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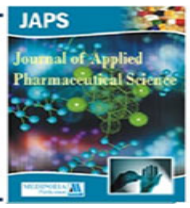

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Hypoglycemic Activity of *Artemisia herba-alba* (Asso.) used in Egyptian Traditional Medicine as Hypoglycemic Remedy

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ABSTRACT

In vitro and *In vivo* screening of hypoglycemic activity of *Artemisia herba-alba* (Asso.) herbs emphasized its activity in hypoglycemic remedy. Further investigation on fractions of 70% ethyl alcohol extract of *Artemisia herba-alba* (Asso.) herbs revealed that the ethyl alcohol extract produces hypoglycemic effect more than any of its fractions. Four compounds were isolated and identified from fractions of 70% ethyl alcohol extract of *Artemisia herba-alba* (Asso.) herbs. These compounds were previously reported for their hypoglycemic activity.

Keywords: Hypoglycemic, Artemisia, Traditional Medicine.

INTRODUCTION

Diabetes mellitus (DM) is a chronic disease caused by inherited and/or acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced. Management of diabetes without any side effects is still a challenge to the medical system (Kameswara Rao *et al.*, 2003). Chronic hyperglycemia during diabetes causes glycation of body proteins that in turn leads to secondary complications affecting eyes, kidneys, nerves and arteries (Sharma, 1993). These may be inhibited or lowered by maintaining blood glucose values close to normal. The therapeutic measurements include use of insulin and other agents like amylin analogues, alpha glycosidase inhibitors like acarbose, miglitol and voglibiose, sulphonylureas, biguanides for the treatment of hyperglycemia. These drugs also have certain adverse effects like causing hypoglycemia at higher doses, liver problems, lactic acidosis and diarrhea. Apart from currently available therapeutic options, many herbal medicines have been recommended for the treatment of diabetes. Traditional plant medicines are used throughout the world. Herbal drugs are prescribed widely because of their effectiveness, less side effects and relatively low cost (Venkatesh *et al.*, 2003). *Artemisia herba-alba* (Asso) has a wide use in traditional medicine, for treatment of gastric disturbances, such as diarrhoea, abdominal cramps and for healing external wounds (Feuerstein *et al.*, 1986).

Uses for diabetes mellitus and other conditions as jaundice are also reported (Marrif *et al.*, 1995). The species is recommended for neurological disorders, and an ethanolic extract has shown activity in the GABAA-benzodiazepine receptor assay (Salah and Jager, 2005).

MATERIALS AND METHODS

Plant material

The dried *Artemisia herba-alba* (Asso.) herbs were purchased from the Egyptian markets and were grinded by electric grinder.

Extraction of plant material

One kilogram of powdered dried *Artemisia herba-alba* (Asso.) herbs was percolated in 70:30 ethanol: water then filtrated using folded muslin. This process was repeated several times until complete exhaustion of the plant materials. The extracts were evaporated to dryness under vacuum and weighed (yield 34.8%).

Fractionation of alcoholic extract of *Artemisia herba-alba* (Asso.) herbs

Fifty grams of the dried 70% ethyl alcohol extract of *Artemisia herba-alba* (Asso.) herbs were dissolved in least amount of water and partitioned several time till complete exhaustion against n. hexane, diethyl ether and ethyl acetate and water soluble fraction. The yields were 6.3%, 1.8%, 1.4% and 89.6%.

Study of the lipoidal matters fractions *Artemisia herba-alba* (Asso.)

Preparation of the n-hexane fraction

Hundred grams of powdered air-dried herbs *Artemisia herba-alba* (Asso.) were extracted with 70% ethyl alcohol at room temperature. The extract was evaporated till dryness (yield = 34.8% w/w) and was dissolved in least amount of water and partitioned with n-hexane. The combined n-hexane fractions were evaporated till dryness (yield = 2.16% w/w of plant material or 6.2% w/w of extract).

Saponification of the n-hexane fraction

The saponification of the n-hexan was carried out according to Tsuda *et al.*, 1960 and the unsaponifible matter were analyzed by GC/MS using Capillary column of fused silica, DB-5 stationary phase, temperature programming of 70-290 °C at a rate of 3 °C /min and mass spectrum detector.

Preparation of fatty acid methyl esters

The preparation of fatty acid methyl esters was carried out according to Finar, 1967and the fatty acid methyl esters were analyzed by GC/MS using Capillary column of fused silica, DB-wax stationary phase, temperature programming of 50-260 °C at a rate of 4 °C /min and mass spectrum detector.

Identification of the constituents analysed by GC/MS was performed by comparison of their spectral fragmentation patterns with those of the available database libraries Wiley (Wiley Int.), USA and /or published data **(Adams, 1995).** Quantitative determination was carried out based on peak area integration.

Study of the volatile constituents of the crude extract

Twenty grams of dried 70% ethyl alcohol extract of herbs *Artemisia herba-alba* (Asso.) were subjected to hydro distillation using Nickerson apparatus. The essential oil obtained was dried over anhydrous sodium sulphate and 1967and analyzed by GC/MS using Capillary column of fused silica, DB-5 stationary phase, temperature programming of 40-260 °C at a rate of 3 °C /min and mass spectrum detector.

Preparation of the polysaccharide (mucilage) fraction

The Polysaccharide (mucilage) of 70 % ethyl alcohol extract of *Artemisia herba-alba* (Asso.) herbs has been isolated and weighed. The yield was 8.68 % w/w of dry extract. The isolated polysaccharide was odourless, soluble in water, insoluble in ethanol, ether and chloroform. It gave positive Molish's test **(Molish, 1886)** and did not reduce Fehling's and Barfoed's solutions. In addition, it gave negative test for proteins **(Plimmer, 1926)** and left no ash on ignition.

