Egyptian Journal of Natural Toxins, Vol. 1: 83-94, Dec. 2004.

SOME EFFECTS OF *GLYCYRRHIZA GLABRA* (LIQUORICE) ROOTS EXTRACT ON MALE RATS

Mostafa A.Shalaby[1*], Hoda S. Ibrahim[2], Elham M. Mahmoud[2] and Amal F. Mahmoud[2]

[1]Pharmacology Dept., Faculty of Veterinary Medicine, Cairo University

[2]Nutrition & Food Science Dept., Faculty of Home Economics, Helwan University, Egypt

ABSTRACT

The present study was carried out to elucidate the effect of oral administration of *Glycyrrhiza glabra* root extract to male rats for 4 weeks on food intake, body weight gain and feed efficiency ratio. Effects of the extract on serum lipid profile, liver enzymes, urea nitrogen and creatinine concentrations were also investigated. In addition, the anti-inflammatory and anti-ulcer activities of the extract were also studied on male rats using other two separate experiments. Results showed that the extract at oral doses of 200, 400 and 800 mg/Kg for 4 weeks induced a significant ($P<0.05$) decrease in food intake and significant increases in body weight gain and feed efficiency ratio as compared to the control. At doses of 400 and 800 mg/Kg, the extract caused significant decreases in total cholesterol and triglycerides associated with non significant reductions in HDLc, LDLc and VLDLc concentrations in the serum. The extract at all doses produced significant decreases in the levels of serum liver enzymes (AST and ALT) and urea nitrogen, while the creatinine concentration significantly decreased by the high dose only. Results also showed that the extract induced marked anti-inflammatory and anti-ulcer effects in male rats.

Key words: *Glycyrrhiza glabra,* Pharmacological effects, Biochemical effects, Anti-inflammatory, Anti-ulcer, Rats.

INTRODUCTION

Glycyrrhiza glabra, family Leguminoseae, is a plant which grows in Egypt and other countries of the world. Its roots possess some nutritive value and medicinal properties. They are widely used as a cold beverage, in preparing some pharmaceutical preparations such as haematinic pills and to disguise the bitter taste of other remedies (Fenwick *et al*., 1990).

* Corresponding author: Dr. Mostafa A.Shalaby, e-mail: drshalabyl@yahoo.com

Phytochemical analysis of *Glycyrrhiza glabra* root extract showed that it contains saponin triterpenes (glycyrrhizin, glycyrrhetinic acid and liquirtic acid), flavonoids (liquirtin, isoflavonoids and formononetin) and other constituents such as coumarins, sugars, amino acids, tannins, starch, choline, phytosterols and bitter principles (Snow, 1996; Fukai *et al*., 1998; Rossi, 1999; Arystanova *et al*., 2001).

Clinical and animal studies on *Glycyrrhiza glabra* total extract and the isolated active constituents revealed that both possess numerous pharmacological effects. Thus the extract has been used for the treatment of different diseases such as Addison`s disease, bronchitis, cough, arthritis, rheumatism, hypoglycemia (Snow,1996), inflammatory and allergic conditions (Chatterjee, 1996), gastric ulcer (Alkofahi and Atta, 1999; Khayyal *et al.*, 2001) and chronic hepatitis B and C (Al - Qarawi *et al*., 2001).

The present study was carried out to investigate some pharmacological and biochemical effects of *Glycyrrhiza glabra* root extract at three doses on male rats.

MATERIALS AND METHODS

Plant and its extraction

Roots of *Glycyrrhiza glabra* plant were purchased from market of Agricultural Seeds and Medicinal Plants, Cairo, Egypt and authenticated at Flora and Phytotaxonomy Unit, Horticultural Research Institute, Dokki. Clean roots (1 Kg) were macerated in methanol 90% for 48 h. and extracted in the Soxhlet apparatus till complete exhaustion. The alcoholic extract was dried under a reduced pressure in a rotatory evaporator. The dried extract was dissolved in a vehicle mixture (carboxymethylcellulose + tween 80, 1:1, v/v) to obtain 10 % liquid extract. This extract was kept in a refrigerator and freshly prepared before each experiment.

