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The Hypoglycemic And Hypolipidemic Activity of Wolfberry (Lycium Barbarum) in Alloxan Induced Diabetic Male Rats

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Abstract: *This study was focused on studying the probable antidiabetic and antilipidemic activity of dry wolfberry fruit powder on Alloxan induced diabetic male rats. In this study, 40 male rats were divided into 4 groups; the first group was the negative control group (G1). The other three groups (30 rats) were intraperitoneally injected with a single dose of Alloxan monohydrate to induce diabetes. The second group (G2) was the positive control diabetic group fed normal diet. The third (G3) and the fourth (G4) groups were treated with 10 g/kg and 20g/kg body weight (b.w.) of dry wolfberry fruit powder in the diet, respectively for 4 weeks. Alloxan induced significantly diabetes in the positive control group. In addition, the antioxidant enzymes, serum immunoglobulins IgE and HDL were significantly decreased as a result of diabetes in the positive control group. In addition, histology of pancreas and kidney showed histopathological changes as a result of diabetes in the positive control group. Treating the diabetic rats with wolfberry at concentration 10 and 20g/kg of b.w. in both G3 and G4, respectively restored the altered biochemical parameters and the histopathological changes and restored them nearly to the normal levels. This study showed that wolfberry administration has considerable antidiabetic and hypolipidemic activity in alloxan induced diabetic male rats.*

Key words: *wolfberry, antidiabetic, hypolipidemic, rats, Alloxan , diabetes.*

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I. Introduction

The deciduous woody perennial plant, *Lycium barbarum* L. (Wolfberries), 1-3 m high, belongs to the family *Solanaceous* and the fruits are sold as a dietary supplement and was widely used as food presenting highly advantageous nutritive and antioxidant properties[1]. Wolfberries are mainly used as fresh fruits, as tea, or as an ingredient in bread or chewy like raisins[2]. The fruits of *L. barbarum* L. contained numerous bioactive constituents including, but not limited to, polysaccharides, carotenoids, flavonoids, betaine, taurine, scopoletin, β-sitosterol, ρ-coumaric acid, and daucosterol and vitamin C content is significantly higher in the fresh (raw) fruits than in most other fruits and vegetables [3,4,5]. Wolfberries have antioxidant and antidiabetic properties and potential health benefits due to the presence of Zeaxanthin and lutein are carotenoids; however, bioavailability data are largely from in vitro and animal studies, whereas human data are very limited[6,7]**.** Moreover, wolfberry showed a moderate antioxidant potential, and a mild antimicrobial activity against Gram-positive bacteria [1,6]**.** Flow cytometry analysis showed also that wolfberry exerted a stimulatory effect on apoptosis of MCF-7 cells, and induced the cell-cycle arrest at the G0/G1 phase, with the observation of intracellular ROS production and DNA damage and reduces oxidation in patients with retinopathy[8,9]. In addition, wolfberry protects mice liver from carbon tetrachloride-induced oxidative stress and necroinflammation by reducing the hepatic necrosis and the serum ALT level induced by CCl_4 intoxication, inhibiting cytochrome P450 2E1 expression, and restoring the expression levels of antioxidant enzymes and decreasing the level of nitric oxide metabolism and lipid peroxidation induced by CCl_4 [10]. It is also useful in controlling blood lipid levels, preventing cardiovascular complications, and adjusting bodily functions in hyperlipidemic patients[11].

Administration of wolfberry to a D-galactose-induced mouse aging model *in vivo* inhibited non-enzymatic glycation[12]. Orally administration of wolfberry via drinking water for 30 days was effective in reducing streptozotocin-induced diabetes in rats by restoring abnormal oxidative indices to near normal levels[13]. Treating diabetic rats with wolfberry polysaccharides showed increased activity of antioxidant enzymes and increased scavenging of oxygen radicals. LBP treatment also resulted in a significant decrease in the concentration of fasting blood glucose levels, total cholesterol and triglyceride in diabetes mellitus mice[14] and rabbits[15].

This study aimed to evaluate the hypoglycemic, antioxidant, immunological and hypolipidemic activity of wolfberry in Aloxan induced diabetic male rats.

II. Materials And Methods

Animal basal diet

Dried wolfberry fruits were purchased from a spice shop in Jeddah, Saudi Arabia. A formal check that this material was wolfberry was carried out by specialists before the experiments were commenced. The conventional animal basal diet was obtained from a grain mill in Jeddah. Each 100 g consists of the following: 12% protein (17.14 g 70% casein), 4 g corn oil (4% fat), 0.3 g methionine (0.3%), 0.2 g choline chloride (00.2 %), 4 g minerals (4% minerals), 1 g vitamin mixture (1% vitamin), 4 g cellulose (4% fiber), and 69.36 g corn starch (69.36%). Wolf berry fruit powder was mixed in the diet in the ratio of 10 g/kg and 20 g/kg b.w. substituted from the corn starch value. The basal diet was stored in a dry place out of direct sunlight.