Hypoglycemic evaluation of plant extract and/or fractions

In Vitro screening for the hypoglycemic activity using α-amylase inhibition technique (Hasenah et al., 2006)

The α-amylase inhibition assay was performed using the chromogenic method adopted from Sigma–Aldrich, adapted from **(Bernfeld, 1955)**. Pancreatic α-Amylase was dissolved in ice-cold distilled water to give a concentration of 4 unit/ml solution. Potato starch (0.5%, w/v) in 20 mM phosphate buffer (pH 6.9) containing 6.7mM sodium chloride, was used as a substrate solution. 40 µl of plant extract or mucilage (20 mg/ml in DMSO), 160 µl of distilled water and 400 µl of starch were mixed in a screw-top plastic tube. The reaction was started by the addition of 200 µl of the enzyme solution. The tubes were incubated at 25 ◦C for a total of 3 min. Final concentrations in the incubation mixture were plant extract, 1 mg/ml, 0.25% (w/v) starch and 1 unit/ml enzyme. After 3 minutes 200 µl mixtures was removed and added into a separate tube containing 100 µl color reagent solution (96 mM 3, 5-dinitrosalicylic acid, 5.31M sodium potassium tartrate in 2M NaOH) and placed into a 85 ◦C water bath. After 15 min, this mixture was diluted with 900 µl distilled water and removed from the water bath. α- amylase activity was determined by measuring the absorbance of the mixture at 540 nm. Control incubations, representing 100% enzyme activity were conducted in an identical fashion replacing plant extract with DMSO (40 µl). For blank incubations (to allow for absorbance produced by the plant extract), the enzyme solution was replaced with distilled water and the same procedure was carried out as above.

$$\text{Percent (\%) of α-amylase inhibition} = \frac{\text{Absorbance control} - \text{Absorbance test}}{\text{Absorbance control}} \times 100$$

In Vivo screening for the hypoglycemic activity of 70% ethyl alcohol extract of Artemisia herba-alba (Asso.) herbs and/or its fractions:

In Vivo study for the hypoglycemic activity of crude extract

Animals

Adult male albino rats weighing 100–120 g obtained from the laboratory animal house of the National Researches Center, Dokki, Egypt. Rats were housed in clean cages and acclimatized to the laboratory condition with temperature (22–24 ◦C), 12-h light: 12-h dark cycle and relative air humidity 40–60%. Rats had continuous access to food and to tap water.

Preparation of diabetic rat

Adult male albino rats were injected with alloxan purchased from Sigma –Aldrich chemical company (USA) intraperitonealy (120 mg/kg.). Nine rats were used in each group. Each animal was used once only in all of experiments. The food and water were removed from cages 12 h before blood sampling. Two ways of regimen were used treatment regimen and prophylactic regimen.

Dose Calculation

The Chosen treatment dose for 70% ethyl alcohol extract of *Artemisia herba –alba* (Asso.) herbs was done as mentioned in (Marrif *et al.,* 1995).

The dose of fractions was calculated as follow

$$\text{Dose} = \frac{(\text{yield \% of fraction}) \times \text{dose of extract}}{100}$$

Blood sampling

Blood samples were drawn from the retro-orbital plexus of the overnight fasted rats (12 hours) as described by **(Schermer, 1967)**.

Determination of blood glucose level

Plasma glucose was determined using enzymatic colorimetric kit (from Vitro Scient, Egypt) according to **(Trinder 1969)**.

Statistical analysis

Differences between vehicle control and treatment groups in biological screening were statistically analyzed by one-way ANOVA technique followed by the least significant difference (L.S.D). Results are expressed as mean ± S.E. Methods of statistical analysis were done according to **Armitage, 1971**.

Determination of the median lethal dose ($L.D_{50}$) of the crude extract

Adult male albino rats with average weight of (100-120) were used for the determination of median lethal dose ($L.D_{50}$) of the crude extract was a single oral dose through a stomach tube. The lethal dose ($L.D_{50}$) of the 70% ethyl alcohol extract of *Artemisia herba-alba* (Asso.) herbs was determined and the mortality was recorded for 72 hours.

The determination of the lethal dose fifty ($L.D_{50}$) for the crude extract was carried-out through two important successive steps according to **(Kärber 1931)** as follow:

Preliminary test for LD_{50} of 70% ethyl alcohol extract of herbs of *Artemisia herba-alba* (Asso.)

Five groups each group contains 4 rats, where the animal groups receiving the following doses of plant extract:

1st group: receive 500 mg/kg of plant extract.
2nd group: receive 1000 mg/kg of plant extract.
3rd group: receive 2000 mg/kg of plant extract.
4th group: receive 3000 mg/kg of plant extract.
5th group: receive 4000 mg/kg of plant extract.

The minimum dose that killed animals in the group called maximum tolerated dose (**MTD**).The following dose that killed animals in the group called lethal dose (**$L.D_{100}$**), where the approximate $L.D_{50}$ (**App. LD_{50}**) is calculated and this dose is used for the determination of median lethal dose (**$L.D_{50}$**) in the following steps.

$$\text{The Approximate } L.D_{50} \text{ (App. } LD_{50}) = \frac{(\text{MTD}) + L.D_{100}}{2}$$

Estimation of the median lethal dose ($L.D_{50}$)

The dose of approximate $L.D_{50}$ is multiplying with constant factor (1.1) and the following groups (6 rats each) taken the following dose:-

1st group: received the dose of (approximate $L.D_{50}$X 1.1) and number of rats died in the group was estimated for 72 hours.
2nd group: received the dose of (approximate $L.D_{50}$X 1.1^2) and number of rats died in the group was estimated for 72 hours.
3rd group: received the dose of (approximate $L.D_{50}$X 1.1^3) and number of rats died in the group was estimated for 72 hours.
4th group: received the dose of (approximate $L.D_{50}$X 1.1^4) and number of rats died in the group was estimated for 72 hours.
5th group: received the dose of (approximate $L.D_{50}$X 1.1^5) and number of rats died in the group was estimated for 72 hours.

