The Effects of *Jasonia montana* (Neheda) on Some Biochemical and Histological Parameters of Diabetic Albino Rats

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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. In Asia, different medicinal plant species are used as a traditional treatment for diabetes mellitus e.g. *Jasonia montana* (Neheda) was one of these plants that was used in a mixture 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 *Jasonia montana* (Neheda) 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 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 diabetic rates with aqueous extract of Neheda 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 Neheda in liver, kidney and pancreatic tissues in comparison to the control group.

Conclusion: It could be concluded that *Jasonia montana* (Neheda) 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, Neheda, Jasonia montana.*

Introduction

Diabetes mellitus is one of the most common endocrine metabolic disorders that is usually associated with micro- and macro-vascular complications[1]. The incidence of diabetes had been increased worldwide in the last years[2]. According to the International Diabetes Federation, the estimated number of diabetic patients was about 30 million in 1985, 150 million in 2000 and then 246 million in 2007[2]. The International Diabetes Federation expects increase in this number up to 380 million patients by 2025[2]. Family Compositae (**Asteraceae**) [locally known as **Neheda**] include many plants such as *Chiliadenus montana* or *Jasonia montana* and *Chrysoco montana* or *Varthemia montana*[3]. *Jasonia montana* had been found in the Mediterranean

and adjacent areas, including the Sinai Peninsulain Egypt[4]. This medicinal plant was traditionally used to treat many diseases like diarrhea, renal troubles, stomachache and chest diseases[4]. High content of essential oils such as camphor, borneol, bornyl acetate, chrysanthemol, intermediol, and 1,8-cineole were found in *Jasonia montana*[5]. Also, many reports confirmed that *Jasonia montana* is rich in flavonoid and methoxylated flavonoids[6]. Moreover, *Jasonia montana* contains high levels of phenolic compounds such as cinnamic and caffeic acids which have excellent antidiabetic, antioxidant and anticholestatic effects[7]. These polyphenols are more potent antioxidants than vitamins C and E[8]. Eighteen phenolic quercetin derivatives like glucuronidejaceidin, and centaureidin were isolated from *Jasonia*

montana[9]. These derivatives may be responsible for the antioxidant activity of *Jasonia montana*[9].Other bioactive compounds such as triterpenes and sterols may be responsible for the anti-inflammatory properties that inhibit the production of pro-inflammatory cytokines[10]. This work was aimed to investigate the antidiabetic, hypolipidemic and antioxidant effects of the aqueous extract of *Jasonia montana* (Neheda) on the alloxan-induced diabetic male albino rats.

Material and Methods

Plant material

The aerial parts of *Jasonia montana* was collected from El-Arbaeen valley, Saint Catherine, Wadi Gebal, South Sinai, Egypt. The plant was grinded and the aqueous extract of neheda was prepared by boiling 2 g of naheda 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[11]. 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 *Jasonia montana* (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**[12].Serum insulin level was measured by coat-A-count radioimmunoassay kits according to **Reeves**[13].While values of HOMA-IR were calculated using the following equation: **HOMA-IR = fasting serum glucose (mg/dl) x fasting serum insulin (µU/ml)/450**[14]. Glucose in mass units mg/dL. And IR is insulin resistance. Creatinine concentrations was determined colorimetrically as described by Junge[15]. Urea concentrations were determined colorimetrically as described by **Patton &Crouch**[16]. Serum uric acid was determined using the uricase-PAP enzymatic colorimetric method[17]. Aspartate amino transferase (AST) and alanine amino transferase (ALT) were assayed according to the method of Schumann[18]. Gammaglutamyl transferase (γGT) assay was performed according to **Kytzia**[19]. Albumin and total protein concentrations were determined colorimetrically[17]. Serum globulin was calculated by subtracting albumin from total protein[17]. Enzymatic determination of serum cholesterol was done as described by **Tietz**[20]. Triglycerides content was determined by the method of **Bucolo and David**[21]. Total lipids (TL) were analysed by the method of **Knight *et al.***[22]. HDL-cholesterol content was determined by applying the method of **Sugiuchi**[23]. LDL-C was calculated using the **Friedwald's formula** when the values of TG were less than 400 mg/dl[24]. VLDL was calculated using the **Friedewald's equation**[24].

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 liver, kidney and pancreas were taken for the histological and histochemical studies. The specimens were prepared *via* fixation in 10% neutral buffered forml solution and Carnoy's fluid. Paraffin sections of5μm thickness were prepared and stained with Harris'shaematoxylin and eosin (H&E)[25]. Polysaccharides were detected using PAS (Periodic acid-Schiff) method[25]. 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, 2 & Table. 1)**. A significant decrease ($p<0.01$) in the levels of serum insulin (-9.16%) accompanied with marked elevation ($p<0.001$) in levels of blood glucose (232.6%) were recorded in diabetic rats (Group II) when compared to the control rats (Group I). *Jasonia montana* showed significant recovery ($p<0.01$) 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 (97.64%), while treatment of diabetic rats with *Jasonia montana* returned HOMA-IR values to the normal level **(Fig. 1, 2 & Table. 1)**. The percent change in the body weight in diabetic rats was significantly decreased (4.16%) **(Fig. 3 & Table2)**. Also, it has been noticed that the percent change of body weight returned to normal weight (9.92%) after treating the

diabetic rats with the plant extract **(Fig. 3 &Table 2)**. Diabetics rats showed a significant increase ($p<0.001$) in serum ALT, AST and γGT activities in diabetic group as compared with the control group **(Table 3)**. While *Jasonia montana* treatment of the diabetic rats significantly decreased these activities when compared with the diabetic group ($p<0.001$) and these activities was returned back to the normal value 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. 4 & Table 4)**. Treatment of the diabetic rats with *Jasonia montana* extract produced significant increase in the serum urea, creatinine and uric acid in comparison with the control group ($p<0.05$), and recorded significant decrease ($p<0.001$) in comparison with the diabetic group **(Fig. 4 & Table 4)**. *Jasonia montana* 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 **(Fig. 5 & Table 5)**. These levels in the diabetic rats were dramatically increased in comparison with the control group **(Fig. 5 & Table 5)**. In addition, diabetics' rats showed marked decline ($p<0.01$) in serum total proteins (-60.2%), albumin (-26.4%) and globulin (-31.o1%) relative to the corresponding controls **(Fig.6 & Table 6)**. Treatment of the diabetic rats with *Jasonia montana* 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 **(Fig.6 & 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. 7A)**. Liver of the diabetic rat showed hepatocytes necrotic changes, ballooning degeneration, pyknotic nuclei and fatty degeneration around the congested central vein **(Fig. 7B)**. H&E stained sections of liver of the *Jasonia montana* treated rats showed that

most of hepatic lobules are almost similar to that of the control group **(Fig. 7C)**. In control group, normal portal triad had been observed **(Fig. 8A)**. The diabetic rats' portal triads showed marked congestion of portal vein and lymphocyte infiltration **(Fig. 8B)**. H&E stained sections of liver of the *Jasonia montana* treated rats showed that the portal triads are almost similar to that of the control group **(Fig. 8C)**. In control group, PAS +ve granules were mainly distributed in most of the hepatocytes **(Fig. 9A)**. The diabetic rats showed marked diminution in mucopolysaccharide content in hepatocytes **(Fig. 9B)**. The group of rats treated with *Jasonia montana* showed that the mucopolysaccharide content more or less similar to the control level **(Fig. 9C)**. Examination of H&E stained sections of pancreas of the control group, revealed normal appearance of the islets of Langerhans **(Fig. 10A)**. Beta cells were the most abundant cells while alpha cells were observed at the periphery of the islets **(Fig. 10A)**. Examination of H&E stained sections of the diabetic group showed marked reduction in islets size and cellularity **(Fig. 10B)**. 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. 10B)**. Examination of H&E stained sections of the diabetic group which was treated with *Jasonia montana* 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. 10C)**. The normal histological structure of the kidney was observed in **Fig. 11A**. The kidney of the diabetic rats showed vacuolar degeneration in some tubular' epithelial cells and cell debris scattered in tubule's lumina **(Fig. 11B)**. Increase in thickness of tubules' epithelial cells with narrowing of lumina, signs of degeneration in the form of karyolysis and karyorrhexis **(Fig. 11B)**. The kidney of the diabetic rats treated with *Jasonia montana* 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. 11C)**. In control group, PAS +ve materials were mainly distributed at the brush border and basement membrane of the renal tubules **(Fig. 12A)**. The

diabetic rats showed marked diminution in mucopolysaccharide content in some tubules, while others showed diffuse stain ability **(Fig. 12B)**. The group of rats treated with *Jasonia montana* showed that the mucopolysaccharide content more or less similar to the control level **(Fig. 12C)**.