Animals and housing conditions

Forty adult male Albino rats (*Rattus norvegicus*) weighing (180-200 g) were obtained from the animal experimental unit of King Fahd Center for Medical Research, King Abdulaziz University. All the animal experiments were carried out under protocols approved by the Institutional Animal House of the University of King Abdulaziz at Jeddah, Saudi Arabia. The plan of this study specifically was approved by our institutional ethics committee at King Abdulaziz University (KAU-1435-15).

The animals were housed 6 in each cage and received normal basal diet and tap water in a constant environment (room temperature 28 ± 2◦C, room humidity 60±5%) with a 12 h light and 12 h dark cycle. The animals were kept under observation for two weeks prior to the start of the experiment to exclude any undercurrent infection.

Experiment design

The 40 rats were distributed into four groups as follows: i-ten normal rats for the first negative control group (G1) fed basal diet. The other 30 rats were intraperitonially injected with Alloxan monohydrate (150 mg/kg b.w.). dissolved in the distilled water) to induce diabetes according to Dash et al.[16]. After five days of injection, rats with blood glucose higher than 200 mg/dl were considered as being diabetic in the fasting state, and then divided into 3 groups as follows: ii- The second positive control group (G2) was diabetic rats fed normal basal diet. iii- The third group (G3) and the fourth group (G4) were diabetic rats fed basal diet supplemented with 10 and 20 g/kg b.w. dry wolfberry fruit powder for 4 weeks.

Physiological Parameters

Food intake (FI), water consumption were calculated every week but body weight gain (BWG) per day and food efficiency ratio (FER) at the end of the experiment were calculated according to the method of Davies and Morris[17].

Blood sample and serum separation

At the end of the experiment, rats were fasted 14–16 hours after their last feeding and blood samples were collected from the heart of each rat under anesthesia with diethyl ether. Blood sample of rats was centrifuged at 2,000 g for 10 minutes at 4◦C, and serum was removed and stored at -80◦C until analysis.

Dissection

Rats were sacrificed using ether anaesthesia by cervical dislocation, and then the abdomen was dissected and one kidney and a piece of the pancreas were dissected out and saved in saline buffer (0.9% NaCl) for histopathological investigations. The other kidney was kept in ice for kidney tissue homogenate preparation.

Kidney tissue homogenate preparation

A piece of kidney was cut into small pieces and washed with phosphate-buffered saline and then grinded in a homogenization buffer consisting of 0.05M Tris-HCl pH 7.9, 25% glycerol, 0.1Mm EDTA, and 0.32M (NH4)2SO4 and containing a protease inhibitor tablet (Roche, Germany). The lysates were homogenized on ice using a Polytron homogenizer. The homogenate was sonicated in an ice bath to prevent overheating for 15 seconds followed by 5 minute centrifugation at 12000 rpm and 4◦C. The supernatant was aliquoted and stored at -80◦C.

Determination of fasting blood sugar (FBS) and glycated hemoglobin Alc

Glucose concentration was estimated in the serum using an enzymatic colorimetric kit, GOD / PAP method according to the method described by Barham and Trinder[18] from Human (Germany). Hemoglobin Alc was estimated according to the method of Simon and Eissler[19] using Glycohemoglobin Reagent Set from POINTE Scientific Inc. (USA).

Determination of immunoglobulins

Immunoglobulins (IgA, IgM, IgE and IgG) were estimated in the serum according to Fahay and mckelevey[20] and Bernne[21] using a kit from Genway Biotech (USA).

Liver enzymes

Alanine aminotransferase (ALT) was estimated according to the method of Thefeld et al.[22] using Human Kit (Germany) according to the instruction of the supplier. The aspartate aminotransferase (AST) was estimated according to the method of Thefeld et al. [22] using Swemed Diagnostics kit (India). Alkaline phosphatase (ALP) was estimated according to the method of Rick et al.[23] using Human kit (Germany) according to the instruction of the supplier. Gamma-Glutamyl Transferase (GGT) was assayed using Architect/Aeroset Systems (USA) according to the method of Thomas[24].

Kidney functions

Urea was estimated in the serum according to the method described by Berthelot[25] and Fawcett and Scott[26] using enzymatic colorimetric kit from Human (Germany). Creatinine was estimated in the serum according to the method described by Bartels et al.[27] using photometric colorimetric kit from Human (Germany). Uric acid (UA) was estimated in the serum according to the method described by Barham and Trinder[18] using enzymatic colorimetric kit from Human (Germany).

Determination of Electrolytes

Sodium (Na^+) was estimated in the serum according to the method of Trinder[28] using colorimetric method from Human (Germany). Potassium (K^+) was estimated according to the method of Terri and Sesin[29] using Human kit from Germany.

Serum lipids

Serum total cholesterol (TC) and serum triglycerides (TG) were estimated according to the method of Young[30] using Spinreact Kit (Spain) according to the instruction of the supplier. Serum high density lipoprotein (HDL) was estimated according to the method of Naito[31] using Spinreact Kit (Spain) according to the instruction of the supplier. Serum low density lipoprotein (LDL) and serum very low density lipoprotein (VLDL) were calculated according to the equation applied by Srivastava *et al*.[32] .

