneous disease of a multifactorial causation. It is characterized by the disturbance in the metabolism of carbohydrates, proteins and sugars and leading to chronic hyperglycemia, muscle wasting and dyslipidemia.

The current reports of the World Health Organization (WHO) warn against the progressive dissemination of the disease and expect that the number of diabetics may hit 380 million people by 2025. Diabetes is highly disseminated among poor and affluent people and is widely distributed in both the developed and the developing countries. The highest incidence was seen in both Asia and Africa [1].

The cost of the modern anti diabetic drugs is beyond the capacity of the most of the health authorities to be able to deliver it to all the diabetic peoples. Furthermore, its incidence constitutes another economic burden as it hinders the performance of the affected workers and add more burdens to the authority to supply the therapy [2]. This dilemma urged the scientists to explore for cheap and reachable ant diabetic products. Lots of recommendation came out from different expert committees suggesting further investigation on the traditional or herbal medicine so as to take into the consideration, the cheapness of the herbal products and the economic constraints [3]. This study is a part of such efforts as it highlighted the protective effects of onion

(*Allium cepa* L. Amaryllidaceae) against the progression of diabetes mellitus and its complication using a positive control model of the alloxanized rats. The model was based on implementing a multiple or a double dosing system to induce both type I and type II diabetes mellitus. Onion or *Allium cepa* L. (Amaryllidaceae) is one of the important medicinal and nutraceutical edible products. It is widely cultivated across the world and is native to the countries of the South East Asia. The plant is made up of a stem measuring about 18-54 cm with a bulb like underground structure which constitutes the eatable crop of the plant [4].

Onion is rich in a plenty of phytochemicals with diverse pharmaceutical and medicinal effects. They include phenolic compounds, such as; quercetin, anthocyanins and flavonoids, phenolic acid derivatives, such as caffeic acid, chlorogenic acid, ferrulic acid, sinapic acid, P-coumaric acid, syringic acid and P-hydroxybenzoic acid., Organosulphur compounds, such as alliin (S-allyl cysteine sulphoxide) and allicin (diallyl sulphide), enzymes (Alliinase enzyme), amino acids, vitamins (vitamin C and vitamin B) and minerals like zinc and selenium [5] and [6]. This made onion as one of the highly consumed crops with lots of putative health benefits.

Previous studies revealed that the extract of *Allium cepa* got a powerful antidiabetic, cardioprotective effect characterized by an anti-platelets and anti-

hypercholesterolemic effects and an antiseptic effect. These actions can be ascribed to the antioxidant and the non-antioxidant effects of the extract content [4].

The phenolic compounds (polyphenols and phenolic acid derivatives) have an antioxidant effect as they can quench the free radicals and interfere with the cascade sequence of oxidative stress. Furthermore, they act as inhibitors for enzymes involved in fatty acid synthesis, mediation of the inflammatory reaction and regulation of cellular proliferation. For instance inhibition of phosphatidylinositol 3-kinase that is responsible for flow of the P<sub>13</sub>K/AKT signaling pathway and the NF- $\kappa$ B which mediates the inflammatory response [7] and [8].

The organo-sulphur compounds confer for the pungency of onion and play a role in replenishing the body with glutathione which is a part of the endogenous antioxidant system of the human body to protect against the oxidative stress and a potent inhibitor for the enzymes involved in fatty acids synthesis [9].

The study aimed at exploring the putative unique effect for the onion extract against alloxan induced diabetes as its diabetogenic effect relies on its ability to generate reactive oxygen species in the presence of glutathione. Alloxan is exposed to a continuous redox cycle that generates dialuric acid as a final reduction product and upon its autooxidation can generate superoxide radicals, hydrogen peroxide and hydroxyl radicals. Obviously, these hydroxyl radicals were blamed for the necrosis (pathological cell death)/ and or apoptosis (physiological programmed death) of the beta cells of islets of Langerhans [10].

## MATERIALS AND METHODS

### Animals

A total of 48 Wistar albino male rats; weighing 150-170 g and aged eight weeks, were procured from the animal house facility of the College of Pharmacy/University of Baghdad. The animals were housed the transient room facility of the Department of Pharmacology and Toxicology and were housed in clean plastic cages under the standard conditions of the animals housing (12-h light:12-h dark cycle), a minimum relative humidity of 40% and at the room temperature 23 $\pm$ 5 °C. First and before of the commencement of the experiment, they were left for 1 week with a free access to the standard rodent's pelleted chow and the drinking water for the acclimatization to the laboratory environment. The diet contained starch (64 %). Protein (12%), fat (10%), sugars (5%), vitamin premixes (1%), salt mixtures (4%) and fibers (4%). All the experiments and procedures were done as per rules and regulations of the Ethical Committee/College of Pharmacy/University of Baghdad.

### Chemicals

Alloxan was purchased from Sigma Chemicals, St. Louis, USA. Meanwhile, the chemicals used for the determination of the malondialdehyde (MDA) (thiobarbituric acid and *n*-butanol) were procured from Sigma Aldrich.

For the blood glucose determination, A glucometer and the glucose measurement strips were obtained from Contour Bayer Germany. Furthermore, the kits for the liver function enzymes, Alkaline Phosphatase (ALP),

Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) were obtained from (Bio-Merieux® Laboratory Reagents and Products, France).

Moreover, the total antioxidant (TAC) and total Oxidant (TOC) capacities that were used for the assessment of the oxidative stress were procured from (Rel Assay Diagnostics®, Gaziantep, Turkey). Meanwhile, each of the reduced form glutathione (r-GSH), glutathione peroxidase (GSH-Px), super oxide dismutase (SOD) and catalase (CAT) enzymes were measured using Randox® diagnostic's kits.

### Onion Extract preparation

Iraqi onion, *Allium cepa* L. (Amryllidaceae) used for the current experiment was procured from the Jamila Market, Baghdad, Iraq. The plants were identified to species level at the Iraqi Natural History Research Center and Museum No: 35/ 21-1-2020, University of Baghdad. According to the methods of Habib *et al.* (2005) [11] with simple modification, the aqueous of extract of *Allium cepa* were prepared. Fresh and healthy *Allium cepa* (1000g) of this plant were washed, trimmed into small pieces and homogenized in a blender. The resulting mixture was immersed in 2 L of distilled water. The mixture was left to stand for 48 hours with multiple shakings. Following filtration, the filtrates were weight and crude extract was obtained. The extract was kept in refrigerator (4°C). After that, the extract was reconstituted in normal saline (0.9 % NaCl) and used as a dose of 1 ml of aqueous *Allium cepa* extract/100 g body weight that was equivalent to (0.45 g/100 g body weight).

### Experimental design

Diabetes was induced by using a model of the double doses of intraperitoneal (I.P) alloxan injection at (75mg/Kg Body weight B.W). The two doses were given at the beginning of the first and the fifth week of the experimental period that continued for 8 weeks.

The animals were subdivided into 4 main groups (n=12), viz; the negative control (Gr 1) that was allowed for the free access to the standard rodents chow and the drinking water for 8 weeks, herbal control group (AACE-Gr) which was given the onion extract orally in a dose of 1 ml of aqueous *Allium cepa* extract/100 g B.W./daily that was equivalent to (0.45g/100 g B.W.), the alloxanized control group (ALX-Gr) which was given alloxan at (75mg/kg B.W) on the first day of the 1<sup>st</sup> and the 5<sup>th</sup> week of the study and the group of the alloxanized rats treated with the herbal extract (ALX+AACE-Gr) which was given alloxan as in the group (ALX-Gr) and the herbal extract as in the (AACE-Gr).

Each of the above mentioned groups was subdivided into two equal parts (n=6) that for one, the study was terminated on day 28 (for the subgroup A) and on day 56 days (for the subgroup B).