Discussion

Diabetes is the most common endocrine disorder affecting millions of people worldwide. More than346 million of people worldwide suffer from this disease[26]. Medicinal plant plays an important role in management of diabetes especially in the developing countries. Among these herbal resources, the plant *Jasonia montana* which is used in the folk medicine in Sinai to treat diabetes. The present results show significant decrease in levels of serum insulin and marked elevation in levels of blood glucose in diabetic rats. This is attributed to the hypo-secretion of insulin by the pancreatic β-cells, as alloxan selectively destroys the pancreatic insulin secreting β-cells and induces hyperglycemia[27]. These findings are in agreement with previous studies which were reported by **Shah and Sivaraj**[28]. However, HOMA-IR recorded highly significant increase in diabetic rats, as high glucose concentrations cause the development of insulin resistance in peripheral tissues owing to impairment of both insulin secretion and insulin sensitivity[29]. The biochemical basis for insulin resistance induced by hyperglycemia is still unclear[30]. It is might be attributed to the modifications in structure of insulin receptors and the glucose transport system, resulting in impaired signal transmission[30]. HOMA-IR has proved to be a robust tool for the surrogate assessment of insulin resistance[31]. Medicinal plants have gained importance for the treatment of diabetes mellitus. Anti-hyperglycemic activity of most medicinal plants is due to their ability to restore the function of pancreatic tissues by causing an increase in insulin output and inhibition of the intestinal absorption of glucose[32]. Neheda extract exhibited significant anti-hyperglycemic and anti-hypoinsulinemia activity in the diabetic animals as compared to the untreated diabetic rats. Recently, **Hussein et al.**[33] studied the antioxidant activities of the ethanolic and aqueous extracts of the aerial parts of *Jasonia montana* in streptozotocin-induced diabetic rats and showed a significant decrease in the fasting blood glucose. This Neheda hypoglycemic

effect may be due to its effect on potentiatingof β-cells glucose-induced insulin release or increasing peripheral uptake of glucose[33]. Moreover, β-trophin hormone was recently described as a potent stimulator of mouse beta cell proliferation that is secreted by liver and adipose tissues[34]. Also, β-trophin hormone promotes β-cells in the pancreas to multiply and produce more insulin[35]. In the present work, the elevation in activities of these enzymes, AST, ALT and γGT in diabetic rats as compared with the corresponding control group. This may be attributed to the excessive release of such enzymes from the damaged liver cells into the blood circulation. The elevation in the activities of ALT, AST and γGT enzymes in diabetic rats reflects a state of hepatocytes injury. These liver enzymes can serve as markers of hepatocellular injury[36].This lesion may be attributed to the insulin resistance that induces excess synthesis of free fatty acids. The excess in free fatty acids is known to be directly toxic to hepatocytes[7]. The treatment of diabetic rats with *Jasonia montana* extract returned these enzymes to nearly the normal level. The essential oils (Camphor, borneol, bornyl acetate, chrysanthemol, intermediol, and 1,8-cineole) are the main components of *Jasonia montana* exhibit anti-hypoinsulinemic effect may be attributed to its protective effect against hepatocyte damage and was shown to have a modulatory effect on the values of HOMA-IR, which may be attributed to enhanced peripheral uptake of glucose[5]. These hepatocytes produced more β-trophin enhancing insulin production by β-cells of pancreas and enhancing body weight. Also, flvonoids components (flavonoid glycosides, flavonoid aglycons) of *Jasonia montana* enhance the functions of the liver and the reduction of body weight by inhibition the pro-inflammatory mediators and protection of hepatocytes[6]. In the current study, significant reduction in the serum total protein, albumin and globulin of the diabetic animal accompanied with slight non-significant elevation in the A/G ratio. Treatment of the diabetic rats with *Jasonia montana* extract resulted in elevating the serum total proteins, albumin and globulin levels. This indicated that *Jasonia montana* had a hepato-protective effect and improved the liver function. These effects may be due to the presence of flavonoids (bioflavonoids) in *Jasonia montana* extract which are natural products. Flavonoids have the capability to modulate the enzymes activity,

affect the behavior of many cell systems and possess many significant antihepatotoxic, antiallergic and anti-inflammatory effects[37]. Therefore our findings are in agreement with **Hussein and Farghaly**[38] who studied the protective activity of *Jasonia* ethanolic extract against liver and kidney damage.

The current study reveals high prevalence of hypercholesterolemia, hypertriglyceridemia, high LDL and low HDL levels in diabetic rats which are well known as risk factors for cardiovascular diseases and affect patients with diabetes[39]. **Kinosian et al.**[40] study showed that the changes in TC/HDL and LDL/HDL ratios were better predictors of coronary heart disease than the changes in only LDL. In the present investigation, diabetic animals showed marked elevation in total lipids, cholesterol, triglycerides and in the ratios of TC/HDL and LDL/HDL in diabetic group when compared with the control group. The current observations are in analogy to earlier results obtained by **Dineshkumar et al.**[41]. Since insulin has a potent inhibitory effect on lipolysis in adipocytes, insulin deficiency is associated with excess lipolysis and increased influx of free fatty acids into the liver[42]. This stimulates the hepatic triglyceride synthesis leading to hypertriglyceridemia as well as over-production of LDL and VLDL by the liver[42]. In the present work results showed significant amelioration of the lipid profiles by reducing the values of TL,TC, TG, LDL, VLDL and ratios of TC/HDL and LDL/HDLand elevating HDL levels in treated animals. Plants such as *Jasonia montana* contain high levels of polyphenols which are excellent antidiabetic, antioxidant and anticholestatic effects[7]. Moreover, flavonols such as quercetinglucuronide and kaempferol3-O-acetyl-glucoside have antioxidant properties that include direct scavenging of reactive free radicals, chelating of trace metal ions involved in free radical formation, inhibition of enzymes involved in free radical production[43]. Quercetin is the most common *Jasonia montana* polyphenols. Also, *Jasonia montana* quercetin derivatives like artemitin, chrysosplenetin, and jaceidin, act as antioxidants *in vitro* by scavenging reactive oxygen and nitrogen species and chelating redoxactive transition metal ions[44]. LDL oxidation results in atherosclerotic plaques formation that leads to the cardiovascular disease[44]. Diabetes mellitus is characterized by hyperglycaemia that is strongly linked to nephropathy. So that, diabetic

patients are at high risk for many renal complications[45]. In the current study, the increased levels of creatinine, urea and uric acid indicate kidney dysfunction in the diabetic rats. *Jasonia montana* extract induced significant improvements in kidney functions in the treated rats. **El-Nashar 2007.**[45] reviewed that polyphenols improved the kidney weight and serum levels of urea, nitrogen, creatinine and creatinine clearance as well as increased the activity of superoxide dismutase in the kidney. While, many authors such **Badary et al.**[46] and **Mohamed et al.**[47] found that flavan one produced significant protection of renal function by significant reduction in serum urea and creatinine concentrations, decreased polyuria and reduction in body weight loss, marked reduction in urinary fractional sodium excretion as well as protected kidney tissues. Finally, **van Hoorn et al.**[48] noticed that flavonoids lowered creatinine and urea concentration, both indicating a better postoperative kidney functions.

In conclusion, the results of the present study suggest that treated diabetic rats with *Jasonia montana* extract resulted in lowering hyperglycemia and improving metabolic abnormalities induced by diabetes also it has an important role through its antioxidant capacity. So, more toxicological studies must be done before recommendation of usage *Jasonia montana* as hyperglycemic drug.

