EFFECT OF WATER EXTRACT OF *ACHILLEA MILLEFOLIUM* AND *SALIX* PLANTS ON SOME BIOCHEMICAL PARAMETERS IN RATS

By

Amin, K. A.

Biochemistry Dept. Fac. Vet. Med. Cairo Univ. Beni- Suef

ABSTRACT

The aim of this work is to investigate the effect of medicinal plants as *salix* and *Achillea millefolium* on body weight, lipid profiles, lipid peroxidation, and glucose of blood. Forty rats were used in this experiment equally divided into 5 groups, 1st group (control), rats giving saline, 2nd group, rats giving 5 mg/kg B.wt/day of *salix* extract, 3rd group, rats giving 10 mg/kg B.wt/day of *salix* extract, 4th group, rats giving 50mg/kg B.wt/day of *Achillea millefolium* and 5th group, rats giving 100mg/kg B.wt/day of *Achillea millefolium*. All groups administered both extracts per Os by using stomach tubes for 6 weeks. Body weight gain and plasma lipid profiles, glucose and malondialdehyde (MDA) were measured. The results showed that both doses (5 and 10 mg/kg b.wt) of *Salix* extract significantly lower body weight gain and plasma triglycerides. While dose (10 mg/kg b.wt) of *salix* induced lowering plasma total cholesterol, LDL and blood glucose. However dose (5 mg/kg b.wt) increase HDL when compared to control. Data indicated that *Achillea millefolium* extract in a dose of (50 mg/kg b.wt) lower significantly plasma total cholesterol, LDL, MDA and glucose levels. While the extract in a dose of (100 mg/kg b.wt) decreases significantly plasma triglyceride, MDA and glucose levels, while elevate HDL concentration compared to control. It could be concluded that both *Achillea millefolium* and *Salix* improve plasma lipid profile (hypolipaemic), maintain a lower blood glucose level and could be advised to used for hyperlipemic patient and also *Achillea millefolium* important as antioxidant and lower lipid peroxidation.

INTRODUCTION

Recently the herbal treatment is widely used in medicine, as the extracts is useful in the treatment of many diseases. The therapeutic properties of plants and their toxic activities in man and animals, usually depend on the presence of what are known as active principles.

Nowadays, there is an increased demand for using plants in therapy "Back to nature" instead of synthetic drugs which have many side effects on the general health condition of the animal **(Fluck *et al.*, 1976)**

Four billion people or 80% of the world's population use the herbal medicine for some aspects of primary health care according to the world health organization **(Elzorba 1993).**

Achillea melliofolium is one of the medicinal plants, extracted by water or ethanol and used for anti-inflammatory **(Zitter-Eglseer *et al.*, 1991 and Abd El-Aal, 2004)**. Of the about 100 species of the *Achillea* genus, *A. millefolium* has been important for a long time as a drug in traditional and modern medical practice **(Chandler *et al.*, 1982).** It is used in folk remedies as an appetizer, wound healer, diuretic, carminative or menstrual regulator **(Baytop, 1999).** To the best of our knowledge, information concerning the antioxidant features of *Achillea millefolium* subsp. *millefolium* little found in the literature.

It has long been recognized that naturally occurring substances in higher plants have antioxidant activity. Recently, there is a growing interest in oxygen-containing free radicals in biological systems and their implied roles as causative agents in the aetiology of a variety of chronic disorders. Accordingly, attention is focused on the protective biochemical functions of naturally occurring antioxidants in the cells of the organisms containing them **(Larson, 1988 and Halliwell, 1997).**

Salix contain salicin that metabolized to salicylic acid **(Baker and Thomas, 1987).** It acts as thrompoplastic agent **(Kudriashov et al., 1986)** and posses activities including anti-inflammatory, analgesic, antipyretic antimicrobial, and purgative **(Schilcher 2000).**

Chemoprevention of free radical-mediated diseases by natural products is an emerging discipline due to its wider applicability and acceptance.

