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# Pharmacological Effects of Ethanol Extract of Artemisia Herba Alba in Streptozotocin-induced Type 1 Diabetes Mellitus in Rats

Heba MI Abdallah<sup>1\*</sup>, Rehab F Abdel-Rahman<sup>1</sup>, Gehad A Abdel Jaleel<sup>1</sup>, Heba AM Abd El-Kader<sup>2</sup>, Salma A El-Marasy<sup>1</sup>, Eman R Zaki<sup>3</sup>, Samir AE Bashandy<sup>1</sup>, Mahmoud S Arbid<sup>1</sup> and Abdel Razik H Farrag<sup>4</sup>

<sup>1</sup>Department of Pharmacology, National Research Centre, Cairo, Egypt

<sup>2</sup>Department of Cell Biology, National Research Centre, Cairo, Egypt

<sup>3</sup>Department of Molecular Biology, National Research Centre, Cairo, Egypt

<sup>4</sup>Department of Pathology, National Research Centre, Cairo, Egypt

## Abstract

**Background:** Diabetes mellitus has been treated orally with herbal remedies based on folk medicine since ancient times. The current study investigates the protective effect of Artemisia herba alba (Ah) against experimentally-induced type 1 diabetes mellitus and its complications.

**Methods:** Diabetes was induced in adult male Wister rats by administration of (Streptozotocin; STZ) at a dose of 52.5 mg/kg, i.p. Animals with diabetes were treated with either Ah ethanolic extract or gliclazide (10mg/kg, p.o.) for 14 days. Biochemical analysis was done such as glucose, insulin, homocysteine, lipid profile (cholesterol, triglyceride), liver function tests (T.bilirubin, AST and ALT), kidney function tests (BUN, sr. creatinine) and oxidative stress biomarkers. In addition, pancreas, liver, kidney, heart and aorta tissues were dissected out for pathological examination. Immuno histochemical study was done on pancreatic tissues for determination of insulin and glucagon immune reactivities. Liver tissues were also separated for genetic analysis.

**Results:** Oral administration of Ah ethanolic extract at a concentration of 400, 200 and 100 mg/kg daily for 14 days results in decreased fasting blood glucose and homocysteine levels as well as enhancement of plasma insulin level as compared with STZ-treated rats. The extract improved lipid profile, liver and kidney function tests. It also increased hepatic and renal contents of GSH, diminished lipid peroxidation, and inhibited pathological alterations induced in the different organs. Treatment with Ah extract increased insulin expression while decreased glucagon immunoreactivity and DNA band polymorphism.

**Conclusion:** Thus, our results show that Ah possesses a promising antihyperglycemic effect that is comparable with gliclazide.

**Keywords:** Medicinal plants; Diabetes; Streptozotocin; Rats

## Introduction

Diabetes mellitus (DM) is a worldwide endocrine disorder that impairs many physiological functions of the body. Type 1 DM is primarily characterized by high blood glucose levels (hyperglycemia) induced by insulin insufficiency [1] due to inherited and/or acquired deficiency in production of insulin by the pancreas. Complications affecting eyes, kidneys, nerves and arteries are produced under the clinical settings of diabetes due to persistent hyperglycemia [2]. Current drugs for treatment of DM are associated with many side effects including obesity, osteoporosis sodium retention, hypoglycemia, and lactic acidosis [3,4]. The beneficial role of medicinal plants in the treatment of DM has been evolved due to their lower cost and lack of serious side effects. Thus, the traditional medicine has been extensively studied for its several therapeutic effects [5-7].

Artemisia herba-alba (Ah) "Shih-balady" is one of the plants that grown in Sinai, the most impressive vegetational regions of Egypt. It is a greyish-white perennial dwarf shrub, with small flowers. It is commonly grown in the central and southern wadis [8]. The plant has been used in folk medicine since ancient times as vermifuge, tonic, diuretic, skin troubles, emmenagogue, stomachic, intestinal, cholagogue, depurative and anthelmintic due to presence of volatile oils [9]. The plant is used also as digestive and analgesic against the rheumatic pains. Herbal tea from this species has been used antibacterial, antispasmodic, and hemostatic agents [10]. It has been used in Moroccan folk medicine to treat arterial hypertension and/or diabetes [11]. Recent studies have reported that aqueous extract of Ah showed hypoglycemic activity

in treated animals [12]. Administration of the extract also caused a reduction of serum lipids [13].

The chemical composition of Ah was previously studied. Several structural types of sesquiterpene lactones were found in the aerial parts of Ah. Eudesmanolides followed by germacranolides seem to be the most abundant types of lactones found in this species [14]. Flavonoids were detected in Ah ranging from common flavone and flavonol glycosides to more unusual highly methylated flavonoids such as Hispidulin and Cirsilineol which possess an anti-proliferative activity against multiple types of cancer cells [15]. In studies of the leaves and stems of Ah collected from Sinai, a total of eight flavonoids O- and C-glycoside were isolated and identified [16]. During a survey for antiulcerogenic principles of Ah, eight polyphenolics and related constituents were isolated. These included chlorogenic acid, 4, 5-O-dicaffeoylquinic acid,

**\*Corresponding author:** Heba Mohammed Ibrahim Abdallah, Department of Pharmacology, National Research Centre, Cairo, Egypt, Tel: 002-01156622592; Fax: 002-33335996; E-mail: [heba21\\_5@yahoo.com](mailto:heba21_5@yahoo.com)

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isofraxidin 7-O-D-glucopyranoside, 4-O-D-glucopyranosylcaffeic acid, rutin, schaftoside, isoschaftoside, and vicenin-2 [17].

The current research was carried out to assess the hypoglycemic effect of the ethanol extract of Ah aerial parts in STZ model of type1 DM in rats. In addition, the protective effects of the extract on the other complications of diabetes were investigated.

## Material and Methods

### Plant material

Artemisia herba-alba (Ah) belongs to family Asteraceae. The dried aerial parts of the plant were purchased from the Egyptian markets and were grinded by electric grinder.

### Preparation of plant extract

The plant powder was soaked in 70% ethyl alcohol for about 3 days, filtered using filter paper and the filtrate was concentrated under vacuum using the rotating evaporator (Rotavap), then percolated several times till exhaustion. The yielded ethanolic extract of Ah (55gm out of 200gm dried powder) was ready for both toxicological and pharmacological studies.

### Toxicological studies (Determination of LD50)

The LD50 was determined as described by [18]. 1/10, 1/20 and 1/40 of the maximum dose (4 gm/kg b.wt.) that did not cause mortalities nor toxic symptoms in rats for the plant extract were chosen to be used for the biological investigation throughout the study.

### Animals and chemicals

Sixty male Wister rats weighing 250-300g were used. The animals were obtained from the animal house colony of the National Research Center and housed in the animal facility of the pharmacology department, National Research Center. The animals were housed in a conditioned atmosphere ( $22^{\circ}\text{C} \pm 2$ , 50-60% humidity and 12h dark and light cycles) and kept on a standard diet pellets (El-Nasr, Abu Zaabal, Egypt) contained not less than 20% protein, 5% fibre, 3.5% fat, 6.5% ash and a vitamin mixture. They received water ad libitum. The experiments were carried out according to the national regulations of animal welfare and Institutional Animal Ethical Committee (IAEC).

Streptozotocin (STZ) was obtained from Sigma Chemical Company (U.S.A.). Gliclazide (standard antidiabetic drug) was purchased from SERVIER subsidiary SERDIA Pharmaceuticals (Canada) in tablet form and ground using a mortar. The powder was dissolved in distilled water and orally administered at a dose of 10 mg/kg body weight per day for the experimental period of 14 days. The dose of gliclazide was based on previous studies [19].

### Phytochemical study

**Determination of total phenolic content (TPC):** Total concentration of phenolic compounds in the extracts was determined using a series of gallic acid standard solutions (2.5- 25  $\mu\text{g/ml}$ ) as described by [20] but with some modifications. Each extract solution (0.1ml) was mixed with 2ml of a 2% (w/v) sodium carbonate solution and vortexed vigorously. The same procedure was also applied to the standard solutions of gallic acid. After 8 min, 0.1 ml of Folin Ciocalteu's phenol reagent was added and each mixture was vortexed again. The absorbance at 765 nm of each mixture was measured, after incubation for 2 hrs at room temperature.

