EFFECT OF SOME PLANTS USED IN ARABIC TRADITIONAL MEDICINE ON BLOOD GLUCOSE, CHOLESTEROL AND TRIGLYCERIDES LEVELS OF NORMAL AND STREPTOZOTOCIN-HYPERGLYCEMIC MICE

Z. Z. Ibraheim[1], N. Boulatova[2], and M. Qato[3]

[1]*Department of Pharmacognosy, Faculty of Pharmacy, Assiut University, Assiut, Egypt*
[2]*Department of Pharmacology, University of Jordan, Amman 11942, Jordan*
[3]*Department of Pharmacology, Al-Isra University, Amman, Jordan*

تمت دراسة تأثير الخلاصة الكحولية لبعض النباتات التى تستعمل فى الطب الشعبى فى منطقة الشرق الاوسط لمعالجة السكرى وهذه النباتات هى الأراك (القشور والخشب) ، الأبهل (الاجزاء الهوائية) والحلتيت (راتنج) باعطائهم عن طريق الفم بجرعات ٣٠٠ مللجرام/كيلوجرام لفئران طبيعية أو مصابة بالسكرى نتيجة حقنها بالاستربتوزوتوسين ، وقد تم قياس مستوى السكر والكولستيرول الكلى وكذا الجليسريدات الثلاثية فى الدم بإستخدام التقدير الطيفى بعد ساعتين من أستعطاء الخلاصة النباتية ، وقد أظهرت النتائج ان مجموعة الفئران المعالجة بالاستربتوزوتوسين (١٢٠ مللجرام/كيلوجرام) حقن داخل بروتونى زاد من مستوى السكر فى الدم (١٢١,٨±١٠,٩ مقارنه بـ ٨٣,٥±٥,٢ فى المجموعة الضابطة ، P> 0.01)، وزيادة نسبة الكولستيرول (١٤٧,٠±١٢,٨ مقارنه بـ ١١٠,١±٨,٠ فى المجموعة الضابطة ، P> 0.05) ، وزيادة نسبة الجلسريدات الثلاثية (١٦٣,٣±٨,٦ مقارنة بـ ٩٨,٩±٥,٤ فى المجموعة الضابطة ، P> 0.001). وقد ادى استعطاء الابهل الى خفض نسبة السكرى لدى الفئران المحدث لها سكرى بإستخدام الاستربتوزوتوسين (٥٦,٧±٥,٠ وهى نسب ٦٧,٩٪ مقارنة بالمجموعة الضابطة، P> 0.001) وليس فى المجموعة الطبيعية. وعلى العكس فان نسبة الكولستيرول قد انخفضت فى حالة الفئران الطبيعية وليست فى المجموعة المحدث لها سكرى (١٠٨,٩±١٠,٣ وهى نسبة ٧٥,٦٪ مقارنة بالمجموعة الضابطة ، P> 0.05). وقد أظهرت خلاصة خشب الأراك إلى نقص فى نسبة السكر فى الدم لدى الفئران الطبيعية (٦٥,٦±٦,٢ وهى نسبة ٦٤,٥٪ مقارنة مع المجموعة الضابطة ، P> 0.01) ولم يعط أى من الخلاصات تأثيرات محسوسة على الجلسيريدات الثلاثية. وقد أظهرت المسح الكيميائى الأولى لنبات الابهل وجود مواد كربوهيدراتية و/أو سكريدات ، استيرولات و/أو تربينات ثلاثية ، زيت طيار ، عفصيات ، فلافونيدات كمكونات أساسية وهذه النتائج تقوى إستعمال الابهل فى حاله علاج السكرى وكذا إستعماله الفعال فى تخفيض نسبة الكولستيرول.

