

The Effects of *Ambrosia maritima*, L. (Damsissa) on Some Biochemical and Histological Parameters of Diabetic Albino Rats

Eman G. E. Helal^{1*}, Nouran Abou-Aouf¹, Sayda M. Khattab¹, Abd EL Razek A. Meselhy², Tamer M. M. Abu-Amara^{3*}

¹Department of Zoology, Faculty of Science (Girls), Al-Azhar University, Cairo, Egypt.

²Anatomy and Embryology Department, Faculty of Medicine, Al-Azhar University, Assiut, Egypt.

³Histology and Cytology Department, Faculty of Medicine, Al-Azhar University, Cairo, Egypt.

*Corresponding authors: Eman G.E. Helal - Tamer M. M. Abu-Amara,

E-mail: emanhelal@hotmail.com - tamer4567@yahoo.com

Abstract

Diabetes mellitus is one of the common and widely distributed metabolic diseases all over the world. This disease is characterized by hyperglycemia that results from defects in insulin secretion, insulin action or both. Different medicinal plant species are used as a traditional treatment for diabetes mellitus e.g. *Ambrosia maritima*, L. (Damsissa) which is one of these plants that its extract was used to treat diabetic patients long times ago.

Aim of the work: This work was aimed to investigate the antidiabetic, hypolipidemic and antioxidant effects of the aqueous extract of *Ambrosia maritima*, L. (Damsissa) on the alloxan-induced diabetic male albino rats.

Material and Methods: This study was performed on thirty male albino rats with an average 100-110 g body weight. The animals were divided into three groups (10 /cage); **Group I** (Control untreated-group), **Group II** (Alloxan-induced diabetic group) and **Group III** (diabetic group treated orally with "28.5 mg/ kg body wt. twice/ day" of the plant extract).

Results: The biochemical results showed marked decline ($p<0.01$) in the levels of the serum insulin, body weight, total proteins, albumin, globulin and HDL accompanied with marked elevation ($p<0.001$) in the levels of fasting blood glucose, levels of HOMA_IR, AST, ALT, GGT, urea, creatinine, uric acid, serum TC, TG, LDL, VLDL and ratios of TC/HDL and LDL/HDL (risk factors) in diabetic rats in comparison with the control group. Daily management of the diabetic rates with aqueous extract of Damsissa showed significant improvement in most of these parameters. Histologically, considerable improvement in the morphological changes that was observed in diabetic groups had been detected after treatment with Damsissa in liver, kidney and pancreatic tissues in comparison to the control group.

Conclusion: It could be concluded that *Ambrosia maritima*, L. (Damsissa) can be used as an antidiabetic drug that can lower blood glucose concentration and guard against the negative effects of diabetes.

Keywords: *Diabetes mellitus*, *Alloxan*, *Hyperglycemia*, *Damsissa*, *Ambrosia maritima*.

INTRODUCTION

Diabetes mellitus is a metabolic disorder that is characterized by hyperglycemia associated with impairment in insulin secretion and/or insulin action as well as aberrations in intermediary metabolism of carbohydrates, proteins and lipids⁽¹⁾. Diabetes mellitus increased risk of developing cardiovascular, nephropathy, retinopathy, reproductive dysfunction, peripheral vascular and cerebrovascular disease^(2, 3). A wide variety of medicinal plants are used in the treatment of diabetes. From these a large number of Egyptian herbs are known to be used in folk medicine⁽⁴⁾. One of these plants is the herb of

Ambrosia maritima, L. (Damsissa) family compositae (Asteraceae), widely grown in Egypt, especially in Sinai⁽⁵⁾. It is a common folk medicine used in the treatment of rheumatic pains, asthma, bilharziasis, diabetes, stomachic, and renal troubles⁽⁶⁾. Alard *et al.*⁽⁷⁾ revealed that no toxic signs were detected after oral administration of dried leaves of such plant. Phytochemical analyses on *Ambrosia maritima* extract have identified the presence of some pseudoguaianolide sesquiterpenes such as; neoambrosin, chloroambrosin, damsinic acid, hymenin⁽⁸⁾. Two new sesquiterpene lactones, characterized as 1'-noraltarnisin and 13

dihydrosilostachyin, were isolated from *Ambrosia maritima*⁽⁹⁾. Triterpenes like (s-amyrin) that is extracted from the damsissa' leaves show molluscicidal activity against the intermediate hosts of shistosoma spp⁽⁹⁾. Some researchers reported the presence of some coumarins like scopoletin, and isoscapoletin in the *Ambrosia maritima* extract⁽¹⁰⁾. In addition, many compounds such as; tannin, alkaloid, saponins, resins and hispidulin were isolated from the *Ambrosia maritima* extract^(11,12). Also, flavonoids such as "apigenin, and β -sitosterol" which are derived from the damsissa exhibit antitumor activity⁽¹³⁾. Essential (volatile) oils like camphor and cineole were extracted from damsissa⁽¹⁴⁾. This study aims planned to evaluate the antioxidant and hypoglycemic effects of aqueous extract of Damsissa (*Ambrosia maritima*) in the alloxan-induced diabetic rats.

Material and Methods

Plant material

The aerial parts of *Ambrosia maritima* was collected from El-Arbaeen valley, Saint Catherine, Wadi Gebal, South Sinai, Egypt. The plant was grinded and the aqueous extract of Damsissa was prepared by boiling 2 g of Damsissa with 200ml of tap water for 15 min, left to cool at room temperature then filtered through filter paper. Later, the extract was stored in a glass container in refrigerator. Fresh extract preparation was done every two days.

Animals

Thirty male adult albino rats (8-10 weeks/ 100-110 g) were used in this experiment. The rats were kept under observation for about 2 weeks before the start of the experiment for adaptation. Diabetes mellitus was induced in animals by single dose of alloxan (120 mg/kg B.W. dissolved in saline) was injected intraperitoneally to induce diabetes mellitus in rats⁽¹⁵⁾. The rats were deprived of food for 16 hours before alloxan injection. After three days of alloxan injection, the rats were deprived of food overnight and they were then given glucose (3 g/kg B.W.) by gastric intubation. After 2 hours of oral glucose administration, blood samples were taken from tail vein and the fasting blood glucose (FBG) concentration was determined by means of one touch ultra-glucometer

(Johnson & Johnson Company, USA) and compatible blood glucose strips. After 2 h of oral glucose administration, the rats' glucose concentrations "ranging from 180 to 300 mg/dl" were considered as mild diabetic animals and included in the experiment.

Experimental design:

Experimental animals were divided into three groups, ten each, as follows:

- **Group I (Control group):** Non-diabetic rats.
- **Group II (Diabetic group):** Rats were injected *intraperitoneally* with a single dose of alloxan (120 mg/kg dissolved in saline solution).
- **Group III (Treated group):** Diabetic rats treated orally with *Ambrosia maritima* extract (28.5 mg/kg twice /day) for 30 days.

Blood sample collection: At the end of the experimental period, the overnight fasted animals (12- 16h) were sacrificed under diethyl ether anesthesia. Blood samples were taken from orbital vein and centrifuged at 3000 rpm for 10 min. The clear non-haemolysed supernatant sera were quickly removed and immediately stored at -20°C till been used for further analysis of biochemical parameters.

Biochemical analyses: Serum glucose was estimated using a commercially available kit according to the method of **Trinder**⁽¹⁶⁾. Serum insulin level was measured by coat-A-count radioimmunoassay kits according to **Reeves**⁽¹⁷⁾. While values of HOMA-IR were calculated using the following equation: **HOMA-IR = fasting serum glucose (mg/dl) x fasting serum insulin (μ U/ml)/450**⁽¹⁸⁾. Glucose in mass units mg/dL. And IR is insulin resistance. Creatinine concentrations was determined colorimetrically as described by **Junge**⁽¹⁹⁾. Urea concentrations were determined colorimetrically as described by **Patton & Crouch**⁽²⁰⁾. Serum uric acid was determined using the uricase-PAP enzymatic colorimetric method⁽²¹⁾. Aspartate amino transferase (AST) and alanine amino transferase (ALT) were assayed according to the method of **Schumann**⁽²²⁾. Gammaglutamyltransferase (γ GT) assay was performed according to **Kytzia**⁽²³⁾. Albumin and total protein concentrations were determined colorimetrically⁽²¹⁾. Serum globulin was calculated by subtracting

albumin from total protein⁽²¹⁾. Enzymatic determination of serum cholesterol was done as described by **Tietz**⁽²⁴⁾. Triglycerides content was determined by the method of **Bucolo and David**⁽²⁵⁾. Total lipids (TL) were analysed by the method of **Knight et al.**⁽²⁶⁾. HDL-cholesterol content was determined by applying the method of **Sugiuchi**⁽²⁷⁾. LDL-C was calculated using the **Friedwald's** formula when the values of TG were less than 400 mg/dl⁽²⁸⁾. VLDL was calculated using the **Friedewald's equation**⁽²⁸⁾.

Friedewald's equation: LDL (mg/dl) = TC - {HDL + [TG/5]}.

VLDL = TG/5

Risk 1 = TC / HDL

Risk 2 = LDL / HDL

Histological and histochemical study:

Therats from the control and treated groups were sacrificed after one month and small pieces of the liver, kidney and pancreas were taken for the histological and histochemical studies. The specimens were prepared *via* fixation in 10% neutral buffered formalin solution and **Carnoy's** fluid. Paraffin sections of 5µm thickness were prepared and stained with Harris's haematoxylin and eosin (H&E)⁽²⁹⁾. Polysaccharides were detected using PAS (Periodic acid-Schiff) method⁽²⁹⁾. Later, the stained sections were examined *via* light microscope, photographed and all the detected variations between the three groups on the level of the microscopic findings had been scientifically discussed.