The antimicrobial and antioxidant activities of the essential oil of *Achillea millefolium* (Asteraceae) extract were investigated. The essential oil identified into 36 compounds constituting 90.8% of the total oil. Eucalyptol, camphor, alpha-terpineol, beta-pinene, and borneol were the principal components comprising 60.7% of the oil. The oil strongly reduced the diphenylpicrylhydrazyl

radical and exhibited hydroxyl radical scavenging effect in Fe(3+)-EDTA-H2O2 deoxyribose system. It also inhibited the nonenzymatic lipid peroxidation of rat liver. The polar phase of the extract showed antioxidant activity **(Candan *et al.*, 2003).**

The aim of this study is to clarify the effect of herbal treatment with water extract of both *Achillea melifolium* and *Salix* on body weight gain, lipid profiles, lipid peroxidation and glucose in blood.

MATERIAL AND METHODS

Animals and dosing:

Forty adult male Albino rats, weighing approximately 120-145 g. and obtained from *the Helwan farm for laboratory animals, ministry of public health,* were used in the present study. The rats were maintained in the department of biochemistry. They were kept and observed one week before use. The animals were given water *ad libitum* and fed a commercial rodent diet. The rats were weighed at the beginning and at the end of the experiment.

The rats were randomly divided into five equal groups: in the 1st group (control), rats giving saline, the 2nd group, rats giving 0.5 ml of *salix* extract for each (5 mg/kg B.wt/day), the 3rd group, rats giving 1 ml of *salix* for each (10 mg/kg B.wt/day), the 4th group, rats rat giving 0.5 ml of *Achillea millefolium* (50 mg/kg B.wt/day), and the 5th group, rats rat giving 1 ml of *Achillea millefolium* (100mg/kg B.wt/day) according to **Montanari *et al.*, (1998)** and **Abd El-Aal, (2004).** All groups administered both extracts per Os by using stomach tubes for successive 6 weeks.

Plant extracts:

Salix: *salix* bark was shade, dried and ground to fine powder and 50 gm of dry powder were boild with 500 ml D.W. and evaporated until giving a past, then 1 gm of dried past dissolved in 1000 ml D.W., according to **Chaplines'ka and Glovokin (1962)** and **Abd El-Aal, (2004),** and rats administered either 0.5 ml dose for each (5 mg/kg body weight/day) or 1 ml dose for each (10 mg/kg body weight/day) per Os by using stomach tube.

Achillea millefolium: The dried flowers were ground to a fine powder in an electrical grinder. This powder (50 g) was extracted with boiling 500 mL of D.W. under continuous stirring. The extract was concentrated by evaporation, the yield of this crude extract was 9%, then 1 gm of dried past dissolved in 100 ml D.W. and rats administered either 0.5 ml dose for each (50 mg/kg body weight/day) or 1 ml dose for each (100 mg/kg body weight/day) and continue as described above for *salix*.

Sampling and analysis:

Blood samples were collected from median canthus of the eye. The blood was collected in clean tubes with heparin as anticoagulant to obtain plasma. Plasma samples were aliquated and stored at -20 C? until analysis. Plasma biochemical variables were analyzed for; total cholesterol (**Allain *et al.*, 1974**), triglycerides (**Fossati, and Prencipe, 1982**), LDL (**Friede Wald, *et al.*, 1972**), HDL (**Burstein, *et al.*, 1970**), glucose (**Trinder, 1969**) and MDA according to the method of **Satoh, (1978)** by using spectrophotometer.

The obtained data were statistically analyzed by "T" test according to **Snedecor and Cochran (1969)** using Slide Write plus for Windows v.3 WSWP program.

RESULTS

The data were analyzed and illustrated in tables as follow:

Table (1) Shows that the mean final body weight of rats administered salix extract was significantly lower than control. While, mean body weight of rats administered *Achillea millefolium* was not significantly different from that of control rats.

Table (2) Demonstrated that rat administered *salix* extract at dose 5 and 10 mg/kg were significantly lower plasma triglycerides. Moreover, *achillea millefolium* at dose 100 mg/kg B.wt/day had significantly lower triglyceride compared to control. *Salix* extract at dose 10 mg/kg were significantly lower plasma cholesterol and LDL. While, *Achillea millefolium* at dose 50 mg/kg lower plasma cholesterol and LDL.