During the study, the fasting glucose level was determined using the tail venous blood and the glucose meter on daily basis. Meanwhile, at the end, the animal's fasting blood was collected via cardiac puncture and were anesthetized using a mixture of Alcohol, diethyl ether and chloroform at a ratio of (1:3:2 for each respectively) [12], and the serum was prepared and kept in deep freezing for the future biochemistry analyses (analyses for

the determination of the oxidative stress and the lipid profile markers and assessment of the liver functions). Eventually, the animals were euthanized through giving them an overdose of the anesthesia and their liver and pancreas were collected and kept in 10% neutral buffer formalin for the histopathology study. The remaining carcasses were wrapped and sent to be disposed according to the regulations of the Animal's Ethics Committee of the College of Pharmacy/University of Baghdad.

#### Blood Glucose Level

The blood glucose was measured daily using the fasting tail venous blood. The measurements were done using the glucometer and the test strips provided by Contour - Bayer Germany. The average of the daily glucose level for each week was calculated and the results for each group were expressed as mean  $\pm$  S.E.M for all the subjects of each group.

#### Serum Biochemical Analyses

The serum samples were used for the assessment of the protein level and the liver function tests (Alkaline Phosphatase (ALP), Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) activities). These enzymes kits were obtained from (Bio-Merieux<sup>®</sup> Laboratory Reagents and Products, France).

#### Measurement of Oxidative/Antioxidative Stress Parameters.

The obtained serum was used to determine the oxidative/antioxidative stress markers like serum total oxidant and antioxidant capacities (TOC and TAC) using the automated techniques described by (Erel, 2004) (Erel, 2005) [13] [14]. Meanwhile, the oxidative stress index (OSI) was calculated through dividing the total oxidant capacity (TOC) to the total antioxidant capacity (TAC). The serum level of malondialdehyde (MDA) was determined using the method; described by Kikugawa *et al.*, 1992 [15]. It was expressed in nmol/ml.

Furthermore, the reduced form of the glutathione was measured in the RBCs hemolysate using the method described by Ellman in (1959) [14] and [16]. On the other hand, the serum activities of glutathione peroxidase (GSH-Px), catalase (CAT) and the superoxide dismutase (SOD) were analyzed as per the spectrophotometric method described by the Randox diagnostic's kits.

#### Histopathological Studies

After the animals were ethically euthanized, decapitated, liver and pancreas were collected and fixed for 72 hours in 10% neutral buffered formalin saline. The tissues were embedded in paraffin and sectioned at 5  $\mu$ m thickness using a rotary microtome. The sections were stained with hematoxylin-eosin (H&E) stain according to Luna (1968) for the light microscopic histopathology [17].

#### Statistical Analysis

The results were expressed as mean  $\pm$  standard error of the mean (SEM). One-way ANOVA followed by post hoc Branferroni test was followed to test the difference between the groups. This test was done using the SPSS version 18 statistical package, 2010, (SPSS Inc. Chicago,

IL, USA). *P*-value of less than 0.05 was considered as statistically significant.

## RESULTS

### Fasting blood Glucose Levels

During the whole period of the experiment, there hasn't been any significant difference in the Fasting Blood Glucose (FBG) levels between (CN-Gr) and (AACE-Gr) ( $P < 0.05$ ).

Concurrently, during the first 4 weeks of the study, both of the (ALX-Gr) and (ALX+AACE-Gr) groups experienced a transient increase in the FBG level during the first week followed by a gradual decline till the end of the 4<sup>th</sup> week. The decline in the (ALX+AACE-Gr) was higher and significantly different as compared to that of the (ALX Gr) ( $P < 0.05$ ) Table 1.

On the other hand, when the second dose of alloxan was given in the 5<sup>th</sup> week of the study (ALX-Gr-sub gr B), the FBG level has increased progressively till the end of the study and the values were significantly different in comparison with that of the negative control ( $P < 0.05$ ) (Table 1).

The aqueous *Allium cepa* extract co treatment in the subgroup B of the (ALX-AACE-Gr) has limited the progression of the alloxan induced hyperglycemia that the sugar levels were significantly less than that of the diabetic control (ALX-Gr) during the last three weeks of the study ( the period from week 5 to week 8) ( $P < 0.05$ ) (Table-1).

### Liver function tests

The liver function tests revealed the absence of any significant difference in the serum level of the hepatic enzymes between (CN-Gr) and (AACE-Gr) ( $P < 0.05$ ) (Table 2). Meanwhile, the deterioration in the hepatic functions was obvious and significantly different in comparison to that of the negative control for the alloxan treated groups (ALX and ALX+AACE groups) on days 28 and 56 ( $P < 0.05$ ) (Table 2) that it was stronger on day 56 as compared to that on day 28 (Table 2). It worth to note that co treatment with the AACE has significantly ameliorated the hepatic dysfunction in the alloxan treated group as seen in the results of the AST and ALT levels (Table 2) but the levels were still significantly higher as compared to that of the negative control ( $P < 0.05$ ) (Table 2). Nevertheless, the results of the ALP showed comparable values for the AACE treated and untreated diabetic rats indicating that the extract has ameliorated the liver dysfunction without amelioration in the serum level of ALP.

### Levels and Activities of Oxidative Stress and Antioxidant Biomarkers

The results revealed a prominent and a significant and progressive increase in the oxidative stress markers along with a decline in the antioxidant enzymes in the rats treated with alloxan in comparison with those in the (CN-Gr) ( $P < 0.05$ ) (Table 3). Meanwhile the co-treatment of the normal non-diabetic rats with the AACE did not produce that much change as compared to that of the (CN-Gr). but the extract treatment has hindered the

alloxan induced deterioration in the oxidative stress as seen in the results of the (ALX-AAEC-Gr) (Tables 3 & 4). The AAEC induced decline was still significantly higher than that of the control for the serum levels of MDA, TOC and OSI but the decline was comparable and insignificantly different to that of the negative control for the TAC values ( $P < 0.05$ ) (Table 3).

Similarly, Co-treatment of the extract to the alloxanized rats produced a decline in the serum level of the antioxidant enzymes and the hemolysate level of glutathione that it was significantly less than that of the extract untreated alloxanized rats and stayed higher than that of the negative control ( $P < 0.05$ ) (Table 4)

#### Changes in the Lipid Profile Findings

The study showed that the two doses of alloxan led to a prominent and a significant hyperlipidemic effect characterized by significant luculent in the serum levels of triglyceride, cholesterol and LDL. Besides, the group experienced a significant decline in the serum level of the beneficial cholesterol (HDL) ( $P < 0.05$ ) (Table 5).

Interestingly, treatment with the AAEC extract produced a prominent hypolipidemic effect in both the alloxanized and the non-alloxanized rats. In the non-alloxanized rats, the extract led to a significant increase in the serum level of the HDL in comparison to the negative control ( $P < 0.05$ ) (Table 5). Besides, it produced a significant hypolipidemic effect in the alloxanized rats characterized by a prominent and a significant decline in the serum levels of cholesterol, triglyceride and LDL and increase in that of the HDL in comparison to that of the ALX group. Nevertheless, the values were still significantly different in comparison to that of the negative control ( $P < 0.05$ ) (table 5).