Statistical analysis

The results were expressed as Mean±SEM of the mean. The data were analyzed by one way analysis of variance (ANOVA) and were performed using the **Statistical Package (SPSS) program, version 20**. The **Kolmogorov-Smirnov test (KS-test)** was used to determine if two datasets differ significantly followed by **Bonferroni** test as multiple comparison method to compare significance between groups. Difference was considered significant when $p < 0.05$.

RESULTS

A. Biochemical Results: The serum insulin and glucose levels in different study groups showed marked decline in the level of serum

insulin accompanied with marked elevation in the level of fasting blood glucose ($p < 0.001$) and the HOMA-IR value ($p < 0.01$) as compared to the controls (**Fig. 1 & Table 1**). A significant decrease ($p < 0.01$) in the levels of serum insulin accompanied with marked elevation ($p < 0.001$) in levels of blood glucose were recorded in diabetic rats (Group II) when compared to the control rats (Group I). Treatment with *Ambrosia maritime* extract showed significant recovery in insulin and glucose levels in comparison with diabetic animals. HOMA-IR values were significantly high ($p < 0.01$) in diabetic rats when compared to the corresponding controls, while treatment of diabetic rats with *Ambrosia maritime* extract returned HOMA-IR values to the normal level (**Table 1**). The percent change in the body weight in diabetic rats was significantly decreased (**Table 2**). Also, it has been noticed that the percent change of body weight returned to normal weight after treating the diabetic rats with the plant extract (**Table 2**). Diabetic rats showed a significant increase ($p < 0.001$) in serum ALT, AST and γ GT activities in diabetic group as compared with the control group (**Table 3**). While *Ambrosia maritime* extract treatment of the diabetic rats significantly decreased these activities when compared with the diabetic group ($p < 0.001$) and these activities were returned back to the normal values after treating the diabetic rats with the plant extract (**Table 3**). On the other hand, biochemical parameters of urea, uric acid and creatinine which are parameters of renal function showed significant increase ($p < 0.001$) in the diabetic group in comparison with the control group (**Fig. 2 & Table 4**). Treatment of the diabetic rats with *Ambrosia maritima* extract produced significant decrease in the serum urea, creatinine and uric acid in comparison with diabetic group ($p < 0.001$) (**Fig. 4 & Table 4**). *Ambrosia maritime* extract treated-group recorded a high significantly inhibition in the total lipids, triglyceride, total cholesterol, LDL, VLDL levels, VLDL, TC/HDL and LDL/HDL values while the HDL value showed a significant increase in comparison with the diabetic group (**Table 5**). These levels in the diabetic rats were dramatically increased in comparison with the control group (**Table 5**). In addition, diabetics' rats

showed marked decline in serum total proteins, albumin and globulin in relative to the corresponding controls ($p < 0.01$) (Table 6). Treatment of the diabetic rats with *Ambrosia maritime* extract resulted in modulation of the measured serum protein profile parameters. The values of A/G ratio showed non-significant changes in the control and the experimental groups (Table 6).

B. Histological and Histochemical Results:

Examination of H&E stained sections of liver of the control group showed normal lobular pattern with a centrilobular vein and radiating irregular branching and anastomosing plates of hepatocytes with intervening sinusoids lined with endothelial cells. Most of the hepatocytes have vesicular nuclei and some of them appear binucleated (Fig. 3A). Liver of the diabetic rat showed hepatocytes necrotic changes, ballooning degeneration, pyknotic nuclei and fatty degeneration around the congested central vein (Fig. 3B). H&E stained sections of liver of the *Ambrosia maritime* extract treated rats showed that most of hepatic lobules are almost similar to that of the control group (Fig. 3C). In control group, normal portal triad had been observed (Fig. 4A). The diabetic rats' portal triads showed marked congestion of portal vein and lymphocyte infiltration (Fig. 4B). H&E stained sections of liver of the *Ambrosia maritime* extract treated rats showed that the portal triads are almost similar to that of the control group (Fig. 4C). In control group, PAS +ve granules were mainly distributed in most of the hepatocytes (Fig. 5A). The diabetic rats showed marked diminution in glycogen content in hepatocytes (Fig. 5B). The group of rats treated with *Ambrosia maritime* extract showed that the glycogen content is more or less similar to the control level (Fig. 5C). Examination of H&E stained sections of pancreas of the control group, revealed normal appearance of the islets of Langerhans (Fig. 6A). Beta cells were the most abundant cells while alpha cells were observed at the periphery of the islets (Fig. 6A). Examination of H&E stained sections of the diabetic group showed marked reduction in islets size and cellularity (Fig. 6B). Some islet cells showed marked degenerative changes with pyknotic nuclei. Furthermore,

marked vascular degenerative changes had been detected at the region of islet suggesting arteritis (Fig. 6B). Examination of H&E stained sections of the diabetic group which was treated with *Ambrosia maritime* extract showed partial return to the normal cellular distribution in the islet of Langerhans and increased cellularity with ill differentiation of the different cell types (Fig. 6C). The normal histological structure of the kidney was observed in Fig. 7A. The kidney of the diabetic rats showed vacuolar degeneration in some tubular epithelial cells and cell debris scattered in the tubule's lumina (Fig. 7B). Increase in thickness of tubules' epithelial cells with narrowing of lumina, signs of degeneration in the form of karyolysis and karyorrhexis (Fig. 7B). The kidney of the diabetic rats treated with *Ambrosia maritime* extract showed some protective effects as compared to the control diabetic group as kidney sections showed mild glomerular degeneration, thickening of Bowman's capsule, cell debris in some tubular lumina and mild cellular infiltration in the interstitial tissue (Fig. 7C). In control group, PAS +ve materials were mainly distributed at the brush border and basement membrane of the renal tubules (Fig. 8A). The diabetic rats showed marked diminution in mucopolysaccharide content in some tubules, while others showed diffuse stain ability (Fig. 8B). The group of rats treated with *Ambrosia maritime* extract showed that the mucopolysaccharide is more or less similar to the control (Fig. 8C).

Discussion

Diabetes mellitus is a serious endocrine disorder that causes millions of deaths worldwide, it is caused by relative or absolute deficiency of insulin⁽³⁰⁾. Medicinal plants provide a natural source of antioxidants that have been used worldwide for treatment of many diseases and are traditionally used for diabetes treatment due to their antidiabetic property^(31, 32). In the present study we assessed the antioxidant and hypoglycemic activities of aqueous extract of *Ambrosia maritime* which is used in traditional folk medicine in Egypt for the treatment of diabetes. In our study, we used alloxan to induce an experimental hyperglycemic state in albino rats. Our

results showed significant decrease in the serum insulin levels with marked significant elevation in the blood glucose levels of the diabetic rats when compared to the control rats. These results agree with the theoretical and practical consideration which postulated that the diabetogenic dose of alloxan could affect β -cells and subsequently insulin secretion that led to elevated blood glucose and impaired glucose tolerance⁽³³⁾. The traditional medicinal plants can provide a useful source as oral hypoglycemic drugs. This is because these plants contain hypoglycemic compounds with antidiabetic, antihyperlipidemic and antioxidant effects such as flavonoids, saponins, alkaloids, and tannins⁽³⁴⁾. Furthermore, the hypoglycemic effect of these herbs maybe due to the increased level of serum insulin and enhancement of glucose peripheral metabolism^(35,36). While treated diabetic rats with *Ambrosia maritime* showed increasing in insuline levels accompanied with decrease in blood glucose levels. Furthermore, these results indicated that the *Ambrosia maritime* possesses a significant hypoglycemic and anti-hypoinsulinemic effects, due to the presence of the sesquiterpene lactones (damsin, ambrosin, hymenin) as well as the presence of some coumarins (scopoletin, umbelliferone, and methoxylated flavones), which are considered as an effective hypoglycemic agents. This findings are in agreement with **Kiliani *et al.***⁽³⁷⁾ who reported that the sesquiterpene lactone isolated from the ethanolic extract of *Ambrosia maritime* is an effective hypoglycemic agent on blood glucose. Induction of diabetes by alloxan caused reduction in increase in body weight in diabetic rats as compared to the weight gain found in the control rats. This is in line with the observation of **Oduye and Adadevo**⁽³⁸⁾, that is associated with excessive tissue catabolism. Treatment of rats with *Ambrosia maritime* extract compensated the reduction of body weight, and caused a significant increase in the body weight of the treated rats. During diabetes mellitus, the increase in blood sugar is accompanied with lacking of sugar in the cells⁽³⁹⁾. This forcing, the cells to use amino acids and fatty acids as a source of energy that leads to the reduction of proteins and fats in the body and subsequent body weight loss⁽³⁹⁾. Moreover,