Rats and preparation of the basal diet

Male albino rats (Sprague Dawley strain) weighing from 150–170 g were obtained from Laboratory Animal Colony, Helwan, Egypt. The basal diet was prepared at the Faculty of Home Economics, Helwan University according to Reeves *et al.* (1993). It consists of casien 20 %, corn oil 10 %, fibers 5 %, salt mixture 4 %, vitamin mixture 1 %, choline chloride 0.2 % and the remainder is corn starch. Rats were fed

on the basal diet during the experimental period and water was provided *ad-libitum*.

Experiments

Effect on body weight gain, feed efficiency ratio and some serum constituents

A total of 28 male rats were allocated into 4 groups, each of 7 rats. The first group was fed on the basal diet and kept as a control. Groups (2), (3) and (4) were fed on the basal diet and orally administered *Glycyrrhiza glabra* root extract at 200, 400 and 800 mg/Kg, respectively. Daily food intake, body weight gain and feed efficiency ratio were calculated according to Chapman *et al*. (1959). At the end of the experiment, all rats were sacrificed and blood samples were taken by puncutre of retro-orbital plexus for biochemical analyses of total cholesterol (Trinder and Ann, 1969), triglycerides (Wahlefield, 1974), lipoproteins (Richmond, 1973), liver enzymes (Reitman and Frankel, 1957), urea nitrogen (Patton and Crouch, 1977) and creatinine (Henry, 1974) concentrations in the serum.

Anti - inflammatory effect

The method of Northover and Subramanian (1962) was used for this study. A total of 35 male rats were allocated into 5 equal groups, each of 7 rats. Group (1) was orally given the vehicle mixture (carboxy methylcellulose + tween 80) and kept as a control. Group (2) was interaperitonally given an anti-inflammatory drug (tenoxicam in a dose of 4 mg/Kg) and left as a standard. Groups (3), (4) and (5) were orally given *Glycyrrhiza glabra* root extract at doses of 200, 400 and 800 mg/Kg, respectively. One hour after treatment, each rat was injected with 0.1 ml of 1 % formalin solution in the *dorsum* of left limb. The paw thickness caused by formalin was measured using skin caliber after 3 and 6 h. post-injection. The anti-inflammatory effect was assessed by the reduction in the thickness of rat`s paw.

Anti - ulcer effect

This effect was determined according to the method of Agrawal *et al*. (2000). Twenty eight rats were allocated into 4 equal groups each of 7 rats. All rats were fasted for 24 h. but only allowed for drinking water. In the morning after a fasting day, group (1) was orally given the vehicle (carboxymethylcellulose + tween 80) and kept as a control. Groups (2), (3) and (4) were orally given *Glycyrrhiza glabra* root extract at 200, 400 and 800 mg/Kg, respectively. After 4 h. of either the vehicle or extract

administration, each rat in all groups was orally given 1 ml of 90 % ethanol. After other 4 h., all rats were sacrificed and their stomachs were opened longitudinally, washed with saline solution and examined under dissecting microscope for measuring the length of gastric ulcer caused by ethanol. The curative ratio from gastric ulceration was then calculated for all treated groups.

Statistical analysis

Data were expressed as means ± SEM. One way analysis of variance (ANOVA) and Student 't' tests according to Armitage and Berry (1987) were used for determining significance between treated groups and the control.

RESULTS AND DISCUSSION

The present investigation was done to study some pharmacological and biochemical effects as well as anti-inflammatory and anti-ulcer activities of *Glycyrrhiza glabra* root extract on male rats.

Results of the present study revealed that oral administration of *Glycyrrhiza glabra* root extract to male rats for 4 weeks caused a significant ($p<0.05$) decrease in food intake accompanied with significant increases in body weight gain and feed efficiency ratio (Table 1).

Table (1): Effect of oral administration of *Glycyrrhiza glabra* root extract on food intake, body weight gain and feed efficiency ratio in rats

Groups (n =7 rats)	Dose (mg/Kg)	Parameters as Means ± SEM		
		Food intake (g/day)	Body weight gain (g)	Feed efficiency Ratio
Control	0	13.56 ± 0.81^{a}	7.43 ± 0.49^{c}	0.54 ± 0.04^{c}
Glycyrrhiza glabra extract	200	10.87 ± 1.58^{b}	7.50 ± 0.73^{bc}	0.69 ± 0.06^{bc}
	400	8.59 ± 0.30^{c}	8.00 ± 0.87^{ab}	0.93 ± 0.10^{ab}
	800	8.30 ± 0.35^{c}	9.57 ± 1.04^{a}	1.15 ± 0.14^{a}

Different superscript letters in each column denote significant differences ($P< 0.05$) between treated groups and the control, while those having similar or partial similar letter superscript denote non-significant differences.