## HISTOPATHOLOGICAL FINDINGS

### Pancreas histopathological findings

During the two periods of this experimental study, there are no observable histological changes in the pancreas of both negative control (CN-Gr) and positive control (AAEC-Gr) groups respectively. In these groups the Islets of Langerhans appeared with normal architecture. These islets display a central gist of  $\beta$ - cells surrounded by an outside, largely continuous sheath of non  $\beta$ -cell cells. Circumferentially, the Islet cells with small, dark nuclei are (alpha-cells), meanwhile, those with light and large nuclei are ( $\beta$ -cells). The islet cells are seen embedded within the exocrine acinar cells and surrounded by a fine connective tissue capsule (Figure-1). Meanwhile, at the end of the 4<sup>th</sup> week after the first alloxan intra-peritoneal injection, the pancreas of (ALX-Gr) has been revealed hydropic cellular degeneration and or cloudy swelling due to an intracytoplasmic accumulation of water and protein that observed in most anastomosing cells of the islets of Langerhans in the diabetic rats with very few fat droplets accumulated as fatty change lesion (steatosis) (Figure-2). Additionally, some of other islets appeared to developed hypertrophy (increase in the size of these islets due to increase in the size of their cells) and or hyperplasia (increase in the size of the islets due to increase in the number of their dividing cells). Obviously, the hypertrophic and hyperplastic islets appear with

irregular projections into the exocrine part of the pancreas (Figure-3).

The histopathologic lesions in the pancreas of alloxan injected and *Allium cepa* treated group (ALX-AAEC-Gr) noticed to be exclusively consist of mild hydropic degeneration only with no other pathognomic lesions.

On day 56, the pancreas of the alloxan double doses treated rats reveals a broad spectrum of histopathological lesions which consist of prominence atrophic islets, extensive cellular vacuolations (mostly hydropic degeneration and rarely fatty infiltration) and partially  $\beta$ -cells depletion together with cellular swellings in the exocrine acinar cells of the pancreas (Figure-4). Rats that underwent couple treatments in (ALX-AAEC Gr) are illustrating semi-characteristic appearance of normal islets criteria with significant regenerative features together with almost no atrophic architecture lesion (Figure-5).

### Liver histopathological findings

The histopathological examination of liver of the rats in both negative control (CN-Gr) and positive control (AAEC-Gr) groups reveals normal hepatic tissue architecture. that appeared to be divided into the classical hepatic lobules. Each lobule has a definitive central vein in the middle and six portal triads at the angle. Every lobule typically formed of cords of hepatocytes extending from the central vein to the periphery of the lobule. The hepatic cords were separated by narrow blood sinusoidal spaces (Figure-6).

Liver of alloxan injected rats at the end of the 4<sup>th</sup> week exhibits reversible degenerative changes ranged between hydropic/and or fatty changes with few and individual necrotic hepatocytes in both portal triad primarily and centrilobular zones occasionally (Figure-7).

The histopathologic examination of liver of double doses alloxan injected rats at the end of the 8<sup>th</sup> week showed severe periportal coagulative necrosis of the hepatocytes in the plural portal triads together with hyperemia and congestion of hepatic artery proper and hepatic portal vein, respectively, as well as multifocal areas of mononuclear cells infiltration (Figure-8). In the periphery of the sinusoids which drain into the centrilobular vein and are lined by an incomplete layer of flattened endothelial cells and a frank prominence of Kupffer's cells was noticed as well. The liver sections further showed occasional centrilobular coagulative necrosis and some degree of pericentral glycogen diminution in the centrilobular zone. Furthermore, pronouncing Kupffer's cells appearance also noticed (Figure-9).

Examination of liver sections of alloxan injected rats that treated with aqueous *Allium cepa* extract at the end of experimental period indicated that the hepatic lobules appeared more or less like the control group with pronounced reversible regenerative changes and active looking well differentiated intermitotic hepatocytes in multiple zones of the liver. Exceptionally and very seldom, some sinusoidal congestion was seen (Figure-10).

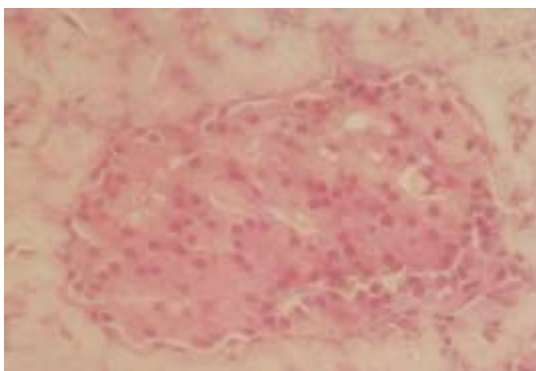


Figure-1: Photomicrograph of islet of Langerhans in pancreas from control negative (CN-Gr) and *Allium cepa* treated (AACE-Gr) groups at the end of the 8<sup>th</sup> week. Normal islet tissue architectural design and endocrine constituents (40X).

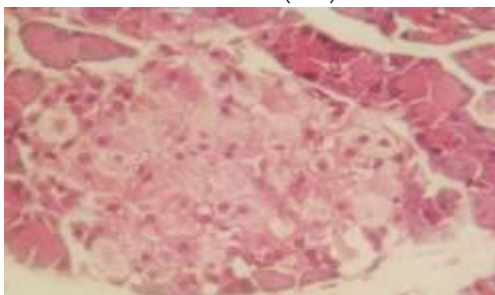


Figure-2: Photomicrograph of pancreas from alloxan treated rat (ALX-Gr) at the end of the 4<sup>th</sup> week. An islet of Langerhans in the endocrine portion exhibits prominence of acute cellular swelling due to both hydropic degeneration and/or cloudy swelling with few fatty change (steatosis) lesions in some endocrine anastomosing cells, giving this islet a hypertrophic appearance. Very few individual cell necrosis is also present (20X).

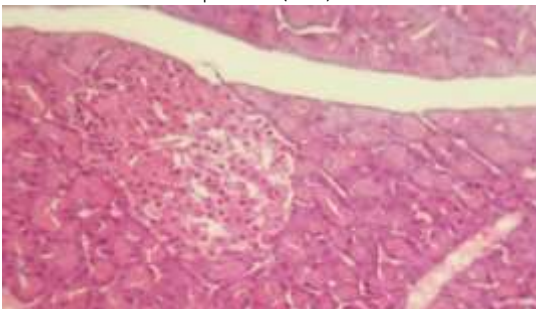


Figure-3: Photomicrograph of pancreas from alloxan treated rat (ALX-Gr) at the end of the 4<sup>th</sup> week. An islet of Langerhans in the endocrine portion exhibits mitotic activity and hyperplasia of some endocrine cells with hydropic degeneration and/or cloudy swelling with few fatty change (steatosis) lesions. (20X).

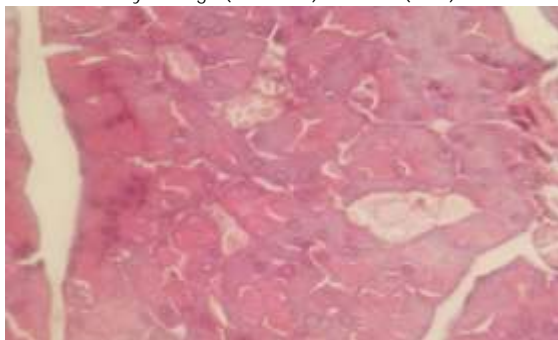


Figure-4: Photomicrograph of pancreas from double doses alloxan treated rat (ALX-Gr) at the end of the 8<sup>th</sup> week. An islet of Langerhans in the endocrine portion seems with severe atrophy due to necrosis and depletion of most of its endocrine cells (20X).