the present data showed significant increase in values of HOMA_IR in diabetic rats when compared to the corresponding controls. These results are in agree with previous studies which confirmed that high glucose concentrations induce insulin resistance in peripheral tissues, impairment of both insulin secretion and insulin sensitivity⁽⁴⁰⁾. After *Ambrosia maritime* administration, the level of HOMA_IR decreased in treated rats and returned to the normal values. HOMA-IR has proved to be a tool for the surrogate assessment of insulin resistance^(41,42). *Ambrosia maritime* extract has variety of hypoglycemic compounds such as saponins, alkaloids and tannins which have an potential action for lowering blood glucose and enhancing the release of insulin⁽⁴³⁾. These compounds such as a Alkaloids that can inhibit α -glucosidase and decrease glucose transport through the intestinal epithelium⁽⁴⁴⁾. This is consistent with the findings of **Eskander and Won-Jun**⁽³⁶⁾. Also, saponins have hypoglycemic activity that may due to the inhibition of liver gluconeogenesis or glycogenolysis⁽⁴⁵⁾. Furthermore, the hypoglycemic effect of *Ambrosia maritime* extract may be exerted through the inhibition of glucose absorption, increase sensitivity of receptors to insulin, insulinase inhibiting effect, stimulation of B cells to secret insulin, stimulation of peripheral tissues uptake of glucose and increases in the level of serum insulin^(35,36). Moreover, Betatrophin hormone, primarily produced in the liver and adipose tissue, has recently been described as a key hormone to stimulate beta-cell mass expansion in response to insulin resistance and obesity in mice⁽⁴⁶⁾. Essential oils components of *Ambrosia maritime* have antioxidant, antibacterial, antiviral, antiparasitic, anti-inflammation and anti-hypoinsulinemic⁽⁴⁷⁾. Therefore, essential oils have protective effect against hepatocyte damage and have a modulatory effect on values of HOMA_IR. This may be attributed to enhanced peripheral uptake of glucose, hypoglycemic effect and increase serum insulin level. So it can lead to produce more betatrophin enhancing insulin production by beta cells of pancreas and enhancing body weight⁽⁴⁷⁾. Hyperglycemia induces oxidative stress and leads to more complicated damages to B- cells and other tissues⁽⁴⁸⁾.

Abnormally high levels of free radicals and the simultaneous decline of antioxidant defence mechanisms may lead to the damage of cellular organelles and enzymes, increased lipid peroxidation and development of insulin resistance⁽⁴⁹⁾. Thus, antioxidant therapy is one of the major strategies for diabetes treatment⁽⁵⁰⁾. Many herbal extracts or derivative with high antioxidant activity are useful for diabetes treatment and other metabolic syndrome⁽⁵⁰⁾.

Serum creatinine, urea and uric acid are often regarded as reliable markers of renal function⁽⁵¹⁾. The present results showed elevations in the levels of serum creatinine, urea and uric acid in the diabetic rats as compared to the normal control group. Elevations in the concentrations of these markers in diabetic rats are indication of renal injury that is resulted due to diabetes and may lead to renal dysfunction⁽⁵²⁾. After administration of the *Ambrosia maritime* extract to the diabetic rats, it reversed these parameters towards normal values. According to these results, *Ambrosia maritime* provide a significant ameliorative effect in renal function and protect against the oxidative renal damage through its capacity to quickly and efficiently scavenge lipid peroxy radicals⁽⁵³⁾. This may be attributed to the presence of some phytochemicals compounds which had antioxidant properties such as saponins, alkaloids, tannins and flavonoids in aqueous extract of Damsissa⁽⁵⁴⁾. Our study showed a significant increase in ASAT, ALAT and GGT enzymes as compared with the control group. Therefore, the elevation in the activities of these enzymes in diabetic rats may due to leakage of these enzymes from the liver into the blood stream as a result of alloxan toxicity that leads to the liver damage⁽⁵⁵⁾. These enzymes serve as markers of the hepatocytes cell injury⁽⁵⁵⁾. These enzymes are located in the cell cytoplasm and emptied into the circulation once the cellular membrane is damaged⁽⁵⁶⁾. On the other hand, daily treatment of diabetic rats with *Ambrosia maritime* significantly abolished the disturbances occurred in the activities of these enzymes. The action of *Ambrosia maritime* may be due to the presence of flavonoids which are the active constituents of the *Ambrosia maritime*

extract. Flavonoids have hypoglycaemic, hypolipidaemic and potent antioxidant actions that attenuate the oxidative stress induced by free radicals^(12,13). Thus, *Ambrosia maritime* can ameliorate the functions of the liver and reduce body weight by inhibition the pro-inflammatory mediators and protection of hepatocytes^(12,13). This results are agreement with **Ahmed and Khater**⁽⁵⁴⁾ results that indicated that *Ambrosia maritime* acts as a hepato-protective and antioxidant agent against the biochemical alterations induced by alloxan effect *via* inhibiting the liver damage, improving the liver function and regeneration of the hepatocytes. In addition diabetic animals showed significant marked decline in the serum total proteins, albumin, globulin and A/G ratio in relative to the corresponding controls. This decrease may be ascribed to hypoinsulinemia induced by alloxan and subsequent increase in the protein catabolism rate that affect albumin and globulin synthesis and secretion^(57,58). While daily administration of *Ambrosia maritime* extract to the diabetic rats maintained the serum protein profile parameters near the normal values. This may be attributed to the presence of flavonoids in *Ambrosia maritime* that suppress the glucose level and increase hepatic glucokinase activity probably by enhancing the insulin release from pancreatic islets^(12,13). Also, saponins stimulate the release of insulin and block the formation of glucose in the blood stream⁽⁵⁹⁾. Our results reveal high prevalence of hypercholesterolemia, hypertriglyceridemia in the diabetic rats with significant elevation in the TL, TC, TG, LDL, VLDL and ratios of TC/HDL (risk-ratio 1) and LDL/HDL (risk-ratio 2) and showed marked decline in HDL when compared to the control group. These results indicate marked hyperlipidemia that characterizes the diabetes and is known as a risk factor for the cardiovascular diseases and atherosclerosis that affect diabetic patients⁽⁶⁰⁾. Diabetes is characterized by hypoinsulinemia and hyperglycemia that may be a result of the uninhibited actions of lipolytic hormones on the fat cells. Also, alloxan cause an increase of the sera lipid profiles that may be due to insulin deficiency⁽⁶⁰⁾. Our results indicates that *Ambrosia maritime* has a potential role in preventing formation of

atherosclerosis and coronary heart disease in diabetic rats. These results may be due to the presence of saponins in *Ambrosia maritime* extract which has a direct saponin antioxidant activity and can potentially decrease Atherosclerosis⁽⁶¹⁾. Also, flavonoids can reduce the cholesterol and triglycerides levels significantly through its antioxidant activity *via* free radical scavenging assay⁽⁶¹⁾.

In conclusion, there is enough evidence to support that the aqueous extract of *Ambrosia maritime* exerted antidiabetic and antihyperlipaemic activities with strong antioxidant activity. So it has protective effects against diabetic damages probably through free radical scavenging activity. More studies must be done to illustrate any toxicological effect of *Ambrosia maritime*.

REFERENCES

- 1- Joseph B, Jini D (2012): An insight in hypoglycemic effect of traditional indian herbs used in the treatment of diabetes. Research Journal of Medicinal plant. 5:352-376.
- 2- Smolek MK, Notaroberto NF, Jaramillo AG, Pradillo LR et al. (2013): Intervention with vitamins. Clinical Ophthalmology. 7:1451- 1458.
- 3- Lidén MK (2013): Prevention and protection in diabetic nephropathy. Lakartidningen. 110(21):1025-1027.
- 4- Abdel Wahab SM, Wassel GM, Ammar NM, Hanna T et al. (1987): Herba Hungarica Tom. 26 (1): 27-31.
- 5- Tackholm, V (1974): Student flora of Egypt. Published by Cairo Univ. 568.
- 6- Reda KM, Hanafi YA, Abdelwahab A, Amin IS, Hegawy A et al. (2000): "Uncultivated Medicinal Plants of Sinai". Arabian Gulf East, Egypt.
- 7- Alard F, Stievenart C, Vanparys P, Thilemans L, Geerts S et al. (1991): Toxicity and mutagenicity of the molluscicidal plant *Ambrosia maritima* L. Drug. Chem. Toxicol. 14(4):353-373.
- 8- Abdelgaleil SAM (2010): Assessment of mosquitocidal, herbicidal and molluscicidal potentials of extracts and phytochemicals isolated from three Egyptian plants. Alexandria Journal of Agricultural Research. 55: 59–73.
- 9- Delgado G, Chaves MA, Alvarez L et al. (1988): Journal of Natural Products. 51:1-83.
- 10- Khalil A, El-Tawill B, Ashy M, Elbeih F, et al (1981): Phytochemical analysis of *Ambrosia maritime* L. Pharmazie. 36(8):569.
- 11- Jenkins D, Goffh D, Leeds A, Alsert K, Wolever T, Gassull M and Hockaday T et al. (1976): Unabsorbable carbohydrates and diabetes: Decreased postprandial hyperglycemia. The Lancet. 24: 172-174.
- 12- Mossa J, Al-Yahya M, Al-Meshal I et al. (1987): Medicinal plants of Saudi Arabia. King Saudi University Libraries. 1: 1-2.
- 13- Chemesova I, Belenovskaya L, Stukov A, et al (1987): Anti-tumour activity of flavonoids from some *Artemisia* species. Restitel'nye Resury. 23(1): 100-103.
- 14- Dominguez XA, Gomez E, Gomez PA, Villareal AN, Rombold C et al. (1970): Physical data on the essential oils of five Compositae plants. Planta Medica. 19:52-54.
- 15- Malaisse WJ (1982): Alloxan toxicity to the pancreatic B-cell. A new hypothesis. Biochem. Pharmacol. 31: 3527-3537.
- 16- Trinder P (1969): Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann Clin Biochem. 6: 24-27.
- 17- Reeves WG (1983): Insulin antibody determination: theoretical and practical considerations. Diabetologia. 24:399- 403.
- 18- Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC et al. (1985): Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 28 (7): 412–419.
- 19- Junge W, Wilke B, Halabi A, Klein G et al. (2004): Determination of reference intervals for serum creatinine, creatinine excretion and creatinine clearance with an enzymatic and a modified Jaffe method. Clin. Chim. Acta. 344:137–148.
- 20- Patton CJ, Crouch SR (1977): Spectrophotometric and kinetics investigation of the Berthelot reaction for the determination of ammonia. Anal. Chem. 49: 464-469.
- 21- Tietz NW (2007): Fundamentals of Clinical Chemistry, 6th Edition, W.B. Saunders, Philadelphia, PA.
- 22- Schumann G, Klauke R (2003): New IFCC reference procedures for the determination of catalytic activity concentrations of five enzymes in serum: preliminary upper reference limits obtained in hospitalized subjects. Clin. Chem. Acta. 327:69-79.