References

1. **Patel DK, Kumar R, Prasad SK, Sairam K, HemALTha S, et al (2011):** Antidiabetic and *in vitro*antioxidant potential of Hybanthus enneaspermus(Linn) F.Muell in streptozotocin-induced diabetic rats. *Asian Pac J Trop Biomed.***1**(4): 316-322.
2. **Samreen R (2009):** Diabetes mellitus. *Scientific Research and Essay.***4** (5): 367-373.
3. **Bhattacharyya B, Johri BM (1998):** Flowering Plants Taxonomy and Phylogeny. Springer-Verlag, Norsa Publishing House, New York, 526.
4. **Merxmuller H, Liens P, Roesseler H, HeywoodJB, HarborneJB, Turner BL, et al (1977):** The Biology and Chemistry of Compositae. Academic Press, New York,590.
5. **Hammerschmidt FJ, Clark AM, Soliman FM, El-Kashoury ESA, Abd El-Kawy MM, El-Fishawy AM, et al (1993):** Chemical composition and antimicrobial activity ofessential oils of *Jasonia candicans* and *Jasonia montana*, *PlantaMedica.***59**:68-70.
6. **Ahmed AA, Ali AA, Mabry TJ, et al (1989):** Flavonoid a glycones from *Jasoniamontana*, *Phytochemistry.***28**:665-7.
7. **Hussein MA, Abdel-Gawad SM, et al(2010):** Protective effect of *Jasonia montana* against ethinyl estradiol induced cholestasis in rats. *Saudi Pharmaceutical Journal.***18**:35-45.
8. **Zhao B, Li X, He R, Cheng S, Xin W, et al(1989):** Scavenging effect of extracts of green tea and natural antioxidants on active oxygen radicals. *Cell Biophys.* **14**:175- 85.
9. **BouktaibM (2002):** Regio- and stereoselective synthesis of the major metabolite of quercetin, quercetin-3-O-b-Dglucuronide. *Tetrahedron Letters.* **43**: 6263-6266.
10. **MohamedAEl-HH(2007):** Jasonone, a nor-sesquiterepene from *Jasonia montana*. Z. Naturforsch. B: *Chem. Sci.* **62b**: 125–128.
11. **Malaisse WJ (1982):** Alloxan toxicity to the pancreatic B-cell. A new hypothesis. Biochem. Pharmacol. **31**: 3527-3537.
12. **TrinderP(1969):** Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. *Ann Clin Biochem*. **6**: 24-27.
13. **Reeves WG (1983):** Insulin antibody determination: theoretical and practical considerations. *Diabetologia*. **24**:399- 403.
14. **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.
15. **Junge W, Wilke B, Halabi A, Klein G, et al(2004):** Determination ofreference intervals for serum creatinine, creatinine excretion and creatinine clearance with an enzymatic and a modified Jaffe method. *Clin. Chim. Acta*. **344**:137–148.
16. **Patton CJ, Crouch SR(1977):** Spectrophotometric andkinetics investigation of the Berthelot reaction for the determination of ammonia. *Anal. Chem.***49**: 464-469.
17. **Tietz NW (2007):** Fundamentals of Clinical Chemistry, 6thEdition, W.B. Saunders, Philadelphia,PA.
18. **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.
19. **KytziaHJ(2005):**Reference intervals for GGT according to the new IFCC 37°C reference procedure.*Clin.Chem .Lab Med*.**43**:69.
20. **Tietz NW(1995):** Clinical Guide to Laboratory Tests, 3rdED. WB Saunders company, Philadelphia: 518-522.

21. **BucoloG, David H(1973):** Enzymatic determination of serum triglycerides quantitatively. *Clin. Chem.* **19**: 475-481.

22. **Knight JA, AndersonS, RawieJM, et al (1972):**chemical basies of the sulfo-phospho-vanilin reaction for estimation total serum lipid .clin.chem.**18**:199-202.

23. **Sugiuchi H (2005):** History of development and technical details of the homogenous assay for HDL and LDL cholesterol. *Eng. J. Med.* **1**:4-11.

24. **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).

25. **Suvarna SK, Layton C, Bancroft JD, et al (2013):** Bancroft's Theory and Practice of Histological Techniques. 7th Ed. Elsevier, Churchill Livingstone, Beijing, China. **Pp** 178-186, 221-224.

26. **Danaei G, Finucane MM, Lu Y, Singh GM, Cowan MJ, Paciorek CJ, et al(2011):** National, regional, and global trends in fasting serum glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2.7 million participants. *Lancet.* **378**: 31-40.

27. **Shah J, Patel M, Patel K, Gandhi T, et al (2007):** Evaluation of Anti-diabetic and Anti-oxidant Activity of *Centratheru manthelmintica* in STZ induced Diabetic Rats. *Internet J Pharmacol.***6:** 1-10.

28. **Sivaraj A, Devi K, Palani S, kumar PV, kumar BS, David E, et al (2009):** Anti-hyperglycemic and Anti-hyperlipidemic effect of combined plant extract of *Cassia auriculata* and *Aeglemarmelos i*n alloxan (alloxan) induced diabetic albino rats. *Int J PharmTech Res.* **1**(4): 1010-1016.

29. **Rossetti L, Giaccari A, Defronzo RA, et al (1990):** Glucose toxicity. *Diabetes Care.***13**: 610–630.

30. **Ordonez P, Moreno M, Alonso A, Fernandez R, Dıaz F, Gonzalez C, et al (2007):** Insulin sensitivity in alloxan-induced diabetic rats treated with different doses of 17β-oestradiol or progesterone. *Exp. Physiol.* **92**(1): 241–249.

31. **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.

32. **Malviya N, Jain S, Malviya S, et al (2010):** Antidiabetic potential of medicinal plants. *Acta. Pol. Pharm.* **67**(2):113-118.

33. **Hussein MA (2008):** Antidiabetic and antioxidant activity of *Jasonia Montana* extract in streptozotocin-induced diabetic rats. *Saudi Pharmaceutical Journal.***16**:214-21.

34. **Yi P, Park JS, Melton DA, et al (2013):** Betatrophin a hormone that controls pancreatic beta cell proliferation. *Cell.*,**153**:747–758

35. **Liu YN, Jung JH, Park H, Kin HS, et al (2014):** Olive Leaf Extract Suppresses mRNA Expression of Pro-inflammatory Cytokines and Enhances IRS-1 Expression in the Rats with Streptozotocin and High-Fat Diet induced Diabetes. *In press.*

36. **Kim KA, Oh SO, Park PW, ParkJY, et al (2005):** Effect of probenecid on the pharmaco kinetics of carbamazepine in healthy subjects. *EurJ. Clin. Pharmacol.* **61**(4):275—80.

37. **Rathee JS, Hassarajani SA, Chattopadhyay S, et al (2006):** Antioxidant activity of Mammealongifolia bud extract. *Food Chem.*, **99**: 436–443.

38. **Hussein MA, Farghaly HS(2010):** Protective Effects of *Jasonia montana* against Lipid Peroxidation in Liver and Kidney of Iron-overloaded Rats. *Australian Journal of Basic and Applied Sciences.* **4**:2004-12.

39. **Ravi K, Rajasekaran S, Subramanian S, et al (2005):** Antihyperlipidemia effect of *Eugenia Jambolana* seed kernel on alloxan induced diabetes in rats. *Food chem. Toxicol.* **43**: 1433-1439.

40. **Kinosian B, Glick H, Preiss L,Puder KL, et al(1995):** Cholesterol and coronary heart disease: predicting risks in men by changes in levels and ratios. *J. Inves. Med* .**43**: 443-450.

41. **Dineshkumar D, AnalavaM, Manjunatha M, et al (2010):** Antidiabetic and hypolipidaemic effects of few common plants extract in Type 2 diabetic patients at Bengal. Int. J .Diabetes &Metab.18: 59-65.

42. **Ohno T, Horio F, Tanaka S, Terada M, NamikawaT, kitoh J, et al (2000):** Fatty liver and hyperlipidemia in IDDM (insulin-dependent diabetes mellitus) of alloxan treated shrews. *Life Sci.***66:** 125-131.

43. **SrokaZZ (2005):** Antioxidative and antiradical proper- ties of plant phenolics. *Natur for sch.* **60**: 833-844.

44. **Yap S, Qin CX, Woodman OL, et al (2010):** Effects of resveratro land flavonols on cardiovascular function: Physiological mechanisms. *Biofactors.***36**: 350–359.

45. **El-Nashar NG (2007):** Development of primary liver cell culture from fish as a valuable tool in nutrition and biotechnology research. Ph.D. Thesis, Faculty of Home Economics, Minoufiya University, Egypt.

46. **Badary OA, Abdel-Maksoud S, Ahmed WA, Owieda G H, et al (2005):** Naringenin attenuates cisplatin nephrotoxicity in rats. *Life Sci.* **76**(18): 2125-35.

47. **Mohamed AF, Ali HAG, Hamamy MI,Abdel-Sattar E, et al(2005):** Antioxidant and hepato protective effects of Eucalyptus maculata. *Med. Sci.Monit*. **11**(11): BR426-31.