Using the method of (**Litchfield and wilcoxon, 1989)**, a curve can be obtained between the administrated doses and number of animal died in the groups. From the curve, the dose which has the ability to kill half number of animals in the group is called the median lethal dose fifty ($L.D_{50}$) can be calculated.

Study of the *In vivo* treatment hypoglycemic activity of the crude extract

Three days after alloxan injection, the rats with fasting blood glucose higher than 180 mg/dl were used for this experiment. Rats were subdivided into 3 groups:

Group.1 Control group: non diabetic rats that orally administered of a daily dose of 1 ml 3% tween 80 in water solution for 60 days.

Group.2 Diabetic control group: alloxanized diabetic rats that orally administered of a daily dose of 1 ml 3% tween 80 in water solution for 60 days.

Group .3 Diabetic-alcoholic extract of 70% ethyl alcohol extract of *Artemisia herba-alba* (Asso.) herbs group: alloxanized diabetic rats that orally administered of a daily dose of 390 mg/kg b.w. for 60 days.

Study of the *In vivo* prophylactic hypoglycemic activity of the crude extract

Normal rats were subdivided into 3 groups.

Group.1 Control group: non diabetic rats that orally administered of a daily dose of 1 ml 3% tween 80 in water solution for 60 days.

Group.2 Diabetic control group: alloxan- that orally administered of a daily dose of 1 ml 3% tween 80 in water solution for 20 days then injected with alloxan (120 mg/kg, i.p.) and oral administration of 1 ml 3% tween 80 in water solution was continued for 15 days.

Group .3 Diabetic prophylactic-alcoholic extract of 70% ethyl alcohol extract of *Artemisia herba-alba* (Asso.) herbs group: rats were subjected for daily oral treatment dose of 390 mg/kg b.w by 70% ethyl alcohol extract of *Artemisia herba-alba* (Asso.) herbs for 20 days then injected with alloxan (120 mg/kg, i.p.) and oral administration of extract was continued for 15 days.

The study of hypoglycemic activity of fractions of the 70% ethyl alcohol extract of *Artemisia herba-alba* (Asso.) (Acute regimen)

Albino mice were injected with alloxan (120 mg/kg, i.p.). Three days after injection, the mice with fasting blood glucose higher than 170 mg/dl were used for the experiments. Eight mice were used in each group. The extracts were administrated orally then blood sampling was carried out after one and two hours.
Mice were subdivided into 6 groups:

Group.1 Diabetic control group: alloxanized diabetic mice that orally administered a dose of 1 ml 3% tween 80 in water solution.

Group .2 Diabetic-crude alcoholic extract of *Artemisia herba-alba* (Asso.) group: alloxanized diabetic mice that orally administered a dose of 390 mg/kg b.w. in 3% tween 80 in water solution.

Group .3 Diabetic-n-hexane fraction of *Artemisia herba-alba* (Asso.) group: Alloxanized diabetic mice that orally administered a dose of 24.18 mg/kg b.w..

Group .4 Diabetic- diethyl ether fraction of *Artemisia herba-alba* (Asso.) group: alloxanized diabetic mice that orally administered a dose of 4.6 mg/kg b.w..

Group .5 Diabetic- ethyl acetate fraction of *Artemisia herba-alba* (Asso.) group: alloxanized diabetic mice that orally administered a dose of 4.6 mg/kg b.w.

Group .6 Diabetic-70% ethyl alcohol fraction of *Artemisia herba-alba* (Asso.) group: alloxanized diabetic mice that orally administered a dose of 3.49 mg/kg b.w.

Safety profile of extract of *Artemisia herba-alba* (Asso.) herbs

Determination of glutamic pyruvic transaminase (GPT) and glutamic oxaloacetic Transaminase (GOT)

The determination of GPT and GOT activities were performed according to Reitman and Frankel 1957.

Determination of Alkaline phosphatase (ALK)

The determination of ALK activity was performed according to Belfield and Goldberg 1971.

Determination of urea

Measurements were made according to the method designated by diamond Kits and referred to Patton and Crouch 1977.

Determination of creatinine

The determination of creatinine was carried out using the biomérieux reagent kits according to Houot 1985.

Histopathology

Slices from liver were taken from the animals and fixed in buffer formalin (10%). Paraffin embedded sections (4µm thick) were taken after fixation and slides were stained using haemoatoxylin and eosin (H&E) by the method of Hirsch *et al.*, 1997.

Phytochemical study of 70% ethyl alcohol extract of herbs of *Artemisia herba-alba* (Asso.)

Investigation of the ethyl acetate fraction of 70% alcoholic extract of herbs of Artemisia herba-alba (Asso.)

The ethyl acetate fraction has been prepared and fractionated by applying 1 gm on 3 mm paper chromatography and subjected to mobile phase 15% acetic acid in water which gave two characteristic. The second band has been eluted, evaporated to dryness under vacuum then, weighed. It is 137 mg. PC profile of the isolated band using BAW 4: 1: 5 as mobile phase gives 3 bands.

RESULT AND DISCUSSION

Hypoglycemic evaluation of plant extract and/or fractions

In Vitro screening of the prepared crude extract and mucilage of Artemisia herba-alba (Asso.) herbs for the hypoglycemic activity using α-amylase inhibition technique

The 70% ethyl alcohol extract of *Artemisia herba-alba* (Asso.) herbs and Mucilage of 70% ethyl alcohol extract of *Artemisia herba-alba* (Asso.) herbs inhibited the activity of α-amylase by 11% and 2% respectively. Thus, their hypoglycemic activity may be duo to inhibition of the activity of α-amylase.
One therapeutic approach for treating diabetes is to decrease the post-prandial hyperglycemia. This is done by retarding the absorption of glucose through the inhibition of the carbohydrate hydrolyzing enzymes, α-amylase and α-glucosidase, in the digestive tract. Inhibitors of these enzymes delay carbohydrate

digestion and prolong overall carbohydrate digestion time, causing a reduction in the rate of glucose absorption and consequently blunting the post-prandial plasma glucose rise (Rhabasa-Lhoret and Chiasson, 2004).