- 23- KytziaHJ(2005):**Reference intervals for GGT according to the new IFCC 37°C reference procedure. Clin. Chem. Lab Med. **43**:69.
- 24-Tietz NW (1995):** Clinical Guide to Laboratory Tests, 3rdED. WB Saunders company, Philadelphia: 518-522.
- 25-Bucolo G, David H (1973):** Enzymatic determination of serum triglycerides quantitatively. Clin. Chem. **19**: 475-481.
- 26- Knight JA, Anderson S, Rawie JM et al. (1972):** chemicalbasies of the sulfo-phosphovanilin reaction for estimation total serum lipid .clin.chem.**18**:199-202.
- 27-Sugiuchi H (2005):** History of development and technical details of the homogenous assay for HDL and LDL cholesterol. Eng. J. Med. **1**:4-11.
- 28-Friedewald WT, Levy RI, Fredrickson DS et al. (1972):** Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin. Chem. **18**:499-502 (Cited in: Clin. Chem. **1999**; **36**:15-19).
- 29-Suvarna SK, Layton C, Bancroft JD et al. (2013):** Bancroft's Theory and Practice of Histological Techniques. 7th Ed. Elsevier, Churchill Livingst one, Beijing, China. Pp 178-186, 221-224.
- 30- Kumar S, Narwal VK, Prakash O et al (2011):** “ α - Glucosidase Inhibitors from Plants: A Natural Approach to Treat Diabetes,” Pharmacogony Review. **5 (9)**:19-29
- 31- Abou El-Soud N, El-Laithy N, El-Saeed G, Wahby M, Khalil M, Morsy F, Shaffie N et al (2011):** Antidiabetic Activities of Foeniculum Vulgare Mill. Essential Oil in Streptozotocin-Induced Diabetic Rats. Macedonian J Med Sci. **4(2)**:139–146
- 32- Chang CL, Lin Y, Bartolome AB, Chen YC, Chiu SC, Yang WC et al. (2013):** Herbal Therapies for Type 2 Diabetes Mellitus: Chemistry, Biology, and Potential Application of Selected Plants and Compounds. Complement Altern. **33**.
- 33- Ahmed OM (2003):** Evaluation of the hypoglycemic and antioxidant effects and the probable mechanism of action of chromium and selenium in streptozotocin-induced diabetic albino rats. J. Egypt Ger. Soc. Zool. **41(A)**:163-193.
- 34-El-Gamal HM, Shaker HK, Po'llmann K, Seifert K et al. (1995) :** Triterpenoidsaponins from zygophyllum species. Phytochemistry **40 (4)**:1233–1236.
- 35- Nagwa MA, Sahar YA, Ibrahim HB et al. (1995):** The hyperglycemic effect of different extracts of *Ambrosia maritima*, L. Compositae. Egypt. J. Pharm. Sci. **36**:107–115
- 36- Eskander EF, Won-Jun H (1995):** Hypoglycemic and hyperinsulinemic effects of some Egyptian herbs used for the treatment of diabetes mellitus (type II) in rats. Egypt. J. Pharm. Sci. **36 (1–6)**: 331–342.
- 37- Kiliani AI, Ibraheem FK, Kamel NAH, Hareedi MA, Ali AA, Shahwan et al. (1990):** Proceedings of Eighth Annual Scientific Conference.
- 38- Oduye OO, Adadevoh BK (1976):** Biochemical values of apparently normal Nigerian sheep. Nig. Vet. J. **5(1)**: 43-50.
- 39- Esonu BO, Emenalom OO, Udedibie ABI, Herbert U, Ekpore CF, Okoli IC Ihukwumere FC et al .(2001):** Performance and blood chemistry of weaner pigs fed raw *Mucuna* beans (Velvet bean) meal. Tropical Animal. **4**:49-54.
- 40- Rossetti L, Giaccari A, Defronzo RA et al.(1990):** Glucose toxicity. Diabetes Care. **13**: 610–630.
- 41- Lann D, LeRoith D (2007):** Insulin resistance as the underlying cause for the metabolic syndrome. Med Clin North Am. **91**:1063–1077.
- 42- Antuna-Puente B, Disse E, Rabasa-Lhoret R, Laville M, Capeau J, Bastard JP et al. (2011):** How can we measure insulin sensitivity/resistance? Diabetes Metab. **37**:179-188.
- 43-Kamel MS, OhtantK, Kurokawa T, Assaf M, El- Shanawany MA, Ali AA, Kasai R, Ishibashi SS, Tanaka O et al. (1991):** Studies on *Balanitesa egyptiaca* fruits, an antidiabetic Egyptian folk medicine. Chem. Pharm. Bull., **39(5)**: 1229–1233.
- 44- Lellau TF, Liebezeit G (2001):** Alkaloids, saponins and phenolic compounds in salt marsh plants from the Lower Saxonian Wadden Sea. – *Sencken bergiana maritima*, **31**: 1-9.
- 45- Nakashima N, Kimura L, Kimura M et al .(1993):** Isolation of pseudoprototimosaponin AIII from rhizomes of *Anemarrhena* as phodeloides and its hypoglycemic activity in streptozotocin—induced diabetic mice. J. Nat. Prod. **56(3)**:345.
- 46- Yi P, Park JS, Melton D A et al. (2013):** “Betatrophin: a hormone that controls pancreatic β cell proliferation,” Cell. **153(4)**: 747–758.
- 47- Bakkali F, Averbeck S, Averbeck D, Idaomar M et al. (2008):** Biological effects of essential oils. Food Chem. toxicol. **46**: 446-475.
- 48- Diogo CV, Suski JM, Lebieczinska M, Karkucinska-Wieckowska A, Wojtala A, PronickiM, Duszynski J, Pinton P, Portincasa P, Oliveira PJ, Wieckowski MR et al. (2013):** .Cardiac mitochondrial dysfunction during hyperglycemia--the role of oxidative stress and p66Shc signaling. International Journal of Biochemistry & Cell Biology. **45(1)**:114-122.
- 49- Yang H, Jin X, Kei Lam CW, Yan SK et al. (2011):** Oxidative stress and diabetes mellitus.

Clinical Chemistry & Laboratory Medicine. **49(11)**:1773-1782.

50- Samad A, Shams MS, Ullah Z, Wais M, Nazish I, Sultana Y, Aqil M et al. (2009): Status of herbal medicines in the treatment of diabetes. *Curr. Diabetes Rev.* **5**: 102-111

51- Davis ME, Berndt WD (1994): Renal methods for toxicology. In: Hayes and A.W. (Eds). Principles and methods of toxicology, 3rd Edition. New York, Raven, USA. 871-894.

52- Adelman RD, Spangler WL, Beasom F, Ishizaki G, Conzelman GM, et al (1981): Frusemide enhancement of neltimicin nephrotoxicity in dogs. *The Journal of Antimicrobial Chemotherapy.* **7(4)**: 431-440.

53- Imai T, Nishimaki K, Shiga T, Kawasaki S, Makuuchi M et al. (1996): Lipid peroxidation and antioxidant activities in regenerating liver after partial hepatectomy in splenectomized rats. *Res. Exp. Med.* **196**:1-7.

54- Ahmed MB, Khater MR (2001): Evaluation of the protective potential of *Ambrosia maritima* extract on acetaminophen induced liver damage. *J. Ethnopharmacol.* **75(2-3)**: 169-174.

55- Ngaha EO, Akanji MA, Madusolumuo MA et al. (1989): Studies on correlation between chloroquine induced tissue damage and serum enzyme changes in the rat. *Experientia.* **45**: 143-146.

56- Lin CC, Huang PC (2000): Antioxidant and hepatoprotective effects of *Acathopanaxsenticosus*. *Phytother. Res.* **14**: 489-494.

57- Chandramohan G, Al-Numair KS and Pugalendi KV et al. (2009): Effect of 3-hydroxymethyl xylitol on hepatic and renal functional markers and protein levels in alloxan-diabetic rats. *Afr. J. Biochem. Res.* **3(5)**: 198-204.

58- Sivajothi V, Dey A, Jayakar B, Raj Kapoor B et al. (2008): Antihyperglycemic, Antihyperlipidemic and Antioxidant Effect of *Phyllanthusrheedii* on Alloxan Induced Diabetic Rats. *Iran. J. Pharm. Res.* **7(1)**: 53-59.