The reduction in food intake produced by *Glycyrrhiza glabra* root extract, in this study, could be possibly attributed to the presence of either a glycoside glycyrrhizin, which is 50-fold sweeter than sugar or bitter principles that may reduce food intake (Snow, 1996). The increase in body weight gain by *Glycyrrhiza glabra* root extract is in accordance with that reported by Miller (1998). The authors of the previously mentioned study explained this finding on the basis that *Glycyrrhiza glabra* inhibits 11 β-hydroxysteroid dehydrogenase and that induces excess release of mineralocorticoids, which causes retention of sodium and water that leads to oedema and increase in body weight. However, the increased body weight gain that reported in this study could be possibly explained by the improvement in feed efficiency reported herein.

Our results showed that oral administration of *Glycyrrhiza glabra* root extract to male rats for 4 weeks induced singnificant ($p < 0.05$) decreases in serum total cholesterol and triglycerides associated with non-significant decreases in concentrations of HDLc, LDLc and VLDLc fractions (Table 2).

The decrease in serum total cholesterol and triglycerides reported in this study following oral administration of *Glycyrrhiza glabra* root to rats for 4 weeks is similar to that reported by Khushbaktova *et al*. (1991) and Fuhrman *et al*. (1999). The authors of the previously mentioned studies attributed the hypocholesterolemic effect of *Glycyrrhiza glabra* to the presence of certain isoflavones, which act as antioxidants via inhibition of LDLc oxidation and that inhibits the local mechanism of atherogenesis. Moreover, Nikitina *et al*. (1995) reported that the glycosides of *Glycyrrhiza glabra* prevent accumulation of cholesterol in cells as well as human blood serum.

Results obtained in this study revealed that oral administration of *Glycyrrhiza glabra* root extract to male rats for 4 weeks produced a significant ($p < 0.05$) reduction in the levels of AST and ALT enzymes in the serum (Fig. 1).

Our results revealed that *Glycyrrhiza glabra* root extract reduced the levels of hepatic enzymes (AST and ALT) in the serum of rats. This finding is consistent with the findings of Fujisawa (1991), Eisenburg (1992) and Tamir *et al*. (2000) who reported that glycyrrhizin of *Glycyrrhiza glabra* reduced the liver enzymes in rats and induced interferon production in patients with chronic hepatitis B and C. The authors explained the hepatoprotective effect of glycyrrhizin by its

inhibitory effect on immuno- mediated cytotoxicity against the hepatocytes.

Table (2) : Effect of oral administration of *Glycyrrhiza glabra* root extract on serum lipid profile in rats.

Groups (n = 7 rats)	Dose (mg/ Kg)	Parameters as Means ± SEM				
		Cholestrol mg/dL	Triglycerides mg/dL	HDL.c mg/dL	LDL.c mg/dL	VLDL.c mg/dL
Control	0	96.29±5.4^{a}	148±11.7^{a}	47.71±4.7^{a}	45.7±3.0^{a}	10.4±0.1^{a}
Glycyrrhiza glabra extract	200	90.14±4.3^{ab}	106±15.7^{b}	41.71±4.1^{a}	38.21±2.2^{a}	9.3±0.5^{a}
	400	79.86±4.7^{b}	88.00±7.5^{b}	41.14±4.2^{a}	37.50±1.1^{a}	8.6±0.4^{a}
	800	70.71±4.3^{b}	83.57±6.7^{b}	40.01±3.9^{a}	34.69±0.7^{a}	7.8±0.7^{a}

Different superscript letters in each column denote significant differences (P< 0.05) between treated groups and the control, while those having similar or partial similar letter superscript denote non-significant differences.