In vivo treatment hypoglycemic activity of the crude extract

It can be concluded from Table (1) and Fig.1 that: Alloxan elevated the plasma glucose level in all animals. The change in the plasma glucose levels in both the control and diabetic control groups are insignificant. The decreases in plasma glucose levels by treatment with the crude extract are significant, which prove the hypoglycemic activity of *Artemisia herba-alba* (Asso.). Also, we can see that the plasma glucose levels decrease at percentages of 72% by 70% ethyl alcohol extract of herbs of *Artemisia herba-alba* (Asso.).

Table. 1: *In vivo* treatment hypoglycemic activity of the crude extract.

Group	Dose mg/kg b.w	Mean ± S.E Before	After	% of change
Control	1 ml 3% tween 80	107±1.4	99±2	7%
Diabetic Control	1 ml 3% tween 80	391±1.36	407±2.6	-4%
Diabetic+ 70% ethyl alcohol extract of *Artemisia herba –alba* (Asso.) herbs	390	391±1.36	109±0.97	72%*

Results are expressed as mean ± S.E (n=9). * Significant. Statistical analysis is carried out using one way analysis of variances (ANOVA) accompanied with pot-hoc SPSS computer program.

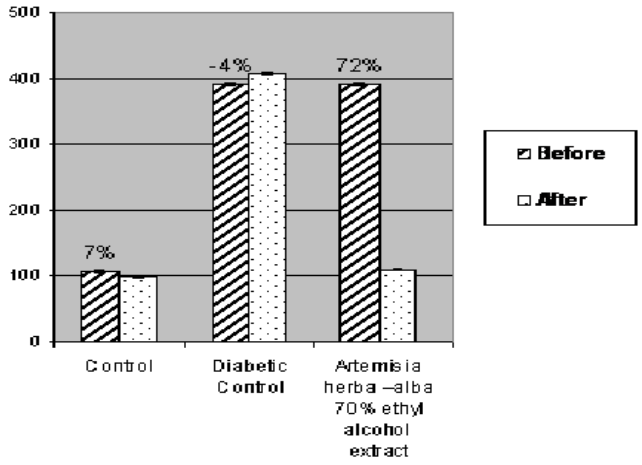

Fig. 1: Blood sugar levels (mg/dl) of diabetic rats before and after treatments with crude extract in vivo prophylactic hypoglycemic activity of the crude extract.

In vivo prophylactic hypoglycemic activity of the crude extract

It can be seen from Table (2) and Fig.2 that: Alloxan elevated the plasma glucose level in all animals. The change in the plasma glucose levels in both the control and diabetic control groups are insignificant. The decreases in plasma glucose levels by treatment with the crude extract are significant, which prove the hypoglycemic activity of *Artemisia herba-alba* (Asso.). Also, we can see that the plasma glucose levels decrease at percentages of 70% by 70% ethyl alcohol extract of herbs of *Artemisia herba-alba* (Asso.). Results did not significantly different from results of the first experiment.

Table. 2: *In vivo* prophylactic hypoglycemic activity of the crude extract.

Group	Dose mg/kg b.w	Mean ± S.E Before	After	% of change
Control	1 ml 3% tween 80	107± 1.4	99± 2	7%
Diabetic Control	1 ml 3% tween 80	391± 1.36	407± 2.6	-4%
Diabetic+ 70% ethyl alcohol extract of *Artemisia herba –alba* (Asso.) herbs	390	391± 1.36	117± 1.91	70%**

Results are expressed as mean ± S.E (n=9).
* Significant
Statistical analysis is carried out using one way analysis of variances (ANOVA) accompanied with pot-hoc SPSS computer program.

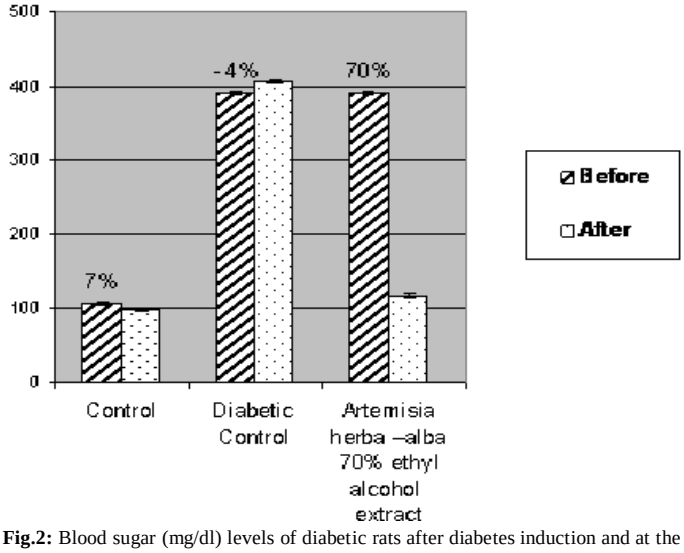

Fig.2: Blood sugar (mg/dl) levels of diabetic rats after diabetes induction and at the end of experiment (prophylactic regimen).

Safety profile of extract of *Artemisia herba-alba* (Asso.) herbs: Determination of the median lethal dose ($L.D_{50}$) of the crude extract

The median lethal dose fifty ($L.D_{50}$) of 70% alcoholic of *Artemisia herba-alba* (Asso.) herbs are found to be 3g/kg of body weight.

Determination of the liver functions in treated rats with the crude extract

Analysis of the liver enzymes (GOT, GPT and ALT) proved that the changes are insignificant relative to the control group which proves the safety of the crude extract on liver as shown in Table (3) and illustrated in Fig. 3.

Table . 3: Effect of the crude extract on the liver enzymes in plasma of the treated rats.