59- Patel DK, Kumar R, Laloo D, Hemalatha S et al (2012): Natural medicines from plant source used for therapy of diabetes mellitus: an overview of its pharmacological aspects. *Asian Pacif. J. Trop. Dis.* **3**:239-250.

60- Lenzen S (2008): The mechanisms of alloxan- and streptozotocin induced diabetes. *Diabetologia.* **51**:216-226.

61- Jadeja RN, Thounaojam MC, Ansarullah Devkar RV, Ramachandran AV et al. (2010): *Clerodendronglandulosum* Coleb. Verbenaceae, ameliorates high fat diet-induced alteration in lipid and cholesterol metabolism in rats. *Brazilian Journal of Pharmacognosy.* **20(1)**: 117-123.

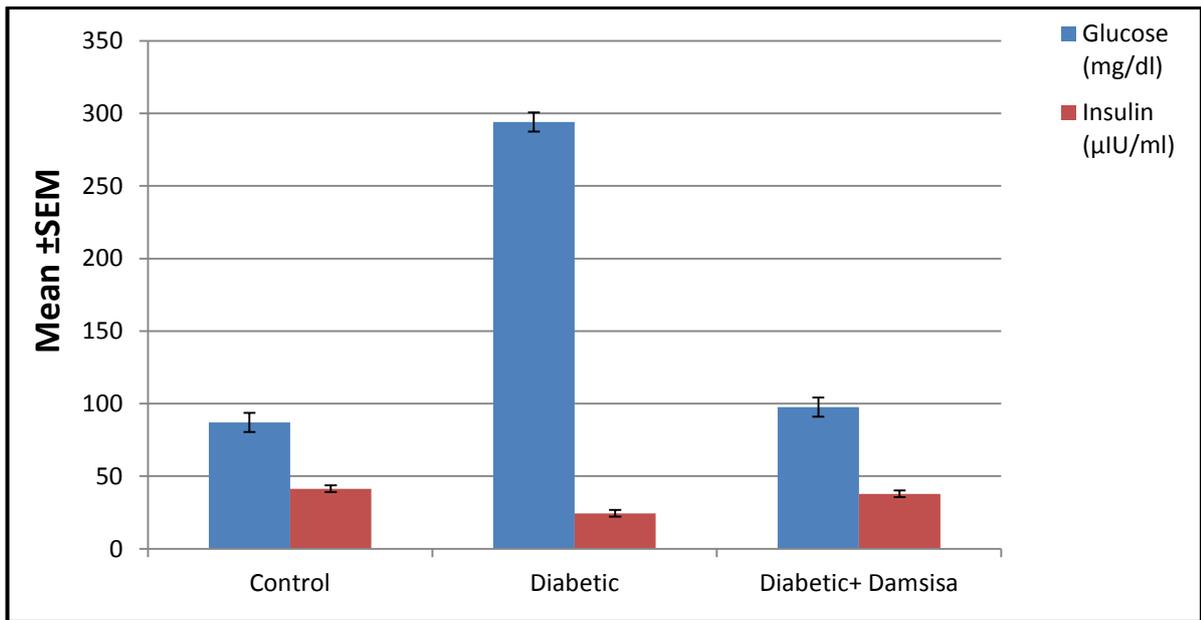


Fig.1: Glucose (mg/dl) and insulin (µIU/ml) levels in the control, diabetic and treated groups.

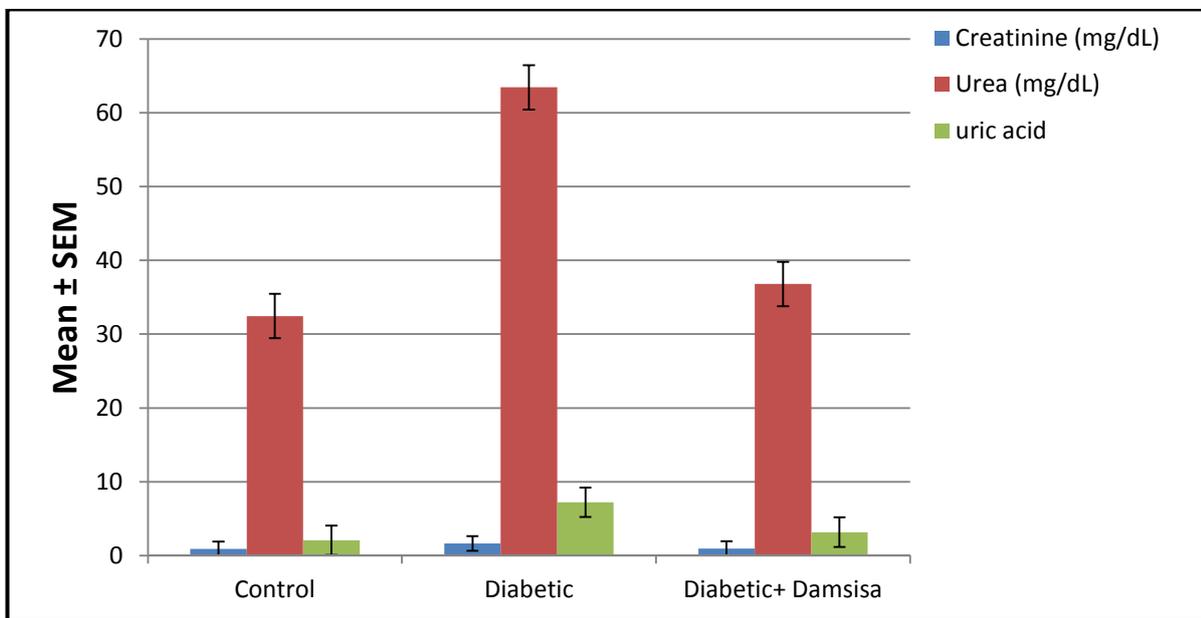


Fig. 2: Levels of creatinine, urea and uric acid in the control, diabetic and treated groups.

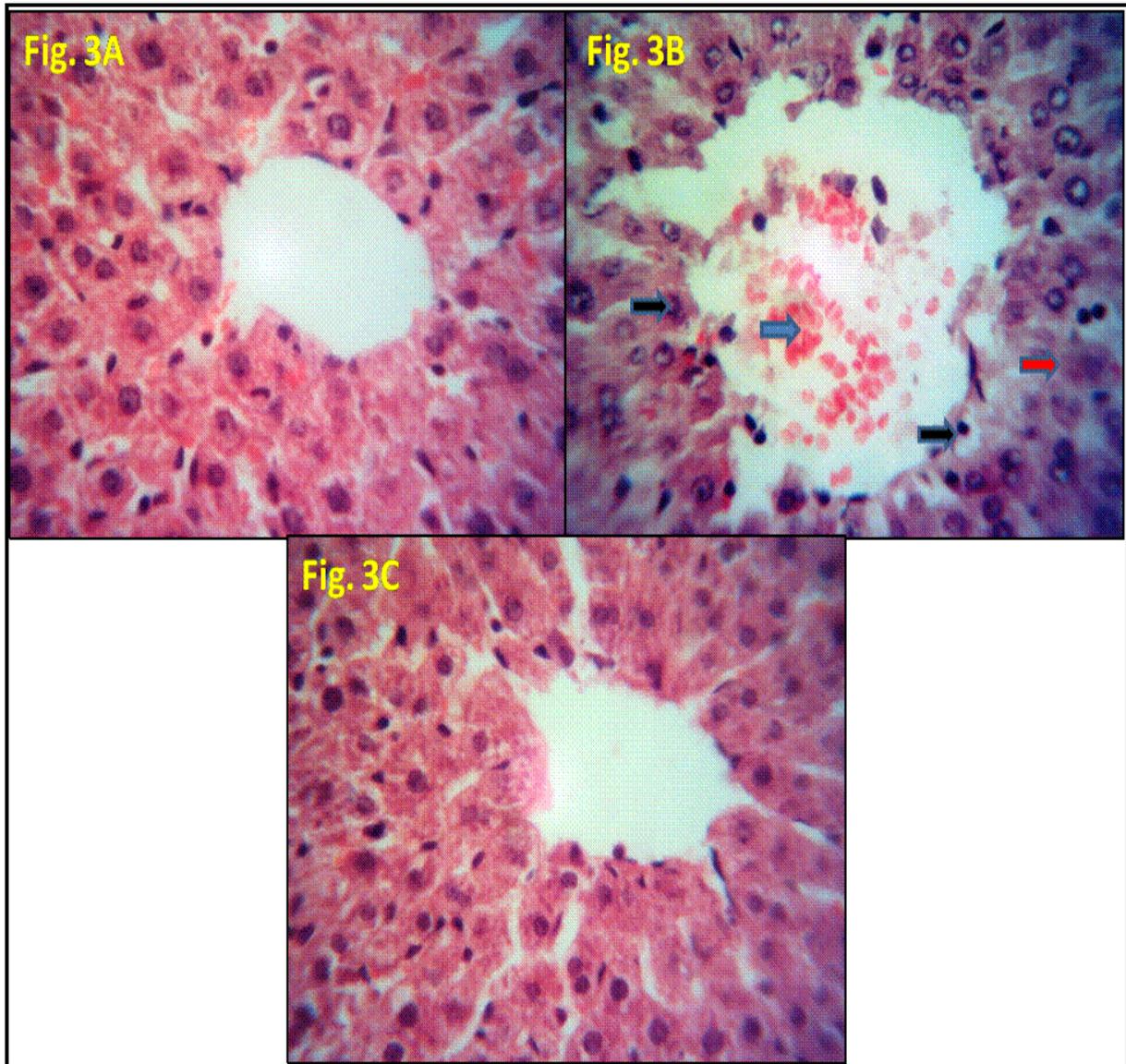


Fig.3. A) A photomicrograph of the control liver of adult albino rat showing normal lobular pattern with a centrilobular vein and radiating irregular branching and anastomosing plates of hepatocytes with intervening sinusoids lined with endothelial cells. Most of the hepatocytes have vesicular nuclei and some of them appear binucleated. B) A photomicrograph of the liver of diabetic adult albino rat showing hepatocytes necrotic changes and congestion of central vein (blue arrow), marked hepatocytes ballooning degeneration (red arrow), pyknotic nuclei and fatty degeneration (black arrows) around the central vein. C) A photomicrograph of the liver of diabetic adult albino rat treated with *Ambrosia maritima* showing that most of hepatic lobules are almost similar to that of the control group. (Hx. & E. x400).