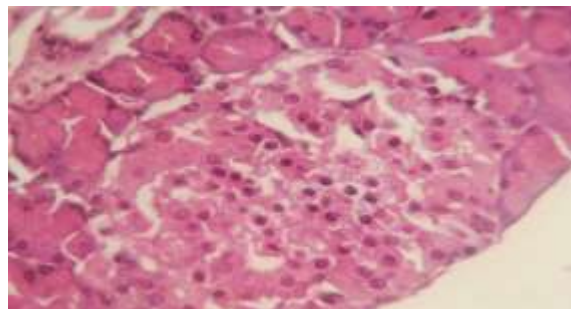


Figure-5: Photomicrograph of pancreas from alloxan injected and *Allium cepa* treated rat (ALX+AACE-Gr) at the end of the 8<sup>th</sup> week clarifying semi-characteristic appearance of normal islets layout with significant regenerative lineaments together with some reversible hydropic degenerative change and almost no atrophic lesion appears (40X).

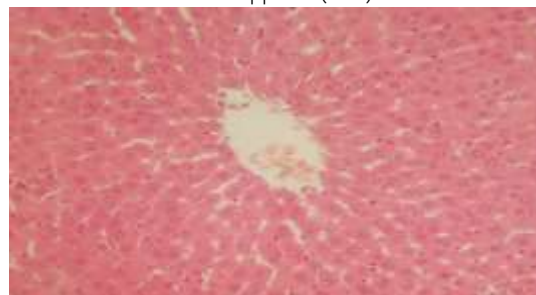


Figure-6: Photomicrograph of liver of rats from both control and aqueous *Allium cepa* extract groups revealed normal tissue mien of hepatocytic cords extending from the central vein to the boundary of the lobule. The hepatic cords are divided by narrow blood sinusoidal spaces (10X).

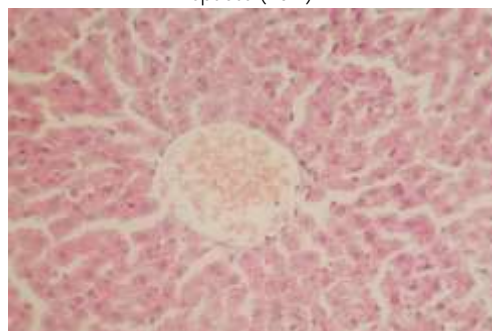
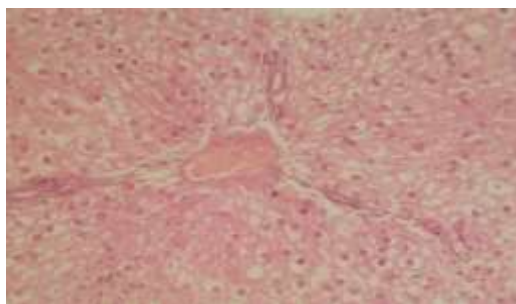
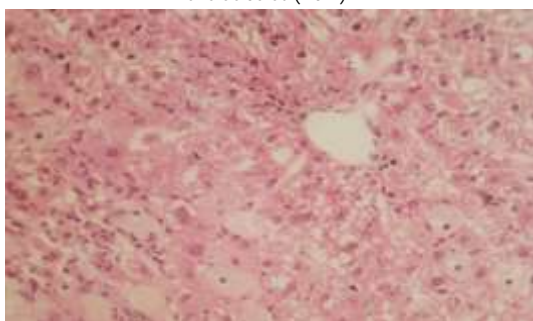


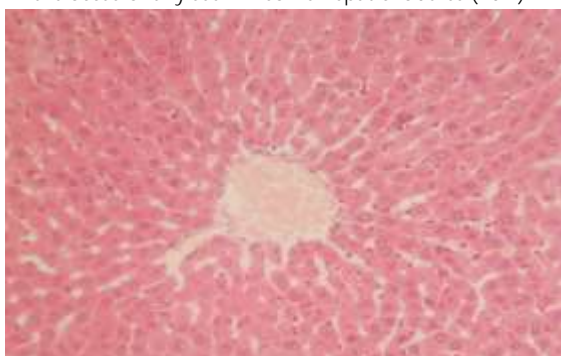
Figure-7: Photomicrograph of liver from alloxan injected rats at the end of the 4<sup>th</sup> week exhibits central vein congestion together with mild to moderate degenerative alterations ranged between hydropic/and or fatty degenerations (steatosis) with few distributive necrotic hepatocytes in the centrilobular and midzonal areas (20X).



**Figure-8:** Photomicrograph of portal (triad) area in liver from alloxan injected rats at the end of the 8<sup>th</sup> week. Portal vein congestion and advanced necrotic features comprise several stages of nuclear changes (Pyknosis, karyorrhexis and karyolysis) and obvious cytoplasmolysis are subsist (10X).



**Figure-9:** Photomicrograph of centrilobular zone in liver from alloxan injected rats at the end of the 8<sup>th</sup> week. Vehement and jumbled degenerative and necrotic changes together with infiltration of mononuclear cells are occasionally seen in some hepatic lobules (10X).



**Figure-10:** Photomicrograph of liver from alloxan injected and aqueous *Allium cepa* extract treated rats at the end of the 8<sup>th</sup> week. Permanence of hepatic lobule architecture with regenerative features of some degenerative changes. Exceptionally, dilatation and congestion of central vein are notice also. (20X).

## DISCUSSION

Alloxan is a pyrimidine derivative with a selective destructive effect on the  $\beta$  cells of Islets of Langerhans in the pancreas. Its similarity with glucose enhance its selective uptake by pancreatic cells as it tends to bind to the GLUT-2 transporters which are responsible for the glucose uptake [18]. Unlike other transporter, it does not depend on insulin for eased glucose diffusion across hepatic and pancreatic cell membranes.

This pyrimidine derivative which also called alloxan is well known as a drug of choice for the induction of diabetes in the animal models. Different dosing protocols were implemented to achieve this aim. The single dosing protocol was used extensively and is based on injecting

150-200 mg/kg (body weight) of alloxan. Smaller doses were used in the multiple dosing protocols to attain diabetes profile different from that of the single dosing protocol. In this study, alloxan was given in two doses at 75 mg/kg leaving a period of 28 days between the two doses. It worth to note that the dosing protocol has an impact on the mechanism of the induced diabetes. According to the mechanism of induction, diabetes is sub-classified into type I and II. The former is characterized by the loss of the functional activity of the pancreas and decline in the insulin release. Meanwhile, type II is mediated by the incidence of glucose intolerance and the loss of the insulin sensitivity in the peripheral cells. Both cases result in hyperglycemia due to the compromised uptake of glucose by the insulin targets in the periphery. Previous studies showed that pathogenesis of type I is more induced by the single dosing protocol. Meanwhile a mixture profile of type I and type II is more induced along with the multiple dosing protocols of the alloxan induced diabetes. [19].

The current study was designed to create a diabetes model characterized by the incidence of the cumulative deleterious effects of two separated sub-diabetogenic doses of alloxan. Furthermore, it aimed at finding the inhibitory impacts of *Allium cepa* extract against the progression of diabetes mellitus pathogenesis in rats alloxanized as per the double dosing protocol.

The pathogenesis of the alloxan diabetogenicity relies on its aptitude to generate reactive oxygen species in the presence of thiols rich glutathione through a cyclic redox reaction. The cycle includes a continuous swap of the alloxan between its original form and its metabolite (Dialuric acid which is its final reduction product). Upon its auto-oxidation, dialuric acid generates superoxide radicals, hydrogen peroxide and hydroxyl radicals that result in higher oxidative stress and consequently higher incidence of necrosis and or apoptosis of the beta cells of Islets of Langerhans [10]. The whole process of the alloxan induced diabetogenicity disturbs the fine balance between the oxidant-antioxidant mechanisms in favor of more free radicals release. From one side, glutathione depletion may occur due to its consumption during the conversion of alloxan to the dialuric acid. On the other hand, the released dialuric acid acts as an oxidant agent that enhance flow of free radicals release. The excessive oxidative stress in the  $\beta$ -cells results in mediation of necrotic or apoptotic damages and loss of their functional activity.