**Determination of total flavonoid content (TFC):** Total

concentration of flavonoid compounds in extracts was determined using a series of standard rutin solutions (2.5-50  $\mu\text{g} / \text{ml}$ ) as described in the aluminum chloride colorimetric method. A known volume of each extract solution was mixed with 5% sodium nitrite solution, vortexed vigorously, then 10% aluminum chloride solution was added and vortexed again. After 6 min, 4.3% of sodium hydroxide solution was added, followed by addition of water, shaken, and left to stand for 15 min before determination. The sample solution without coloration was used as reference solution and the colour was read at 510 nm wavelength [21].

### Induction of diabetes and Animal grouping

Type 1 DM was induced by a single i.p. injection of a freshly prepared solution of STZ (52.5 mg/kg body weight) in 0.1 M citrate buffer (pH 4.3) after a fasting period of 24 h [22]. On the third day of STZ injection, diabetes in surviving rats was confirmed by measuring the glucose level of blood obtained from the tail vein. Rats with a plasma glucose level of 180 mg/dl or greater and exhibited polyuria were accepted as diabetic and included in this study.

After induction of diabetes, rats were divided into six equal groups (ten rats per group). Group I (control negative group) comprises rats that receive 1 ml saline. Group II (control positive) comprises rats that receive streptozotocin (STZ) as mentioned above. Group III comprises rats that receive STZ and gliclazide (standard antidiabetic drug). Groups IV, V and VI comprise rats that receive STZ and oral administration of the Ah extract at the three selected doses. Treatment with either the standard drug or Ahextract was started 3 days after STZ injection and lasted for 14 days. Blood Samples will be drawn from the tail veins of fasted rats for recording fasting blood glucose (FBG) each group on days 3 (beginning of diabetes induction) and 17 (last day of the experiment, after treatment) of the study. Blood glucose was measured using glucose kit (Stanbio Laboratory, USA) according to Trinder [23].

### Sampling and processing

At the end of the experiment, animals were anaesthetized by ether and blood samples were collected via the retro-orbital venous plexus and left for 20min to allow clotting. Serum samples were obtained by centrifugation at 3000 rpm for 10 min using the cooling centrifuge (Sigma and laborzentrifugen, 2k15, Germany). It was used for biochemical measurements. Animals were sacrificed by cervical dislocation. Kidney and liver tissues were dissected out and washed with ice-cold saline. Tissues were homogenized in saline solution and stored at  $-80^{\circ}\text{C}$ .

**Biochemical analysis:** The collected sera were used for determination of insulin hormone level was evaluated by the enzymatic immunoassay method according to Eastham [24]. Homocystiene was estimated using a microplate enzyme immunoassay (Glory Science Co., USA).Serum triglycerides level was determined according to Fossati and Lorenzo [25] and total Cholesterol was evaluated according to Abell et al. [26]. Total bilirubin was estimated colorimetrically according to the method of Walter and Gerade [27]. AST and ALT were determined according to the method of Reitman and Frankel [28].

**Antioxidant activity:** The concentration of reduced glutathione (GSH) and lipid peroxides were estimated in liver and kidney tissue homogenates. GSH was estimated by the method of Ellman [29]. Lipid peroxides were assayed using thiobarbituric acid reactive substances (TBARS) method according to Ohkawa et al. [30].

**Histopathological examination:** After blood samples were obtained, the histopathological samples (pancreas, liver, Kidney, heart and aorta) were excised and fixed in 10% buffered neutral formalin. The paraffin sections were taken at 5  $\mu$ m thickness processed in alcohol-xylene series and was stained with alum hematoxylin and eosin. The sections were examined microscopically for histopathology changes.

**Immunocytochemistry staining:** The pancreas was removed immediately and placed in 10% formalin in phosphate-buffered saline (PBS), pH 7.4, for 18 hrs before paraffin embedding. Tissues were routinely processed through a graded series of alcohols, cleared in xylol and embedded in paraffin. 5 $\mu$ m thick sections were obtained and processed for immunohistochemical staining for immunocytochemical localization of glucagon and insulin. Immunohistochemical staining was carried out by the peroxidase linked avidin-biotin complex (ABC) method as in the instruction manual provided with the kit (Mouse extravidin peroxidase staining kit Stock No EXTRA-2, Sigma, USA) [31].

**Molecular analysis:** The genomic DNA was isolated from liver tissue of mice using phenol/chloroform extraction and precipitation method. The concentrations of the extracted DNAs were measured at 260 nm as described by John et al. [32].

**RAPD-PCR analysis:** Primer screening for RAPD analysis was performed using six commercially available decamer random primers (Operon, Alameda, CA, USA). Four out of six primers amplified clear and reproducible bands, the other two primers amplified monomorphic bands : OPA02 (5'-TGCCGAGCTG-3'), OPA03(5'-AGTCAGCCAC-3'), OPA05 (5'-AGGGGTCTTG-3'), OPB13 (5'- TTCCCCCGCT-3'). The PCR protocol for RAPD analysis was followed as described by Williams et al. [33].

## Results

Phytochemical investigation showed that 70% ethanolic extract of Artemisia herba alba contains high content of total phenolics (248.6  $\pm$  20.4) mg gallic acid/g dry extract and flavonoids (62.15  $\pm$  5.8) mg rutin/g dry extract. Results are represented as the mean values of three replicates of the same sample  $\pm$ SE. Statistical analysis was performed using one way analysis of variance.

## Biochemical analysis

FBG and insulin levels of groups are shown in Table 1. In the STZ (control positive) group, a significant increase in FBG level was observed on days 3 (start of treatment) and 17 (end of the experiment) as compared to the control negative (normal) group. Treatment with Ah extract significantly decreased the elevated FBG levels as compared to the control positive group at the end of the experiment. The extract at doses 400, 200, 100mg/kg exhibited % change in FBG: 65, 64.9 and 69%, respectively before and after treatment. This anti-hyperglycemic effect was comparable to that of the standard drug, gliclazide (71% changes in FBG). STZ induced a significant decrease in serum insulin level reached 64% of normal group. Treatment of diabetic rats with Ah for 14 days normalized serum insulin level as compared to STZ-treated group. Homocysteine level showed significant decreased in diabetic rats group compared to normal control. Ah extract administration increased the level of homocysteine significantly (Figure 1).

STZ-induced diabetic rats showed non-significant increase in the level of serum t. cholesterol as compared to the negative control. However, gliclazide therapy significantly decreased serum cholesterol by 31% as compared to STZ-treated group. Similarly, Ah extract at doses of 400, 200 and 100mg/kg showed a significant decrease in serum

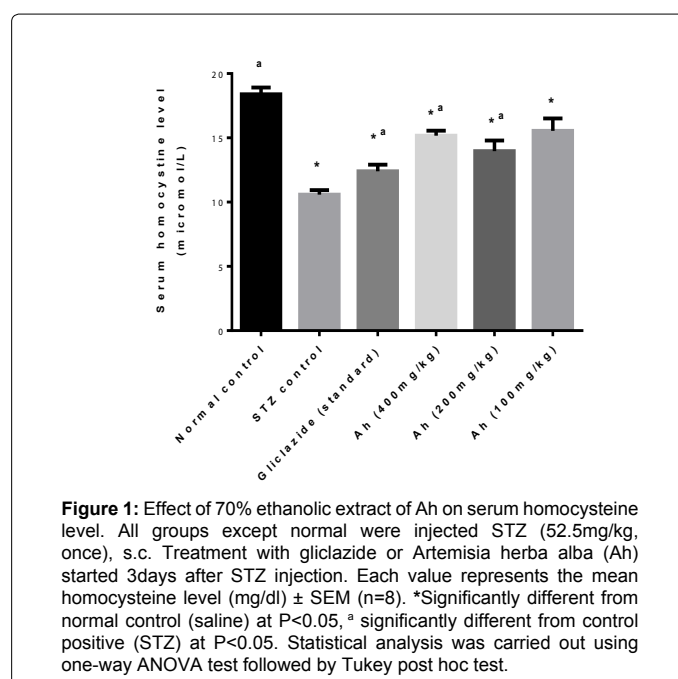
cholesterol level by 43.2, 42.8 and 34.7% as compared to STZ-treated group. This positive impact is, however, more pronounced in the rats treated with 100 mg/kg body weight extract. Treatment with both the standard gliclazide and Ah extract reduced t. cholesterol level below the normal value. A significant increase in serum triglycerides level was shown in diabetic rats, with an increase of 33.5% compared to the negative control group. Gliclazide treatment significantly decreased serum triglycerides level by 23.2% as compared to STZ-treated group. Treatment with Ah extract significantly reduced serum triglycerides level below the normal value. It showed a decrease of 39.5, 39.2 and 34.4% compared to STZ-positive control group (Table 2).