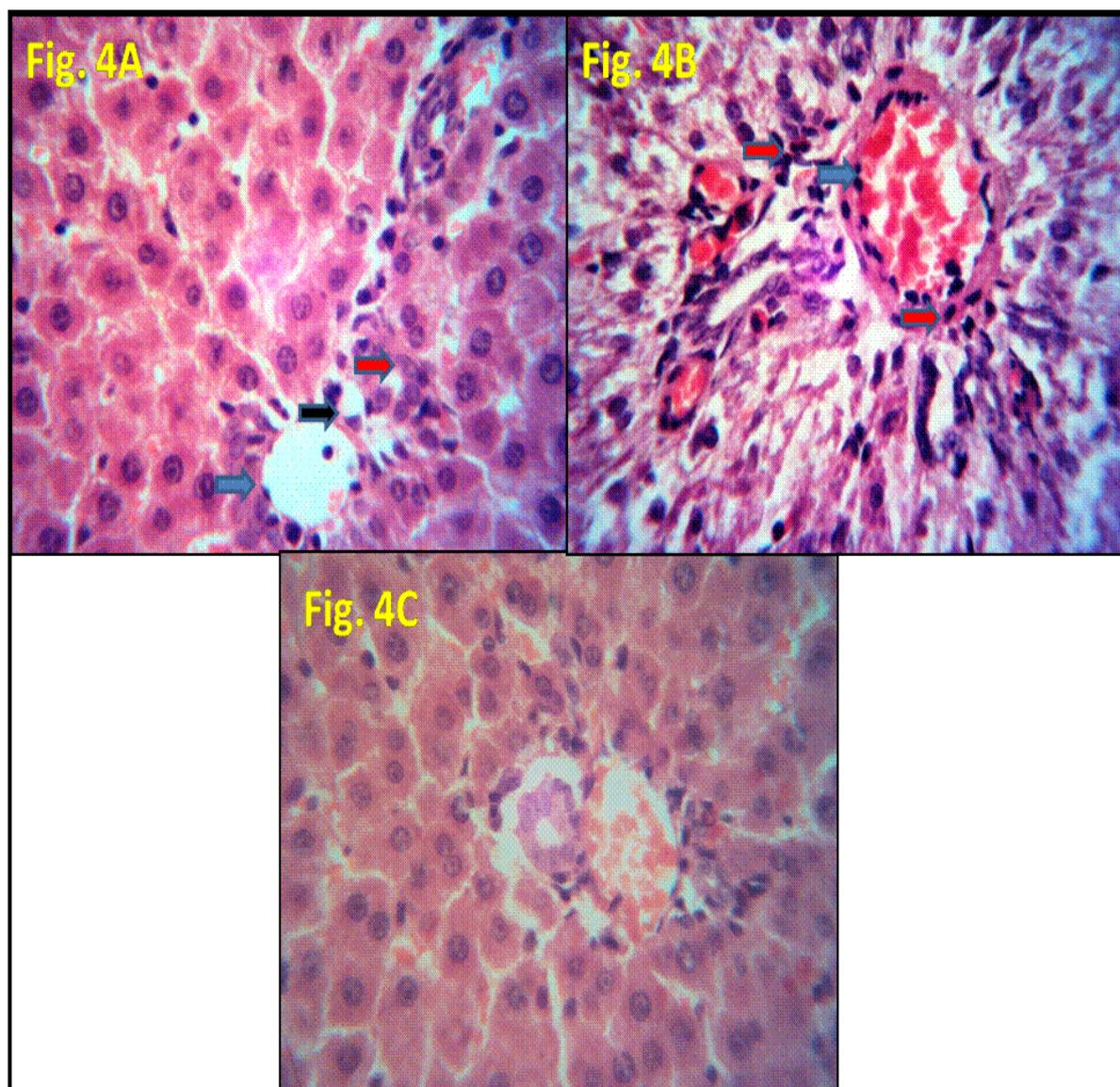


Fig.4. A) A photomicrograph of the control liver of adult albino rat showing normal portal triad consisting of a branch of portal vein (blue arrow), branch of the hepatic artery (black arrow) and bile ductule (red arrow). B) A photomicrograph of the liver of diabetic adult albino rat showing marked congestion and thickened wall of the portal vein (blue arrow) and lymphocyte infiltration (red arrows). C) A photomicrograph of the liver of diabetic adult albino rat treated with *Ambrosia maritima* showing that the portal triads are almost similar to that of the control group.(Hx. & E. x400).

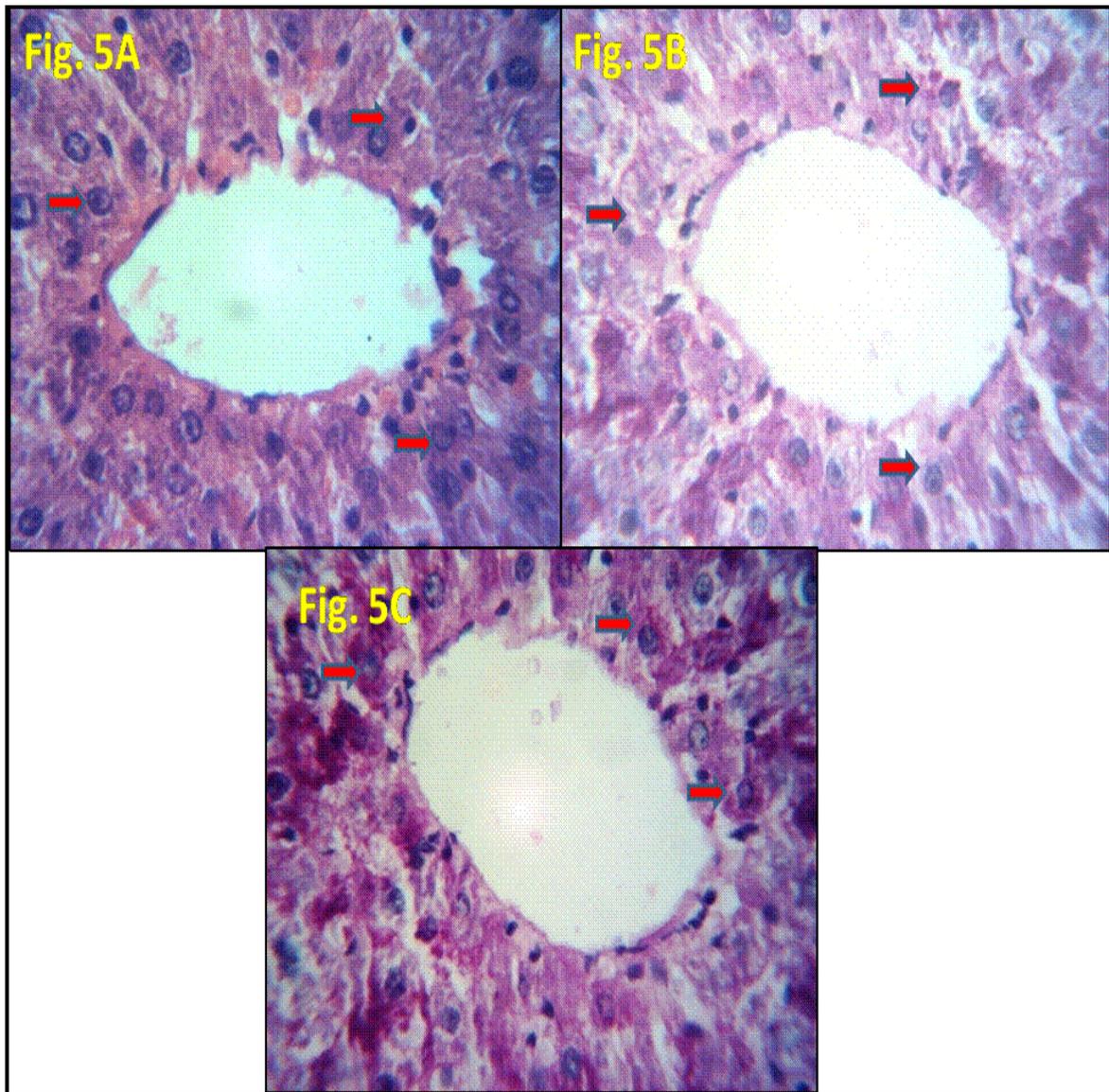


Fig. 5. **A)** A photomicrograph of the control liver of adult albino rat showing strong PAS +ve granules in most of the hepatocytes (red arrows). **B)** A photomicrograph of the liver of diabetic adult albino rat showing weak PAS +ve granules in most of the hepatocytes (red arrows). **C)** A photomicrograph of the liver of diabetic adult albino rat treated with *Ambrosia maritima* showing strong PAS positive granules in most of the hepatocytes. (PAS. x400).