The ethanol extracts of three plants used for folkloric treatment of diabetes mellitus in the Middle East: Salvadora persica L (bark and wood), Savine (Juniperus sabina L.) (aerial parts), Asafetida (Ferula foetida L) (oleo-gum-resin) were administered orally in dose 300 mg/kg body weight to normal and streptozotocin (STZ)-induced diabetic mice. The blood serum glucose, total cholesterol and triglycerides levels were measured spectrophotometrically two hours after administration of plant extracts. Intraperitoneal administration of STZ (120 mg/kg body weight) induced hyperglycemia (121.8±10.9 versus 83.5±5.2 in control, p< 0.01), hypercholesterolemia (147.0±12.8 versus 110.1±8.0 in control, p< 0.05), and hypertriglyceridemia (163.3±8.6 versus 98.9±5.4 in control, p< 0.001). Savine extract reduced blood serum glucose level in STZ-diabetic mice (56.7±5.0, which is 67.9% of control level, p< 0.001) but not in normal mice. In contrast, total cholesterol serum level was decreased by Savine in normal (108.9±10.3, which is 75.6% of control level, p< 0.05), but not in diabetic animals. Salvadora persica (wood) extract produced hypoglycemia only in

normal mice (65.6±6.2, which is 64.5% of control level, p < 0.01). Neither of plant extracts caused significant changes in triglycerides levels. The preliminary phytochemical screening of Savine extract showed the presence of carbohydrates and/or glycosides, sterols and/or triterpenes, volatile oil, tannins and flavonoids as the main active constituents in addition to traces of alkaloids. The results support the use of Savine in patients with diabetes mellitus and indicate its potential usefulness as a cholesterol-lowering drug.

INTRODUCTION

Herbal drugs still represent the main medicinal form in many developing countries. Among the diseases agonizing many patients is diabetes mellitus (D.M.). About 5% of the population in the developing societies are suffering from diabetes and the high cost of insulin as well.[1,2] Many investigators have done much effort for screening various plants and isolated compounds, yet none of the examined plants afforded available drug for the treatment of D.M.[3-13] However, the majority of patients with type II DM still prefer a herbal daily dose over the regular pharmaceutical forms.

These facts encouraged us to undertake a preliminary phytochemical and pharmacological studies of some plants used in folk medicine for antidiabetic properties as *Salvadora persica*, *Savine* and *Ferula foetida*.

Salvadora persica (S. persica), *Juniperus sabina (J. sabina)* and *Ferula asafoetida (F. asafetida)* are among the most commonly used plants in Arabic traditional medicine.[14] *S. persica* have been mentioned as having antidiabetic effect (Table 1).

The aim of the present work is to study the effect of *S.persica* bark, *S. persica* wood, *J. sabina* and *F. asafetida* extracts on blood glucose, cholesterol and triglycerides level in normal and STZ-induced hyperglycemic.

MATERIALS AND METHODS

Chemicals
- Streptozotocin (STZ) ["Sigma" (USA)].
- Glucose, cholesterol and triglycerides kits ("Deltapharm Diagnostic", Germany).

Plant materials and parts of plants used.
Plants
Plants were collected in Jeddah, Saudi Arabia (1998). The plants were authenticated by Prof. Dr. D.M.H. Al-Eisawi, Professor of Botany, Faculty of Science, Jordan University, Amman, Jordan. A voucher specimen has been deposited in the herbarium of Al-Isra University.

Extraction
Certain weight of each dried plant material was grounded and extracted with 70% ethanol by maceration and percolation for 24 h. This process was repeated 3 times, the combined ethanolic extract was filtered, the filtrate was concentrated by evaporation under vacuum in rotatory evaporator at 40°. The solvent-free residue was weighted. The percent yield of each extract is listed in Table (2).

Preparation of extracts for administration
Weighted amounts of plant extracts (3 g) were dissolved in 50 ml of distilled water by adding small amount of polyethylene glycol 400 (PEG). The volume was completed to 100 ml with distilled water. A control PEG solution was prepared using the same amount of PEG (placebo).

Animals
Adult male albino mice obtained from Jordan University weighing 20-25 g were used. Mice were kept in standard boxes (10 mice per box) and fed laboratory diet *ad libitum* and allowed free access to drinking water; they were kept at 12/12 hours light/dark cycle. Before determination the blood glucose, cholesterol and triglycerides levels, mice were fasted overnight, however, they still had free access to water.