As shown in Table 2, serum activities of ALT and AST enzymes were significantly increased in STZ-treated group reached 129.5 and 119.1%, respectively as compared to the normal group. Treatment with Ah extract decreased ALT and AST activities as compared to the control positive group. Doses of 400, 200 and 100 mg/kg of the plant extract recorded a decrease in ALT serum activity of 31.4, 31.4 and 34.7%, respectively compared to STZ-treated group. The same doses showed a decrease in AST serum activity of 11.2, 12.1 and 13% compared to

Treatment	Fasting Blood glucose (mg/dl)			Insulin ( $\mu$ IU/ml)
	Before	After	% change	
Normal control	98.2 $\pm$ 4.0 <sup>a</sup>	99.5 $\pm$ 5.9 <sup>a</sup>	-1.3	4.52 $\pm$ 0.13 <sup>a</sup>
STZ control	385.0 $\pm$ 15.5 <sup>*</sup>	333.2 $\pm$ 16.6 <sup>*</sup>	13.5	2.90 $\pm$ 0.04 <sup>*</sup>
Standard (gliclazide)	382.5 $\pm$ 24.9 <sup>*</sup>	109.7 $\pm$ 8.9 <sup>a</sup>	71.3	4.27 $\pm$ 0.31 <sup>a</sup>
Ah extract (400mg/kg)	347.5 $\pm$ 20.1 <sup>*</sup>	121.7 $\pm$ 8.4 <sup>a</sup>	65.0	4.74 $\pm$ 0.37 <sup>a</sup>
Ah extract (200mg/kg)	320.0 $\pm$ 26.4 <sup>*</sup>	112.2 $\pm$ 7.1 <sup>a</sup>	64.9	4.81 $\pm$ 0.12 <sup>a</sup>
Ah extract (100mg/kg)	365.0 $\pm$ 19.4 <sup>*</sup>	113.1 $\pm$ 6.0 <sup>a</sup>	69	4.75 $\pm$ 0.14 <sup>a</sup>

All groups except normal were injected STZ (52.5mg/kg, once), s.c. Treatment started 3 days after STZ injection. Blood glucose was measured twice; after 3days of STZ injection (before treatment) and at the end of the experiment (after treatment). Each value represents the mean glucose level or insulin level (mg/dl)  $\pm$  SEM (n=10). \*Significantly different from control negative (saline) at P<0.05, <sup>a</sup> significantly different from control positive (STZ) at P<0.05. Statistical analysis was carried out using one-way ANOVA test followed by Tukey post hoc test.

**Table 1:** Effect of 70% ethanolic extract of Ah on blood glucose level before (induction) and after treatment (end of experiment) and serum insulin level in rats:



Treatment	T.bilirubin mg/dl	ALT U/L	AST U/L	BUN mg/dl	S. creatinine mg/dl	Cholesterol mg/dl	Triglycerides mg/dl
Control negative (normal)	1.91 ± 0.07	37.3 ± 0.6 <sup>a</sup>	107.1 ± 2.1 <sup>a</sup>	14.7 ± 0.7 <sup>a</sup>	0.57 ± 0.03 <sup>a</sup>	83.7 ± 2.8	86.0 ± 2.6 <sup>a</sup>
Control positive (STZ)	2.23 ± 0.11	48.3 ± 1.1 <sup>*</sup>	127.5 ± 1.1 <sup>*</sup>	23.8 ± 2.2 <sup>*</sup>	1.29 ± 0.02 <sup>*</sup>	93.8 ± 4.6	114.8 ± 6.9 <sup>*</sup>
Standard (gliclazide)	1.60 ± 0.06 <sup>a</sup>	31.9 ± 3.0 <sup>a</sup>	110.7 ± 3.5 <sup>a</sup>	18.1 ± 1.8 <sup>a</sup>	0.86 ± 0.03	63.8 ± 4.2 <sup>a</sup>	88.1 ± 1.9 <sup>a</sup>
Ah extract (400mg/kg)	1.59 ± 0.07 <sup>a</sup>	33.1 ± 1.2 <sup>a</sup>	113.1 ± 3.8 <sup>a</sup>	13.8 ± 0.5 <sup>a</sup>	0.69 ± 0.01 <sup>a</sup>	53.3 ± 2.5 <sup>a</sup>	69.4 ± 2.1 <sup>a</sup>
Ah extract (200mg/kg)	1.63 ± 0.10 <sup>a</sup>	33.1 ± 1.6 <sup>a</sup>	112.0 ± 2.1 <sup>a</sup>	13.5 ± 0.7 <sup>a</sup>	0.75 ± 0.05 <sup>a</sup>	53.1 ± 3.0 <sup>a</sup>	69.8 ± 3.7 <sup>a</sup>
Ah extract (100mg/kg)	1.54 ± 0.14 <sup>a</sup>	31.5 ± 1.0 <sup>a</sup>	110.4 ± 2.8 <sup>a</sup>	14.5 ± 0.8 <sup>a</sup>	0.74 ± 0.16 <sup>a</sup>	61.2 ± 4.6 <sup>a</sup>	75.3 ± 3.6 <sup>a</sup>

All groups except normal were injected STZ (52.5mg/kg, once), s.c. Treatment with gliclazide or Artemisia herba alba (Ah) started three days after STZ injection. Each value represents the mean ± SEM (n=8). <sup>\*</sup>Significantly different from control negative (saline) at P<0.05, <sup>a</sup> significantly different from control positive (STZ) at P<0.05. Statistical analysis was carried out using one-way ANOVA test followed by Tukey post hoc test.

**Table 2:** Effect of 70% ethanolic extract of Ah on selected biochemical parameters:

	Liver		Kidney	
	GSH (μmol/g tissue)	MDA (nmol/g tissue)	GSH (μmol/g tissue)	MDA (nmol/g tissue)
Normal (saline)	6.98 ± 0.12 <sup>a</sup>	155.3 ± 8.8 <sup>a</sup>	4.60 ± 0.03 <sup>a</sup>	192 ± 13.6 <sup>a</sup>
STZ control	4.32 ± 0.09 <sup>*</sup>	474.6 ± 25.0 <sup>*</sup>	3.39 ± 0.03 <sup>*</sup>	390.9 ± 14.7 <sup>*</sup>
Gliclazide (10mg/kg)	5.98 ± 0.05 <sup>a</sup>	247.8 ± 17.6 <sup>a</sup>	4.33 ± 0.40 <sup>a</sup>	216.7 ± 7.3 <sup>a</sup>
Ah extract (400mg/kg)	6.25 ± 0.03 <sup>a</sup>	262.4 ± 8.5 <sup>a</sup>	4.28 ± 0.06 <sup>a</sup>	225.0 ± 11.6 <sup>a</sup>
Ah extract (200mg/kg)	6.23 ± 0.05 <sup>a</sup>	204.4 ± 4.4 <sup>a</sup>	4.33 ± 0.01 <sup>a</sup>	248.1 ± 16.5 <sup>a</sup>
Ah extract (100mg/kg)	6.45 ± 0.15 <sup>a</sup>	220.1 ± 15.2 <sup>a</sup>	4.25 ± 0.05 <sup>a</sup>	247.4 ± 10.5 <sup>a</sup>

herba alba (Ah) started 3days after STZ injection. Each value represents the mean GSH or MDA level (mg/dl) ± SEM (n=8). <sup>\*</sup>Significantly different from normal control (saline) at P<0.05, <sup>a</sup> significantly different from control positive (STZ) at P<0.05. Statistical analysis was carried out using one-way ANOVA test followed by Tukey post hoc test.

All groups except normal were injected STZ (52.5mg/kg, once), s.c. Treatment with gliclazide or Artemisia

**Table 3:** Effect of 70% ethanolic extract of Ah on oxidative stress biomarkers:

STZ-treated group. The protective effect of the plant extract on liver enzymes was comparable to the standard drug, gliclazide (33.9% for ALT, 13% for AST).