Estimation of Antioxidants Enzymes activity

Superoxide dismutase (SOD) activity was estimated in the serum and in the kidney tissue homogenate according to the method described by Nishikimi et al.[33] Catalase (CAT) activity was estimated in the serum and in the kidney tissue homogenate according to the method described by Aebi [34] while Glutathione-S-transferase (GST) was estimated in the serum and in the kidney tissue homogenate according to the method described by Habig et al.[35]

Determination of lipid peroxide

Lipid peroxide was estimated by determination of malondialdehyde (MDA) in the serum and in the kidney tissue homogenate according to the method described by Ohkawa et al.[36]

Histopathological examinations

Kidney and pancreatic tissues were collected after animal sacrifice, fixed in 10% formalin, processed routinely, and embedded in paraffin. 5 μm thick sections were prepared and stained with hematoxylin and eosin (H&E) dye for microscopic investigation Drury et al.[37]. The stained sections were examined and photographed under a light microscope.

III. Statistical Analysis

Values were analyzed using SPSS program to calculate the t-test and the mean ± SD and then analyzed using one way analysis of variance (ANOVA, $p<0.05$) using a protected least significant difference (LSD) test using SAS software.

IV. Results

Table (1) showed the effect of treating Alloxan induced diabetic male rats with wolf berry for 4weeks on water consumption. Alloxan induced diabetes in the positive control group significantly increased the consumed water compared with the negative control group in the 1st and 2nd weeks and non significantly increased it in the 3rd and the 4th weeks. Treating the diabetic rats with dry wolf berry fruit powder in the ratio of 10 and 20 g/kg b.w. in the 3rd and the 4th group, respectively significantly decreased the consumed water in the first two weeks and non significantly decreased it in the 3rd and the 4th weeks compared with the positive control.

Table (2) showed the effect of treating Alloxan induced diabetic male rats with wolf berry for 28 days on physiological evaluation. Food intake decreased non significantly as a result of diabetes in all groups compared with the negative control group. BWG in the 4 weeks, BWG per day and BWG% were decreased in G2 as a result of diabetes and gradually increased as a result of treating diabetic rats with wolfberry in G3 and G4, respectively. Food efficiency ratio were also decreased in G2 as a result of diabetes and gradually increased as a result of treating diabetic rats with wolfberry in G3 and G4, respectively.

Table (3) showed the effect of treating Alloxan induced diabetic male rats with wolfberry for 28 days on fasting blood glucose and hemoglobin A1C. Alloxan induced diabetes significantly increased fasting blood glucose and hemoglobin A1C in the positive control group compared with the negative control group. Treating the diabetic rats with 10 and 20 g/kg b.w. wolfberry in both G3 and G4, respectively very high significantly ameliorated the FBG and HbA1C % levels and restored them nearly to the normal levels as in G1.

Table (4) showed the effect of treating Alloxan induced diabetic male rats with wolfberry for 28 days on serum immunoglobulins (IgG, IgA and IgM). Alloxan induced diabetes very high significantly increased all serum immunoglobulins (IgG, IgA, IgM and IgE) in the positive control group. Treating these diabetic rats with 10 g/kg BW and 20g/kg BW wolfberry in both G3 and G4, respectively very high significantly ameliorated all serum immunoglobulins (IgG, IgA, IgM) and restored them approaching the normal levels as in G1. In contrast, serum IgE very high significantly decreased in G2 as a result of induced diabetes and significantly affected with treatment with wolfberry in G3 and G4.

Table (5) showed the effect of treating Alloxan induced diabetic male rats with wolf berry for 4weeks on serum liver enzymes (AST, ALT, ALP and GGT). Neither induction of diabetes in G2 nor treating diabetic rats with wolfberry in G3 and G4 significantly affected serum AST, ALT and ALP while ALP was significantly increased as a result of diabetes in G2 and decreased by treating diabetic rats with wolfberry in G3 and G4 for 4weeks. Bilirubin was not significantly affected either by diabetes or treating with wolfberry. Table (6) showed the effect of treating Alloxan induced diabetic male rats with wolf berry for 4weeks on kidney function and serum electrolytes (Na^+ and K^+). Alloxan induced diabetes very high significantly increased kidney function parameters (urea, creatinine and uric acid) in the positive control group. Treating these diabetic rats with 10 and 20g/kg b.w. wolfberry in both G3 and G4, respectively very high significantly ameliorated all kidney function parameters (urea, creatinine and uric acid) and restored them approaching the normal levels as in G1. In addition, serum sodium and potassium ions were not affected either by induction of diabetes or treating with wolfberry for 28 days, in the rats under study. Table (7) showed the effect of treating alloxan induced diabetic male rats with wolf berry for 28 days on serum lipids. It was noticed that alloxan induced diabetes in male rats of the positive control group (G2) very high significantly increased triglycerides, total cholesterol low density lipoproteins, very low density lipoprotein, and decreased the high density lipoproteins. However, treating the diabetic rats with wolfberry in G3 and G4 very high significantly ameliorated the protein profile by lowering the mean values of TG, TC, VLDL and VLDL and raising the mean values of HDL. Table (8) showed antioxidant enzymes and lipid peroxide. In the positive control diabetic group (G2), catalase, super oxide dismutase and glutathione-s-transferase were very high significantly decreased as a result of diabetes, whereas the mean values of lipid peroxide were very high significantly increased. Treating the diabetic rats of G3 and G4 with wolfberry very high significantly ($p<0.001$) increased the antioxidant enzymes activity (Cat, SOD and GST) and also decrease lipid peroxidation.