Table 1: The results of preliminary phytochemical screening, known major constituents and medicinal uses of plants under the study.

Plant	Results of preliminary phytochemical screening	Major constituents reported	Major uses in folk and modern medicine
S. Persica	- Carbohydrates and/or glycosides - Sterols and/or triterpens - Tannins - Saponins - Flavonoids - Volatile oils - Traces of alkaloids	Flavonoids, lignans, sulphur glycosides volatile oil and saponins[15-18]	Cleaning of teeth ,prevention of preciptation of plaque and caries, antibacterial and mild hypoglycemic[15-21]
(Savine) *Juniperus sabina*	- Carbohydrates and/or glycosides. - Sterols and/or triterpens - Flavonoids - Volatile oil - Tannins - Alkaloids	volatile oil, tannins and resin	Powerful emmenagogue rubefacient, stimulant. Used in treatment of hypertension, as hypnotic, fungicidal and bactericidal, employed in gout, rheumatism and skin diseases[22-25]
Ferula foetida	- Sterols and/or triterpens - Flavonoids - Coumarins - Volatile oil	Volatile oil containing sulpur compounds, ferulic acid, umbelleferone and gum	Hypoglycemic, Carminative, expectorant, for asthma ,bronchitis and whooping cough[14,26]

Table 2: % yield of 70 % alcohol extract of different plants under investigation.

Plant name	Part used	Wt. of plant (g)	Wt. of extract (g)	% yield
Salvadora persica	Root bark	97	17.17	17.9
Salvadora persica	Heart wood	238	29.31	12.3
Juniperus sabina	Total herb	300	80	26.7
Ferula foetida	Oleo-gum-resin	100	42.03	42.0

To make mice hyperglycemic, STZ freshly dissolved in citrate buffer (pH 4.5) was injected intraperitoneally (120 mg/kg).[27] Blood serum glucose, cholesterol and triglycerides levels were spectrophotometrically checked on the seventh day after injecting STZ.[28-30]

Plant extracts were administered orally via the stomach tube at the dose: 300 mg/kg in a volume 0.1 ml/10 g weight.

Grouping of mice

In the first series of experiments normal mice were used. Group 1 were normal mice treated with placebo, group 2 received *S. persica* bark extract, group 3 were treated with *S. persica* wood extract, group 4 were given *J. Sabina* extract, and group 5 were treated with *F. asafetida* extract.

The second series of experiments was performed in hyperglycemic mice. Group 1 served as a normal control receiving placebo, group 2 were hyperglycemic mice treated with placebo, group 3 were hyperglycemic treated with *S. persica* bark extract, group 4 were hyperglycemic mice treated with *S. persica* wood extract, group 5 were hyperglycemic treated with *J. Sabina* extract and group 6 were hyperglycemic mice given *F. asafetida* extract.

Statistical analysis

All values were expressed in mg/dl as mean ± S.E. The Student's, t-test for unpaired observation between the control (normal and diabetic) and the experimental samples was carried out for statistical evaluation of the differences. P values of 0.05 or less were considered as significant.

RESULTS

The effect of *S. persica* bark, *S. persica* wood, *J. Sabina*, *F. asafetida* on blood serum glucose, cholesterol and triglycerides levels of normal and STZ-hyperglycemic mice are shown in Figs. 1-6.

I. Effect of plants extracts on serum glucose, cholesterol and triglycerides in normal mice

Fig. 1 shows the effects of the plants extracts on blood glucose levels in normal mice. As seen in the figure, *S. persica* wood extract significantly decreased blood glucose level in normal mice (P< 0.01), while other plant extracts did not change it.

Fig. 2 shows the effects of plants extracts on serum cholesterol level in normal mice. As seen in the figure, *S. persica* bark extract insignificantly and *J. Sabina* extract significantly (p< 0.05) reduced, while *S. persica* wood and *F. asafetida* extracts did not affect serum cholesterol level in normal mice.