48. **van Hoorn DE, Nijveldt RJ, Boelens PG, et al (2006):** Effects of preoperative flavonoid supplementation on different organ functions in rats. *J Parenter Enteral Nutr*. **30**(4):302-8.
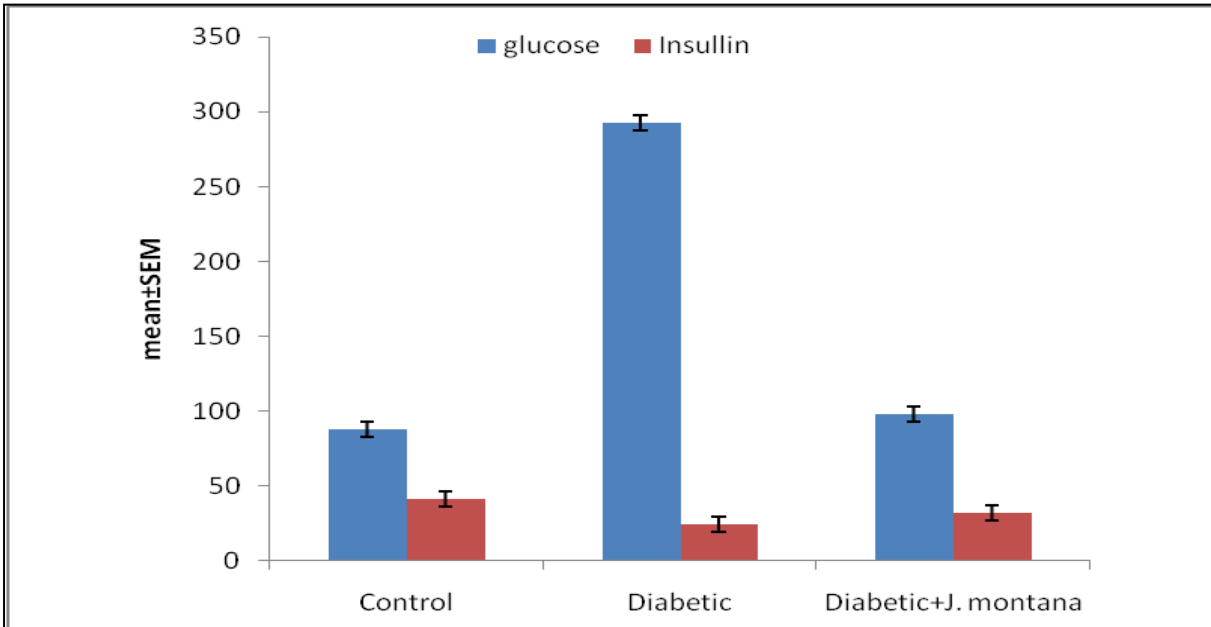
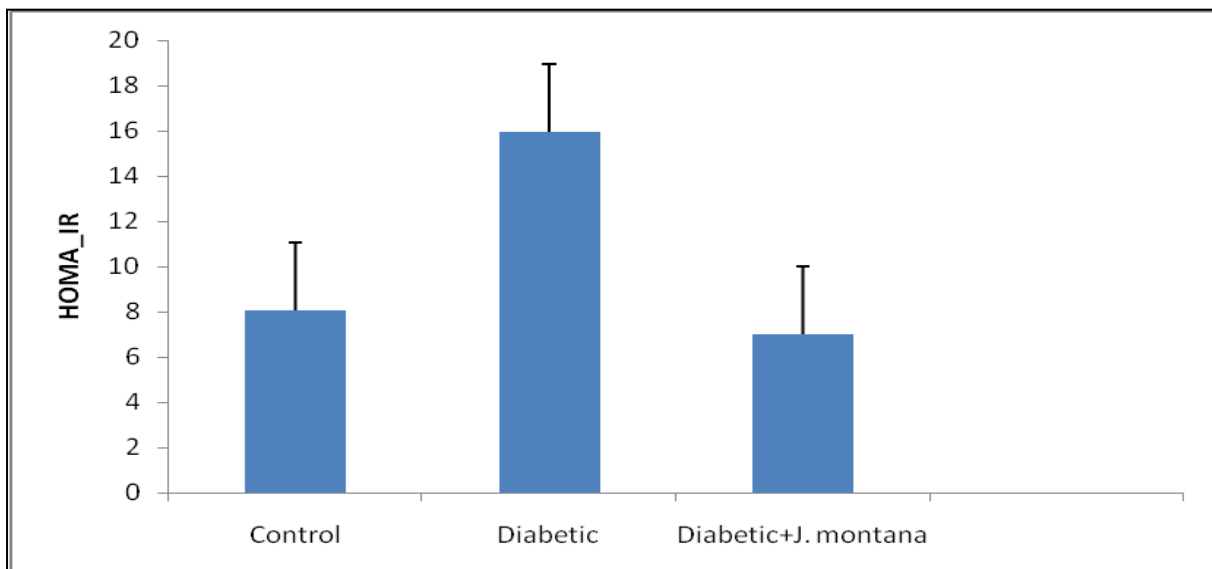**Fig.1: Glucose (mg/dl) and insulin (µIU/ml) levels in the control, diabetic and treated groups.**