On the other hand, *salix* 5 mg/kg B.wt/day and *Achillea millefolium* 100 mg/kg had significantly higher plasma HDL respectively when compared with control.

Table (3) displays that rats administered *Achillea millefolium* extract at dose 50 or 100 mg/kg had significantly lower plasma MDA and plasma glucose level compared to control. While those administered *salix* extract had no significant difference. But *salix* at dose 10 mg/kg had significantly lower plasma glucose.

DISCUSSION

The obtained results showed significant lower in body weight in rats administered *salix* extract (Table 1) and this finding is in accordance with results of **(Han *et al.*, 2003 a).**

The present data showed that *salix* extract administration were significantly lower plasma triglycerides and total cholesterol level in comparison with control (table 2). These results agree with that of **Han *et al.*, (2003 a)** who found that the inhibitory effects of the flavonoid glycoside fraction of *Salix* on plasma lipid might be due to the inhibition of carbohydrate and lipid absorption from small intestine through the inhibition of ?-amylase and palmitic acid uptake into small intestinal brush border membrane or by accelerating fat mobilization through enhancing norepinephrine-induced lipolysis in fat cells. Moreover the polyphenols of *Salix* significantly reduced the hepatic total cholesterol content **(Han *et al.*, 2003 b)**.

This work showed significant lower triglyceride level in rat administered *achillea millefolium* at dose 100 mg/kg in addition lower plasma cholesterol and LDL with the dose of 50 mg/kg (table 2). These results agree with those of **Asgary *et al.*, (2000)** who stated that *Achillea millefolium* (*Asteraceae)* plant is full of flavonoids and sesquiterpene lactones, which have been shown to be effective in lowering blood lipids (triglycerides, total cholesterol and LDL). Meanwhile the results showed significant higher levels of HDL after treatment with *Achillea millefolium* at dose 100 mg/kg in accordance with **Asgary *et al.*, (2000).**

Achillea millefolium extract 50 and 100mg /kg had significant lower plasma glucose levels in comparison with control (table 3). These results consistent with those obtained by **Petlevski *et al.*, (2001)**. The future studies will be focused on the search of active principles of the extracts.

Higher glucose level explained by **Petlevski *et al.*, (2003)** who mentioned that higher glucose level was responsible for the development of oxidative stress (via glucose auto-oxidation and protein glycation), which is characterized by increased lipid peroxide production (MDA is a lipid peroxidation end product).

Data of the current work showed that rats administered *Achillea millefolium* at dose 50 and 100mg /kg had significantly lower plasma MDA (marker for lipid peroxidation) compared to control (table 3). These results agree with those of **Petlevski *et al.*, (2003)** indicating that the decrement in lipid peroxidation may be attributed to the presence of antioxidants in the essential oils of *Achillea millefolium* which is important in the stabilization of free fatty acids **(Six, 1994; Baldioli *et al.*, 1996).** The antioxidant activity of phenols and

other compounds present in oils has been described by others **(Visioli *et al.*, 1998** and **Yoshida and Takagi, 1999).**

Plasma MDA decrease indicated the strong ability of the essential oil of *Achillea millefolium* to act as a donor for hydrogen atoms or electrons. This could be assigned to the presence of some phenolic compounds where its main components e.g. eucalyptol, camphor, ?-pinene, borneol, terpinen-4-ol, these essential oils are antioxidant in nature. The antioxidative effectiveness in natural sources was reported to be mostly due to phenolic compounds **(Hayase and Kato, 1984).** Phenolic compounds were reported to play an important role in inhibiting autoxidation of the oils **(Candan *et al.*, 2003)**.

Further studies are suggested to isolate the water extracts active principles and for the determination of the mechanism of action of these extracts. It could be advised that both *Achillea millefolium* and *Salix* plants is useful for antioxidant, maintaining lower blood glucose levels and treating hyperlipaemia.

REFERENCES

Abd El-Aal, M. F. (2004): "Pharmacodynamic studies on Achillea Millefolium and *Salix* plants" MV.Sc. Thesis Fac..Of Vet. Med., Beni- Suef Cairo Univ.