Fig. 3 shows effect of plant extracts on serum triglycerides level in normal mice. As seen in the figure, *S. persica* wood extract insignificantly elevated (p> 0.05) while *J. sabina* extract in significantly decreased blood triglycerides level in normal mice. *S. persica* bark and *F. asafetida* extracts did not change triglycerides level.

II. Effect of plant extracts on serum glucose, cholesterol and triglycerides level in STZ-hypermice

Fig. 4 shows the effect of plants extracts on blood glucose level in STZ-hyperglycemic mice. As seen in the figure, administration of STZ produced significant hyperglycemia (p< 0.01). *J. sabina* extract significantly reduced (p< 0.001), while *S. persica* bark, *S. persica* wood and *F. asafetida* extracts did not change blood glucose level in hyperglycemic mice.

Fig. 5 shows the effect of plants extracts on serum cholesterol level in STZ-hyperglycemic mice. As seen in the figure, STZ produced significant hypercholesterolemia. From extracts used, only *S. persica* bark extract produced insignificant reduction, while other plants did not change blood cholesterol level in STZ-hypercholesterolemic mice.

Fig. 6 shows the effect of plants extracts on serum triglycerides level in STZ-treated mice. As seen in the figure, STZ produced highly significant hypertriglyceridemia (p< 0.001). Among four plants studied, only *S. persica* wood extract produced insignificant reduction of triglycerides level in STZ-treated mice.
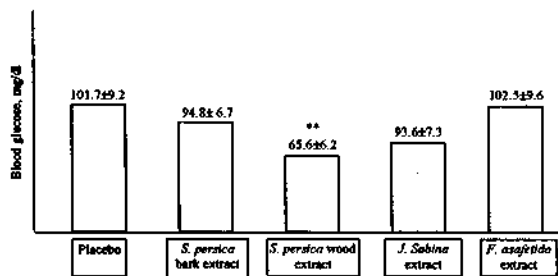
Fig. 1: Effect of *S. persica* bark, *S. persica* wood, *J. sabina* and *F. asafetida* extracts on serum glucose level in normal mice.

Results are expressed as mean ± S.E., n= 6-8
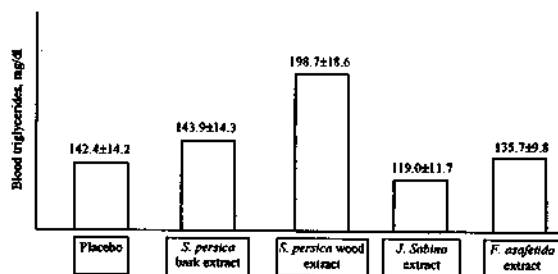**p< 0.01 compared to group tested with placebo.

Fig. 3: Effect of *S. persica* bark, *S. persica* wood, *J. sabina* and *F. asafetida* extracts on serum triglycerides level in normal mice.
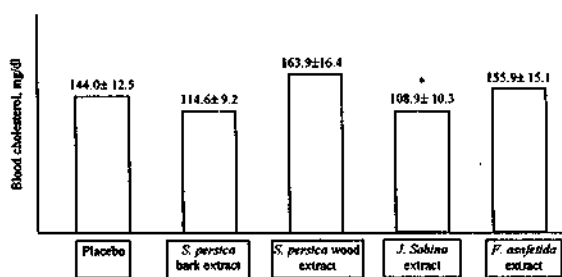
Results are expressed as mean ± S.E, n= 6-8.

Fig. 2: Effect of *S. persica* wood, *S. persica* bark, *J. sabina* and *F. asafetida* extracts on serum cholesterol level in normal mice.

Results are expressed as measn ± S.E., n= 6-8.
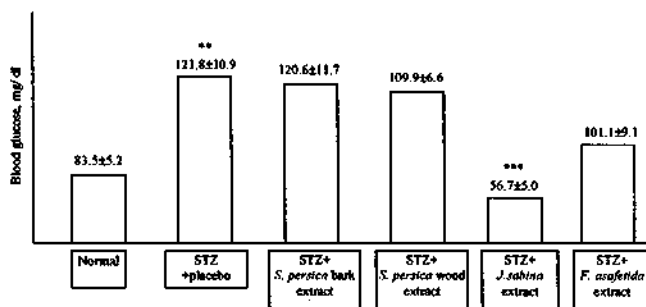*p< 0.05 compared to placebo-treated group.