Fig. 2: HOMA-IR in the control, diabetic and treated groups.
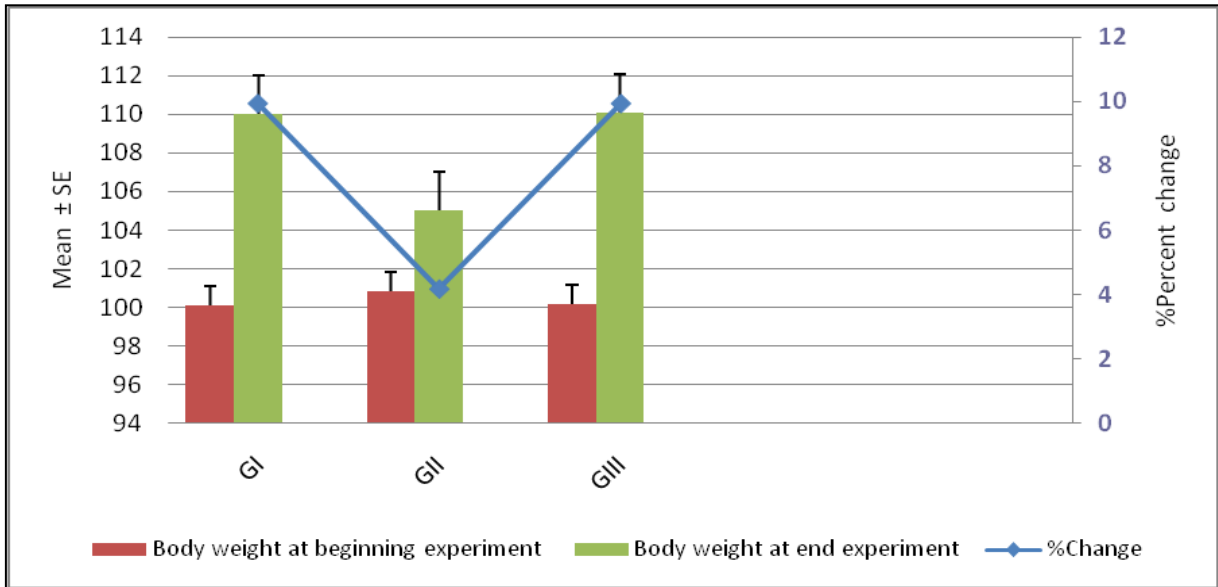
Fig.3: Changes in the body weight (kg) of the control, diabetic and treated groups at the beginning and the end of the study.
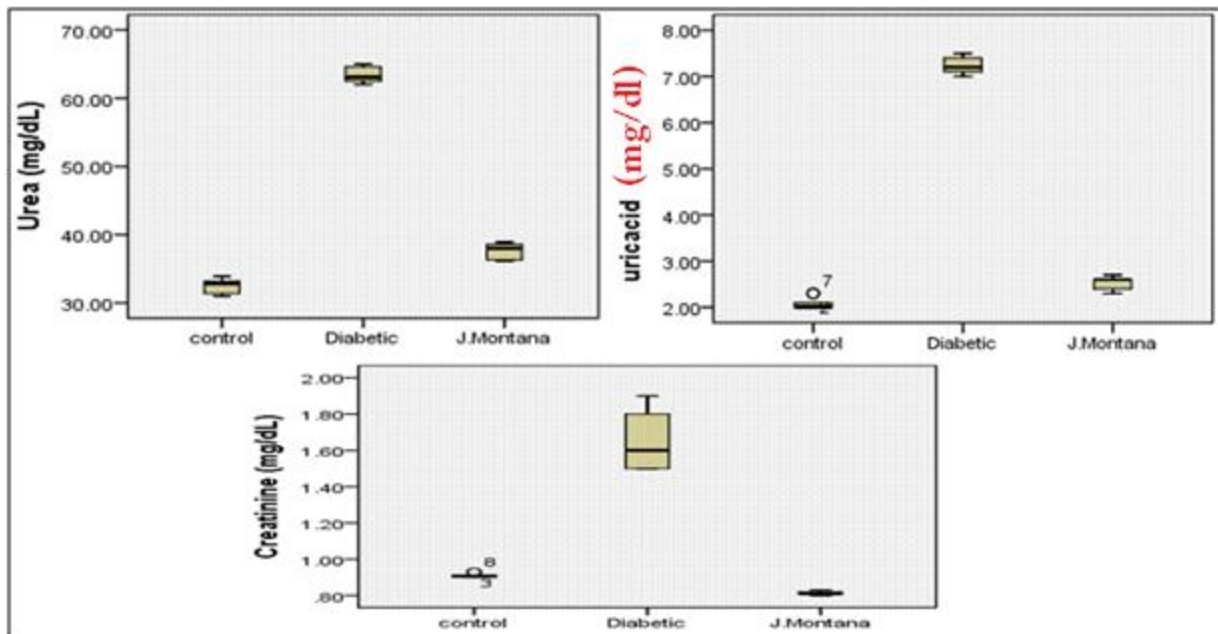
Fig. 4: Levels of creatinine, urea and uric acid in the control, diabetic and treated groups.
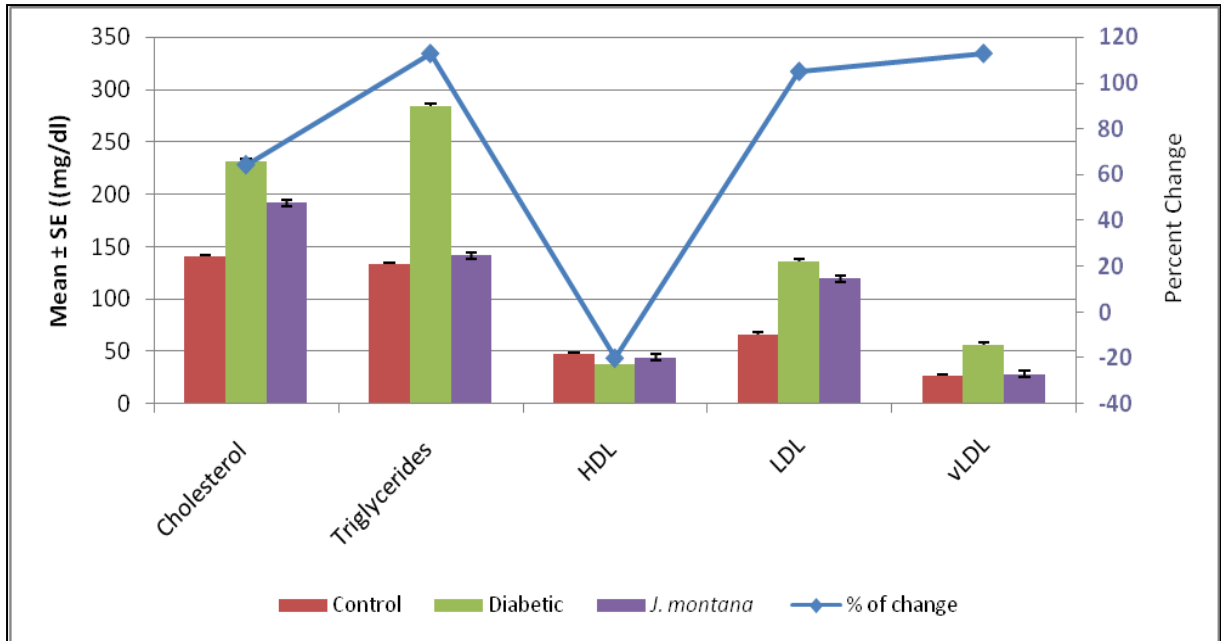
Fig.5: Changes in triglyceride (TG), total cholesterol (TC), HDLcholesterol (HDL-C), LDL-cholesterol (LDLC) and vLDL-cholesterol (vLDLC) parameters in the control, diabetic and treated groups.
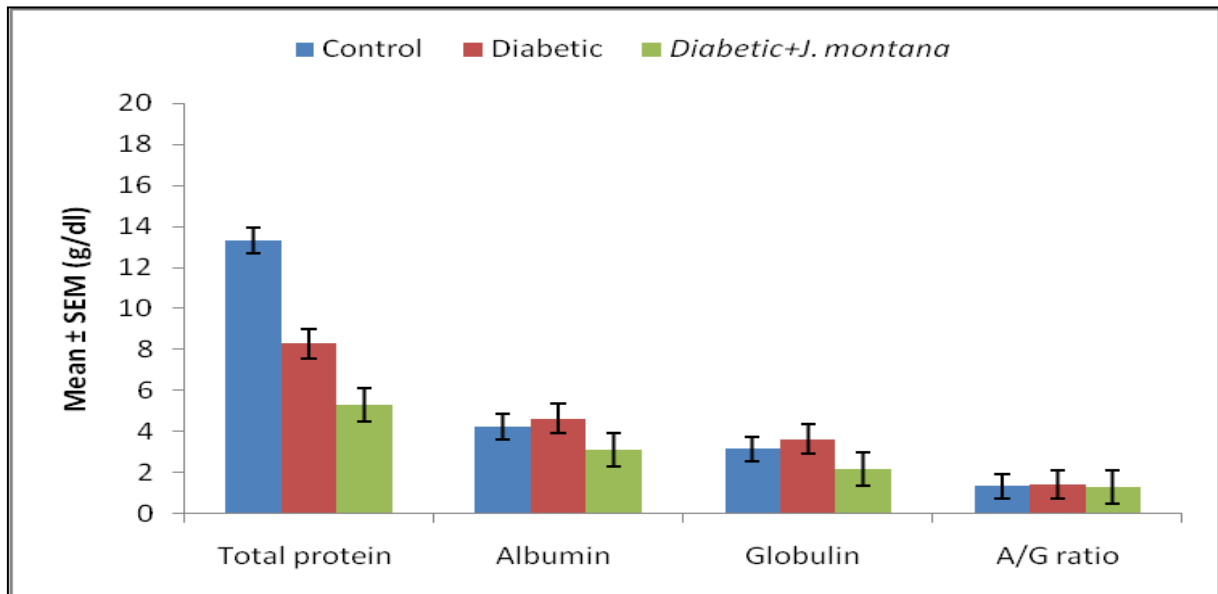