Group	GOT	GPT	ALK
Control	60.14±0.70	35±0.50	74.86±1.47
Diabetic	60.89±0.52	36.13±0.79	91±0.90
70% alcoholic extract of herbs of *Artemisia herba-alba* (Asso.)	61.4±0.81	35.80±0.91	90±0.65

Results are expressed as mean ± S.E (n=9).
* Significant
Statistical analysis is carried out using one way analysis of variances (ANOVA) accompanied with pot-hoc SPSS computer program.

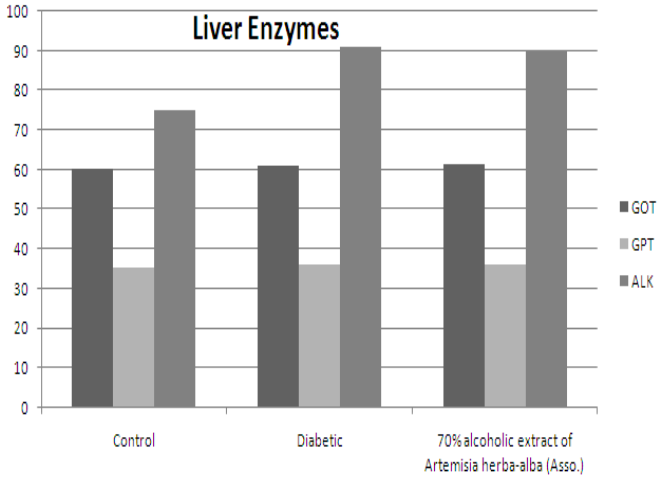

Fig.3: Effect of the crude extract on the liver enzymes of plasma of the treated rats.

Determination of the kidney functions in treated rats with the crude extract

Analysis of urea and creatinine proved that the changes are insignificant relative to the control group which proves the safety of the crude extract on kidney as shown in Table (4) and illustrated in Figs. 4.

Table. 4: Effect of the crude extract on creatinine and urea in plasma of the treated rats.

Group	Urea	Creatinine
Control	19.57±0.90	0.76±0.035
Diabetic	19.86±0.60	0.89±0.03
70% alcoholic extract of herbs of *Artemisia herba-alba* (Asso.)	19±0.65	0.81±0.038

Results are expressed as mean ± S.E (n=9).
* Significant.
Statistical analysis is carried out using one way analysis of variances (ANOVA) accompanied with pot-hoc SPSS computer program.

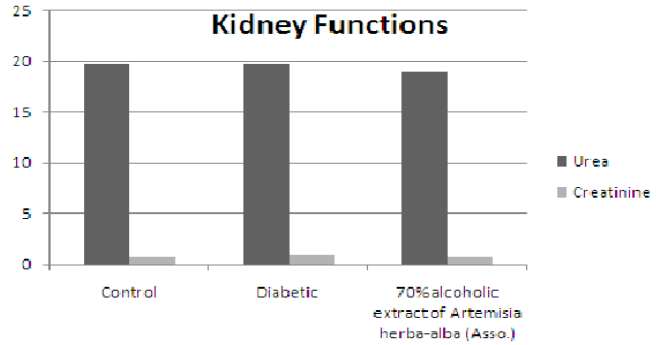

Fig.4: Effect of the crude extract on creatinine and urea in plasma of the treated rats.

Histopathology

In the present study, the light microscopic examination of the liver sections of normal rats showed that the hepatic lobules formed of radially arranged cords of normal liver cells that radiated from the central vein to the periphery of the lobule. These observations were in accordance with (Bailey *et al.*, 2009) who founded the same architecture of the normal hepatic cells. On the other hand the diabetic control group liver tissue show the damaging effect of alloxan on the liver tissue in the form of multiple foci of cellular infiltrate and diffuse infiltrate in the dilated blood sinusoids. Lison of alloxan injection is dilatation and congestion of the main blood vessels with marked fibrosis around, that extends in between the hepatocytes as shown in Fig.5.

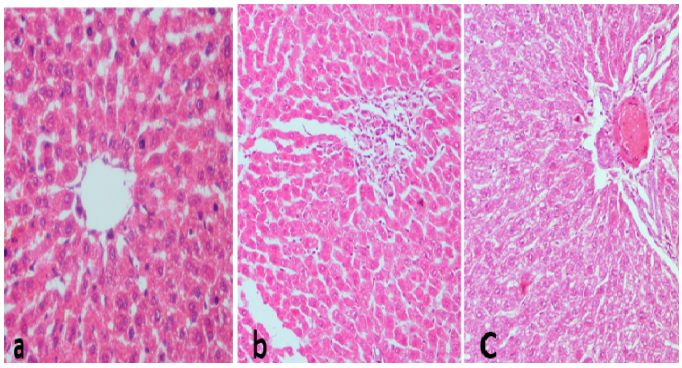

Fig.5: Histopathological profile of normal group and alloxanized liver sections.
(a) The normal architecture of the liver tissue.
(b) A section of the liver tissue showing the damaging effect of alloxan on the lever tissue in the form of multiple foci of cellular infiltrate and diffuse infiltrate in the dilated blood sinusoids.
(c) Another lesion of alloxan injection is dilatation and congestion of the main blood vessels with marked fibrosis around, that extends in between the hepatocytes.

In the 70% ethyl alcohol extract of *Artemisia herba-alba* (Asso.) herbs group, a very good effect of the drug, where the liver tissue appeared quite normal, but a few cellular infiltrate was seen at the portal area diffusing from it along the periphery of the lobule as shown in Fig.6**.** The improvement occurred in the histopathological picture of liver and also confirmed our results.