Fig. 4: Effect of *S. persica* bark, *S. persica* wood, *J. sabina* and *F. asafetida* extracts on serum glucose level in STZ-hyperglycemic mice.

Results are expressd as mean ± S.E., n= 6-8
**p< 0.01 compared to normal mice
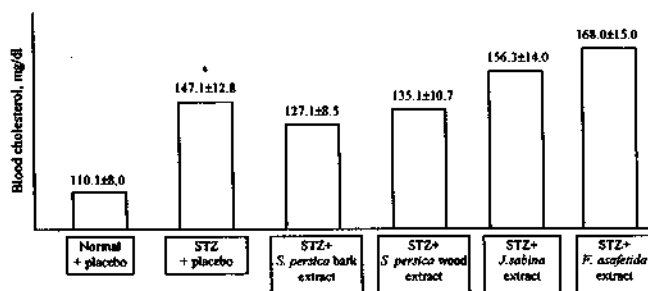***p< 0.001 compared to group receiving STZ + placebo.

Fig. 5: Effect of *S. persica* bark, *S. persica* wood, *J. sabina* and *F. asafetida* extracts on serum cholesterol level in STZ-treated mice.

Results are expressed as mean ± S.E., n= 6-8
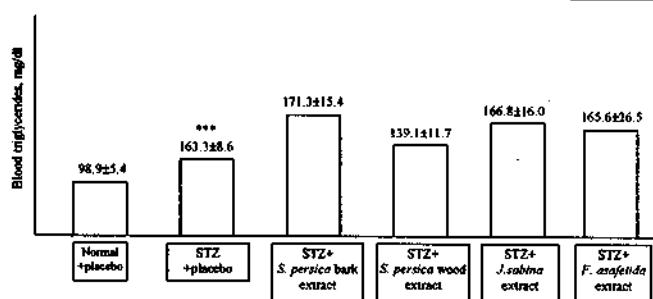*p< 0.05 compared to group tested with placebo.

Fig. 6: Effect of *S. persica* bark, *S. persica* wood, *J. sabina* and *F. asafetida* extracts on serum triglycerides level in STZ-treated mice.

Results are expressed as measn ± S.E., n= 6-8.
***p< 0.001 compared to normal mice.

DISCUSSION

S. persica, J. sabina and *F. asafetida* are very popular medicinal plants in the Middle East.[14] There is no experimental data concerning the use of these plants in the treatment of diabetes mellitus and its complications.

In our study, we measured serum glucose, cholesterol and triglycerides levels after oral administration of different plant extracts to normal and STZ-treated animals, since diabetes is commonly associated with hyperlipidemia.

The results showed that *J. sabina* extract lowered serum glucose levels in STZ-hyperglycemic, but not in normal mice. In intact mice, however, this plant produced significant reduction of cholesterol level. It seems that glucose-lowering effect does not parallel the cholesterol-lowering effect of this extract.

S. persica wood extract produced significant decrease in sereum glucose level in normal, but not in STZ-treated mice. Such effect must be remembered while using stems of this plant as toothbrush, very popular in Arabic World. Lack of glucose-lowering effect of *S. persica* wood in STZ-treated animals disgrees with previous reports.

F. asafetida did not produce any changes in normal and STZ-treated animals.

We suggest different mechanisms of action of *S. persica* and *J. Sabina*, since the former decreased glucose level in normal and this is could be due to increase the insulin secretion from the ß-cells or inhibit the α-cells or hypoglycemic factor, and the latter lowered glucose level in diabetic mice and this could be due to an enhancement of peripheral metabolism of glucose even if an increase of insulin release cannot be excluded.[2,31]

In conclusion, our data suggest the antidiabetic and hypocholesterolemic properties of *J.sabina* extract.