Allain, C. C; Poon, I. S.; Chain, C. S; Richmond, W. and Fu, P. C (1974): "Enzymatic determination of total serum cholesterol" Clin. Chem., 20 (4): 470-475.

Asgary S, Naderi, G.H.; Sarrafzadegan, N.; Mohammadifard, N.; Mostafavi, S. and Vakili, R. (2000): "Antihypertensive and antihyperlipidemic effects of *Achillea wilhelmsii*" Drugs Exp Clin Res.; 26(3):89-93.

Baker, S. and Thomas, P. S. (1987): "Herbal medicine precipitating massive hemolysis" Lancet; 1, 1039-1040.

Baldioli, M.; Servili, M.; Perretti, G. and Montedoro, G. F., (1996): "Antioxidant activity of tocoferols and phenolic compounds of virgin olive oil." Journal of American Oil Chemistry Society 73, 1589-1593.

Baytop, T. (1999): "treatment with plants in Turkey" Istanbul University Publications No.: 3255:40, Istanbul, p. 176.

Burstein, M.; Scholnick, H. R. and Morfin, R. (1970): "Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions" J. Lipid Res., 11(6):583-95.

Candan F, Unlu, M.; Tepe, B.; Daferera, D.; Polissiou, M.; Sokmen, A.; and Akpulat, H.A. (2003): "Antioxidant and antimicrobial activity of the essential oil and methanol extracts of *Achillea millefolium* subsp. *millefolium* Afan. (*Asteraceae*)" J Ethnopharmacol.; 87(2-3):215-20.

Chandler, R. F.; Hooper, S. N. and Harvey, M. J. (1982): Econ. Botany 36,203.

Chaplines'ka, M. G. and Glovokin, V. A. (1962): "Antimicrobial action of some extract" Farma tsevt. Zh. 18(2):56-60.

Elzorba, H. (1993): "pharmaco-toxic studies on *cleome baserifolia* and *centaurium* spicatum herbs used in floke medicine" MV.Sc. Thesis Fac. Of Vet. Med., Cairo Univ.

Fluck, H.; Scib, R. and Rowson, J. M. (1976): Medicinal plants and their uses W. Foulsham and Co. Ltd, England ,p. 168.

Fossati, P. and Prencipe, L. (1982): "Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide" Clin. Chem., 28 (10):2077-2080.

Friede Wald, W.T.; Levy, R. J. and Fredrickson, D. S. (1972):"Estimation of the concentration of low denisity lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge" Clin. Chem.,18:499-502.

Halliwell, B. (1997): "Antioxidants and human disease: a general introduction" Nutrition Revews 55, S44–S52.

Han, L. K.; Sumiyoshi, M.; Zhang, J.; Liu, M.X.; Zhang, X.F.; Zheng, Y.N.; Okuda, H. and Kimura, Y. (2003 a): "Anti-obesity action of *Salix matsudana* leaves (Part 1). Anti-obesity action by polyphenols of *Salix matsudana* in high fat-diet treated rodent animals" Phytother Res.;17(10):1188-94.

Han, L.K.; Sumiyoshi, M.; Zheng, Y. N.; Okuda, H. and Kimura, Y. (2003 b): "Anti-obesity action of *Salix matsudana* leaves (Part 2). Isolation of anti-obesity effectors from polyphenol fractions of *Salix matsudana*" Phytother Res.;17(10):1195-8.

Hayase, F. and Kato, H. (1984): "Antioxidative components of sweet potatoes" Journal of Nutritional Science and Vitaminology 30, 37–46.

Kudriashov, B. A.; Azieva, L. D. and Liapina, L. A. (1986): "Hemostatic system function as effected by thromboplastic agents from higher plants" Nauchnye, Doki, Vyss-Shkoly, Biol. Nauki, 4:58-61.

Larson, R. A., (1988): The antioxidants of hinger plants. Phytochemistry 27, 969–978.

Montanari, T.; Ernesto de Carvalho, J. and Dolder, H. (1998): "Antispermatogenic effect of *Achillea millefolium* L. in mice" CONTRACEPTION; 58:309–313.