Unlike in the single dosing protocol, a partial reversible damage is expected in the  $\beta$ -cells of Islets of Langerhans along with the multiple dosing protocol as the intensity of the damage is controlled by the degree of the oxidative stress mediated. This coincides with the results of the experiment that showed a biphasic hyperglycemic response after the 1<sup>st</sup> and 2<sup>nd</sup> alloxan injections. Frankly, the hyperglycemic nature during the first 4 weeks was of regressive rat in opposite to the accelerating and progressive type after the second dose of alloxan injection. Despite these two types of hyperglycemia it is well known that diabetes does not occur without advance  $\beta$ -cell dysfunction. The hyperglycemia reversal during the first month of the study suggests that the oxidative stress was only sufficient for the induction of reversible

degenerative effects without showing any tendency to develop necrotic irreversible effects in the  $\beta$ -cells of the islets of Langerhans.

The higher dose of the single dosing protocol is sufficient to induce the due oxidative stress for having a complete necrotic effect on the  $\beta$ -cells of islets of Langerhans. This fact explains the reason behind the predominance of the type I pathogenesis along with the single dosing protocol and the incidence of a mixed form of type I and type II diabetes along with the multiple dosing one [20]. Consequently, this model is advantageous in screening the antidiabetogenic effects of drugs as it shows their aptitude to enhance insulin insensitivity or release. Both targets are crucial for the treatment of diabetes mellitus

The regressive type panel of the hyperglycemia; that was noticed during the first 28 days and before the second dose of alloxan, can be ascribed to the ability of the regenerative system to restore the glucose homeostasis after the first injection of alloxan. Meanwhile, the progressive profile that was noticed during the second month indicates the incapability of the regenerative system to restore the homeostasis and to counteract the flow of free radicals. this observation can be linked to results of the antioxidant enzyme profile on days 28 and 56. The profile showed a mild decline in the glutathione peroxidase, superoxide dismutase and catalase on day 28 while the decline was stronger on day 56. These enzymes are crucial for prevention of the oxidative stress which plays an important role in progression of the alloxan induced diabetes. Furthermore, the longer period of exposure to the oxidative stress can be set as another enhancement factor for the deterioration of the glucose homeostasis.

The incidence of the mild hyperglycemia during the first 4 weeks of the study may result in higher cellular uptake of the free fatty acids as a main source of energy in the mitochondria leading to a stronger oxidative stress and more superoxide production [21]. As a compensatory mechanism, most Islets of Langerhans during this period underwent hypertrophy and or hyperplasia due to hyperphagia, insulin resistance, or most commonly, a combination of these two criteria as seen in the results of the histopathology study. This can be ascribed to the incidence of hyperphagia, insulin resistance, or most commonly, a combination of these two criteria.

Previous studies showed that there is a shift in the source of energy from carbohydrates to the fats during the incidence of diabetes or metabolic syndrome [22]. The overflow of more fatty acids into the mitochondria results in generation of more free radicals as fatty acids are metabolized by the process of  $\beta$ -oxidation. Firstly, this stress triggers the cells to undergo the compensatory mechanisms as free radicals can act as signaling molecules for some biological processes but then at their excessive level, they turn deleterious and lead to cellular degeneration and necrosis [23].

An advance-stage of diabetes was developed after 56 day suggesting the incidence of a time and dose dependent necrotic or apoptotic lesions of the pancreatic beta cells leading to insulinopenia and subsequently to hyperglycemia. Apparently, the alloxan treated-rats at this time showed marked reduction in the  $\beta$ -cells with

atrophic appearance of islet architecture together with fat deposition as a sequela of the pronounced dyslipidemia. Unfortunately, the deposition of intra-islet amyloid that described by one of the previous studies (Elizabeth, *et. al.*, 2019) [24] has not been seen in current study. The regenerative capacity in both liver and pancreas treated with the extract can be ascribed to the intermitotic activity of these organs by the antioxidative capacity and restoring of redox system in favor of reduction status done by this plant [25]. The discrepancy between day 28 and 58 can be ascribed to the longer period of exposure to the oxidative stress and the observed decline in the antioxidant enzymes that protect against the flow of free radicals.

Besides, the alloxanized rats experienced marked decline in the liver function. The decline was higher after the second dose as compared with that after the first. This stronger deterioration can be ascribed also to the longer period of exposure to the oxidative stress along with the prominent dysfunction of the endogenous antioxidant system that is represented by the antioxidant enzymes. Although the oxidative stress parameters on day 56 were slightly less than that on day 28 (Table 5) but this persistent decline suggests a longer period of exposure to the free radicals which may enhance the dysfunction in different organs.

The liver function profile showed a prominent increase in the serum level of alkaline phosphatase enzyme in the ALX-Gr. It was increased by about 1.7 times on day 28 and 2.6 time on day 56. This increase suggests a deterioration in the integrity of the biliary hepatocytes whose membrane microvilli is endowed with a plenitude of this enzyme. Alkaline phosphatase helps in breakdown of the organic phosphates and facilitates their uptake by cells. It is found abundantly in bone, placenta, biliary hepatocytes and renal tubules. Since, it is a nonspecific indicators for the liver dysfunction, the study does not exclude the presence of other dysfunctions that might have enhanced its over-release into the plasma [26]. The results suggest the incidence of an intrinsic damage in the plasma membrane of the cells that bear this enzyme that biliary hepatocytes constitute an important type of such cells

Furthermore, the study also showed a significant increase in the plasma levels of the hepatic enzymes; ALT and AST. Both enzymes are important for transamination of the amino acids and creation of metabolites important for the citric acid cycle. Their presence is crucial for the progression of different metabolic reactions. AST is found abundantly inside the mitochondria while ALT is found more in the cytosol. In most of the cases of the liver dysfunction, there is a higher increase in the level of ALT as compared to AST ( lower AST/ALT ratio) [27], [28]and [39]. With smaller ratios, the muscle rather the hepatic dysfunction should be considered. In this study, the ratio was about 4.2 in the control rats but it declined to about 3.4 after 28 days of the first dose of alloxan and showed a further decline on day 56 of the study to about 1.7. This progressive decline in the ratio suggests the incidence of the hepatic dysfunction and the liver has experienced a prominent loss of the integrity of its plasma membrane that led to leakage of the hepatic enzymes into the plasma. This observation can be linked

to the higher oxidative stress that was noticed on days 28 and 56. The higher dysfunction on day 56 can also be ascribed to the longer exposure period to the oxidative stress.

In the current study, after 28 days of the first dose, alloxan triggered excessive release of free radicals due to their excessive generation in  $\beta$  cells of islets of Langerhans and due to the incidence of diabetes [29], which may be sublethal yet and remained diabetogenic. Steatosis was the prominent histopathological change in the livers of alloxan treated rats [29]. The accumulation of fat droplets push the nuclei a side and displaced the cytoplasm of the liver which occurred in two forms; (microvesicular steatosis) which appear after day 28 that then progressed to the (macrovesicular steatosis) on day 56 wherein multiple small fat droplets coalesce to form one large well delineated droplet of neutral fat. The droplet was sufficient enough to distort and push the nucleus aside.

Histopathologically, the fatty steatosis that was seen as a cardinal degenerative change of alloxan injected rats is induced by the hypertriglyceridemia that the alloxan treated rats had experienced [30]. Beside steatosis, hydropic degeneration with wide accumulation of water vacuoles with hazy boundaries was seen as well. It is a reversible change that hydropic degeneration was developed due to the partial cessation of energy production as a consequence of the oxidative stress mediated mild dysfunction of mitochondria.