Fig. 6: Changes in the levels of serum proteins profile (g/dl) and A/G ratio in the control, diabetic and treated groups.
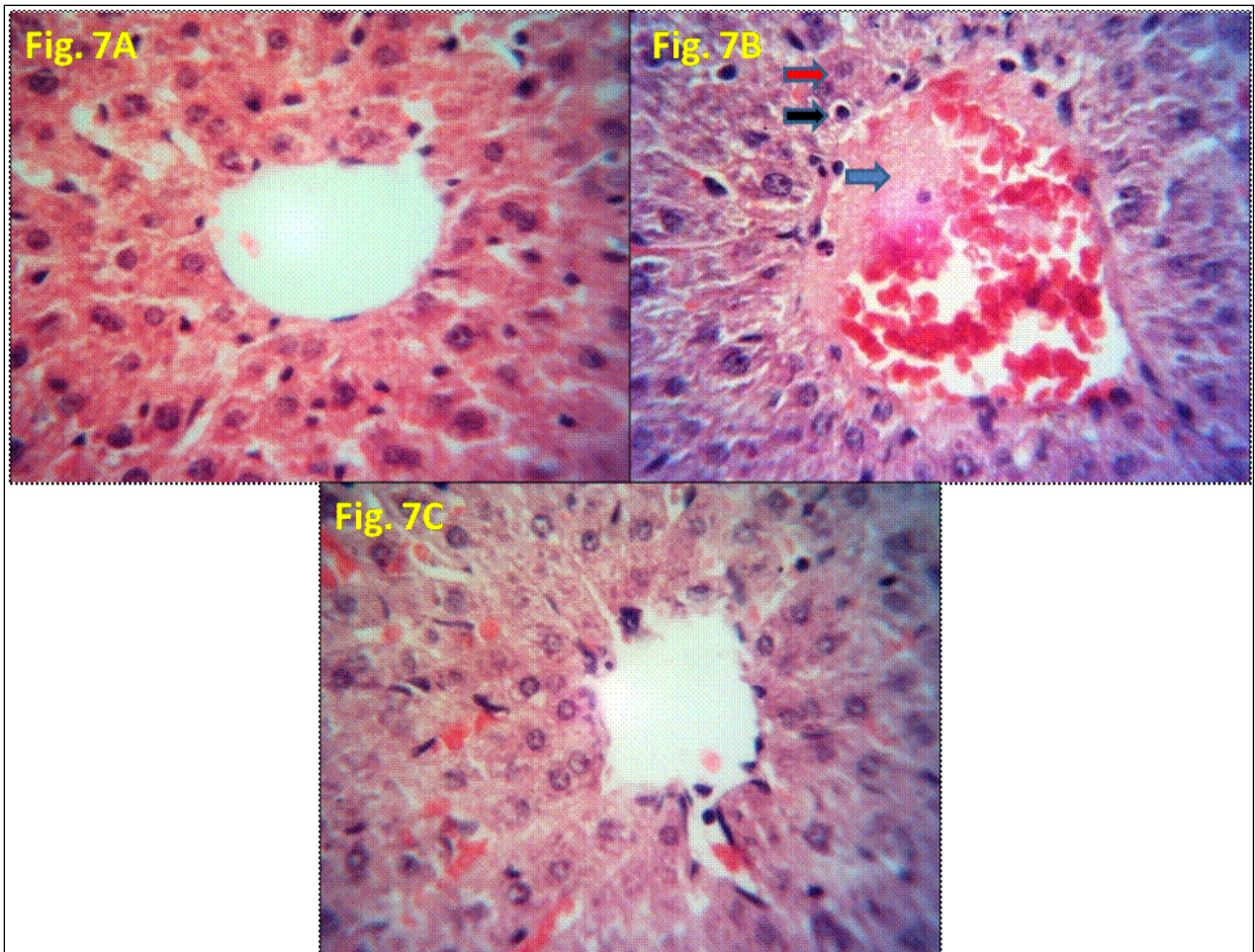
Fig.7. A) A photomicrograph of the control liver of adult albino rat showing normal lobular pattern with a centrilobular vein and radiating irregular branching and anastomosing plates of hepatocytes with intervening sinusoids lined with endothelial cells. Most of the hepatocytes have vesicular nuclei 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 arrow) around the central vein. **C)** A photomicrograph of the liver of diabetic adult albino rat treated with *Jasonia montana* showing that most of hepatic lobules are almost similar to that of the control group. **(Hx. & E. x400)**.
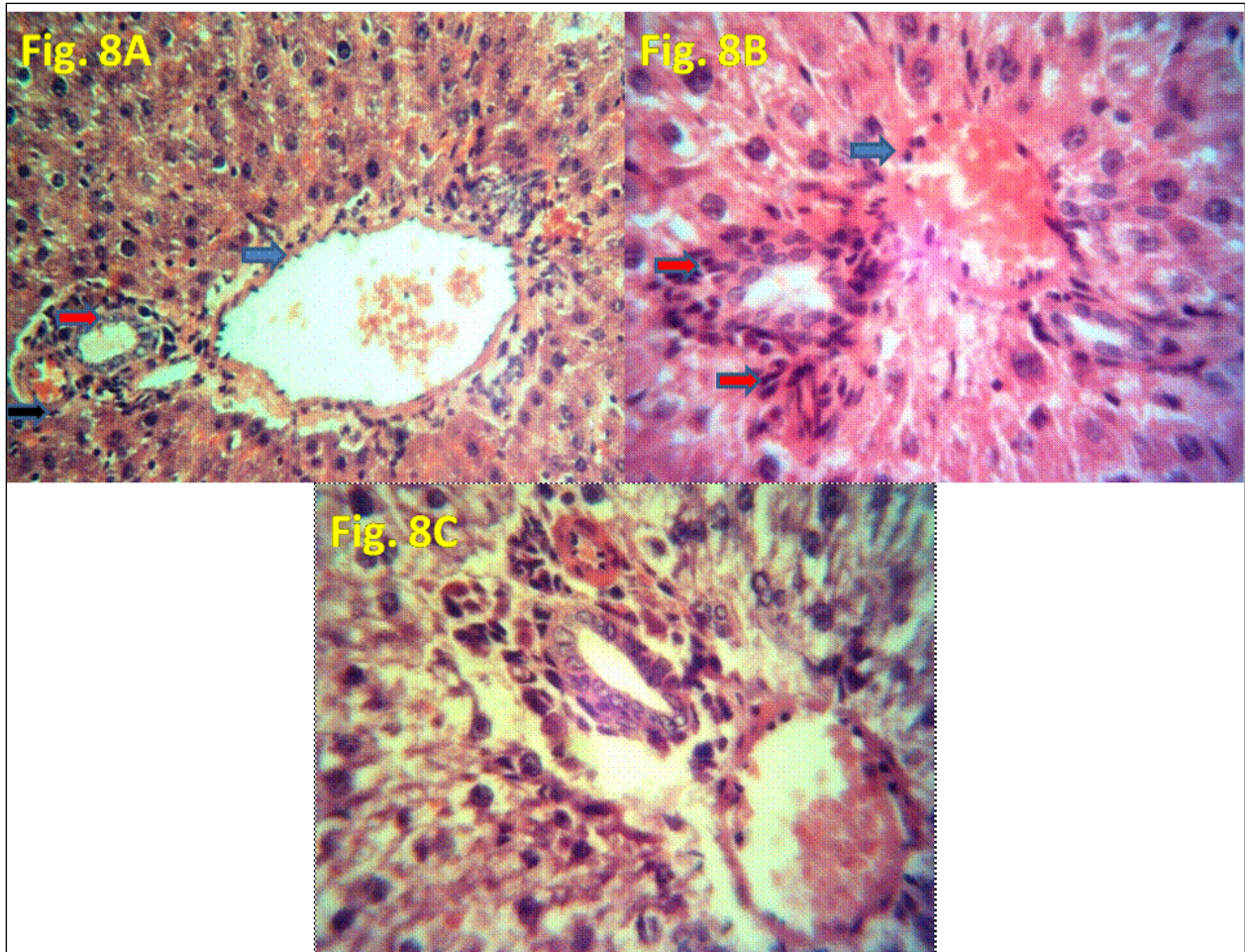
Fig. 8. **A)** A photomicrograph of the control liver of adult albino rat showing normal portal triad consisting of a branch of portal vein (blue arrow), branch of the hepatic artery (black arrow) and bile ductule (red arrow). **B)** A photomicrograph of the liver of diabetic adult albino rat showing marked congestion of portal vein (blue arrow) and lymphocyte infiltration (red arrows). **C)** A photomicrograph of the liver of diabetic adult albino rat treated with *Jasonia montana* showing that the portal triads are almost similar to that of the control group. **(Hx. & E. x400)**.
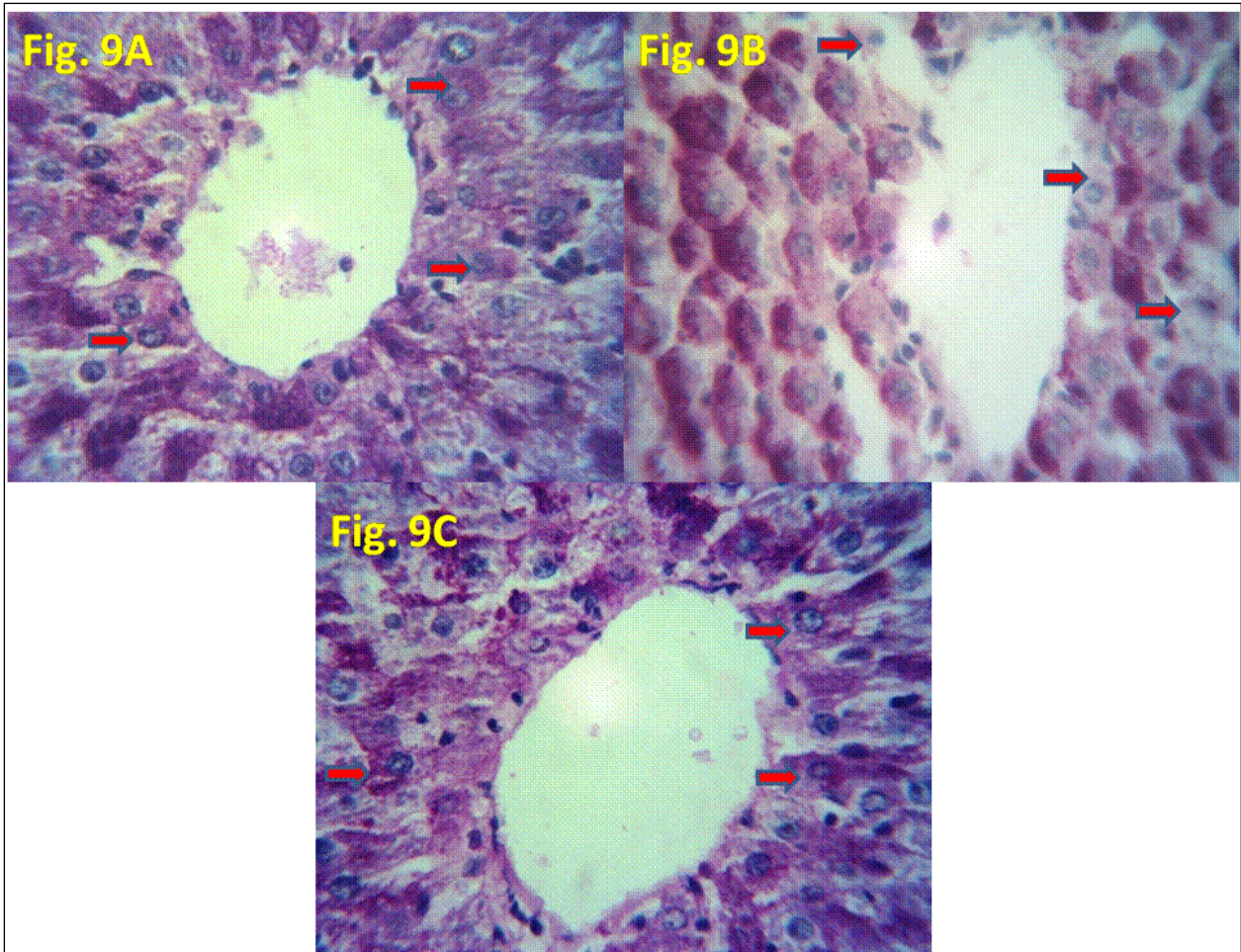
Fig. 9. A) A photomicrograph of the control liver of adult albino rat showing strong PAS+ve granules in most of the hepatocytes (red arrows). **B)** A photomicrograph of the liver of diabetic adult albino rat showing weak PAS+ve granules in most of the hepatocytes (red arrows). **C)** A photomicrograph of the liver of diabetic adult albino rat treated with *Jasonia montana* showing strong PAS+ve granules in most of the hepatocytes. **(PAS. x400)**.
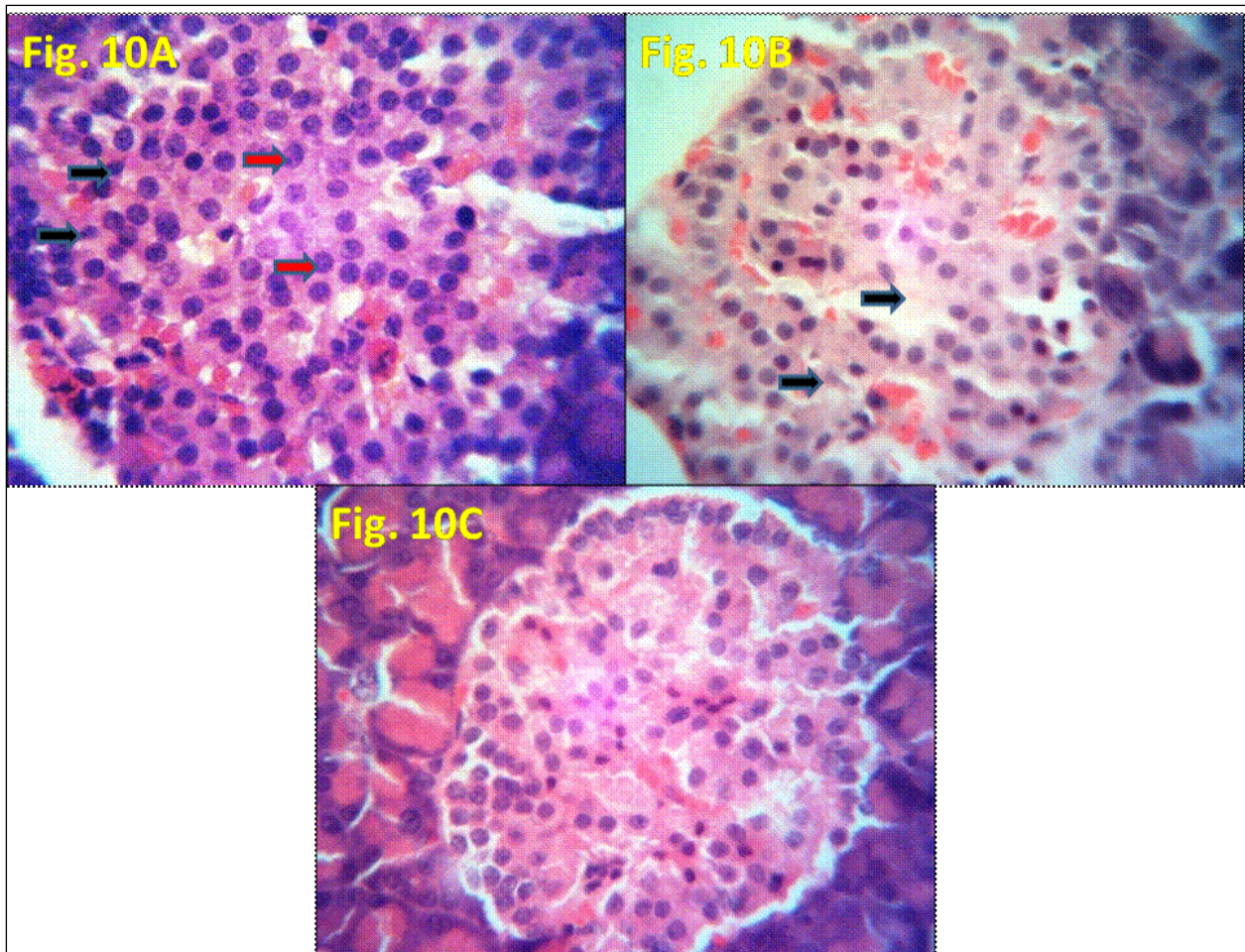