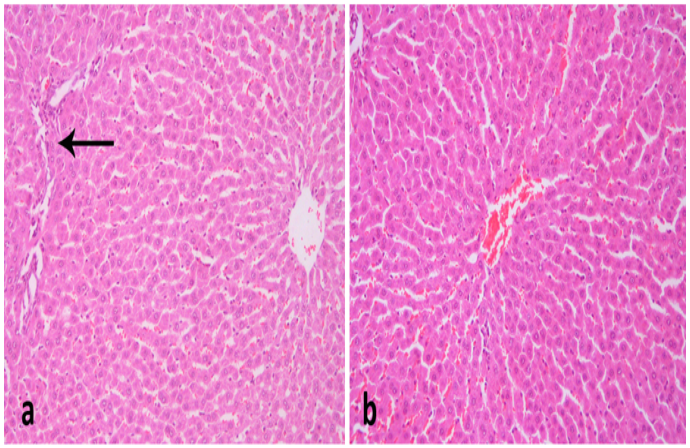

Fig.6: Histopathological profiles of 70% ethyl alcohol extract of *Artemisia herba-alba* (Asso.) group liver sections.
(a) A very good effect of the drug, where the liver tissue appeared quite normal. Only, a very few cellular infiltrate was noticed at the portal area diffusing from it along the periphery of the lobule.
(b) the same as in the previous section except for mild congestion in the central vein. The hepatocytes appeared normal.

The hypoglycemic activity of fractions of the 70% ethyl alcohol extract of *Artemisia herba-alba* (Asso.) (Acute treatment)

As shown in table (5) and Fig.7, the 70 % ethyl alcohol extract of *Artemisia herba-alba* (Asso.) decreases the plasma glucose level by 27% after 1 hour and by 34% after 2 hours. The n-hexane fraction decreases the plasma glucose level by 8% after 1 hour and by 26% after 2 hours, while the diethyl ether

Table. 5: Blood sugar levels (mg/dl) of diabetic mice after one hour and two hours of administrations of treatments with fractions of *Artemisia herba –alba* (Asso.) (n=8).

Group	Dose mg/kg b.w	Mean ± S.E		% of change from control	
		After one hour	After two hour	After one hour	After two hour
Control	1 ml 3% tween 80	174±10.36	172±9.89	---	---
Diabetic+ 70% ethyl alcohol extract of *Artemisia herba –alba* (Asso.)	390	127±1.12	113±3.82	27%	34%**
Diabetic+ Hexane fraction of *Artemisia herba –alba* (Asso.)	24.18	160±10.19	129±2.41	8%	26%**
Diabetic+ Ether fraction of *Artemisia herba –alba* (Asso.)	4.6	109±0.59	155±12.79	37%	10%*
Diabetic+ Ethyl acetate fraction of *Artemisia herba –alba* (Asso.)	4.6	152±10.96	177±6.51	13%	-3%
Diabetic+ 70% ethyl alcohol fraction (water soluble fraction) of *Artemisia herba –alba* (Asso.)	3.49	129±8.32	128±2.86	26%	25%**

Results are expressed as mean ± S.E (n=8).
* Significant & ** High significant.
Statistical analysis is carried out using one way analysis of variances (ANOVA) accompanied with pot-hoc SPSS computer program.

fraction decreases the plasma glucose level by 37% after 1 hour and by 10% after 2 hours. The ethyl acetate fraction decreases the plasma glucose level by 13% after 1 hour and raises it by 3% after 2 hours. Also, the 70% ethyl alcohol fraction (water soluble fraction) decreases the plasma glucose level by 26% after 1 hour and 25% after 2 hours.

Finally, the 70% ethyl alcohol extract of *Artemisia herba-alba* (Asso.) herbs produces hypoglycemic effect more than its fractions.

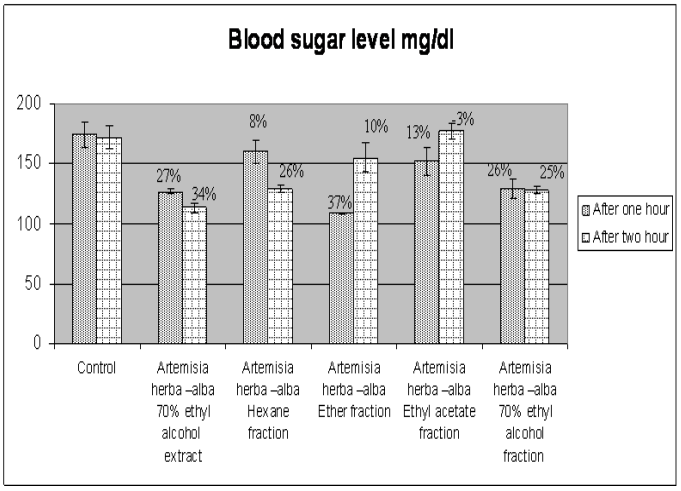

Fig.7: Blood sugar levels (mg/dl) of diabetic mice after one hour and two hours of administrations of treatments with fractions of *Artemisia herba –alba* (Asso.)

Chemical specification of the crude extract of their isolated fraction of *Artemisia herba-alba* (Asso.) herbs

Phytochemical screening of the crude extract of *Artemisia herba-alba* (Asso.) herbs proved to contain Volatile constituents, Sterols &/or triterpenes, Carbohydrates &/or glycosides, Flavonoids, Coumarins and Alkaloids &/or nitrogenous compounds. The percentage of Carbohydrates, Flavonoids and total phenolics were determined according to (Dubois *et al.,* 1956, Aline *et al,*. 2005) and was found to be 20.82, 1.37% and 6.4% respectively Results are illustrated in Figs. 8&9

GC/MS of unsaponifible matters

The result of GC/MS analysis of unsaponifible matters are shown in table (6) revealed that twenty three compounds have been identified, representing 90.64 % of the total unsaponifiable content. The analysis revealed that the identified components consist of 25.39% unoxygenated compounds and 65.25% oxygenated compounds (41% hydroxylated compounds, 4.86% ketones and 19.39% steroidal). Butylated hydroxy toluene (25.81%) is the major compound in the USM, followed by Phytol (14.29%) and Triacontane (8.46%).