Petlevski, R.; Hadzija, M.; Slijepcevic, M. and Juretic, D. (2001): "Effect of 'antidiabetis' herbal preparation on serum glucose and fructosamine in NOD mice" Journal of Ethnopharmacology 75, 181–184

Petlevski, R.; Hadzija, M.; Slijepcevic, M.; Juretic, D. and Petrik, J. (2003): "Glutathione S-transferases and malondialdehyde in the liver of NOD mice on short-term treatment with plant mixture extract P-9801091" Phytother Res.;17 (4):311-4.

Satoh, K. (1978): "Serum lipid peroxides in cerebrovascular disorders determined by a new colorimetric method" Clin. Chim. Acta 90:37-43.

Schilcher, H. (2000): "Efficient phytotherapy anti-inflammatory herbal remedies" Herba Polonica, 46:2, 105-110.

Six, P. (1994): "Current research in natural food antioxidants" International News on Fats, Oils & Related Materials 5, 679–688.

Snedecor, G. W. and Cochran, G. (1969): In "Statistical methods" 6th ed. Iowa state Univ. Press. Anes Iowa, USA, P. 70.

Trinder, P. (1969):"Deteminartion of glucose in blood using glucose oxidase with alternative oxygen acceptor" Ann. Clin. Biochem., 6:24-27.

Visioli, F.; Bellomo, G. and Galli, C. (1998): "Free radical-scavenging properties of olive oil polyphenols" Biochemical and Biophysical Research Communications 247, 60–64.

Yoshida, H. and Takagi, S. (1999): "Antioxidative effects of sesamol and tocopherols at various concentrations in oils during microwave heating" Journal of the Science of Food and Agriculture 79, 220–226.

Zitter-Eglseer, K.; Jurenitsch, J.; Korhammer, S.; Haslinger, E.; Sosa, S.; Della-Loggio, R.; Kubelka, W. and Franz, C. (1991): "Sesquiterpene Lactones of *Achillea setacea* with antiphlogistic activity" Planta-Med. 57 (5) :444-446.

Table (1): Effect of administration of *Salix* and *Achillea millefolium* extract on mean final body weight , and mean body weight gain (g) in rats.

	Mean initial body weight (g)	Mean final body weight (g)	Mean body weight gain (g)
Control	128.75?9.65	182.83?4.02	55.2?2.94
***Salix* 5 mg/kg B.wt**	129.4?7.83	167.83?4.49 *	36.5?3.47 *
***Salix* 10 mg/kg B.wt**	134.6?9.14	169.5?3.08*	34.67?3.09 *
***Achillea* 50 mg/kg B.wt**	132?8.60	178.66?2.72	48.33?4.60
***Achillea* 100 mg/kg B.wt**	130?10.80	174?4.16	44.8?3.88

* Significant at P< 0.05 ** Significant at P< 0.001

Table (2): Effect of administration of *Salix* and *Achillea millefolium* extract on plasma lipid profiles in rats.

	Triglycerides (mg/dl)	Cholesterol (mg/dl)	LDL (mg/dl)	HDL (mg/dl)
Control	102.4?3.47	113.33?3.11	55.74?4.13	36.02?2.15
***Salix* 5 mg/kg B.wt**	81.87?2.15 **	110.59?2.42	52.13?3.70	42.87?1.19 *
***Salix* 10 mg/kg B.wt**	90.23?2.17 *	101.51?3.84 *	43.39?2.96*	39.59?2.85
***Achillea* 50 mg/kg B.wt**	106.65?5.75	97.94?2.25 *	41.05?1.82 *	37.26?2.51
***Achillea* 100 mg/kg B.wt**	92.5?2.05 *	113.19?0.69	51.5?2.43	43.05?1.68 *

* Significant at P< 0.05 ** Significant at P< 0.001

Table (3): Effect of administration of *Salix* and *Achillea millefolium* extract on plasma malondialdehyde and glucose levels in rats.