Figure (1) showed the histopathology of pancreas, group 1 (the negative control group) had normal histology without any histopathological changes. Cells of Group 2 (the positive control diabetic group) had vacuolation of islets of Langerhan's and dilatation of pancreatic duct. Fig. (1 C) shows a pancreas of rat from group 3 with no histopathological changes. Fig. (1 D): Pancreas of rat from group 4 showing no histopathological changes (H & E X 400).

Fig. (2) showed histology of kidney and the negative control group (G1) with normal histological structure of renal parenchyma and glomeruli while Fig. (2 B) showed cells with vacuolation of epithelial lining renal tubules and atrophy of glomerular tuft. Fig. (2 C) shows Kidney of rat from group 3 with protein cast in the lumen of renal tubules. Fig. (2 D) shows kidney of rat from group 4 with no histopathological changes and normal glomeruli (H & E X 400).

V. Discussion

Wolfberry has been used as a traditional Chinese medicine (TCM) to nourish liver and kidney, and brighten the eye[38]. It contains polysaccharides (LBP) with a Glycan-O-Ser glycopeptide structure that gives wolfberry the efficacy on aging, neuroprotection, general well-being, fatigue/endurance, metabolism/energy expenditure, glucose control in diabetics, glaucoma, anti-oxidant properties, immunomodulation, anti-tumor activity and cytoprotection.

Induction of diabetes by single dose of alloxan intraperitoneally injected lead to damaging of insulin secreting β-pancreatic cells, reduction of endogenous insulin release, and lowering intake of glucose by the cells

which leads to increase blood sugar level[39,40]. However, alloxan induced diabetes in the positive control group significantly increased fasting blood glucose, glycated hemoglobin, serum immunoglobulins (IgG, IgA, IgM), lipid profile (total cholesterol, Triglycerides, LDL, VLDL), lipid peroxide and kidney functions (due to the diabetic nephropathy), whereas serum liver enzymes (AST, ALT, ALP and GGT), bilirubin (total, direct and indirect) and serum electrolytes (Na^+ and Ca^+) were nonsignificantly affected compared with the negative control group. In addition, the antioxidant enzymes, serum immunoglobulins IgE and HDL were significantly decreased as a result of diabetes in the positive control group. In addition, histology of pancreas and kidney showed histopathological changes as a result of diabetes in the positive control group. This result is also consistent with Sayed[41] and Al-Malki and El Rabey[42]. Treating the diabetic rats with 10 g/kg BW and 20g/kg BW wolfberry in both G3 and G4, respectively treated diabetes, adjusted the other altered biochemical parameters and treated the diabetic nephropathy, and the histopathological changes and restored them nearly to the normal levels due to the fact that wolfberry has several phenolic compounds, such as chlorogenic, p-coumaric and ferulic acids, isoquercitrin, rutin and quercitrin as assessed by an HPLC/MS method wolfberry [1,6,12,13]. The polysaccharides, carotenoids and total phenolic (betaine, and taurine which are proposed to have enhanced anti-oxidant activity) are the predominant bioactive constituents in wolfberries that regulates hyperlipidemia and hyperglycemia[1,12,13,43]. On the other hand, Bondia-Pons et al. [44] reported that wolfberries characterized by significantly higher levels of several flavonol glycosides, such as quercetin and kaempferol glycosides; isomers of dicaffeoylquinic acid and phenolic acids such as coumaric acid.

Wolfberry administration succeeded in treating the elevated serum immunoglobulins (IgG, IgA, IgM) and increasing the lowered IgE, due to its possession of potential and mild antimicrobial activity against Gram-positive bacteria and lacking against *Escherichia coli*[1].

In this study, wolfberry showed hypolipidemic property by increasing the HDL and lowering total cholesterol, triglycerides, LDL and VLDL. This is consistent with Tian et al.[43] and Xie et al.[11] who stated that that wolfberry is used for weight reduction due its possession of inhibitors for fatty acid synthase and its hypolipidemic activity. The resulted antioxidant activity of wolfberry in G3 and G4 illustrated in increasing the antioxidant enzymes (Cat, SOD, GST) [13,14] and lowering lipid peroxidation (MDA) is consistent with the study of He et al.[8], He et al.[9] and Mocan et al.[1].