GC/MS of fatty acids methyl esters

The result of GC/MS analysis of unsaponifible matters are shown in Fig.8 revealed that the fatty acid methyl ester derivatives could be identified; representing 69.89% of the total composition. The major fatty acids are Octadecenoic acid (oleic acid) acid (26%), followed by 9, 11-octadecadienoic acid (21.6%), Hexadecanoic acid (13.47%), Octadecanoic acid. (3.26%). Saturated fatty acids represent (20.39%) of the total fatty acids content, while mono-, di- and tetra-unsaturated fatty acids represent (26%) , (21.6%) and (1.9%) of the total fatty acids content respectively.

Isolation and identification of some compounds from unsaponifible fraction by TLC technique

The unsaponifible matter (0.5 gm) was subjected to preparative TLC using silica gel plates and solvent system benzene: Ethyl acetate 8:2 alongside with available authentic samples. Visualization was carried out by spraying with 10% H_2So_4.

Three compounds have been isolated: β- sitosterol (R_f 0.58) 43 mg. It was purified several times by PTLC to give crystalline substance from methanol. It was identified by MS analysis. It was identical with authentic β- sitosterol (Co TLC, m.p. & m.m.p.).

9, 19-cyclolanost-24-en-3-ol (Cycloartenol) (R_f 0.62) 34 mg. It was purified several times by PTLC to give crystalline substance from methanol. It was identified by MS analysis by co-chromatography with authentic, m.p. & m.m.p.

9, 19-Cyclo-9-lanostan-3-ol-24-methylene (R_f 0.66) (24-methylenecycloartanol) 22mg. It was purified several times by PTLC to give crystalline substance from methanol, it was identified by co TLC with authentic substance and by MS analysis.