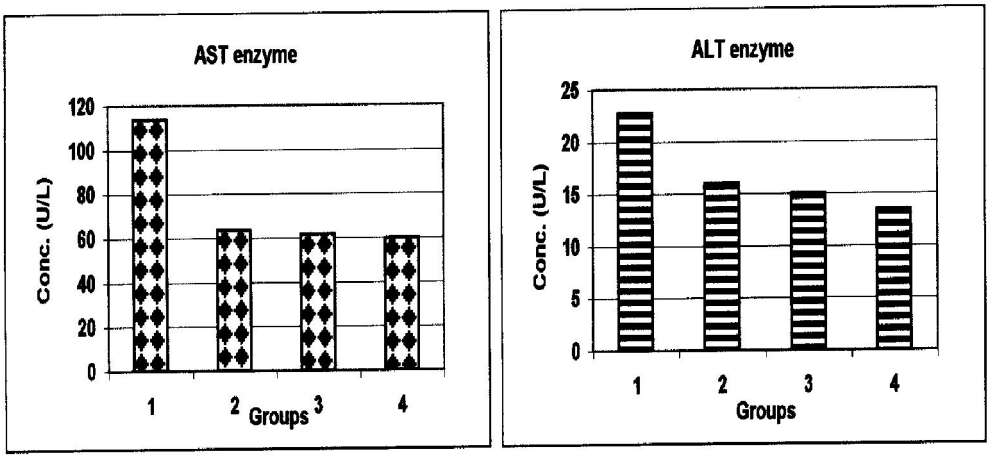

Figure (1): Effect of oral administration of *Glycyrrhiza glabra* root extract on the serum levels of AST and ALT enzymes in rats (n = 7)

1: control, 2, 3 and 4 : groups given the extract at 200, 400 and 800 mg/kg, respectively

Results of the present study revealed that oral administration of *Glycyrrhiza glabra* root at all tested doses to male rats caused a significant ($p<0.05$) reduction in urea nitrogen concentration associated with a significant decrease in creatinine concentration at the high dose only (Fig. 2).

The reduction in urea nitrogen and creatinine concentrations in the serum of rats given *Glycyrrhiza glabra* root extract reported in this study is similar to that reported by Yokozawa *et al*. (2000) in hepatotoxic rats and by Kumar *et al.* (2002) in alloxan-diabetic rats. This result indicates that *Glycyrrhiza glabra* root extract may be useful in patients with renal insufficiency. On contrast, Rossi (1999) concluded that intake of large amounts of glycyrrhizin is sometime associated with renal insufficiency. Such discrepancy could be attributed to the effect of large amounts of glycyrrhizin used by Rossi (1999), while in the present study therapeutic doses of *Glycyrrhiza glabra* root extract, which contain relatively small amounts of glycyrrhizin were used.

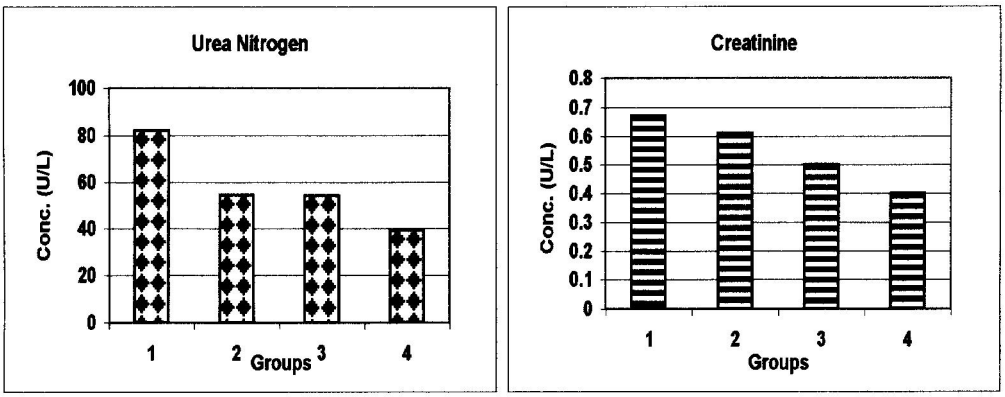

Figure (2): Effect of oral administration of *Glycyrrhiza glabra* root extract on serum urea nitrogen and creatinine concentrations in rats (n= 7)
1: control, 2, 3 and 4 : groups given the extract at 200, 400 and 800 mg/kg, respectively

Our results showed that oral administration of *Glycyrrhiza glabra* root extract to rats caused a significant reduction in pedal inflammation and swelling induced by formalin compared to the control group (Table 3). The Anti-inflammatory effect produced by all doses of *Glycyrrhiza glabra* root extract was less marked than that produced by tenoxicam (a standard anti-inflammatory drug).