Levels of BUN and sr.creatinine increased significantly in diabetic groups (23.8±2.2mg/dl and 1.29±0.02mg/dl for BUN and sr.creatinine vs 14.7±0.7mg/dl and 0.57±0.03mg/dl in normal rats). Gliclazide treatment recorded a 39% decrease in BUN level and 33% decrease in sr.creatinine level as compared to STZ-treated group. The ethanol extract of Ah improved the kidney function indices of diabetic rats by significantly reducing the concentrations of BUN and sr.creatinine. It showed a decrease of 42, 43 and 39% in BUN levels at the doses, 400, 200, 100mg/kg, respectively and 46.5, 41.9 and 42.6% in sr.creatinine level compared to STZ-treated group (Table 2).

### Antioxidant activity

The results are summarized in Table 3. As shown, STZ injection increased hepatic and renal contents of TBARs and decreased their GSH contents as compared to the normal control group. On the other hand, Ah extract at doses of 400, 200 and 100 mg/kg and gliclazide treatment decreased TBARs content by 44.7%, 56.9%, 53.6%, 47.8%; respectively in the liver tissue and by 42.4% , 36.5%,36.7%,44.6%; respectively in the kidney tissue as compared to STZ control group. All treatment groups increased GSH content that reached 144.7%, 144.2%, 149.3%, 138.4%; respectively in the liver tissue and 126.2%, 127.7%, 125.4%, 127.7%; respectively in the kidney tissue as compared to STZ control group.

### Histopathological findings

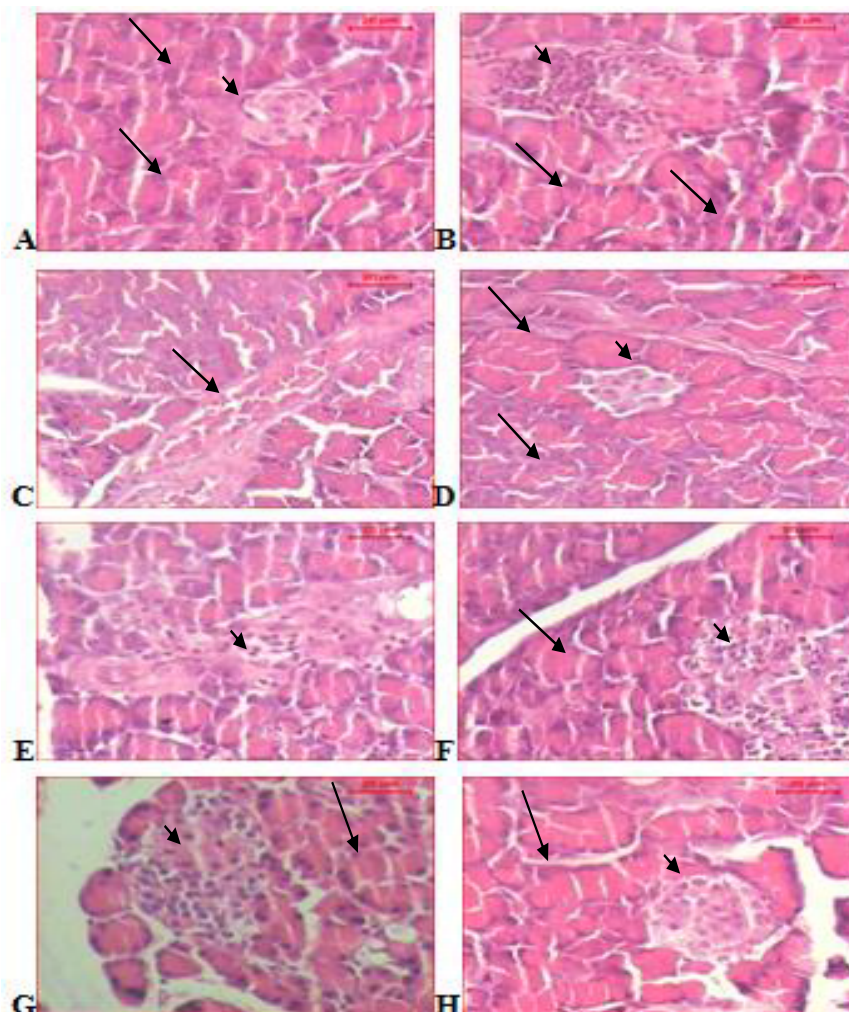
Sections of the normal pancreas of rats showed the exocrine component of the pancreas that consisted of closely packed acini. The interlobular duct, surrounded with the supporting tissue. The endocrine tissue of the pancreas, islets of Langerhans, scattered throughout

the exocrine tissue (Figure 2A).In case of pancreas of diabetic rats, histopathological examination showed the acinar cells around the islets does not look classical. The islets were largely occupied by a uniform eosinophilic material and few atrophic cells (Figure 2B). Pancreas of diabetic rats also showed congested blood vessel and inter acinar haemorrhage (Figure 2C). Diabetic rats treated with the standard anti-diabetic, gliclazide, showed normal acinar cells and islets which were present with a smaller volume as compared with control (Figure 2D). In some examination islets were present with a very scanty inflammatory cells infiltration (Figure 2E). Similarly, diabetic rats treated with Ah (400 mg/kg) showed normal acinar cells were seen to be normal. The islets were present with a large proportion of islet cells though smaller than control (Figure 2F). Treatment with Ah extract (200 mg/kg) showed relatively larger islets of Langerhans than the control one (Figure 2G), whereas, rats treated with Ah (100 mg/kg) showed smaller acinar cells and islets than normal control (Figure 2H).

Liver of normal rats showed a normal structure of the hepatic lobule. The central vein is surrounded by the hepatocytes with eosinophilic cytoplasm and distinct nuclei. The hepatic sinusoids are shown between the hepatocytes (Figure 3A). However, liver of diabetic rats showed inflammatory infiltration and periportal necrosis of the hepatocytes appeared around the portal area was also shown (Figure 3B). Focal necrosis of the hepatocytes and some pyknotic nuclei of the hepatocytes were also shown (Figure 3C). Rats treated with gliclazide, showed hepatic lobules appeared more or less like control (Figure 3D). In some rats, focal necrosis associated with inflammatory infiltration were seen (Figure 3E). Treatment with Ah extract (400, 200 and 100 mg/kg) showed the hepatocytes appeared more or less as the control (Figure 3. F-H; respectively).

Histological examination of sections of kidney of control rats showed normal structure of distal convoluted tubules that could be differentiated from the proximal convoluted tubules as having a larger





**Figure 2:** Sections of pancreas of A) control rat shows dense-staining acinar cells (arrows) and a light-staining islet of Langerhans (arrowhead) just right of the center of the field, B): a diabetic rat shows normal acinar cells (arrows). The islets are occupied by a uniform eosinophilic material (arrowhead), C): a diabetic rat shows congested blood vessel and inter acinar haemorrhage (arrow), D): a diabetic rat treated with the standard anti-diabetic, gliclazide, shows the acinar cells and islets are seen to be normal. The islets are present with a smaller volume (arrowhead) as compared with control, E): a diabetic rat treated with gliclazide shows the islets is present with a very scanty inflammatory cell infiltration (arrowhead), F): diabetic rat treated with *Artemisia* extract (400 mg/kg) shows acinar cells are seen to be normal (arrow). Islets are present with a large proportion of islet cells (arrowhead) as compared with control, G): a diabetic rat treated with *Artemisia* extract (200 mg/kg) shows islets of Langerhans that appeared relatively larger than the control one (arrowhead). Exocrine pancreas appeared more or less as control (arrow), H): a diabetic rat treated with *Artemisia* extract (100 mg/kg) shows acinar cells and islets are seen to be normal (arrow). Islets are present with a large proportion of islet cells though with a smaller volume as compared with control (arrowhead) (H & E, Scale Bar: 20  $\mu$ m)

and well defined lumina, and glomerulus (Figure 4A). Sections of kidney of diabetic rat showed the development of necrosis of epithelial cells of some proximal tubules. Some cellular debris and hemorrhage in the dilated interstitial space were noticed (Figure 4B). In some rats, lobulation and hypercellularity of glomeruli and large interstitial hemorrhagic were shown (Figure 4C). Treatment with gliclazide showed normal structure of the renal corpuscles and renal tubules (Figure 4D). In some cases, lobulated glomeruli, partially degenerative glomeruli, and haemorrhagic areas were shown (Figure 4E). Ah treatment (400, 200, 100 mg/kg), microscopic examination showed normal renal corpuscles and renal tubules (Figure 4 F-H; respectively).