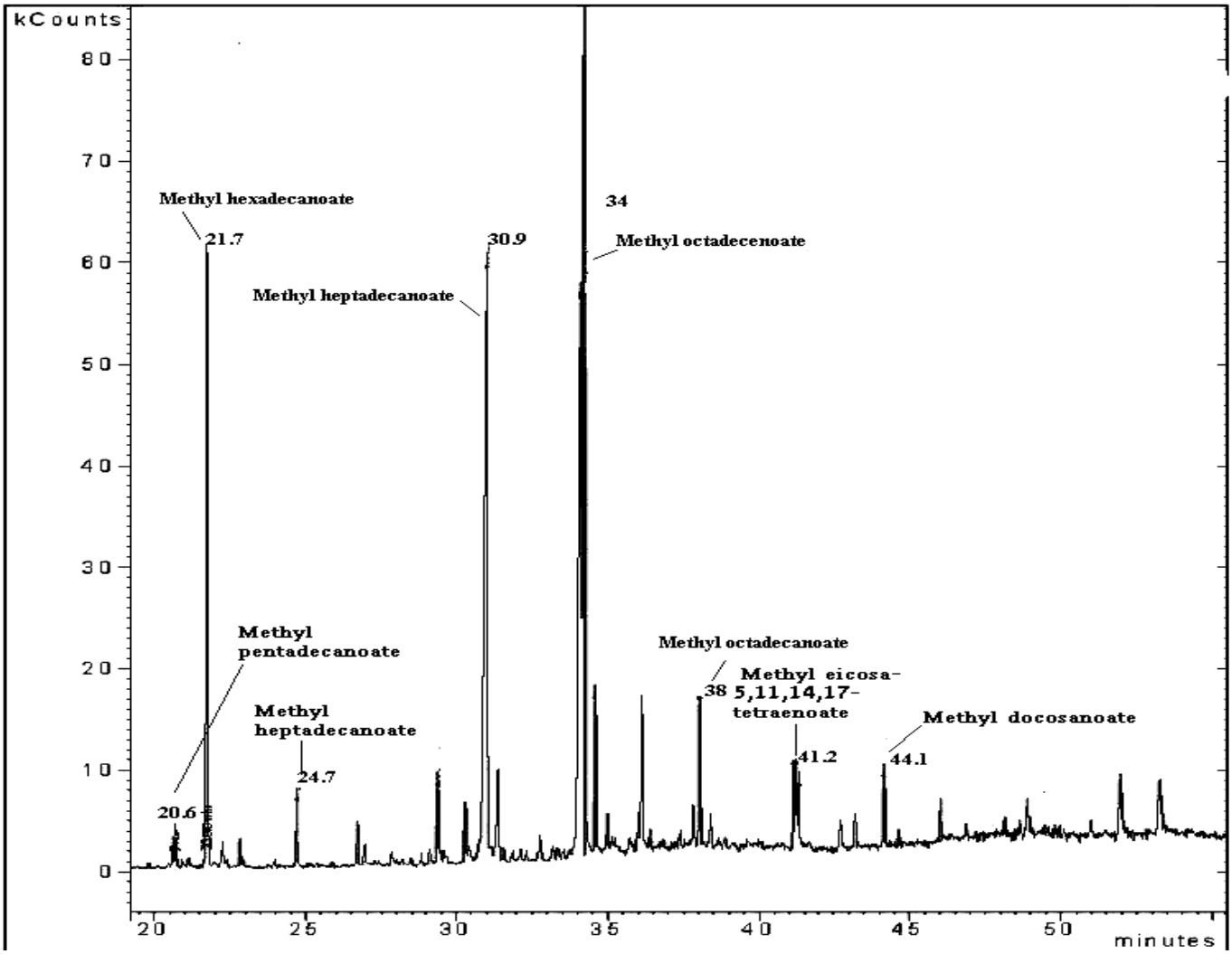

Fig. 8: Chromatogram of GC/MS analysis of FAME

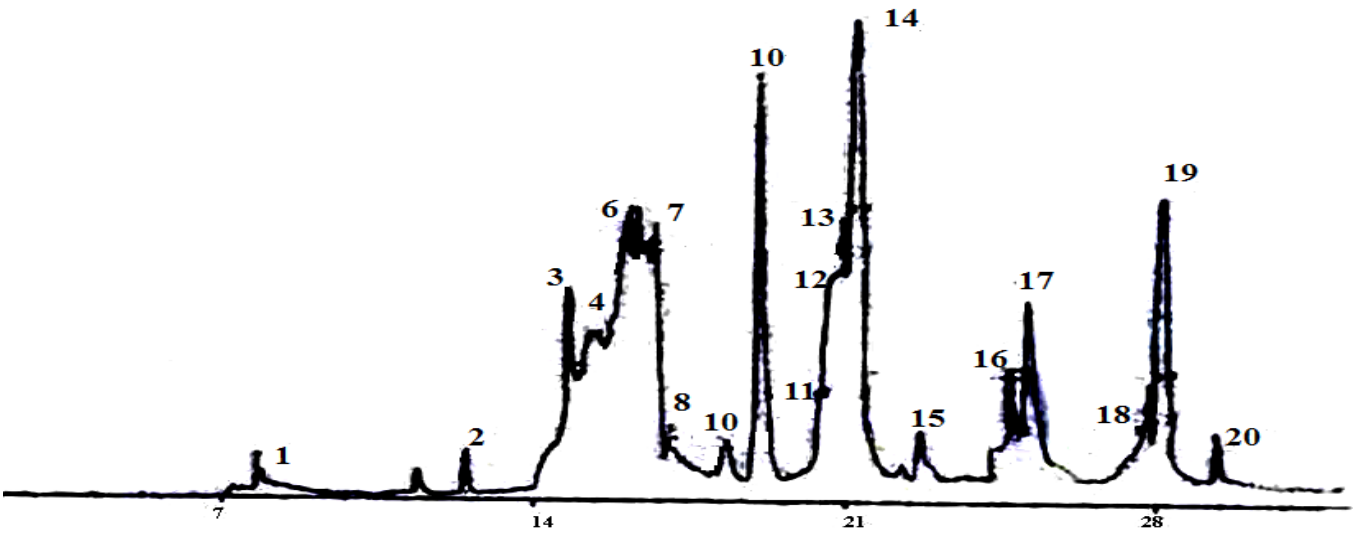

Fig. 9: Chromatogram of GC/MS of the isolated volatile constituents from 70% ethyl alcohol extract of *Artemisia herba-alba* (Asso.).

It is to be mentioned that from the reported data

1. β- sitosterol administration increased circulating insulin levels via stimulation of insulin secretion which led to hypoglycemic effect (Ivorra *et al.,* (1988).
2. Cycloartenol and 24-methylenecycloartanol produced significant hypoglycemic effect via β-cell protective effects and inhibitory effects on glucose-absorption speed and a modulation of liver enzymes (Tanaka *et al.,* 2006; Eriko *et al.,* 2008).

Investigation of the ethyl acetate fraction of 70% alcoholic extract of *Artemisia herba-alba* (Asso.) herbs

Yellow amorphous powder (14 mg) melting point was 352 °C, R_f value was 0.64 in solvent system BAW (4: 1: 5 v/v). It appeared as dull spot under UV and gave yellow-green fluorescence on spraying with $AlCl_3$ reagent.

The compound was identified as be apigenin on the basis of its co-chromatographic behavior with authentic apigenin (Rf values, fluorescence under UV, melting point and mass spectrum (Markham, 1982). On complete acid hydrolysis, PC profile proved that no sugar in the aqueous phase and apigenin is present as an aglycone in the organic phase. Apigenin was previously isolated from *Artemisia herba-alba* (Asso.) (Myun-Ho *et al.,* 2005). A reported data about the hypoglycemic activities of apigenin prove that it increases insulin secretion and it has insulinomimetic effect (Cazarolli *et al.,*2009).

Study of the isolated volatile constituents from 70% ethyl alcohol extract of *Artemisia herba-alba* (Asso.) herbs

Quantitative determination was carried out based on peak area integration, and the results are illustrated in Fig.9 and Table (7).

Twenty compounds of the volatile components of 70% ethyl alcohol extract of *Artemisia herba-alba* (Asso.) (representing about 95.13 % of the oil) were identified. The unoxygenated sesquiterpenoid compound Germacrene B is representing 17.25%. Nineteen compounds (77.88%) are oxygenated, including ketones (2.91%), aldehydes (16.35%), hydroxylated compounds (10.66%), esters (42.34%), other terpenoids (2.58%), and Acids (3.04%). The major compound was Geranyl N-butyrate (26.72%) followed by Germacrene B (17.25), 9-Octadecenal (14.38%) and Nerolidol (7.59%).

CONCLUSION

Diabetes mellitus is a disease due to abnormality of carbohydrate metabolism and it is mainly linked with low blood insulin level or insensitivity of target organs to insulin. It is the most prevalent chronic disease in the world affecting nearly 25% of the population and 10% in Egypt. From literature review it has been revealed that 15–20% of diabetic patients are suffering from insulin-dependent diabetes mellitus (IDDM) (Urger and Foster, 1998).

Investigation on fractions of 70% ethyl alcohol extract of *Artemisia herba-alba* (Asso.) herbs revealed that the ethyl alcohol extract produces hypoglycemic effect more than any of its fractions. Four compounds were isolated and identified from fractions of 70% ethyl alcohol extract of herbs of *Artemisia herba-alba* (Asso.). These compounds were previously reported for their hypoglycemic activity.

This study proved that folk medicine is still widely practiced by the population in Egypt, and the use of medicinal plants constitutes the common legacy of all Egyptians. Despite the penetration of the modern medicine, traditional medicine continues to be a viable health alternative for the large underprivileged section of the Egyptian population. Thus, it is important to document and restore the remains of ancient medical practices that still exist in Egypt and other parts of the world, and preserve this knowledge for future generations.

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