

Full Length Research Paper

Biological study of the effect of licorice roots extract on serum lipid profile, liver enzymes and kidney function tests in albino mice

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This study was carried out to elucidate the effects of oral administration of *Glycyrrhiza glabra* root extract on serum lipid profile, liver enzymes, kidney function test, and glucose concentration in albino mice when compared with ten male mice used as control. 40 male mice were treated for one month and equally allocated into four groups: the first group (G1) was used as the control group. The second (G2), third (G3) and fourth groups (G4) were treated with 1 ml of 0.2, 0.7 and 1 mg mL⁻¹ day⁻¹, respectively. There was statistically high significance difference between treated and untreated groups for all biochemical parameters, showing a remarkable effect on serum lipid profile, enzymes and kidney function test. *G. glabra* root extract at low dose act as anti-lipidaemic agent, has hepatoprotective activity for liver cell, prevents renal failure and is an anti-hyperglycemic agent.

Key words: *Glycyrrhiza glabra*, liver enzymes, serum lipid profile, kidney function test, glucose concentration.

INTRODUCTION

Glycyrrhiza glabra (GG) (Licorice or sweet wood, Fabaceae-Papilionaceae) is a traditional medicinal herb that grows in various parts of the world and it has ethnobotanical history. Its roots have some nutritive value and medicinal properties. The dried roots of this plant were employed by Iraqi, Egyptian, Chinese, Greek, Indian, as food and medicinal remedies for thousands of years (Olukoga and Donaldson, 1998; Ross, 2001) Phytochemical analysis of *G. glabra* root extract showed that it contains saponin, triterpenes (glycyrrhizin, glycyrrhetic acid and liquiritic acid), flavonoids (liquiritin, isoflavonoids and formononetin) and other constituents such as coumarins, simple sugar and polysaccharide like starch, pectin, amino acids, tannins, choline, phyto-sterols, mineral salts and various other substance (Fukai et al., 1998).

The more important compounds are glycyrrhizin and

glycyrrhizic acid, which are believed to be partly responsible for anti-ulcer, anti-inflammatory, anti-diuretic, anti-epileptic, anti-hepatotoxic, anti-viral activities, anti-allergic and anti-oxidant property of the plant as well as their ability to fight low blood pressure (Ross, 2001; Arystanova et al., 2001; Al Qarawi et al., 2001).

Furthermore, *G. glabra* extract have been shown to possess anti-depressant-like, memory enhancing activities and produce anti-thrombotic effects. On other hand, the root extracts are reported to exhibit anti-angiogenic activities and radio-protective effects (Vaya et al., 1997; Belinky et al., 1998). The other important compound is glabridin, it is the major flavonoid, present specifically in licorice; it has various physiological activities such as cytotoxic, anti-tumor promoting, anti-microbial, estrogenic and anti-proliferative activity against human breast cancer cells. It also affects melanogenesis, inflammation, low density lipoprotein (LDL) oxidation and protection of mitochondria functions from oxidative stresses (Khatta and Simpson, 2010). Glabridin is reported to be a potent anti-oxidant towards LDL oxidation (Vaya et al., 1997; Belinky et al., 1998), where-

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as isoliquiritigenin is known to have vasore-laxant effect, anti-platelet, anti-viral, estrogenic activity and has protective potential against cerebral ischemic injury (Zhan and Yang, 2006). Licorice roots contains flavonoids, which have lipophilic characteristic and anti-oxidative properties (Rice-Evans et al., 1996), among several flavonoid and isoflavan glabridin that protect LDL from oxidation induced by free radical generating system (Vaya et al., 1997; Zhan and Yang, 2006). The anti-oxidant activity of flavonoids is related to their chemical structure (Rice-Evans et al., 1996). Consumption of polyphenolic flavonoids in the diet was inversely associated with morbidity and mortality from coronary heart disease (Hertog et al., 1993). Polyphenolic flavonoids may prevent coronary artery disease by reducing plasma cholesterol levels and their ability to inhibit LDL oxidation (Fuhrman and Aviram, 2001; Fuhrman et al., 2002). Anti-hyperlipidaemic and anti-hypertriglyceridaemic properties of *G. glabra* have also been reported (Sitohy et al., 1991). The liver damage caused by pathogens as well as chemical agents is of similar nature and a proper treatment regime or plan is absent for both. The fact that reliable liver drugs are explicitly inadequate in allopathic medicine urged the scientists to explore herbal remedies (Trivedi and Rawal, 2000). In traditional medical practices, followed throughout the world, herbs play a major role in the management of various liver disorders.