The study also showed a prominent hyperlipidemia in the alloxanized rats; characterized by hypertriglyceridemia and Hypercholesterolemia after the alloxan treatment. The deterioration was stronger on day 56 as compared with that on day 28. The results of the lipid profile showed that each of the triglyceride, cholesterol and LDL were increased by (1.6 and 2.55), (2.1 & 2.7) & (2.75 & 4.5) times as compared to that of the negative control on days 28 and 56 respectively. This progressive increment can be ascribed to the deterioration in the glucose homeostasis and to the persistent decline in the oxidative stress. The longer period of exposure to the oxidative stress might have played a role in the stronger deterioration in the lipid profile. The lipid profile study showed also a decrease in the serum level of HDL; the beneficial form of cholesterol. The decline was more prominent for the LDL as it was seen previously that LDL is highly affected by the oxidative stress as it transforms into the oxidized form LDL<sub>ox</sub> which has a high propensity to deposit in the blood vessels and induce atheroma.

The study revealed a hypoglycemic effect for the extract of *Allium cepa* when it was administered to the alloxanized rats. It failed to show any effect in the non alloxanized rats indicating that the extract does not have any impact on the basal secretion of insulin or the basal level of insulin sensitivity. The hypoglycemic impact was seen after the first and the second dose of the alloxan and the impact was stronger after the second. This was confirmed in lots of studies [20], [31] and [32].

The diabetogenic impact of alloxan was milder and regressive after the first dose as it induced changes in the pancreas that dwindled the insulin release. The study does not exclude a negative impact on the glucose

intolerance as the rats experienced a marked elevation in the oxidative stress which is incriminated in the induction of the glucose intolerance and [21]. The extract could have hindered the progression of the hyperglycemia during this stage although the values were still significantly higher than that of the negative control. During the second month of the study, the impact of the extract was stronger as it decreased the glucose level to be about 61% of that of the positive control. In this occasion and as per the previous studies, the rats might have experienced both type I and type II [20]. The histology study revealed an irreversible necrotic damage in the pancreas which is confirmatory for the incidence of type I and showed a marked increase in the oxidative stress which is culminated in the incidence of type II. The impact of the extract was stronger during this period and the extract showed marked regenerative changes in the pancreas. This action can be ascribed to the phytochemical content of the extract as it is rich in several types of polyphenols, such as ; quercetin, anthocyanin, flavones and different phenolic acids derivatives. Furthermore, it is endowed with a great deal of organo-sulphur compounds which are rich in the cysteine residues that supplies the body with the essential elements for glutathione synthesis [33] and [25]. Glutathione plays an important role in the pathogenesis of the alloxan induced diabetes mellitus. Alloxan depletes the  $\beta$ -cells of the Islets of Langerhans of its content of glutathione through induction of the cyclic redox mechanism. The redox cycle evolve oxidative metabolites that generate plenty of free radicals [10]. Supplementation of *Allium cepa* helps in replenishing the body with the lost glutathione and by this it helps in restoration of the endogenous glutathione-based antioxidant system. This may explain the regenerative capacity of *Allium cepa* for the pancreas of the alloxanized rats. Furthermore, the endowed polyphenols suppress the alloxan induced flow of free radicals resulting in halting the oxidative stress induced damage within the pancreas. The study also suggests a synergistic impact for the polyphenols with the organosulphur compounds in the prevention of the alloxan induced diabetes. Further investigations are recommended to confirm this notion.

Previous studies revealed that the content of the *Allium cepa* extract can interfere with hyperglycemia through different mechanism. One of the study set *Allium cepa* as a powerful inhibitor for the  $\alpha$ -glucosidase enzyme which is responsible for the digestion of carbohydrate in the intestine [34] and by this it can control the spikes of the postprandial glucose. This action was ascribed to the flavonoid content of the extract [34]. Besides, it is expected that the extract might enhance the activity of the glucose transporters in muscles and liver and by this it helps in pumping glucose toward the intracellular compartment and hindering the progression of the glucose intolerance [35]. These actions for the extract can be ascribed mainly to the polyphenol contents of the extract especially quercetin which exists abundantly in the extract and is highly incriminated in these actions [35].

Furthermore, it was found that the organosulphur compounds of the onion extract can compete with



insulin toward the insulin inactivation site in liver resulting in the elevation of the level of free insulin and by this it can halt the alloxan induced hypoinsulinemia [36]. On the other hand, another study that they play a role in regulation of the enzymes involved in the glycogen synthesis and this role was ascribed to their enhancing potential for glutathione production and storage [37].

It worth to note that the extract played a crucial role in modulating the crosstalk between the oxidative stress, inflammation and glucose intolerance. Over release of free radicals lead to activation of the transcription factors which suppress the genes responsible for the glucose tolerance and activate the genes related to the flow of the inflammatory cytokines. Cytokines like TNF- $\alpha$  or IL-6 were found as a potent initiators of the glucose intolerance [38]. This cross talk is highly affected by the onion extract as it is rich with the antioxidants that halt one of the cornerstones of this cross talk. Furthermore, the polyphenol content of the extract is also culminated in a series of non-antioxidant actions characterized by direct modulation of the flow of the intracellular signaling molecules and the transcription factors related to the production of the inflammatory cytokines or regulation of the glucose level [39].

The extract also produced a prominent antihyperlipidemic effect and the action was significant starting from the early stage of the study. This powerful impact can be ascribed to the cooperative action of the polyphenols and the organo-sulphur compounds. Onion extract is known for its content of hypolipidemic polyphenols like quercetin or dihydro-quercetin. They are famous for their unique inhibitory action against the enzymes involved in the fatty acid synthesis and the induction of fats metabolism. Furthermore, they are famous for their hypocholesterolemic effect that was attributed to their positive impact for production of apoproteins like APO-A and APO-B and activation of HMG\_CO-reductase enzyme. Furthermore, the antihyperglycemic effect might contribute in enhancing their antihyperlipidemic effect. On the other hand, the antioxidant effect of the extract can be set as another mechanism for its antihyperlipidemic effect. The antioxidants hinder oxidation of the LDL and by this they prevent formation of its oxidized form LDLox which can precipitate in the wall of the blood vessels and lead to atherosclerotic plaque formation [40].

Pancreas histopathological findings at the end of first period (28 days) after single dose of alloxan injections revealed the genesis of degenerative and hyperplastic/ and or hypertrophic lesions as a compensatory mechanism to amends the insulin insufficiency. Meanwhile and controversially, the pancreatic lesions at the end of the entire experimental period (56 days) after 2 doses of alloxan injections unveiled post mitotic atrophy and irreversible necrotic changes of the islets of Langerhans. These results are in consistency with the regressive type hyperglycemia noticed in first period and the progressive type in the second period. Furthermore, when the diabetic rats were treated continuously with aqueous extract of *Allium cepa*, the cytoarchitecture of liver and these islets appeared restored significantly. These ameliorative effects were strongly linked with the

potential activation of the estimated anti-oxidoreductive parameters in this study. Obviously, these findings asserted and confirmed the role of oxidoreductive stress in the pathogenesis of alloxan diabetogenicity [32] and [41]. Additionally, the active ingredients of onion especially the allyl and propyl disulfide compounds are chemically competing with a disulfide insulin structure for insulin suppressing areas in the liver and ultimately, may evoke free insulin production [31]. It should be mentioned also that *Allium cepa* could induce hypoglycemia by promoting remarkable glycogen storage [32] and thus prevent the glycogen depletion caused by alloxan toxicity and subsequently restores the arrangement of liver parenchymatous architecture.