This study showed that wolfberry administration at a dose of 10 g/kg BW and 20g/kg BW dry wolfberry fruit powder in the diet in G3 and G4, respectively for 28 days has considerable antidiabetic and hypolipidemic activity in alloxan induced diabetic male rats. It adjusted the altered biochemical parameters nearly to the normal levels and treated the injured kidney and pancreas tissues and returned them back to the normal state. Moreover, the higher dose of wolfberry in G4 was more effective than the lower dose in G3. The results of this study reflected the importance of wolfberry as hypoglycemic and hypolipidemic. So, wolfberry can be used in ameliorating human health.

Competing interests

The author declares that he has no competing interests.

Ethics approval and consent to participate

The plan of this study specifically was approved by our institutional ethics committee at King Abdulaziz University (KAU-1435-15).

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Table 1. Effect of treating alloxan induced diabetic male rats with wolf berry for 4 weeks on water consumption (ml/day).

water ml/day	G1 Negative Control	G2 Positive Control	G3 Treated with 10 g/k b.w	G4 Treated with 20 g/k b.w	Statistics
Adaptation week	33.33±1.05^{b}	42.50±1.11^{a}	36.33±0.88^{b}	36.33±0.88^{b}	LSD 0.05=3.257
1st week	35.33±1.17^{b}	42.50±1.11^{a}	34.83±0.90^{b}	37.16±0.79^{b}	LSD 0.05=2.943
2nd week	29.16±1.53^{b}	42.50±1.11^{a}	26.66±1.66^{b}	26.66±1.05^{b}	LSD 0.05=3.725
3rd week	27.50±1.11^{a}	29.16±1.53^{a}	28.00±1.00^{a}	27.50±1.11^{a}	LSD 0.05=3.489
4th week	26.66±1.05^{a}	30.83±2.00^{a}	29.16±1.53^{a}	30.83±2.00^{a}	LSD 0.05=5.377

Data are represented as mean ± SE. T-test values ***: significant at P<0.001. ANOVA analysis: within each row, means with different superscript (a, b, c or d) are significantly different at P<0.05, whereas means superscripts with the same letters mean that there is no significant difference at P<0.05. LSD: least significant difference, N.S: Non significant.

Table 2. Effect of treating Alloxan induced diabetic male rats with wolf berry for 4 weeks on some physiological evaluation.

Biological evaluation	G1 Negative control	G2 Positive control	G3 Treated with 10 g/k b.w	G4 Treated with 20 g/k b.w	Statistics
FI g/day	16.433±0.316[a]	16.233±0.252[a]	16.100±0.199[a]	16.066±0.208[a]	LSD 0.05=0.542
BWG g /4 week	34.833±2.329[a]	-7.833±1.013[c]	32.666±0.954[a]	29.666±1.054[b]	LSD 0.05=4.251
BWG g /day	1.161±0.077[a]	-0.261±0.033[c]	1.089±0.031[a]	0.989±0.035[b]	LSD 0.05=0.141
BWG %	23.580±1.793[a]	-5.291±0.653[c]	18.865±0.665[b]	16.263±0.619[b]	LSD 0.05=2.946
FER g/day	0.070±0.004[a]	-0.016±0.002[c]	0.067±0.002[ab]	0.061±0.002[b]	LSD 0.05=0.086
FER %	7.065±0.472[a]	-1.608±0.208[b]	6.763±0.197[a]	6.154±0.218[a]	LSD 0.05=3.011

Data are represented as mean ± SE. T-test values ***: significant at P<0.001. ANOVA analysis: within each row, means with different superscript (a, b, c or d) are significantly different at P<0.05, whereas means superscripts with the same letters mean that there is no significant difference at P<0.05. LSD: least significant difference, N.S: Non significant.

Table 3. Effect of treating alloxan induced diabetic male rats with wolf berry for 28 days on fasting blood glucose and hemoglobin A1C.

Parameters	G1 Negative Control	G2 Positive Control	G3 Treated with 10 g/k b.w	G4 Treated with 20 g/k b.w	Statistics
FBG mg/dl	87.33±2.70[d]	276.16±1.47[a]	134.33±1.30[b]	115.33±1.87[c]	LSD 0.05 =6.355
HbA1C %	5.333±0.176[b]	7.933±0.055[a]	5.516±0.153[b]	5.450±0.143[b]	LSD 0.05 =0.468

Data are represented as mean ± SE. T-test values ***: significant at P<0.001. ANOVA analysis: within each row, means with different superscript (a, b, c or d) are significantly different at P<0.05, whereas means superscripts with the same letters mean that there is no significant difference at P<0.05. LSD: least significant difference, N.S: Non significant.

Table 4- Effect of treating alloxan induced diabetic male rats with wolf berry for 28 days on serum immunoglobulins (IgG, IgA, IgM and IgE).