The anti-inflammatory effect reported herein by *Glycyrrhiza glabra* root extract in rats is consistent with that recorded by Teelucksingh *et al*. (1990) and Fugh-Berman (2000). The authors attributed the anti-inflammatory effect of *Glycyrrhiza glabra* total extract to either an inhibition of PGE2 (an inflammatory mediator) or inhibition of 5 β-reductase enzyme that delays the metabolism of cortisol and potentiates the action of topical and oral corticosteriodes.

Table (3): Effect of oral administration of *Glycyrrhiza glabra* root extract on the paw`s thickness of rats after induction of pedal inflammation by formalin

Groups (n = 7 rats)	**Dose (mg/Kg)**	**Mean ± SEM of paw`s thickness (mm), after:**	
		3 hours	**6 hours**
Control (vehicle)	0	6.11 ± 0.10^{a}	6.57 ± 0.13^{a}
Standard (Tenoxicam)	4	4.43 ± 0.20^{c}	5.22 ± 0.29^{b}
***Glycyrrhiza glabra* extract**	200	5.43 ± 0.19^{b}	5.60 ± 0.19^{b}
	400	5.40 ± 0.11^{b}	5.86 ± 0.09^{b}
	800	5.20 ± 0.08^{b}	5.61 ± 0.08^{b}

Different superscript letters in each column denote significant differences ($P<0.05$) between treated groups and the control.

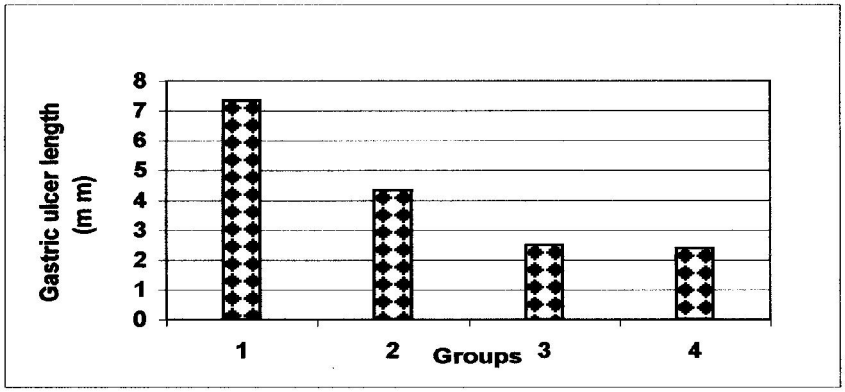

Figure (3): Effect of oral administration of *Glycyrrhiza glabra* root extract on the length of ethanol-induced gastric ulcer in rats . (n= 7)

1: control, 2, 3 and 4 groups given the extract at 200, 400 and 800 mg/Kg, respectively

The present study revealed that oral administration of *Glycyrrhiza glabra* root extract caused a significant ($p < 0.05$) reduction in the length of gastric ulcer induced by ethanol in rats (Fig. 3). The curative ratios from gastric ulceration were 40.0 , 65.9 and 67.3 % in groups of rats given the extract at 200 , 400 and 800 mg/Kg, respectively.

The anti-ulcerogenic activity of *Glycyrrhiza glabra* root extract reported in this study is in accordance with that reported by Alkofahi and Atta (1999) and Khayyal *et al*. (2001). The authors attributed the anti-ulcer effect of *Glycyrrhiza glabra* to the inhibitory effect of glycoside glycyrrhizin on the release of PGE2, which is responsible for gastric ulceration. The resulting effect may promotes the production of stomach mucus and decreases the production of gastric acid secretion thus consequently protects against gastric ulceration.