The hepatoprotective of *Allium cepa* was characterized by halting the over release of the liver enzymes [AST (SGOT) and ALT (SGPT)]. Furthermore, the study also showed a significant increase in the plasma levels of the hepatic enzymes; ALT and AST. Both enzymes are important for transamination of the amino acids and creation of metabolites important for the citric acid cycle. Their presence is crucial for the progression of different metabolic reactions. Aspartate aminotransferase (AST) is found abundantly inside the mitochondria while ALT is found more in the cytosol. In most of the cases of the liver dysfunction, there is a higher increase in the level of ALT as compared to AST (lower AST/ALT ratio) [27]. With smaller ratios, the muscle rather the hepatic dysfunction should be considered. In this study, the ratio was about 4.2 in the control rats but it declined to about 3.4 after 28 days of the first dose of alloxan and showed a further decline on day 56 of the study to about 1.7. This progressive decline in the ratio suggests the incidence of the hepatic dysfunction and the liver has experienced a prominent loss of the integrity of its plasma membrane that led to leakage of the hepatic enzymes into the plasma. This observation can be linked to the higher oxidative stress that was noticed on days 28 and 56. The higher dysfunction on day 56 can also be ascribed to the longer exposure period to the oxidative stress.

The antioxidant effect of the extract might have played a role in the maintenance of the integrity of the plasma membrane of the hepatocytes [42]. This effect was seen obviously in the results of the serum levels of each of AST and the ALT enzymes. It succeeded to restore the AST/ALT ratio to 2.7. Meanwhile, it was 1.7 in the positive control group. This indicates that the efflux of ALT from the hepatocytes was decreased and the integrity of the hepatocyte's plasma membranes was improved. On the other hand, this impact was not seen for the alkaline phosphatase enzyme and the results shows a persistent increment in the serum level of the enzyme [25]. This can be ascribed to the inability of the extract to counteract the damage in the biliary hepatocytes or due to the longer half-life of the enzyme that gives a persistent elevation in its level.

## CONCLUSION

Taken together, the histopathological and biochemical results of the current study revealed that the pathogenesis of diabetes mellitus is a dose and time dependant process mediated by oxidoreductive stress. Furthermore, the

Iraqi aqueous *allium cepa* extract exerts a potent and distinctive antioxidant capacity thereby prohibiting hyperglycemia and normalizing the constituents of lipid profile together with switching liver and pancreas pathology caused by diabetes mellitus to almost normal architecture.

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**Table-1:** Weekly means of the daily fasting blood glucose levels (FBG) (mg/dl), during the experimental study period for all groups. (The results are expressed as mean  $\pm$  SEM)

Groups	1 <sup>st</sup> week N=12	2 <sup>nd</sup> week N=12	3 <sup>rd</sup> week N=12	4 <sup>th</sup> week N=12	5 <sup>th</sup> week N=6	6 <sup>th</sup> week N=6	7 <sup>th</sup> week N=6	8 <sup>th</sup> week N=6
CN-Gr	112.55 <sup>A</sup>	110.20 <sup>A</sup>	111.23 <sup>A</sup>	107.37 <sup>A</sup>	111.04 <sup>A</sup>	113.12 <sup>A</sup>	109.82 <sup>A</sup>	110.42 <sup>A</sup>
AACE-Gr	110.55 <sup>A</sup>	109.22 <sup>A</sup>	109.82 <sup>A</sup>	107.35 <sup>A</sup>	109.53 <sup>A</sup>	110.95 <sup>A</sup>	110.55 <sup>A</sup>	111.65 <sup>A</sup>
ALX-Gr	170.65 <sup>B</sup>	162.34 <sup>B</sup>	143.10 <sup>B</sup>	134.42 <sup>B</sup>	171.13 <sup>B</sup>	174.51 <sup>B</sup>	191.10 <sup>B</sup>	218.22 <sup>B</sup>
ALX+ AACE-Gr	168.84 <sup>B</sup>	159.28 <sup>B</sup>	141.82 <sup>B</sup>	124.65 <sup>C</sup>	170.25 <sup>B</sup>	163.53 <sup>C</sup>	147.32 <sup>C</sup>	134.31 <sup>C</sup>

(CN-Gr), (AACE-Gr), (ALX-Gr) and (ALX+AACE-Gr) represent the negative control, AACE (Aqueous *Allium cepa* Extract) treated, Alloxan treated and Alloxan+AACE treated groups respectively.

Each value that is not sharing a common superscript letter signifies a statistically significant difference with ( $P < 0.05$ ).

The sample size is 12 for the subgroups A (the rats that stayed for 28 days) and 6 rats for the subgroups (that continued for 56 days).

**Table-2:** levels of hepatic enzymes (Alkaline Phosphatase (ALP), Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) in serum of rats of all groups at two periods of the study. (The results are expressed as mean  $\pm$  SEM).

Groups	ALP (IU/L)		AST (IU/L)		ALT (IU/L)	
	28 Days N=6	56 Days N=6	28 Days N=6	56 Days N=6	28 Days N=6	56 Days N=6
CN-Gr	117.55 $\pm$ 4.73 <sup>A</sup>	116.24 $\pm$ 4.33 <sup>A</sup>	121.15 $\pm$ 4.16 <sup>A</sup>	123.35 $\pm$ 5.10 <sup>A</sup>	26.33 $\pm$ 1.70 <sup>A</sup>	28.66 $\pm$ 1.94 <sup>A</sup>
AACE-Gr	115.71 $\pm$ 3.55 <sup>A</sup>	115.42 $\pm$ 2.99 <sup>A</sup>	119.22 $\pm$ 3.50 <sup>A</sup>	122.55 $\pm$ 2.90 <sup>A</sup>	25.90 $\pm$ 1.05 <sup>A</sup>	26.33 $\pm$ 1.10 <sup>A</sup>
ALX-Gr	191.27 $\pm$ 5.85 <sup>B</sup>	318.15 $\pm$ 8.88 <sup>B</sup>	146.13 $\pm$ 2.75 <sup>B</sup>	193.43 $\pm$ 5.22 <sup>B</sup>	47.88 $\pm$ 1.33 <sup>B</sup>	112.24 $\pm$ 3.87 <sup>B</sup>
ALX+AACE-Gr	189.71 $\pm$ 4.32 <sup>B</sup>	316.25 $\pm$ 5.90 <sup>B</sup>	130.23 $\pm$ 3.11 <sup>C</sup>	144.31 $\pm$ 3.45 <sup>C</sup>	37.65 $\pm$ 2.11 <sup>C</sup>	52.23 $\pm$ 2.33 <sup>C</sup>

(CN-Gr), (AACE-Gr), (ALX-Gr) and (ALX+AACE-Gr) represent the negative control, AACE (Aqueous *Allium cepa* Extract) treated, Alloxan treated and Alloxan +AACE treated groups respectively.

Each value that is not sharing a common superscript letter signifies a statistically significant difference with ( $P < 0.05$ ).

The sample size is 6 rats for each group.