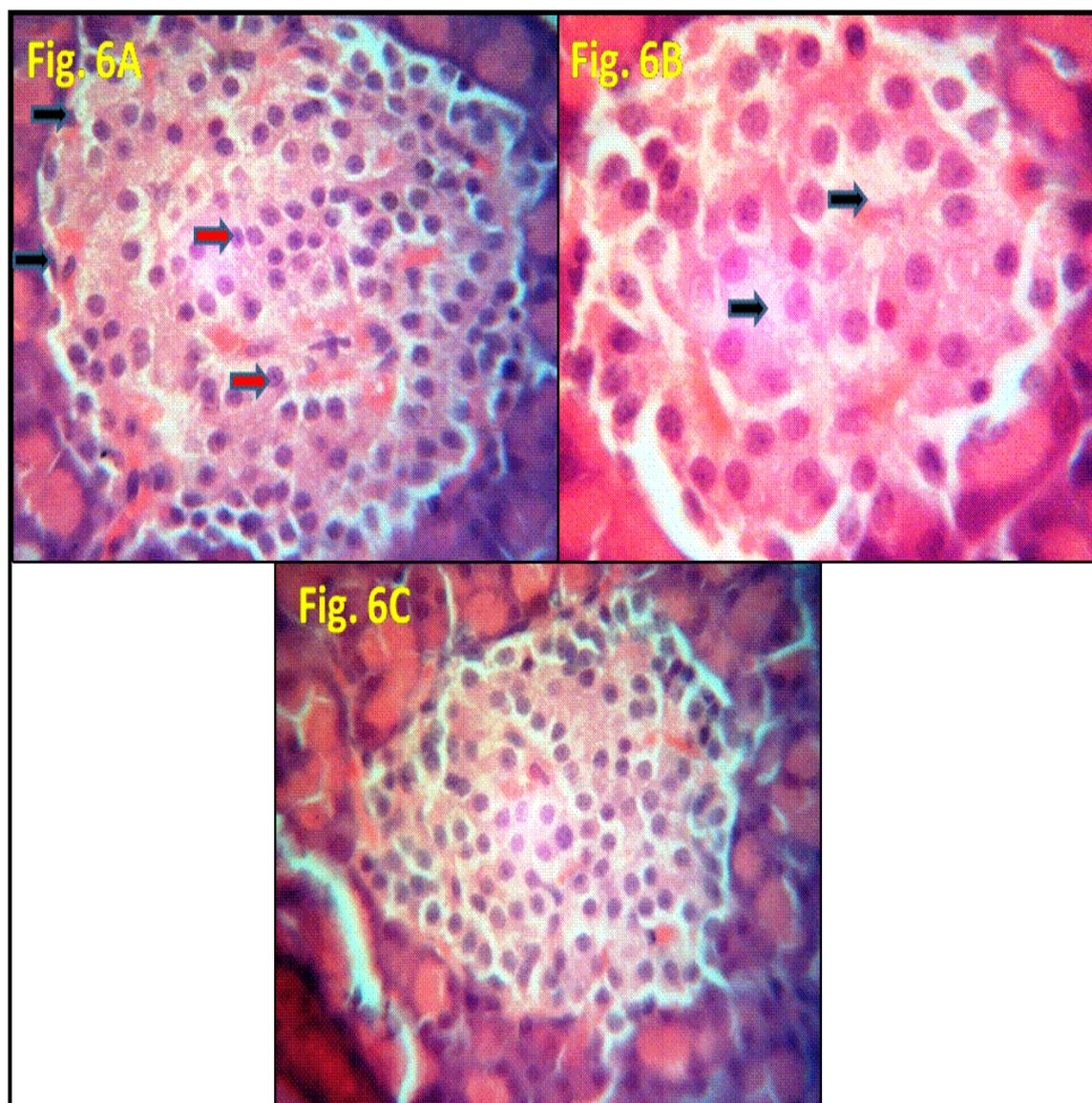


Fig.6. A) A photomicrograph of a control pancreas of adult albino rat showing the normal cellular distribution in the islet of Langerhans, the red arrows show beta cells while the black arrows show the alpha cells. B) A photomicrograph the pancreas of diabetic adult albino rat showing marked degenerative changes in the islet (black arrows)with decreased islet cellularity. C) A photomicrograph the pancreas of diabetic adult albino rat treated with *Ambrosia maritime* showing partial return to the normal cellular distribution within the islet of Langerhans. (Hx. & E. x400).

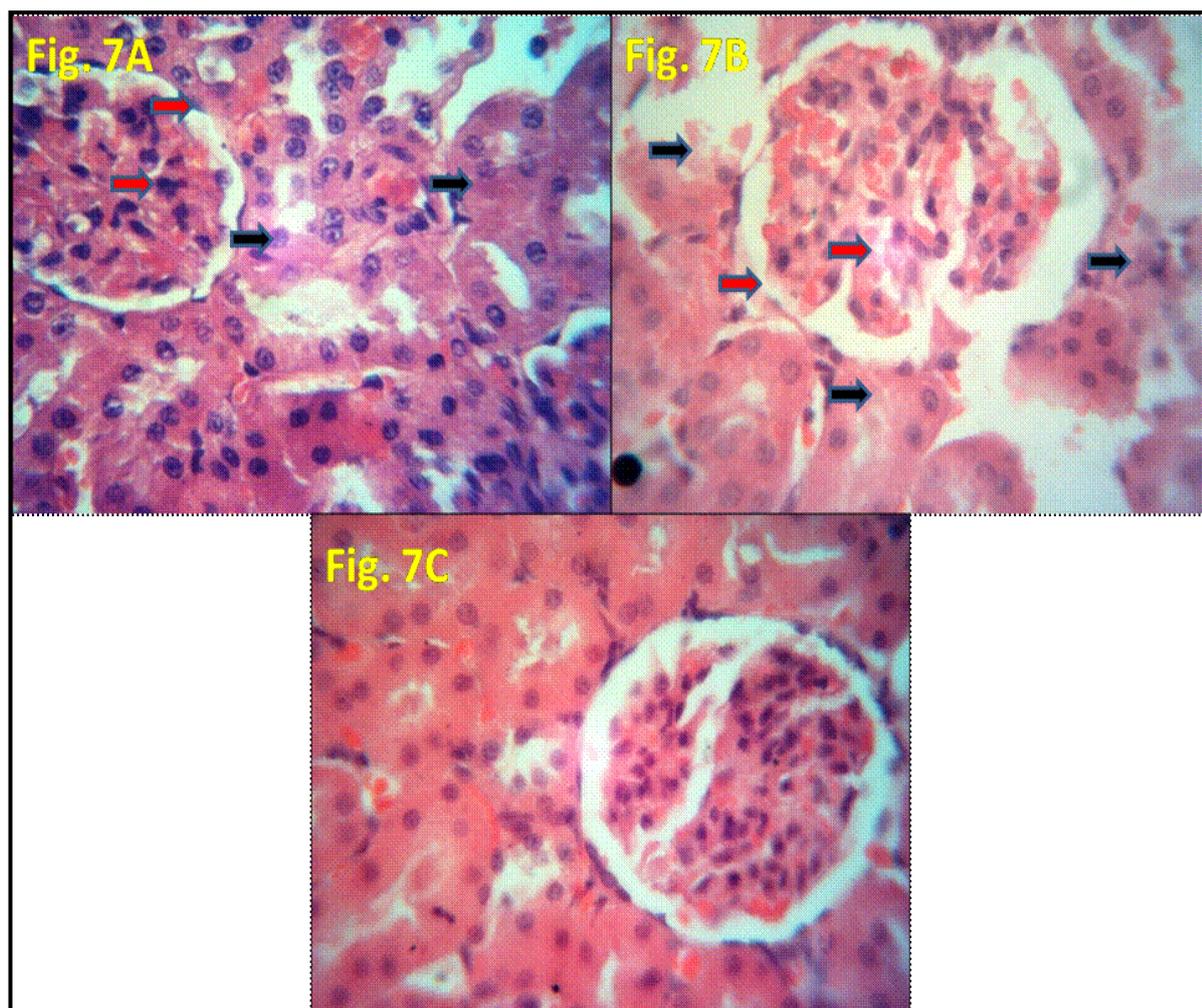


Fig. 7. A) A photomicrograph of a control kidney of adult albino rat showing normal glomerular tufts, normal glomeruli with normal Bowman's capsules (red arrows) and normal ascending and descending tubules (black arrows). **B)** A photomicrograph of the kidney of diabetic adult albino rat showing glomerular degenerative changes and thickening of Bowman's capsule (red arrows), vacuolar degeneration in some tubular epithelial cells (ascending and descending) and cell debris scattered in tubular lumina, thickened tubular epithelial cells with narrowing of lumen and degenerative changes in the form of karyolysis and karyorrhexis (black arrows). **C)** A photomicrograph of the kidney of diabetic adult albino rat treated with *Ambrosia maritima* showing some protective effects as compared to the diabetic group in the form of lesser degree of degenerative changes in glomeruli, Bowman's capsule and tubules. (Hx.&E. x400).