Diabetes mellitus is a group of syndromes characterized by hyperglycemia: altered metabolism of lipids, carbohydrates and proteins, as well as an increased risk of complications from vascular diseases (Yoshinari et al., 2009). The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction and failure of various organs (Lyra et al., 2006).

Phytochemicals isolated from plant sources are used for the prevention and treatment of several medical problems including diabetes mellitus (Waltner-Law et al., 2002). There are more than 800 plant species showing hypoglycemic activity. The aim of this study was to demonstrate the effect of biochemical parameters of *G. glabra* root extract at three different doses on the serum, lipid profile, liver enzymes, pancreatic enzyme, kidney function test and glucose concentration in albino male mice.

MATERIALS AND METHODS

The roots of *G. glabra* were purchased from the local herbal merchandise, Baghdad, Iraq and were air dried, ground to powder and stored overnight at 4°C.

Extraction

250 g of crushed *G. glabra* were weighted and added to 500 ml ethanol (30%) in soxhlet apparatus at 50°C for 60 min, then left to cool with continuous slow mixing and then the solution was

filtered in the rotary evaporator at 60°C until a thick solution was gotten. After that, the solution was dried in the incubator at 37°C for one to two days until it became a crushed dried, then it was taken and stored in the refrigerator at 4°C. The resulted deposit was dissolved in distilled water to prepare the doses.

Laboratory animals and sample collection

Albino male mice were obtained from the Laboratory Animal Production Unit of Biotechnology Division, University of Technology. All mice were kept under constant environmental conditions (24 to 26°C and 55 to 60% humidity) with a 12-hour light/dark cycle. They were housed in polypropylene cages with wood dust and given free access to food and tap water *ad libitum*. The animals were procured, maintained, and used in accordance with 'Guide for the Care and Use of Laboratory Animals in Biotechnology Division, and approved by the University of Technology, Animal Ethical Committee'. Experimental groups were organized as four groups that included ten animals each.

The first group (G1) was the control groups which did not receive the extract. G2, G3 and G4 included the animals which were orally administered *G. glabra* root extract at 0.2, 0.7 and 1 mg mL⁻¹ day⁻¹, respectively. At the end of the experiment, after 30 days of receiving the extract, all the animals were sacrificed and blood samples were taken by puncture for biochemical analysis of serum, total cholesterol, triglyceride, high density lipoprotein-cholesterol (HDL-c), very low density lipoprotein-cholesterol (VLDL-c), low density lipoprotein-cholesterol (LDL-c), liver enzymes [gamma glutamyl transpeptidase (GGT) and alkaline phosphatase (ALP)], pancreatic enzyme amylase, glucose concentration and kidney function test (urea, uric acid, creatinine concentrations).

Biochemical assay

Serum cholesterol, triglyceride, HDL, GGT, ALP, amylase, glucose, urea, uric acid and creatinine levels were measured by commercially available kits in spectrophotometer.

Statistical analysis

Data were presented as mean ± standard deviation (SD). To get such data, the individual values were tabulated in a sheet of the statistical programme GraphPad Prism version 5.01 (GraphPad software, Inc., La Jolla, CA, USA). The difference between means was assessed by Duncan's test, in which P ≤ 0.05 was considered significant.

RESULTS

The result of this present study revealed that oral administration of *G. glabra* root extract to the animals for one month at three different doses, 0.2, 0.7 and 1 mg mL⁻¹ day⁻¹ show significant decrease in total cholesterol and triglyceride concentration, and a significant increase in HDL-c concentration when compared with the untreated group. Very low density and low density lipoprotein was markedly reduced in the treated group in comparison with the untreated, as shown in Table 1 as compared with the control group values.