Fig. 10. A) A photomicrograph of a control pancreas of adult albino rat showing the normal cellular distribution in the islet of Langerhans, the red arrows show beta cells while the black arrows show the alpha cells. **B)** A photomicrograph the pancreas of diabetic adult albino rat showing marked degenerative changes in the islet (black arrows) with decreased islet cellularity. **C)** A photomicrograph the pancreas of diabetic adult albino rat treated with *Jasonia montana* showing partial return to the normal cellular distribution within the islet of Langerhans. **(Hx. & E. x400)**.
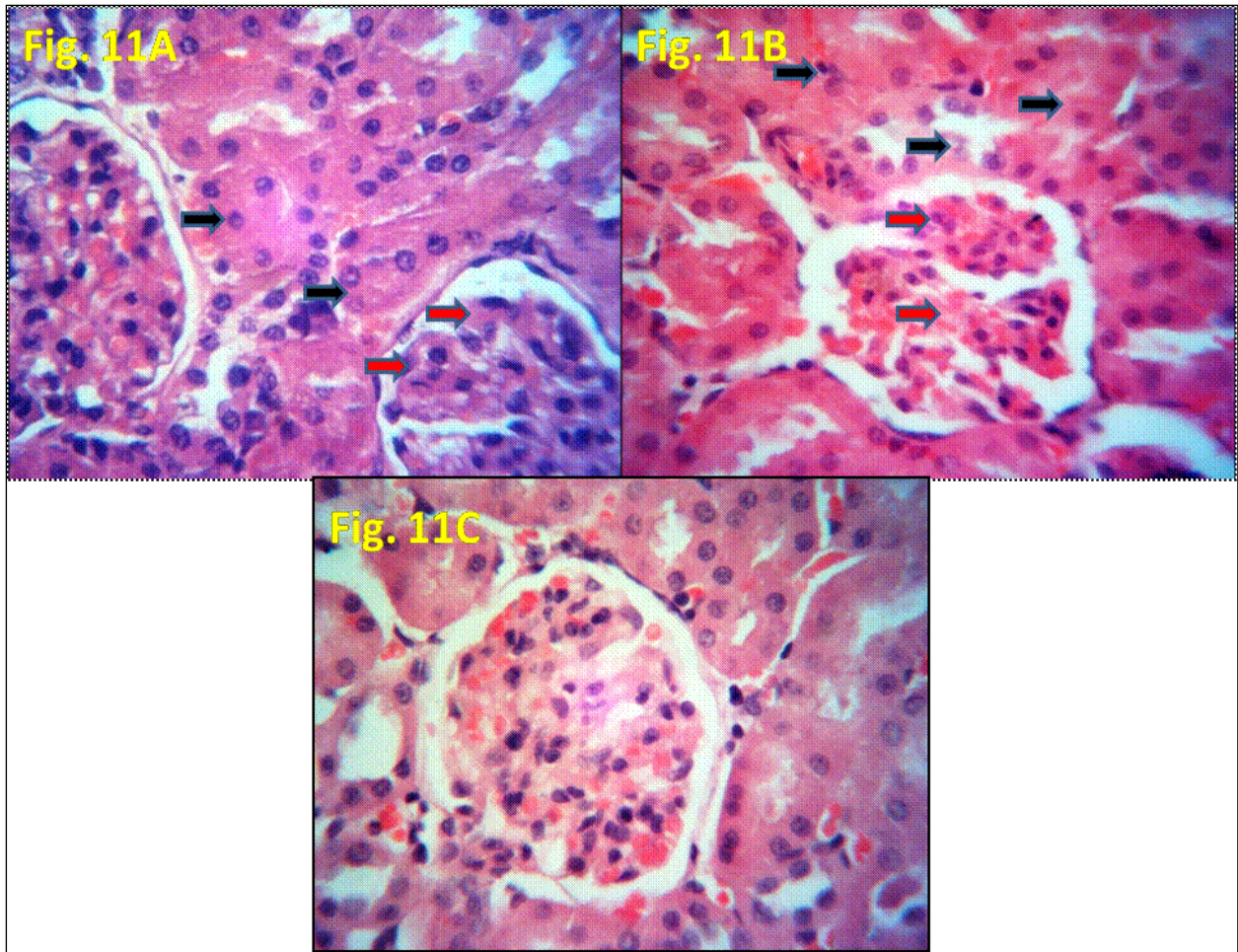
Fig. 11. A) A photomicrograph of a control kidney of adult albino rat showing normal glomerular tufts, normal glomeruli with normal Bowman's capsules (red arrows) and normal ascending and descending tubules (black arrows). **B)** A photomicrograph of the kidney of diabetic adult albino rat showing glomerular 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 *Jasonia montana* 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).**
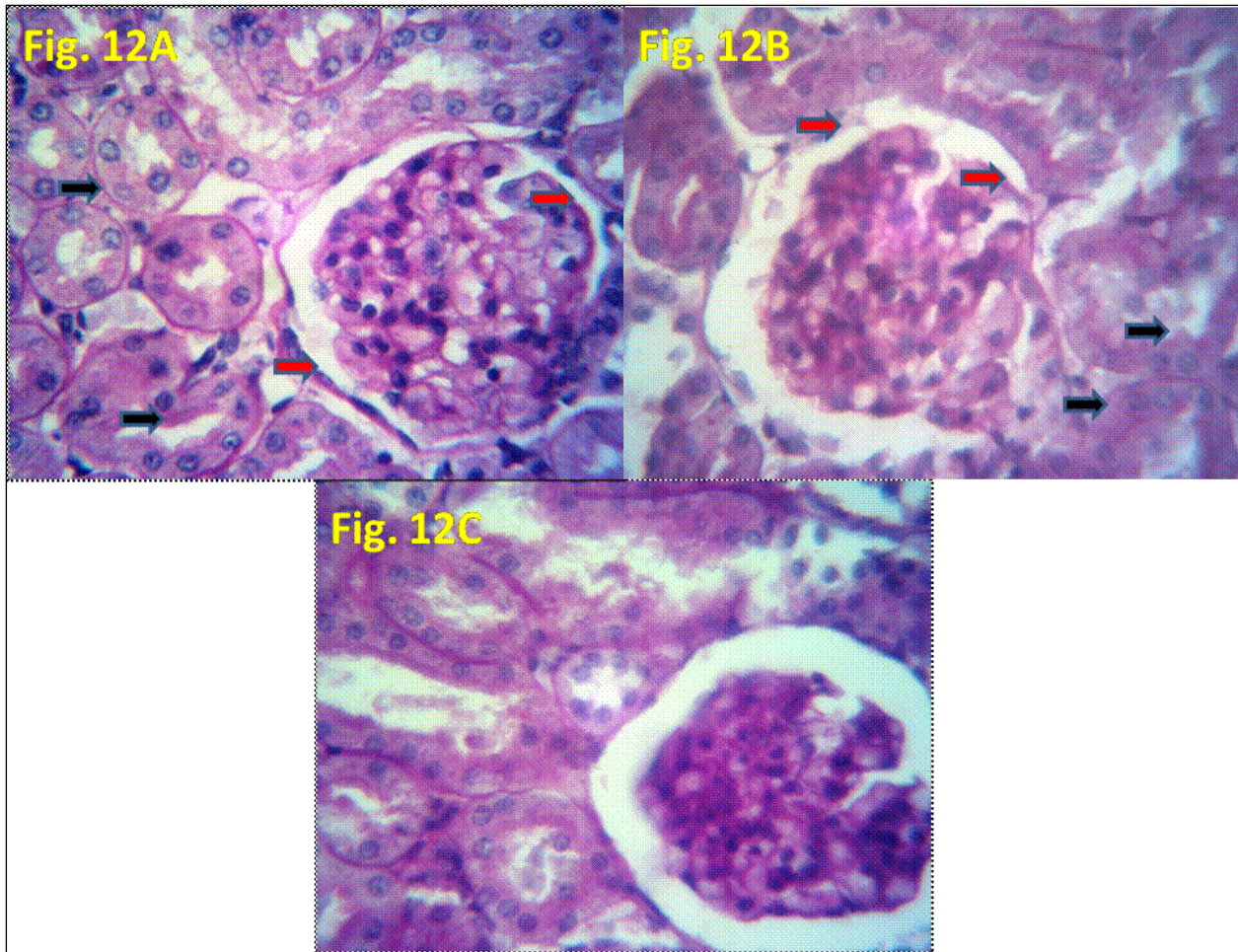
Fig. 12. A) A photomicrograph of a control kidney of adult albino rat showing strong PAS+ve granules in the glomeruli basement membrane(red arrows), basement membrane and brush borders of the ascending and descending tubules(black arrows). **B)** A photomicrograph of the kidney of diabetic adult albino rat showing decrease stainability of PAS +ve granules in both glomeruli (red arrows)and ascending and descending tubules (black arrows). **C)** A photomicrograph of the kidney of diabetic adult albino rat treated with *Jasonia montana* showing that the mucopolysccharide 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 + *Jasonia. montana*
Glucose (mg/dl)	87.44±0.77	292.20±0.84***	98.15±0.35[a]
Insulin (µIU/ml)	41.36±0.45	37.72±0.77**	24.56±0.24[a,b]
HOMA-RI	8.03±0.23	24.54±0.36**	6.97±0.11