In conclusion, the present study revealed that *Glycyrrhiza glabra* root extract possesses various effects on male rats viz: increase in body weight gain, improvement of feed efficiency, reduction in serum total cholesterol, triglycerides, liver enzymes and urea nitrogen concentrations as well as marked anti–inflammatory and anti-ulcer effects. This study recommends the use of *Glycyrrhiza glabra* root extract for patients with chronic hepatitis, hypercholesterolemia, gastric ulcer and some local inflammatory conditions. Moreover, fortification of some staple foods with *Glycyrrhiza glabra* root extract should be taken in consideration.

REFERENCES

Agrawal, A.; Roo, V.; Sairman, K.; Joshi, V. and Goel, R. (2000): Effect of *Piper longum* L., *Zingiber officinal* L. and ferule species on gastric ulcerartion and secretion in rats. *Exper. Biol*., **38**: 994-998.

Alkofahi, A. and Atta, A. (1999): Pharmacological screening of the anti-ulcerogenic effects of some Jordanian plants in rats. *J. Ethnopharmacol.*, **67(3):** 341-345.

Al Qarawi, A.; Abdel Rahman, H. and El Mougy, S. (2001): Hepatoprotective activity of liquorice in rat liver injury models. *J. Herb. Sp. Med.*, **8:** 7-14.

Armitage, P. and Berry, G. (1987): "Statistical Methods in Medical Research. Blackwell", pp. 93-213, Oxford.

Arystanova, T.; Irismetov, M. and Sophekova, A. (2001): Chromatographic determination of glycyrrhizinic acid in *Glycyrrhiza glabra* preparation. *Chem. Nat. Com*., **37:** 89-91.

Chapman, D.; Castilla, R. and Cambell, J (1959): Evaluation of protein in foods: Method for the determination of feed efficiency ratio. *Can. J. Biochem. Phys*., **37:** 679-686.

Chatterjee, S. (1996): Mechnism of anti-inflammatory action of *Glycyrrhiza glabra* extract. *Ind. J. Ind. Med*., **18(2):** 183-186.

Eisenburg, J. (1992): Treatment of chronic hepatitis B. part 2: Effect of glycyrrhizinic acid on the course of illness. *Forschr. Med*., **110:** 395-398.

Fenwick, G.; Lutomski, J. and Nieman, C. (1990): *Glycyrrhiza glabra* L. (Liquorice): Composition, uses and analysis. *Food Chem*., **38(2):** 119-143.

Fugh-Berman, A. (2000): Herb-drug interactions. *Lancet*, **55:** 134-138.

Fuhrman, B.; Vaya, J.; Belinky, P. and Aviram M. (1999): The isoflavone glabridin inhibits LDL oxidation. Proc. 2nd Intern. Conf. Nat. Antioxidants, Finland, pp. 161-165.

Fujisawa, K. (1991): Interferon therapy in chronic hepatitis C (HCV): Clinical significance of pretreatment with glycyrrhizin. *Trop. Gastroentrol.*, **12:** 176-179.

Fukai, T.; Baosheng, C.; Maruno, K.; Migakawa, Y.; Konoshi, M.; Nomura, T. and Cai, B. (1998): An isopernylated flavonone from *Glycyrrhiza glabra* and re-assay of liquorice phenols. *Phytochem*., **49:** 2005-2013.

Henry, R.J. (1974): Creatinine measurement with colorimetric method, cited in "Clinical Chemistry: Principles and Technics" 2 nd Ed., pp. 93-213 Haper and Row Publishers.

Khayyal, M.; El Ghazaly, A.; Kenaway, S.; Seif El Nasr, M.; Mahran, L.; Kafafi, Y. and Okpanyi, S. (2001): Anti-ulcerogenic effect of some gastro-intestinally acting plant extracts and their combinations. *Arzneimittel Forschung*, **51:** 545-553.

Khushbaktova, Z.; Syrov, V. and Batirov, E. (1991): Effect of flavonoids on the course of hyperlipidemia and atherosclerosis under experimental conditions. *Khimiko Farmatsevticheskii Zhurnal*, **25:** 54-57.

Kumar, T.; Stanley, V.; Lal, A. and Bala, S.M. (2002): Biochemical evaluation of multiple herbal treatment in alloxan-diabetic rats. *J. Environ. Biol*., **23:** 407-410.

Miller, I. (1998): Herbal medicine: Selected clinical considerations focusing on known or potential drug-herb interaction. *Arch. Inter. Med.*, **158:** 2200-2211.