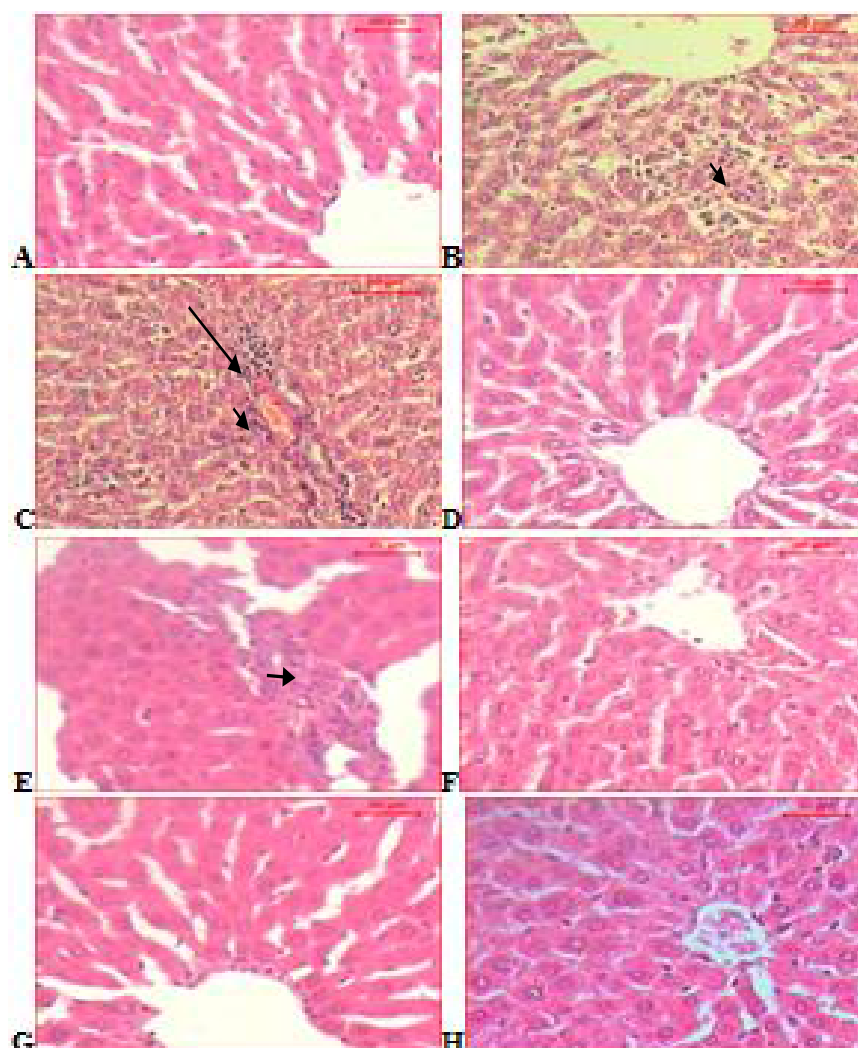
Examination transverse section normal rat aorta showed the normal histological structure of the tunica intima, tunica media and tunica adventitia (Figure 5A). Diabetic rats aorta showed a significant atherosclerosis, vacuolation in the cells of the tunica media (Figures

5B,C). In diabetic rats treated with gliclazide, Ah extract (400,200,100 mg/kg) aorta showed the normal histological structure of the tunica intima, tunica media and tunica adventitia (Figure 5 D-G, respectively).

### Immunohistochemical results

Glucagon immunoreactive cells were distributed normally in the marginal zone of the islets but some positive cells in the central zones as brown color (Figure 6). The diabetic rats show much more abundant and ubiquitous immunostaining for glucagon as compared with normal control. Treatment with gliclazide shows distribution of glucagon that appears more or less like normal. Similarly, Ah extract at the different doses shows reduction of glucagon staining but it did not reach to the normal control.

Figure 7 shows Insulin immunoreactive cells were detected with a high frequency in the central regions of the pancreatic islets of normal



**Figure 3.** Sections of liver of A) control rat shows the architecture of a hepatic lobule, B): a diabetic rat shows focal necrosis of the hepatocytes. Some of the nuclei of the hepatocytes are pyknotic (arrowhead), C): a diabetic rat shows the periportal tract with dilated and congested vein (arrow) and periportal necrosis of the hepatocytes (arrowhead), D): diabetic rat treated with gliclazide shows hepatic lobule that appears more or less like control, E): a diabetic rat treated with the standard anti-diabetic, gliclazide, shows focal necrosis associated with inflammatory infiltration (arrowhead), F): a diabetic rat treated with *Artemisia* extract (400 mg/kg) shows the hepatocytes appear more or less as the control, G): a diabetic rat treated with *Artemisia* extract (200 mg/kg) shows the hepatocytes appear more or less as the control, H): a diabetic rat treated with *Artemisia* extract (100 mg/kg) shows the hepatocytes appear more or less as the control (H & E, Scale Bar: 20  $\mu$ m).

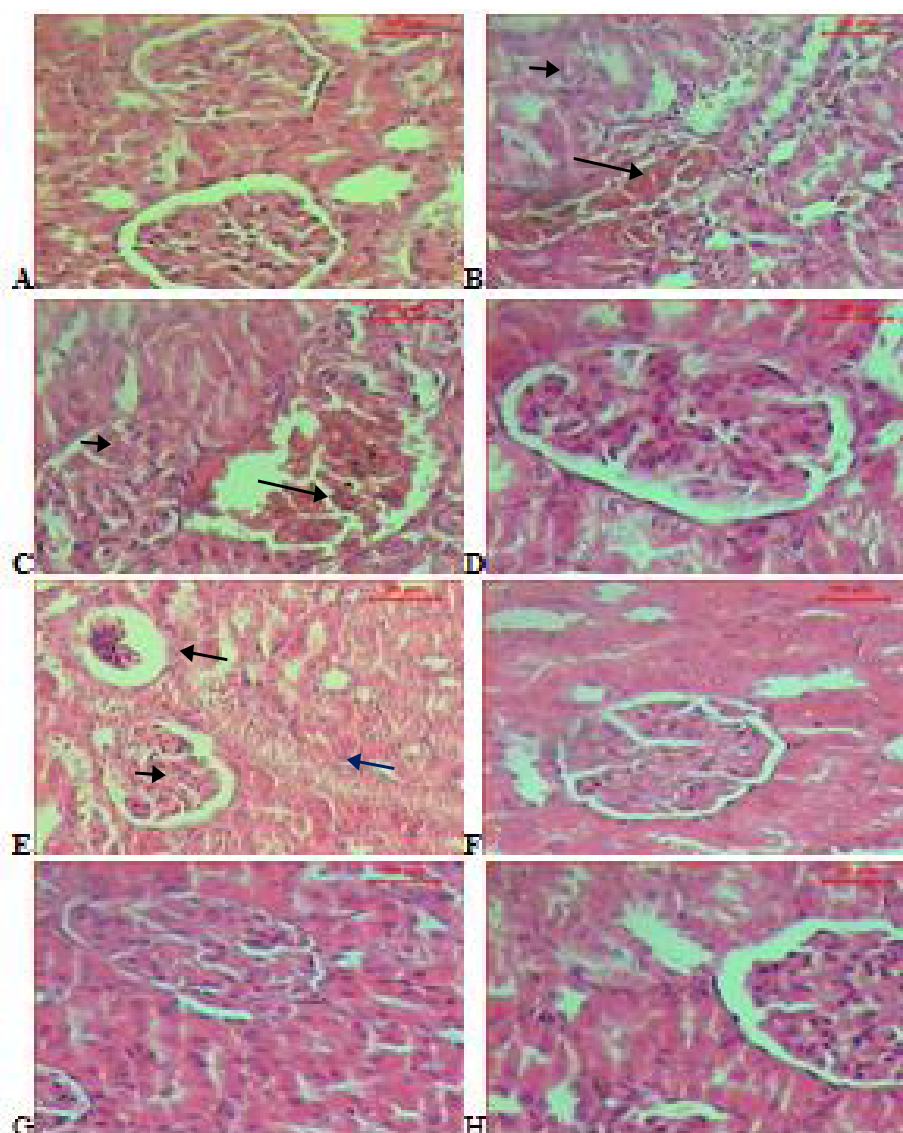
control rat appeared in brown color. No insulin positive cells were found in the peripheral regions or in the exocrine portions. On the other hand, the diabetic rat shows weaker immunostaining for insulin. However, diabetic rats treated by gliclazide or Ah extract appear with more dense insulin immunoreactivity as compared to the STZ-diabetic rats.