*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+ *Jasonia. montana*
Body weight at beginning experiment	100.1±0.04	100.82±0.36	100.16±0.06
Body weight at end experiment	110.02±0.01	105.01±0.02	110.09±0.07
%Change	9.92	4.98**	9.92

*** $p<0.01$ 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+ *Jasonia. montana*
ALT (IU/L)	22.46±0.55	33.52±0.40***	27.52±0.47[a]
AST (IU/L)	37.02±0.59	53.24±0.71***	37.96±0.38[a]
γGT(IU/L)	2.28±0.11	9.66±0.054***	4.52±0.04[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+ *Jasonia. montana*
Creatinine (mg/dL)	0.91±0.005	33.52±0.060***	27.52±0.47[a]
Urea (mg/dL)	37.02±0.54	53.24±1.07***	37.96±0.38[a]
uric acid	2.28±0.10	9.66±0.08***	4.52±0.04[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 + *Jasonia. montana*	% of change
Total lipids (mg/dl)	474.0±0.05	1430.0±0.07***	624.0±0.04ᵃ	201.68%
TC (mg/dl)	141.08±0.37	231.52±0.52***	191.84±0.35ᵃ	64.28%
TG (mg/dl)	133.10±0.77	283.96±0.86***	141.73±0.42ᵃ	112.74%
HDL (mg/dl)	47.88±0.48	38.08±0.38**	44.25±0.29ᶜ	-19.94%
LDL (mg/dl)	66.56±0.47	136.40±0.59***	119.43±0.10ᵃ	104.92%
VLDL (mg/dl)	26.62±0.16	50.80±0.16***	28.32±0.083ᵃ	112.78%
TC/HDL	3.00±0.00	6.08±0.03***	3.21±0.01ᵃ	168.34%
LDL/HDL	1.38±0.02	3.58±0.03***	2.71±0.02 ᵃ	157.55%

*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 + *Jasonia. montana*	% of Change
Total protein (g/dL)	7.04±0.08	5.30±0.11**	8.28±0.05ᶜ	-24.72%
Albumin (g/dL)	4.24±0.07	3.12±0.05**	4.64±0.05ᶜ	-26.4%
Globulin (g/dL)	3.16±0.05	2.18±0.06**	3.64±0.08ᶜ	-31.01%
A/G ratio	1.34±0.05	1.22±0.02	1.42±0.02	----

*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. ᶜ$p<0.01$: significant increase in the parameters levels in the treated group in comparison to the diabetic group.