Immunoglobulins mg/dl	G1 Negative control	G2 Postive control	G3 Treated with 10 g/k b.w	G4 Treated with 20 g/k b.w	Statistics
IgG	535.00±3.01[c]	744.33±1.70[a]	559.50±3.35[b]	543.66±2.40[c]	LSD0.05=8.729
IgM	125.83±2.24[d]	359.33±4.37[a]	204.66±4.07[b]	136.66±24.81[c]	LSD0.05=16.400
IgA	103.50±3.64[d]	343.66±6.13[a]	155.66±3.35[b]	134.33±2.21[c]	LSD0.05=13.074
IgE	15.56±0.57[a]	14.06±0.44[a]	13.91±0.42[a]	14.01±0.84[c]	LSD0.05=1.56

Data are represented as mean ± SE. T-test values ***: significant at P<0.001. ANOVA analysis: within each row, means with different superscript (a, b, c or d) are significantly different at P<0.05, whereas means superscripts with the same letters mean that there is no significant difference at P<0.05. LSD: least significant difference, N.S: Non significant.

Table 5: Effect of treating alloxan induced diabetic male rats with wolf berry for 28 days on serum liver enzymes (AST, ALT, ALP and GGT) and bilirubin (total, direct and indirect).

Liver enzymes U/l	G1 Negative control	G2 Positive control	G3 Treated with 10 g/k b.w	G4 Treated with 20 g/k b.w	Statistics
AST U/l	21.16±0.70[a]	21.0±1.98[a]	21.33±1.05[a]	21.50±1.28[a]	LSD0.05=4.217
ALP U/l	162.16±10.08[b]	186.5±10.98[a]	122.50±2.44[c]	126.50±3.81[c]	LSD0.05=21.254
GGT	25.83±2.2[a]	22.3±1.56[a]	25.16±1.74[a]	24.33±2.81[a]	LSD0.05=6.617

U/l					
Total Bilirubin mg/dl	0.56 ± 0.04^{b}	0.56 ± 0.033^{b}	0.550 ± 0.034^{b}	0.766 ± 0.061^{a}	LSD 0.05 =0.152
Direct Bilirubin mg/dl	0.15 ± 0.02^{a}	0.18 ± 0.016^{a}	0.133 ± 0.021^{b}	0.216 ± 0.030^{a}	LSD 0.05 =0.076
Indirect Bilirubin mg/dl	0.41 ± 0.04^{a}	0.38 ± 0.040^{b}	0.416 ± 0.016^{a}	0.550 ± 0.042^{a}	LSD 0.05 =0.116

Data are represented as mean ± SE. T-test values *: significant at P<0.05, ***: significant at P<0.001. ANOVA analysis: within each row, means with different superscript (a, b, c or d) are significantly different at P<0.05, whereas means superscripts with the same letters mean that there is no significant difference at P<0.05. LSD: least significant difference, N.S: Non significant.

Table 6- Effect of treating alloxan induced diabetic male rats with wolf berry for 28 days on kidney function and serum electrolytes (Na^{+} and k^{+}).

Parameter	G1 Negative control	G2 Positive control	G3 Treated with 10 g/k b.w	G4 Treated with 20 g/k b.w	Statistics
Urea	22.33 ± 1.49^{b}	49.83 ± 1.42^{a}	27.00 ± 0.93^{b}	25.16 ± 2.21^{b}	LSD 0.05 =4.949
Creatinine	0.600 ± 0.05^{b}	1.550 ± 0.056^{a}	0.733 ± 0.033^{b}	0.750 ± 0.05^{b}	LSD 0.05 =0.163
Uric acid	4.066 ± 0.223^{a}	3.833 ± 0.180^{a}	3.116 ± 0.222^{b}	4.500 ± 0.531^{a}	LSD 0.05 =0.945
Na^{+}	131.33 ± 2.95^{a}	137.50 ± 1.25^{a}	135.66 ± 1.76^{a}	138.33 ± 2.18^{a}	LSD 0.05 =7.024
K^{+}	9.233 ± 0.13^{a}	9.416 ± 0.21^{a}	9.266 ± 0.09^{a}	9.083 ± 0.08^{a}	LSD 0.05 =0.392

Data are represented as mean ± SE. T-test values *: significant at P<0.05, ***: significant at P<0.001. ANOVA analysis: within each row, means with different superscript (a, b, c or d) are significantly different at P<0.05, whereas means superscripts with the same letters mean that there is no significant difference at P<0.05. LSD: least significant difference, N.S: Non significant.

Table 7. Effect of treating alloxan induced diabetic male rats with wolf berry for 28 days on serum lipids.