Nikitina, N.; Khalilov, E.; Tertov, V. and Orekhv, A. (1995): *In vitro* decrease in the atherogenicity by polyunsaturated phosphotidylcholine. *Byu. Eksper. Biol. Med.*, **119:** 497-501.

Northover, B. and Subramanian, G. (1962): Pedal inflammation induced by chemical agents. *Br. J. Phramacol.*, **18:** 346-349.

Patton, C. and Crouch, S. (1977): Enzymatic colorimetric method for determination of urea in serum. *Anal. Chem.*, **49:** 494-499.

Reeves, P.; Nielson, F. and Fahmy, G. (1993): Purified diets for laboratory rodents: Final report of the American Institute of Nutrition on the reformulation of rodent diet. *J. Nutri.*, **123:** 1939-1951.

Reitman, S. and Frankel, S. (1957): Determination of glutamate pyruvate transferase. *Amer. J. Clin. Path.*, **28:** 32-33.

Richmond, N. (1973): Colorimetric method of determination of total cholesterol and lipoproteins. *Clin. Chem.*, **19:** 1350-1356.

Rossi, I. (1999): "Medicinal Plants of the World". Vol. 2: Chemical constituents, traditional and modern medicinal uses. Human Press, Otawa, USA.

Snow, J. (1996): *Glycyrrhiza glabra* Monograph. *J. Bot. Med.*, **1(3):** 9-14.

Tamir, S.; Eisenburg, J.; Somjen, D. and Izrael, S. (2000): Natural compounds for medicinal use. *J. Steroid. Biochem. Mol. Biol.*, **78:** 291-298.

Teelucksingh, S.; Mackie, A. and Burt, D. (1990): Potentiation of hydrocortisone activity in skin by glycyrrhetinic acid. *Lancet*, **5:** 1060-1063.

Trinder, P. and Ann, A. (1969): Colorimetric determination of total cholesterol. *Clin. Biochem.*, **6:** 24-25.

Wahlefield, A. (1974): "Methods of Enzymatic Analysis", 5th Ed., pp. 1831-1835, Academic Press, USA.

Yokozawa, T.; Lin, Z. and Chen, C. (2000): Protective effect of *Glycyrrhiza glabra* in renal hypoxy-reoxygenation model. *Phytomed.*, **6:** 439-445.

بعض تأثيرات خلاصة جذور نبات العرقسوس على ذكور الجرذان

مصطفى عباس شلبي[١]، هدى سلامة إبراهيم[٢]، إلهام محمد محمود[٢]، أمل فوزي محمود[٢]

[١] قسم الفارماكولوجي- كلية الطب البيطري – جامعة القاهرة

[٢] قسم التغذية وعلوم الأطعمة – كلية الاقتصاد المنزلي – جامعة حلوان

تم إجراء هذه الدراسة لمعرفة تأثير إعطاء خلاصة جذور نبات العرقسوس عن طريق الفم لذكور الجرذان لمدة أربعة أسابيع على معدل استهلاك الغذاء، ومعدل الزيادة في وزن الجسم وكفاءة تحويل الغذاء. كما تم دراسة تأثير هذه الخلاصة على صورة الدهون ومستوى إنزيمات الكبد ونيتروجين اليوريا والكرياتينين بمصل الدم، بالإضافة إلى دراسة التأثير المضاد للالتهاب والمضاد لقرحة المعدة في ذكور الجرذان.

وأظهرت نتائج الدراسة إن إعطاء خلاصة جذور نبات العرقسوس بجرعات ٢٠٠، ٤٠٠ و ٨٠٠ مجم/كجم من وزن الجسم عن طريق الفم لمدة أربعة أسابيع أدى إلى نقص معنوي في معدل استهلاك الغذاء وزيادة معنوية في وزن الجسم وتحسن في كفاءة تحويل الغذاء في ذكور الجرذان. كما أدت هذه الخلاصة إلى حدوث نقص معنوى في تركيز الكوليسترول والجلسريدات الثلاثية وكذلك تركيز إنزيمات الكبد ونيتروجين اليوريا والكرياتينين في مصل الدم. كما كان لخلاصة جذور هذا النبات تأثير مضاد للالتهاب ولقرحة المعدة في ذكور الجرذان.