### Molecular analysis

The results of RAPD analysis were determined by considering the bands which appeared in the control STZ sample as the criterion of the judgment. Polymorphism observed in RAPD profiles included disappearance of a normal control band and appearance of a new band. As illustrated in Table 4, four random primers generated a total of 12 bands in normal group. The molecular size of these bands ranged from 944 to 264 bp. The highest number of band appearance and disappearance was determined in control STZ group (25), the lowest

number was determined in Ah extract at doses 200 and 100 groups (20). The Percentage of polymorphic bands recorded in standard gliclazide group was (88%), percentages of polymorphic bands determined in Ah extract at doses 400, 200 and 100 groups were (88%, 80% and 80%, respectively). Changes in RAPD profiles were significantly increased in control STZ group compared to normal group. The polymorphic bands percentages were decreased in the groups treated by standard gliclazide and Ah extract at all doses compared to Control STZ group.

On the other hand, the total number of bands generated by four primers in six groups was (121) with band sizes ranged from (1177-244bp) and the total number of polymorphic bands was (109). Primer OPA03 recorded the highest number of polymorphic bands with band size ranged (944-276) among the six groups while the lowest number of polymorphic bands was generated by primer OPA02 with band size ranged (539-265) (Figure 8)

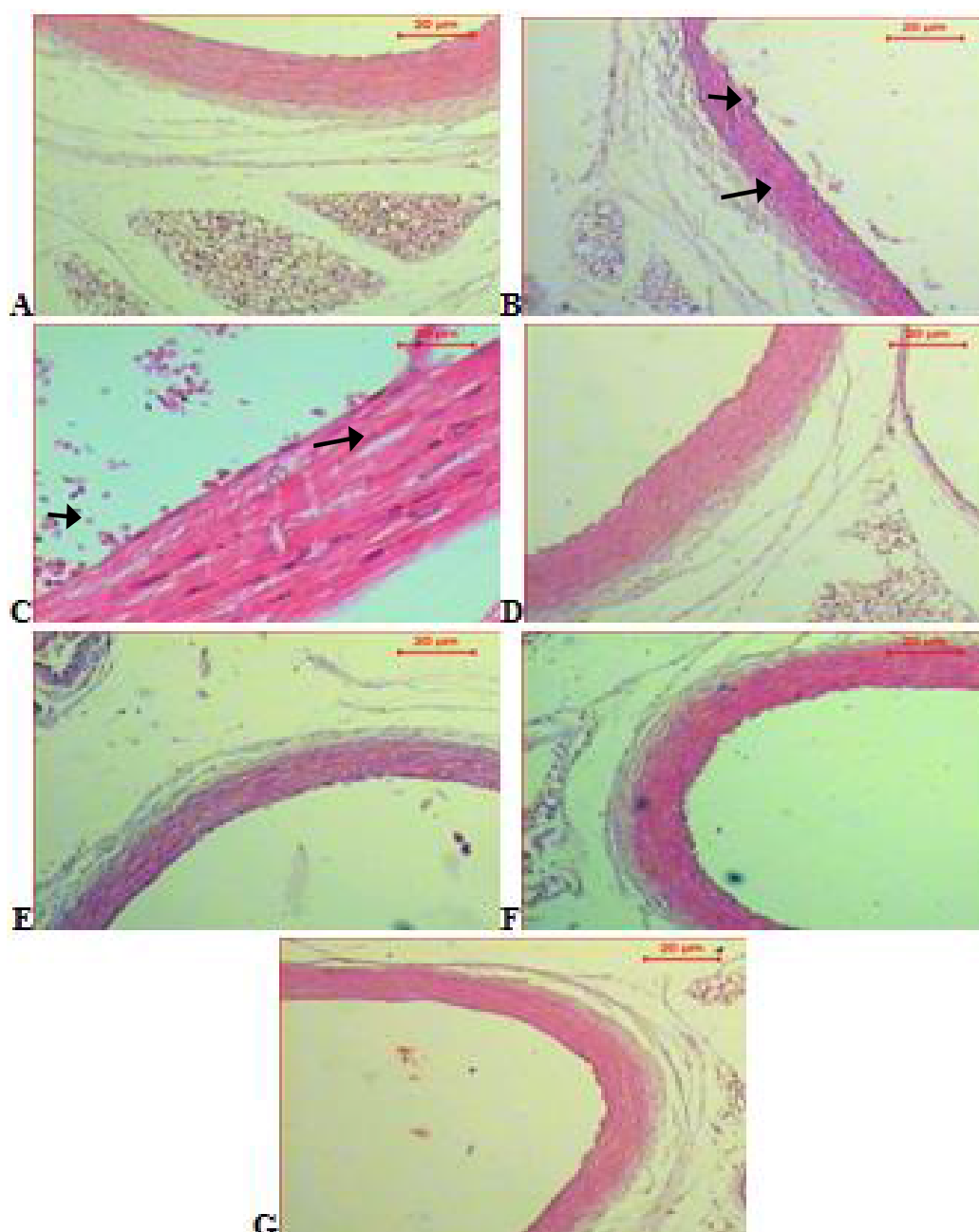


**Figure 4.** Sections of kidney of A): control rat shows normal structure of glomeruli and renal tubules, B): a diabetic rat shows the development of necrosis (arrowhead) of epithelial cells of some proximal tubules. Some cellular debris and hemorrhage in the dilated interstitial space (arrow), C), a diabetic rat shows lobulated and hypercellularity glomerulus (arrow). Large interstitial hemorrhagic is noticed (arrowhead), D): a diabetic rat kidney treated with the standard anti-diabetic, gliclazide, shows normal structure of the renal corpuscles and renal tubules, E): a diabetic rat treated with gliclazide shows the lobulated glomerulus (arrowhead), partially degenerative glomerulus (arrow), and haemorrhagic areas (blue arrow), F): a diabetic rat treated with *Artemisia* extract (400 mg/kg) show normal renal corpuscles and renal tubules, G): a diabetic rat treated with *Artemisia* extract (200 mg/kg) shows normal renal corpuscles and renal tubules, H): a diabetic rat treated with *Artemisia* extract (100 mg/kg) shows normal renal corpuscles and renal tubules (H & E, Scale Bar: 20  $\mu$ m).

## Discussion

The present study investigates the different pharmacological effects of Ah ethanolic extract in rats with type 1 diabetes. STZ injection induced hyperglycemia and decreased serum insulin level. STZ-induced diabetes is widely used as type-1 like diabetic animal model to investigate hyperglycemia [34]. It has been reported that STZ induce an autoimmune process that results in the destruction of the Langerhans islets beta cells. Symptoms of diabetes type 1 are clearly seen in rats within 2-4 days following single intravenous or intraperitoneal injection of STZ [35, 36]. Ah extract lowered plasma glucose concurrently with an

increase of plasma insulin in STZ-diabetic rats. Its hypoglycemic effect was comparable with that of the standard drug, gliclazide. Gliclazide is known to produce a hypoglycemic activity by pancreatic (stimulating insulin secretion by blocking  $K^+$  channels in the pancreatic  $\beta$  cells) and extra pancreatic (increasing tissue uptake of glucose) mechanisms [37, 38]. Hence, it can be postulated that Ah extract produce similar effects as gliclazide. In addition, hypoglycemic effects of Ah could, possibly, be due to increased peripheral glucose utilization. Recently, Tastekin et al. [39] has concluded that Ah could reduce the absorption of glucose from the intestine and inhibit the absorption of glucose by the kidney tubules thus lowers blood glucose.