Parameter	G1 Negative Control	G2 Positive Control	G3	G4	Statistics
TG mg/dl	99.16 ± 1.01^{d}	272.83 ± 1.44^{a}	126.33 ± 1.35^{b}	115.16 ± 1.75^{c}	LSD 0.05 =3.725
TC mg %	132.50 ± 2.17^{d}	293.66 ± 1.74^{a}	194.66 ± 1.83^{b}	173.50 ± 2.60^{c}	LSD 0.05 =5.348
HDL mg/dl	51.16 ± 1.47^{a}	25.83 ± 0.83^{d}	42.66 ± 0.66^{c}	47.16 ± 1.24^{b}	LSD 0.05 =2.772
LDL mg/dl	63.13 ± 2.88^{a}	210.46 ± 1.93^{a}	127.15 ± 2.29^{b}	106.50 ± 0.55^{c}	LSD 0.05 =6.917
VLDL mg/dl	19.83 ± 0.20^{d}	54.56 ± 0.28^{a}	25.26 ± 0.27^{b}	23.03 ± 0.35^{c}	LSD 0.05 =0.745

Data are represented as mean ± SE. T-test values ***: significant at P<0.001. ANOVA analysis: within each row, means with different superscript (a, b, c or d) are significantly different at P<0.05, whereas means superscripts with the same letters mean that there is no significant difference at P<0.05. LSD: least significant difference.

Table 8-Effect of treating Alloxan induced diabetic male rats with wolf berry for 4 weeks on antioxidant enzymes and lipid peroxide.

Parameter	Statistics	G1 Negative Control	G2 Positive Control	G3	G4
(CAT) U/g.	Mean±SE	4.018 ± 0.048^{a}	0.418 ± 0.006^{d}	2.813 ± 0.061^{c}	3.230 ± 0.040^{b}
	LSD 0.05 =0.133				
	T.test	-	77.22^{***}	-41.36^{***}	-65.38^{***}
(SOD) U/g.	Mean±SE	1116.21 ± 6.59^{a}	422.88 ± 3.49^{d}	941.67 ± 7.33^{c}	1005.63 ± 7.83^{b}
	LSD 0.05 =20.973				
	T.test	-	73.29^{***}	-60.93^{***}	-56.71^{***}
GST U/g.	Mean±SE	7727.66 ± 28.19^{a}	3172.50 ± 21.06^{d}	6659.83 ± 69.16^{c}	7271.16 ± 19.99^{b}
	LSD 0.05 =127.695				

	T.test	-	102.38***	-55.33***	-182.81***
MDA nmol/ g. liver tissue	Mean±SE	2.861±0.152[d]	911.883±0.31[a]	35.248±0.12[b]	64.001±0.12[c]
	LSD 0.05 = 0.677				
	T.test	-	-20.13***	16.11***	18.04***

Data are represented as mean ± SE. T-test values ***: significant at P<0.001. ANOVA analysis: within each row, means with different superscript (a, b, c or d) are significantly different at P<0.05, whereas means superscripts with the same letters mean that there is no significant difference at P<0.05. LSD: least significant difference.