276

REFERENCES

1- T. M. Sarg, A. S. Salem, A. M. Ateya, M. E. El-Sayed and T. A. M. El-Qersh, Bull. Fac. Pharm. Cairo Univ., 29 (2), (1991).

2- Atta-ur-Rahman and Z. Khurshid, J. Ethnopharmacol., 26, 1 (1989).

3- Y. Hammouda, A. K. Rashid and S. A. Amer, J. Pharmacol., 16 (2), 83 (1964).

4- M. A. Ajabnoor, M. A. Al-Yahaya, M. Tariq and A. A. Jayyab, Fitoteropia, 55, 227 (1984).

5- M. S. Akhtar and M. R. Ali, Planta Medica, 51, 81 (1985).

6- F. M. Al-Awadi, M. A. Khattar and K. A. Gumma, Diabetologia, 28, 432 (1985).

7- R. M. Amin, A. S. Abdul-Ghani and M. S. Suleiman, Planta Med., 54, 286 (1988).

8- B. K. Chakravarthy, S. Gupta, S. S. Gambhir and K. P. Gode, The Lancet, 2, 759 (1981).

9- J. S. Mossa, Int. J. Crude Drug Research, 22, 137 (1985).

10- H. Rahman, M. Tyeb and M. Saleem, Indian J. Med. Res., 81, 418 (1985).

11- Atta-ur-Rahman, J. Sci. Med., 28, 3 (1985).

12- C. Day, T. Cartwright, J. Provost and C. J. Bailey, Planta Med., 56, 426 (1990).

13- M. D. Ivorra, M. Paya and A. Villar, J. Ethnopharmacol., 27, 2432 (1989).

14- S. A. Ghazanfar, Handbook of Arabian Medicinal Plants, CRC Press, (1994).

15- M. A. Al-Yahya, I. A. Al-Meshal, J. S. Mossa, A. A. Al-Badr and M. Tarq, Saudi Plants, A Phytochemical and Biological Approash, GDRGP, KACST, Riyadh (1990).

16- M. S. Kamel, Ph.D. Thesis, Assiut University, Assiut, Egypt (1991).

17- M. S. Kamel, K. Ohtani, M. H. Assaf, R. Kasai, M. A. El-Shanawany, K. Yamasaki, A. A. Ali and O. Tanaka, Phytochemistry, 2469 (1992).

18- M. I. H. Farooqi and J. D. Srivastava, Quart. J. Crude Drug Research., 8, 1297 (1978).

19- K. Almas, Indian J. Dent. Res., 10, 23 (1999).

20- S. M. Abdel-Wahab, M. A. Selim and N. M. El-Fiki, Conference of Pharmaceutical Sciences, Dar-El-Hekma, Cairo, 89 (1990).

21- E. M. Galati, M. T. Monforte, A. M. Forestieri, N. Miceli, A. Bade and A. Trovato, Phytomedicine, 6, 181 (1999).

22- R. Kompf, Pharm. Acta Helv., 50, 350 (1975).

23- The Wealth of India (Raw Materials), CSIK, New Delhi, India Vol. 5, 306 (1951).

24- R. N. Chopra, S. L. Nayar and I. C. Chopra, Glossary of Indian Medicinal Plants, CSIR, New Delhi, 146 (1956).

25- W. Dallimove and A. B. Jackson, A Handbook of Coniferae and Ginkgoaceae, 4th ed. (Revised by S. G. Harisess) (1980), p. 234.

26- E. F. Steinmetz, Materia Medica Vegetabilis, Holland (1945).

27- C. Day, T. Cartwright, J. Provost and C. J. Bailey, Planta Med., 56, 426 (1990).

28- D. Bartram and P. Trindler, Analyst, 97, 142 (1972).

29- P. Roeschlau, E. Bernt and W. Grubert, J. Clin. Chem. Biochem., 12, 403 (1974).

30- M. J. Jacobs and P. J. Van Denamark, Arch. Biochem. Biophys., 88, 250 (1960).

31- F. Skim, A. Kaaya, J. T. Jaouhari, H. B. Lazrek, M. Jana and H. Al-Amri, Fitoterapia, 70, 382 (1999).