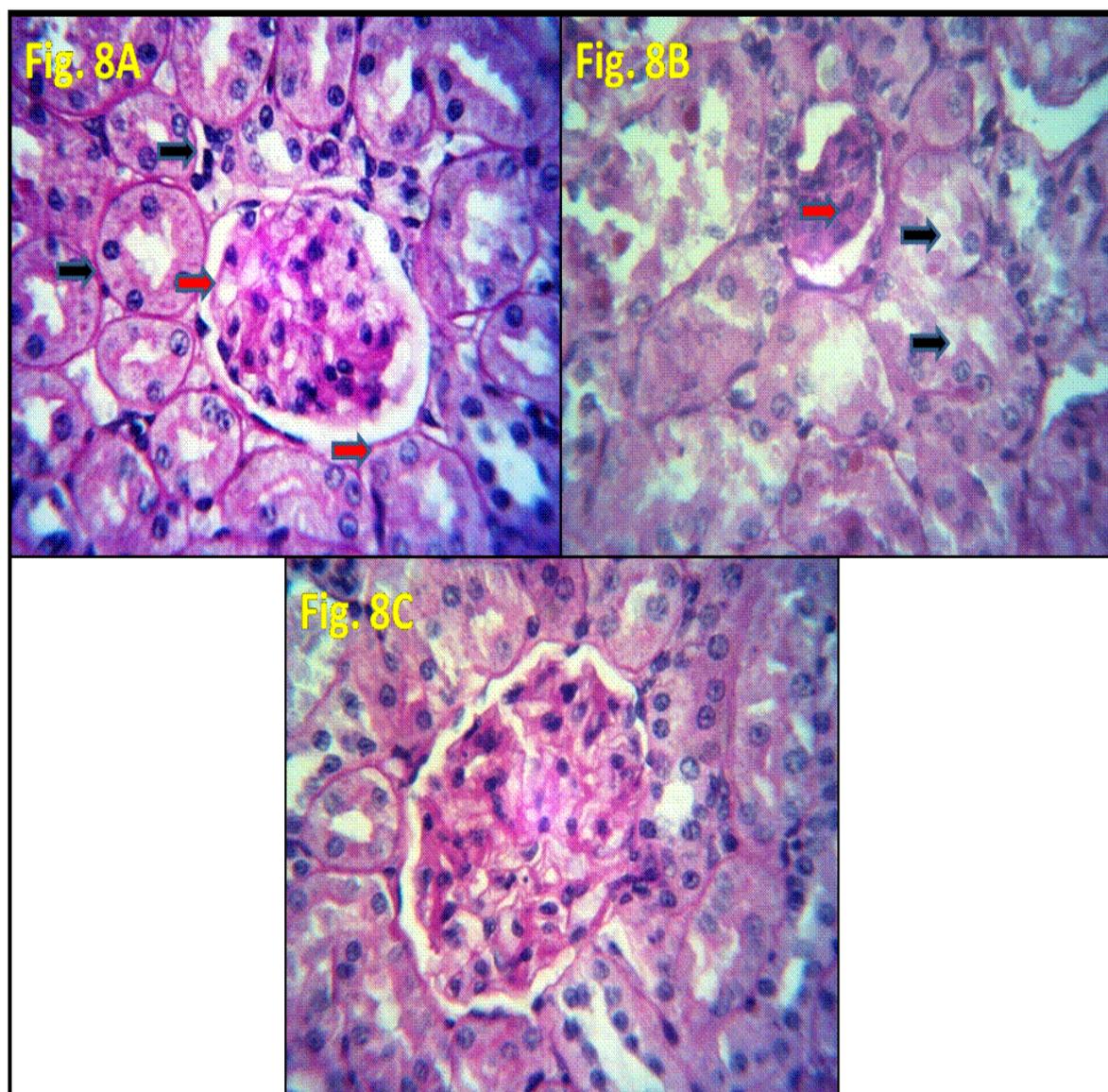


Fig.8. A) A photomicrograph of a control kidney of adult albino rat showing strong PAS +ve granules in the glomeruli basement membrane (red arrows), basement membrane and brush borders of the ascending and descending tubules (black arrows). B) A photomicrograph of the kidney of diabetic adult albino rat showing decrease glomeruli size (red arrow) and decrease stainability of PAS +ve granules in both ascending and descending tubules (black arrows). C) A photomicrograph of the kidney of diabetic adult albino rat treated with *Ambrosia maritime* showing that the mucopolysaccharide content more or less approximated to the control. (PAS. x400).

Table (1): Serum insulin and glucose levels in the control, diabetic and treated groups.

Group Parameters	Control	Diabetic	Diabetic + <i>Ambrosia maritima</i>
Glucose (mg/dl)	87.44± 0.77	292.20± 0.84 ^{***}	97.61± 0.36 ^a
Insulin (µIU/ml)	41.36± 0.45	37.72± 0.77 ^{**}	27.84± 0.35 ^{a,b}
HOMA-RI	8.03± 0.23	24.54± 0.36 ^{**}	8.21± 0.086

*Values are represented as mean±SE for groups of ten animals. ^{***} $p < 0.01$: significant increase in the parameters levels of the diabetic group in comparison to the control group. ^{**} $p < 0.01$: significant decrease in the parameters levels of the diabetic group in comparison to the control group. ^a $p < 0.01$: significant decrease in the parameters levels of the treated group in comparison to the diabetic group. ^b $p < 0.01$: significant decrease in the parameters levels of the treated group in comparison to the control group.

Table (2): Changes in body weight (g) in the control, diabetic and treated diabetic groups.

Groups Parameters	Control	Diabetic	Diabetic+ <i>Ambrosia maritima</i>
% of Change	9.92± 7.41	4.98± 3.54 ^{**}	9.98±0.17

^{**} $p < 0.01$ significant decrease in the parameters levels of the diabetic group in comparison to the control group.

Table (3): Changes in the ALT, AST and γGT activities in the control, diabetic and diabetic groups.

Groups Parameters	Control	Diabetic	Diabetic+ <i>Ambrosia maritima</i>
ALT IU/L)	22.46±0.55	33.52±0.40 ^{***}	23.54±0.41 ^a
AST (IU/L)	37.02±0.59	53.24±0.71 ^{***}	36.56±0.54 ^a
γGT(IU/L)	2.28±0.11	9.66±0.054 ^{***}	3.59±0.07 ^a

*Values are represented as mean±SE for groups of ten animals. ^{***} $p < 0.01$: significant increase in the parameters levels of the diabetic group in comparison to the control group. ^a $p < 0.01$: significant decrease in the parameters levels of the treated group in comparison to the diabetic group.

Table (4): Changes in creatinine, urea and uric acid levels in the control, diabetic and treated groups.

Groups Parameters	Control	Diabetic	Diabetic+ <i>Ambrosia maritima</i>
Creatinine (mg/dL)	0.91± 0.005	33.52± 0.060 ^{***}	0.96± 0.01 ^a
Urea (mg/dL)	32.46± 0.10	53.24± 1.70 ^{***}	36.80± 0.38 ^a
uric acid	3.26± 0.04	9.66± 0.08 ^{***}	3.18± 0.37 ^a

*Values are represented as mean±SE for groups of ten animals. ^{***} $p < 0.01$: significant increase in the parameters levels of the diabetic group in comparison to the control group. ^a $p < 0.01$: significant decrease in the parameters levels of the treated group in comparison to the diabetic group.

Table(5):Changes in total lipid(TL) , triglycerides (TG), total Cholesterol (TC), HDL cholesterol (HDL-C), LDL-cholesterol (LDLC) and VLDL- cholesterol (VLDLC)parameters in the control, diabetic and treated groups.

Groups Parameters	Control	Diabetic	Diabetic + <i>Ambrosia maritima</i>
Total lipids (mg/dl)	475.0±0.05	1435.0±0.07***	930.0±0.05 ^a
TC (mg/dl)	141.08±0.37	231.52±0.52***	187.22±0.39 ^a
TG (mg/dl)	133.10±0.77	283.96±0.86***	142.70±0.55 ^a
HDL (mg/dl)	47.88±0.48	38.08±0.38**	44.75±0.36 ^c
LDL (mg/dl)	66.56±0.47	136.40±0.59***	114.36±0.63 ^a
VLDL (mg/dl)	26.62±0.16	50.80±0.16***	28.25±0.26 ^a
TC/HDL	3.00±0.00	6.08±0.03***	4.18±0.03 ^a
LDL/HDL	1.38±0.02	3.58±0.03***	2.56±0.03 ^a

* Values are represented as mean±SE for groups of ten animals *** $p < 0.01$: significant increase in the parameters levels of the diabetic group in comparison to the control group ** $p < 0.01$: significant decrease in the parameters levels of the diabetic group in comparison to the control group. ^a $p < 0.01$: significant decrease in the parameters levels of the treated group in comparison to the diabetic group. ^c $p < 0.01$: significant increase in the parameters levels in the treated group in comparison to the diabetic group.

Table(6): Changes in the serum proteins profile (g/dl) and A/G ratio in the control, diabetic and treated groups.

Groups Parameters	Control	Diabetic	Diabetic + <i>Ambrosia maritima</i>
Total protein (g/dL)	7.40±0.08	5.30±0.11**	7.07±0.07
Albumin (g/dL)	4.24±0.07	3.12±0.05**	4.19±0.07
Globulin (g/dL)	3.16±0.5	2.18±0.06**	2.88±0.05
A/G ratio	1.34±0.05	1.22±0.02	1.45±0.05

* Values are represented as mean±SE for groups of ten animals. ** $p < 0.01$: significant decrease in the parameters levels of the diabetic group in comparison to the control group.