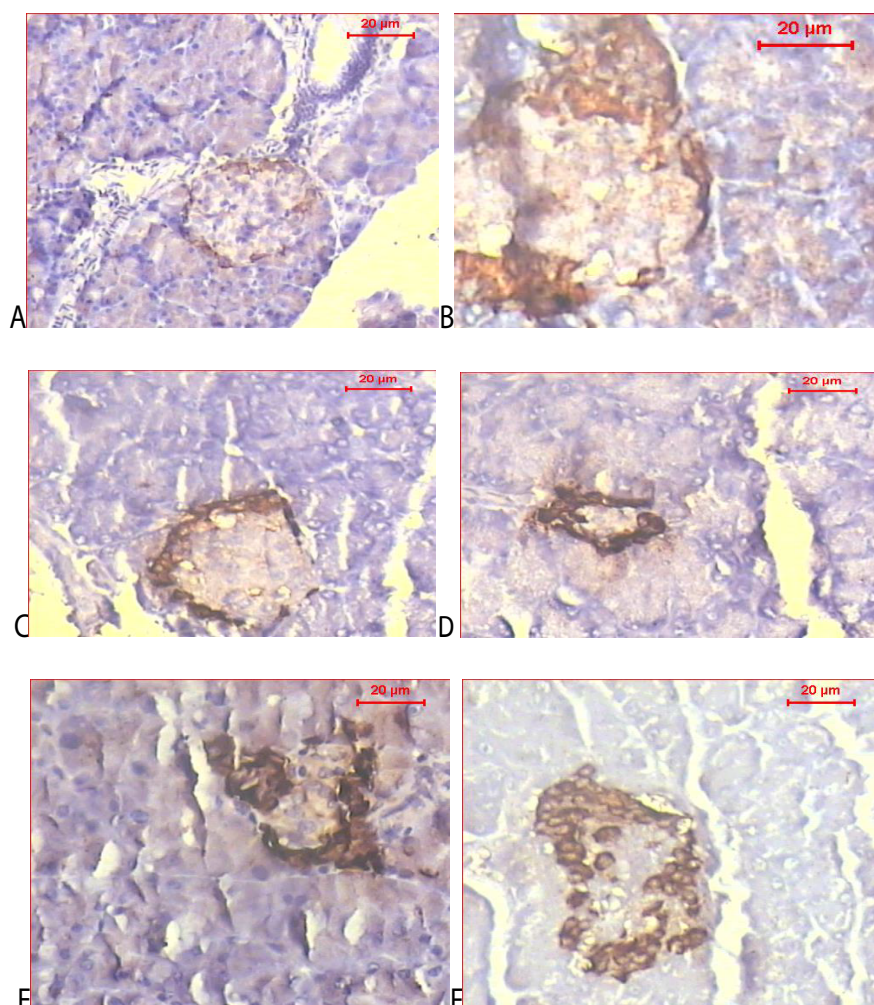


**Figure 5.** Transverse sections of aorta of rat **A**): control shows the normal histological structure, **B**): a diabetic rat shows a significant atherosclerosis (arrowhead), vacuolation in the cells of the tunica media (arrow), **C**): Magnification of **(B)** shows a significant atherosclerosis (arrowhead), vacuolation in the cells of the tunica media (arrow), **D**): a diabetic rat treated with gliclazide shows the normal histological structure of the tunica intima, tunica media and tunica adventitia, **E**): a diabetic rat treated with *Artemisia* extract (400 mg/kg) shows the normal histological structure, **F**): a diabetic rat treated with *Artemisia* extract (200 mg/kg) shows the normal histological structure, **G**): a diabetic rat treated with *Artemisia* extract (100 mg/kg) shows the normal histological structure (H & E, Scale Bar: 20  $\mu$ m).

Alterations in homocysteine metabolism have also been observed in diabetic patients. Elias and Eng [40] recorded that plasma homocysteine levels have been elevated in patients with diabetes. However, the plasma concentration of homocysteine in patients with diabetes is further confounded by the use of medication used to treat the disease and by the development of renal impairment. In the current study, STZ-treated rats presented decreased serum level of HCY and

this reduction was normalized by Ah extract and gliclazide treatment. In agreement, Gursu et al., [41] found that homocysteine levels were reduced in STZ-induced diabetic rats. This reduction was normalized by insulin in a dose-dependent manner. HCY is converted to methionine by remethylation and cysteine by transsulfuration. Insulin administration reduced activities of transsulfuration and remethylation enzymes and hence prevented conversion of Hcy to methionine





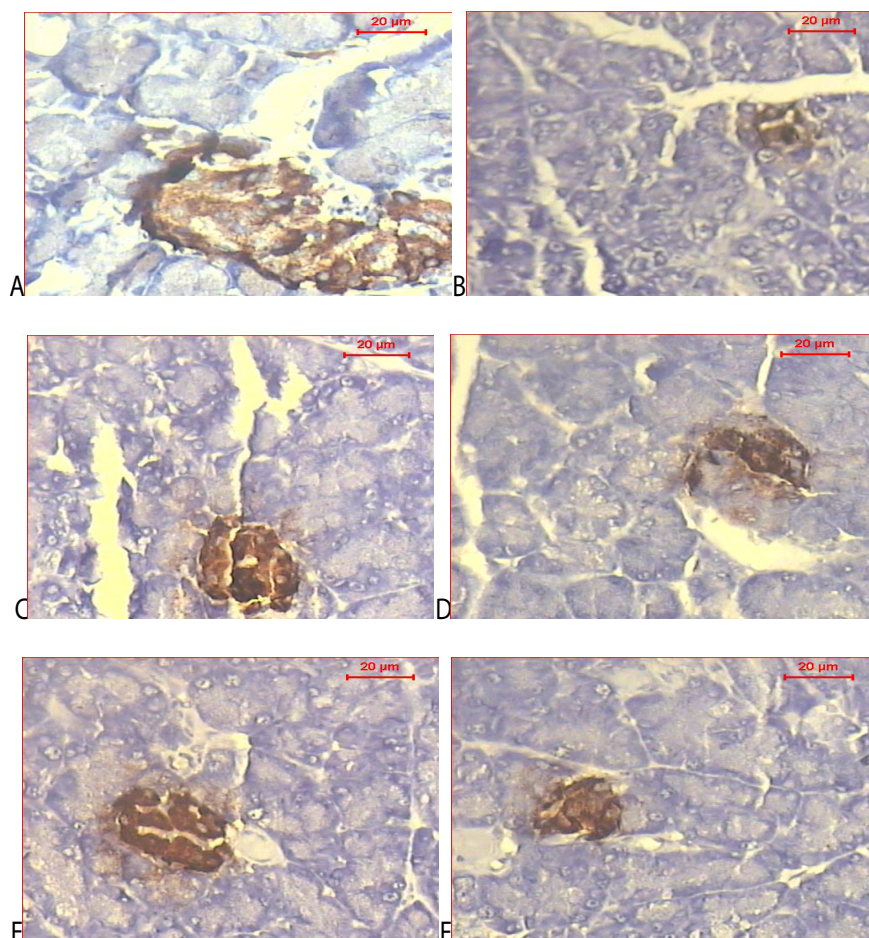
**Figure 6.** Immunohistochemical staining for glucagon antibody to identify the alpha cells in the islets of Langerhans of pancreas in (A): control rat B): a diabetic rat C): a diabetic rat treated with gliclazide D): a diabetic rat treated with Ah (400 mg/kg) E): and F): a diabetic rat treated with Ah (200 and 100mg/kg; respectively) (Immunoreactivity for insulin, Scale Bar: 20 µm).

and cysteine. Similarly, Jacobs and colleagues [42] observed a 30% reduction in plasma homocysteine in the diabetic rats. Given the above insulin results, it could be postulated that the treatment with Ah extract corrected HCY metabolism via increasing insulin level back to normal.

Diabetes is also associated with hyperlipidemia [43]. Serum lipids concentration is raised during diabetes due to increased mobilization of fatty acids from peripheral depots, since insulin inhibits the hormone sensitive lipase [44]. Excess fatty acids in the serum of diabetic rats are converted into phospholipids and cholesterol in the liver. These two substances along with excess triglycerides formed at the same time in the liver may be discharged into the blood in the form of lipoproteins [45]. In the present study, increased levels of serum triglycerides observed in STZ-induced diabetic rats were in accord with other studies [46, 47]. Administration of Ah at the three dose level normalized serum triglycerides and decreased serum cholesterol level even below the normal value. These results are in agreement with that of Abass [48] who found significant reductions were recorded in serum cholesterol upon treatment of normoglycemic rats with Ah aqueous

extract. It was concluded that this extract could present a good adjuvant to hyperlipidemia classical therapy compared to consuming the crude herb without any further modification like extraction. Mansi et al. [13] also reported that administration of Ah water extract (0.39 g/kg bwt) to alloxan-induced diabetic rats for 14 days showed considerable lowering of serum total cholesterol, triglycerides, LDL cholesterol, TC/HDL-C and an increase in HDL cholesterol.