	Plasma MDA (nmol/ml)	Plasma glucose (mg/dl)
Control	8.89?0.44	113.31? 5.89
***Salix* 5 mg/kg B.wt**	12.00?1.82	101.39?9.92
***Salix* 10 mg/kg B.wt**	11.34?3.01	96.82? 2.15 *
***Achillea* 50 mg/kg B.wt**	6.91?0.22 *	94.81? 4.93 *
***Achillea* 100 mg/kg B.wt**	5.74? 0.51 *	95.02?4.63 *

* Significant at P< 0.05 ** Significant at P< 0.001

الملخص العربي

تأثير المستخلص المائي لنباتي الاخيليا والصفصاف على بعض القياسات الكيميائية الحيوية في الفئران

كمال عادل أمين

قسم الكيمياء الحيوية - كلية الطب البيطري ببني سويف- جامعة القاهرة

تهدف هذه الدراسة إلى بحث تأثير النباتات الطبية مثل نباتي الاخيليا)ذات الألف ورقة) والصفصاف على وزن الجسم، ومستوى الدهون والأكسدة الفوقية للدهون ومستوى السكر في الدم.

وقد تم استخدام أربعين من الفئران قُسمت بالتساوي إلى خمس مجاميع :أعطيت فئران المجموعة الأولى (الضابطة) محلول ملح فسيولوجي، وأعطيت فئران المجموعة الثانية جرعة 5 مجم/كجم من وزن الجسم خلاصة نبات الصفصاف، وأعطيت فئران المجموعة الثالثة جرعة 10 مجم/كجم من وزن الجسم خلاصة نبات الصفصاف، وأعطيت فئران المجموعة الرابعة جرعة 50 مجم/كجم من وزن الجسم خلاصة نبات الاخيليا، وأعطيت فئران المجموعة الخامسة جرعة 100 مجم/كجم من وزن الجسم خلاصة نبات الاخيليا، وتم إعطاء المستخلصين لكل المجاميع عن طريق الفم بواسطة أنبوب معدي لمدة 6 أسابيع.

ولقد تم قياس الزيادة في وزن الجسم ومستوى الدهون)الجلسريدات الثلاثية، الكوليسترول الكلى والبروتينات الدهنية عالية ومنخفضة الكثافة) ومستوى المالونداياألدهايد والجلوكوز فى بلازما الدم.

وقد أوضحت النتائج أن جرعة 5مجم/ كجم و 10مجم/ كجم من وزن الجسم من خلاصة نبات الصفصاف أحدثت انخفاض معنوي في وزن الجسم المكتسب والجلسيريدات الثلاثية في بلازما الدم ، بينما أحدثت جرعة 10مجم / كجم من وزن الجسم انخفاضاً معنوياً في مستوى الكوليسترول الكلى والبروتينات الدهنية منخفضة الكثافة وجلوكوز الدم، وكذلك أحدثت جرعة 5 مجم/ كجم من وزن الجسم زيادة معنوية في مستوى الكوليسترول الكلى والبروتينات الدهنية عالية الكثافة مقارنة بالمجموعة الضابطة.

وقد أشارت النتائج أيضا أن جرعة 50مجم/ كجم من وزن الجسم من خلاصة نبات الاخيليا أحدثت انخفاض معنوي في مستوى الكوليسترول الكلى والبروتينات الدهنية منخفضة الكثافة ومستوى المالونداياألدهايد والجلوكوز في بلازما الدم، وكذلك أحدثت جرعة 100مجم /كجم من وزن الجسم من خلاصة نبات الاخيليا انخفاض معنوي في مستوى الجلسيريدات الثلاثية ومستوى المالونداياألدهايد والجلوكوز فى بلازما الدم، بينما أدت إلى ارتفاع مستوى البروتينات الدهنية عالية الكثافة.

ومن هذه النتائج يمكن الاستنتاج أن استخدام خلاصة نباتي الصفصاف والاخيليا يحسن أيض الدهون في الدم ويخفض مستوى الجلوكوز وينصح باستخدامهما كأعشاب لمرضى ارتفاع الدهون في الدم وأن والاخيليا ضروري كمضاد للأكسدة وخافض للأكسدة الفوقية للدهون.