**Table 3:-** Biochemical Serum levels of Malondialdehyde (MDA), Total Antioxidative Stress Capacity (TAC), Total Oxidant capacity (TOC) and Oxidative Stress Index (OSI) for all groups during the experimental period. (The results are expressed as mean  $\pm$  SEM)

	At 28 day (n=6)				At 56 day (n=6)			
	MDA nmol/ml.	TOC ( $\mu$ mol H <sub>2</sub> O <sub>2</sub> Eq/L)	TAC (TroloxEq $\mu$ mol/L)	OSI (TOC\TAC)	MDA nmol/ml.	TOC ( $\mu$ mol H <sub>2</sub> O <sub>2</sub> Eq/L)	TAC (TroloxEq $\mu$ mol/L)	OSI (TOC\TAC)
CN-Gr	16.54 $\pm$ 1.11 <sup>A</sup>	12.2 $\pm$ 3.42 <sup>A</sup>	1.51 $\pm$ 0.26 <sup>A</sup>	0.78 $\pm$ 0.80 <sup>A</sup>	16.88 $\pm$ 1.30 <sup>A</sup>	12.8 $\pm$ 3.67 <sup>A</sup>	1.43 $\pm$ 0.21 <sup>A</sup>	0.84 $\pm$ 0.75 <sup>A</sup>
AACE-Gr	15.96 $\pm$ 1.44 <sup>A</sup>	11.9 $\pm$ 2.68 <sup>A</sup>	1.90 $\pm$ 0.45 <sup>B</sup>	0.62 $\pm$ 0.67 <sup>B</sup>	16.50 $\pm$ 1.05 <sup>A</sup>	13.5 $\pm$ 2.94 <sup>A</sup>	1.81 $\pm$ 0.52 <sup>B</sup>	0.70 $\pm$ 0.66 <sup>B</sup>
ALX-Gr	24.35 $\pm$ 2.35 <sup>B</sup>	18.4 $\pm$ 3.54 <sup>B</sup>	0.99 $\pm$ 0.54 <sup>C</sup>	1.70 $\pm$ 0.65 <sup>C</sup>	34.39 $\pm$ 2.13 <sup>B</sup>	21.4 $\pm$ 3.60 <sup>B</sup>	0.83 $\pm$ 0.22 <sup>C</sup>	2.51 $\pm$ 0.95 <sup>C</sup>
ALX+AACE-Gr	19.54 $\pm$ 1.58 <sup>C</sup>	12.3 $\pm$ 4.25 <sup>A</sup>	1.42 $\pm$ 0.96 <sup>A</sup>	0.91 $\pm$ 0.77 <sup>D</sup>	21.27 $\pm$ 1.52 <sup>C</sup>	16.3 $\pm$ 3.05 <sup>C</sup>	1.41 $\pm$ 0.20 <sup>A</sup>	1.14 $\pm$ 0.84 <sup>D</sup>

(CN-Gr), (AACE-Gr), (ALX-Gr) and (ALX+AACE-Gr) represent the negative control, AACE (Aqueous *Allium cepa* Extract) treated, Alloxan treated and alloxan +AACE treated groups respectively.

Each value that is not sharing a common superscript letter signifies a statistically significant difference with ( $P < 0.05$ ).

The sample size is 6 rats for each group

**Table 4:** Serum levels of Glutathione peroxidase (GSH-Px), Super oxide dismutase (SOD) and Catalase (CAT) ) together with the RBCs hemolysate reduced glutathione (r-GSH) concentrations on days 28 and 56 of the experimental period. (The results are expressed as mean  $\pm$  SEM).

	At 28 day				At 56 day			
	r-GSH ( $\mu$ mol/ml)	GSH-Px (U/ml)	SOD (U/ml)	CAT (U/ml)	r-GSH ( $\mu$ mol/ml)	GSH-Px (U/ml)	SOD (U/ml)	CAT (U/ml)
CN-Gr	21.07 $\pm$ 1.10 <sup>A</sup>	114.4 $\pm$ 5.6 <sup>A</sup>	85.53 $\pm$ 2.5 A	3.09 $\pm$ 0.13 A	22.05 $\pm$ 1.15 <sup>A</sup>	115.5 $\pm$ 5.1 A	87.83 $\pm$ 2.66 <sup>A</sup>	2.99 $\pm$ 0.16 <sup>A</sup>
AACE-Gr	21.20 $\pm$ 1.13 <sup>A</sup>	115.7 $\pm$ 6.2 <sup>A</sup>	86.22 $\pm$ 2.8	3.10 $\pm$ 0.16	22.25 $\pm$ 1.18 <sup>A</sup>	117.4 $\pm$ 6.5	88.13 $\pm$ 2.13 <sup>A</sup>	3.01 $\pm$ 0.11 <sup>A</sup>

			A	A		A		
ALX-Gr	19.98±1.08 <sup>A</sup>	110.9±4.4 <sup>B</sup>	67.83±1.5 <sup>B</sup>	2.22±0.18 <sup>B</sup>	11.23±1.08 <sup>B</sup>	74.9±5.4 <sup>B</sup>	44.33± 2.33 <sup>B</sup>	1.01± 0.18 <sup>B</sup>
ALX+AACE-Gr	20.25±1.75 <sup>A</sup>	114.8±3.3 <sup>A</sup>	78.66±2.4 <sup>C</sup>	2.19±0.13 <sup>B</sup>	15.74±1.01 <sup>C</sup>	106.9±3.3 <sup>C</sup>	69.45± 2.20 <sup>C</sup>	2.01 ± 0.18 <sup>C</sup>

(CN-Gr), (AACE-Gr), (ALX-Gr) and (ALX+AACE-Gr) represent the negative control, AACE (Aqueous *Allium cepa* Extract) treated, Alloxan treated and alloxan +AACE treated groups respectively. r-GSH, GSH-Px, SOD and CAT refer to the blood hemolysate reduced glutathione level, serum level of the glutathione peroxidase, superoxide dismutase and catalase respectively. Each value that is not sharing a common superscript letter signifies a statistically significant difference with ( $P<0.05$ ). The sample size is 6 for each group

**Table 5:** Measurements of the lipid profile (Triglyceride (T.G), Total cholesterol, Low density lipoprotein (LDL) and High-Density Lipoprotein (HDL) on days 28 and 56 of the experiment periods. (The results are expressed as mean ± SEM)

	At 28 day				At 56 day			
	Triglyceride (TG) mg/dl	Total cholesterol mg/dl	LDL-cholesterol mg/dl	HDL-cholesterol mg/dl	Triglyceride (TG) mg/dl	Total cholesterol mg/dl	LDL-cholesterol mg/dl	HDL-cholesterol mg/dl
CN-Gr	81.8±1.90 <sup>A</sup>	70.21±2.10 <sup>A</sup>	20.57±0.88 <sup>A</sup>	41.18±1.18 <sup>A</sup>	79.8±1.23 <sup>A</sup>	73.55±2.61 <sup>A</sup>	22.37±0.85 <sup>A</sup>	42.15±1.13 <sup>A</sup>
AACE-Gr	80.3±1.42 <sup>A</sup>	69.57±1.91 <sup>A</sup>	19.59±0.67 <sup>A</sup>	48.16±1.31 <sup>B</sup>	78.3±1.11 <sup>A</sup>	72.11±2.43 <sup>A</sup>	21.45±1.01 <sup>A</sup>	56.72±2.02 <sup>B</sup>
ALX-Gr	132.7±4.35 <sup>B</sup>	150.36±3.41 <sup>B</sup>	54.59±1.77 <sup>B</sup>	29.21±1.15 <sup>C</sup>	199.4±3.24 <sup>B</sup>	201.10±5.71 <sup>B</sup>	89.18±1.77 <sup>B</sup>	25.55±1.13 <sup>C</sup>
ALX+AACE-Gr	88.8±1.66 <sup>C</sup>	103.51±2.86 <sup>C</sup>	32.71±1.03 <sup>C</sup>	36.22±1.17 <sup>D</sup>	98.9±2.17 <sup>C</sup>	145.33±3.88 <sup>C</sup>	40.71±1.63 <sup>C</sup>	33.12±1.14 <sup>D</sup>

(CN-Gr), (AACE-Gr), (ALX-Gr) and (ALX+AACE-Gr) represent the negative control, AACE (Aqueous *Allium cepa* Extract) treated, Alloxan treated and alloxan+AACE treated groups respectively. Each value that is not sharing a common superscript letter signifies a statistically significant difference with ( $P<0.05$ ). The sample size is 6 rats for each group