In the current study, the significant increase in serum ALT and AST levels that was observed in STZ-induced diabetic rats represents liver damage compared to control rats. Serum enzyme activities can be used as useful biomarkers for monitoring the cytotoxicity of xenobiotics including STZ. Similar to our results, some earlier studies noted increases in serum ALT and AST activities in STZ-diabetic rats [49]. Significant reductions in serum activities of these enzymes were currently observed in A. *Herba alba* extract-treated groups. It is most likely that leakage of enzymes from tissues to serum was reduced after supplementation with this extract. It might have provided muscle integrity and ameliorated injuries of liver and heart tissues in diabetic



**Figure 7.** Immunohistochemical staining for insulin antibody to identify the beta cells in the islets of Langerhans of pancreas in (A): control rat (B): a diabetic rat (C): a diabetic rat treated with gliclazide (D): and (E): and (F): a diabetic rat treated with Ah (400,200,100mg/kg; respectively) (Immunoreactivity for insulin, Scale Bar: 20 µm).

rats. In accord with present results, Farhad et al. [50] demonstrated the reduction the complications of diabetes brought on liver after treatment with the hydro-alcoholic extract of Ah (200 and 300mg/kg bwt).

The present data revealed elevations in BUN and sr.creatinine in STZ-induced diabetic rats as expected. A similar effect was recorded previously [51]. Deficiency of insulin and consequent inability of glucose to reach the extrahepatic tissues stimulate gluconeogenesis as an alternative route of glucose supply [52]. This route is sustained by increased proteolysis which releases free glucogenic amino acids into the plasma that are deaminated in the liver with the consequence of increased urea in the blood. Creatinine is produced from creatine, a molecule of major importance for energy production in muscles. The kidneys maintain the blood creatinine in a normal range; hence, abnormally high levels of creatinine indicate impairment of renal function. [53]. In the current study, administration of ethanol extract of Ah produced a reduction in the levels of these metabolites, thereby conferring protection against impairment due to diabetes. It is worthy to mention that this herb showed better improvement of kidney function than the standard gliclazide.

Diabetes mellitus (hyperglycemia) is a metabolic disorder that

results in excessive production of free radicals which leads to severe oxidative damage of cell components like lipids, proteins and DNA. In the present study, Ah extract showed strong antioxidant activity via enhancement of GSH concentration and inhibition of lipid peroxidation in hepatic and renal tissues. It has been documented that Ah plant contain wide variety of antioxidant molecules, such as phenolic acids, flavonoids and other natural antioxidants [54]. Additional studies have shown the antioxidant effect of Ah. The phenolic compounds have been reported to be significantly associated with the antioxidant activity of plant and food extracts [55]. It has been also found that Ah essential oils have some antioxidant abilities for preventing the linoleic oxidation and to reduce DPPH radicals [56].

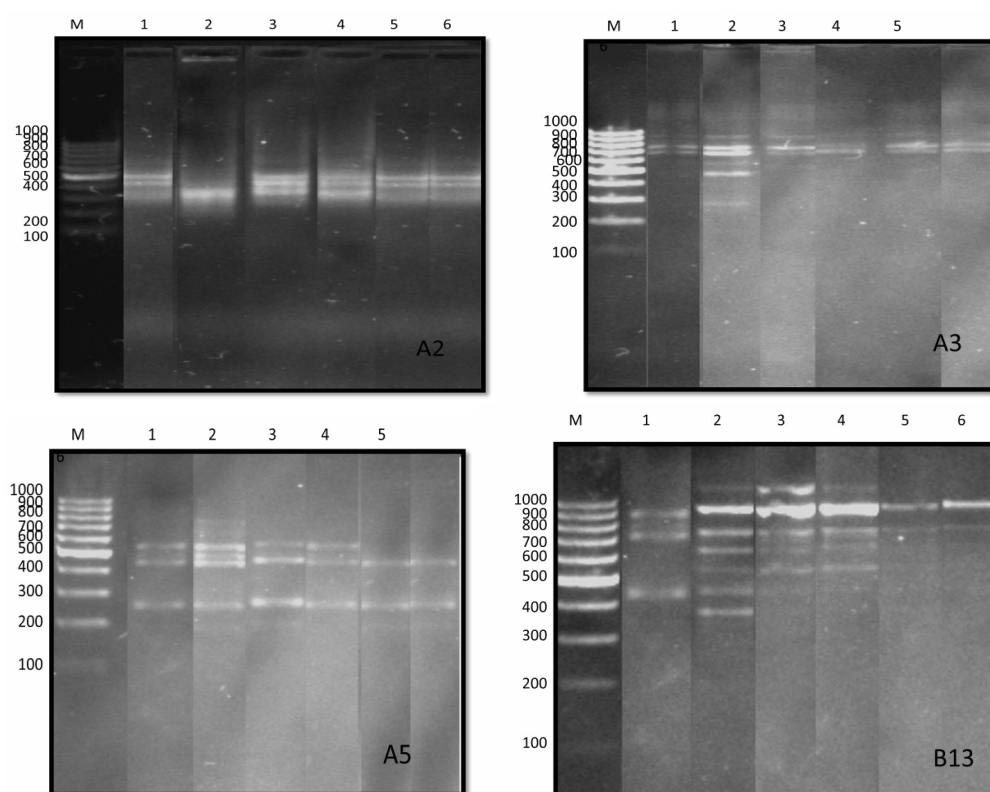
The histopathological findings of STZ-treated rats further indicated the presence of pathological alterations in the pancreatic tissue as well as the other organs (liver, kidney, heart, aorta) revealing progression of diabetic complications. Treatment with Ah extract restored the morphological changes and began to recover the normal tissues histology. Hence, the pancreatic, hepatic, renal, cardiac and aortic lesions induced by STZ were significantly diminished by administration of Ah at lower to higher doses. The current Immunohistochemical results showed that the plant could reverse the catabolic features of



Primer	Normal Control	STZ Control		Gliclazide (Standard)		Ah ext (400mg/kg)		Ah ext (200mg/kg)		Ah ext (100mg/kg)	
		a	b	a	b	a	b	a	b	a	b
OPA02	3	2	2	3	3	2	2	2	3	1	2
OPA03	3	4	1	1	4	2	6	2	6	2	6
OPA05	3	5	1	1	5	1	5	0	4	0	4
OPB13	3	7	3	1	4	1	3	0	3	2	3
Total	12	18	7	6	16	6	16	4	16	5	15
a+b		25		22(88%)		22(88%)		20(80%)		20(80)	

a:appearance of new bands, b:disappearance of control bands , a+b: polymorphic bands.

**Table 4:** Changes of total bands in control, polymorphic bands and varied bands in samples:



**Figure 8:** RAPD-PCR fingerprints generated by OPA02, OPA03, OPA05 and OPB13 primers for genomic DNA isolated mice and DNA fragmentation. Lane M: ladder DNA (1000-100pb). Lane1 (normal control), Lane2 (STZ control), Lane3 (gliclazide), Lane4 (Ah, 400mg/kg) Lane 5 (Ah, 200mg/kg), Lane 6 (Ah, 100mg/kg).

insulin deficiency, decrease the release of glucagon and increase that of insulin which may be via direct stimulation of glycolysis in peripheral tissues, increase glucose removal from blood or reduce glucose absorption from the gastrointestinal tract [57].

In the current study, STZ-induced diabetes caused DNA damage in rat liver, which was decreased in gliclazide and Ah extract treated groups as compared to diabetic one. This is in accordance with Iriadam et al. [58] who found that Ah possesses antidiabetic effects. Sliwinska et al. [59] found that gliclazide has antioxidant properties and diminished DNA damage induced by free radicals. Araki and Nishikawa [60] and Mckillop and Schrum [61] reported that oxidative stress is one of the main etiologies for complications of diabetes, which involves the formation of highly reactive OH and leads to severe oxidative damage of the cell's components like DNA. Therefore, the current study reveals

that besides having a beneficial effect on reducing oxidative stress in diabetic rats through the scavenging of free radicals, Ah extract may be used as protective agent against DNA damage.

## Conclusion

Oral administration of ethanol extract of Ah for 14 days showed hypoglycemic activity in STZ-induced diabetes in Wistar rats. The findings indicated the beneficial effects of this herb in reducing hyperlipidemia accompanying diabetes. Other complications such as hepatic and renal impairment were also improved.

Additional investigations are warranted to study the active principles of Ah that are responsible for these pharmacological activities. Further studies are also needed to know more about the mechanisms of hypoglycemic action of this plant.

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