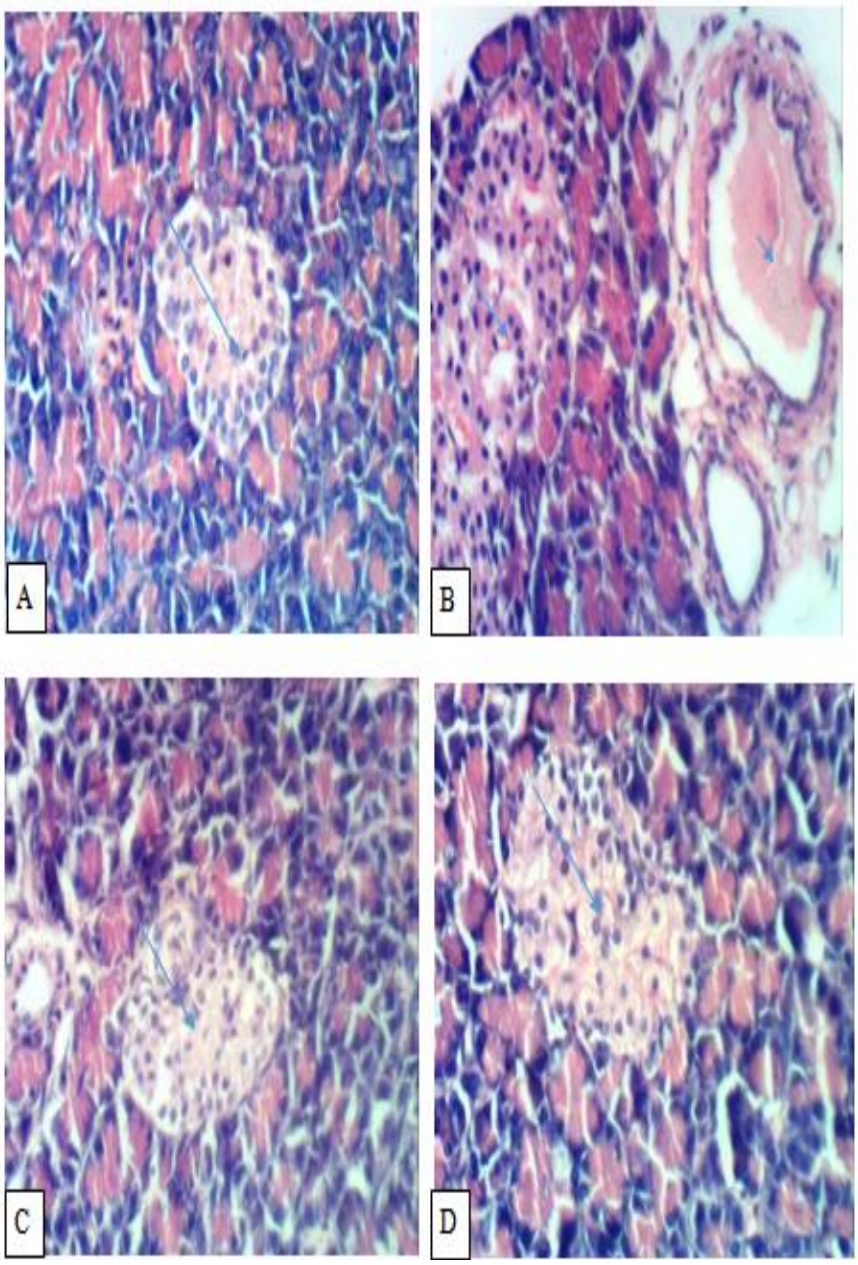

Figure 1: (A) Pancreas of rat from group 1 showing no histopathological changes and normal islets of Langerhan's (long arrow), (B) Pancreas of rat from group 2 showing vacuolation of islets of Langerhan's (medium arrow) and dilatation of pancreatic duct (short arrow), (C) Pancreas of rat from group 3 showing no histopathological changes, (D) Pancreas of rat from group 4 showing no histopathological changes (H & E X 400).

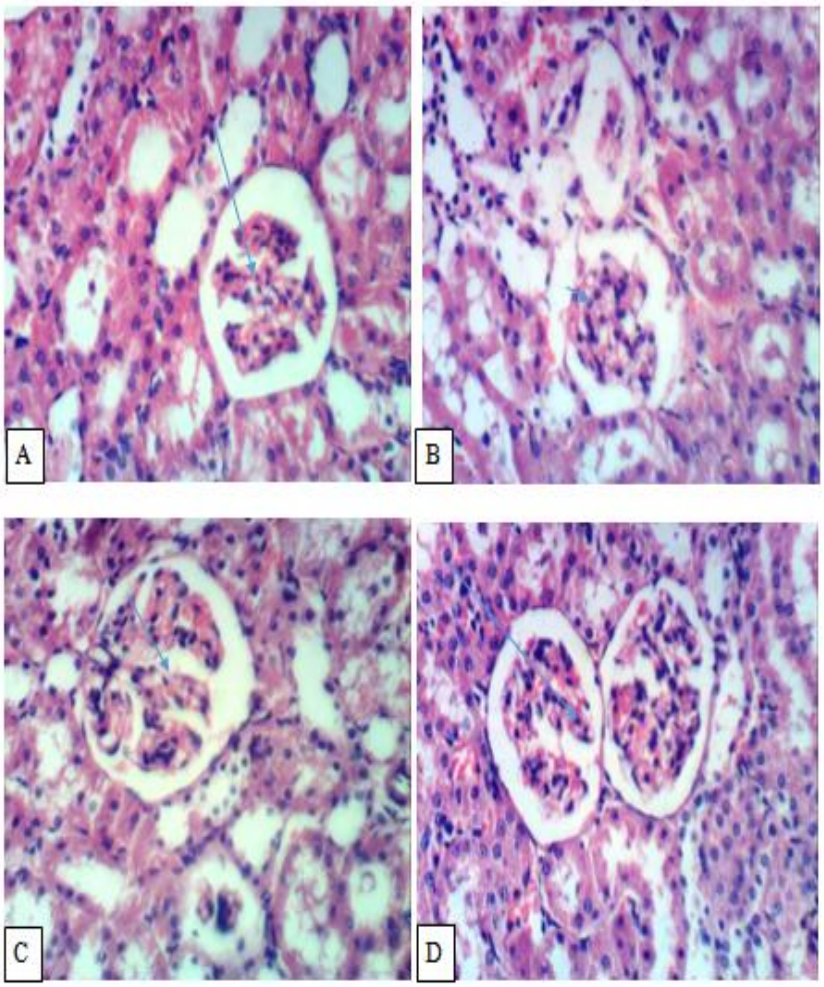

Figure 2: (A) Kidney of rat from group 1 showing the normal histological structure of renal parenchyma and glomeruli (long arrow), (B) Kidney of rat from group 2 showing vacuolation of epithelial lining renal tubules and atrophy of glomerular tuft (short arrow), (C) Kidney of rat from group 3 showing protein cast in the lumen of renal tubules (medium arrow), (D): Kidney of rat from group 4 showing no histopathological changes and normal glomeruli (H & E X 400).

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