Table 1. Effect of *G. glabra* root extract on serum lipid profile in mice (parameters as mean \pm SD).

Parameter (mg dL ⁻¹)	Dose (mg mL ⁻¹ day ⁻¹)			
	Control	G1 (0.2)	G2 (0.7)	G3 (1)
Cholesterol	211.10 \pm 6.65 ^A	182.10 \pm 5.19 ^B	166.10 \pm 3.81 ^C	150.40 \pm 5.12 ^D
Triglyceride	153.80 \pm 3.29 ^A	133.60 \pm 2.91 ^B	114.70 \pm 5.16 ^C	90.00 \pm 4.10 ^U
HDL -c	40.70 \pm 4.11 ^A	54.50 \pm 3.97 ^B	63.60 \pm 2.71 ^C	74.70 \pm 4.00 ^U
VLDL-c	30.76 \pm 3.45 ^A	26.72 \pm 2.23 ^B	22.94 \pm 2.11 ^C	18.00 \pm 1.24 ^U
LDL -c	139.64 \pm 4.52 ^A	100.88 \pm 3.48 ^D	79.56 \pm 3.78 ^U	57.7 \pm 2.90 ^U

Different capital letters show significant difference ($P \leq 0.05$) between means of rows; HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein.

Table 2. Effect of *G. glabra* root extract on the liver enzymes, pancreatic enzyme and glucose in mice (parameters as mean \pm SD).

Parameter	Dose mg mL ⁻¹ day ⁻¹			
	Control	G1 (0.2)	G2 (0.7)	G3 (1)
GGT (UL ⁻¹)	38.20 \pm 4.61 ^A	27.60 \pm 1.42 ^B	19.50 \pm 1.08 ^C	14.80 \pm 1.31 ^D
ALP (UL ⁻¹)	41.60 \pm 2.27 ^A	37.40 \pm 1.57 ^B	28.80 \pm 1.31 ^C	20.10 \pm 2.13 ^U
Amylase (UL ⁻¹)	107.30 \pm 4.52 ^A	83.30 \pm 3.43 ^B	68.10 \pm 2.64 ^C	54.30 \pm 3.23 ^U
Glucose (mg dL ⁻¹)	77.80 \pm 3.61 ^A	59.10 \pm 5.48 ^B	49.40 \pm 3.13 ^C	34.70 \pm 3.09 ^U

Different capital letters show significant difference ($P \leq 0.05$) between means of rows.

Table 3. Effect of *G. glabra* root extract on the kidney function tests in mice (parameters as mean \pm SD).

Parameter (mg dL ⁻¹)	Dose mg mL ⁻¹ day ⁻¹			
	Control	G1 (0.2)	G2 (0.7)	G3 (1)
Urea	45.30 \pm 3.19 ^A	34.00 \pm 3.23 ^B	24.90 \pm 2.92 ^C	23.10 \pm 2.13 ^D
Uric acid	5.80 \pm 0.78 ^A	4.50 \pm 0.70 ^B	3.30 \pm 0.48 ^C	1.8 \pm 0.63 ^U
Creatinine	16.10 \pm 0.99 ^A	13.50 \pm 0.97 ^B	9.30 \pm 0.82 ^C	6.90 \pm 0.73 ^U

Different capital letters show significant difference ($P \leq 0.05$) between means of rows.

Table 2 demonstrates the effect of oral administration of *G. glabra* root extract on liver enzyme (GGT and ALP) and pancreatic enzyme (amylase); significant reduction were observed in all these enzymes and a decrease in glucose concentration was also observed when compared with the control group values.

Table 3 illustrates the effect of oral administration of *G. glabra* root extract on kidney function test, urea, uric acid and creatinine concentrations; it shows significant decrease in the concentration of the treated group when compared with the control group values.

DISCUSSION

Dyslipidemia, which can range from hypercholesterolemia to hyperlipoproteinemia, is one of the many modifiable risk factors for coronary artery disease (CAD), stroke and peripheral vascular disease (Chong and Bachheimer, 2000). High level of total cholesterol

is one of the major risk factors for coronary heart diseases and it is well known for hyperlipidemia and the incidence of atherosclerosis and increase in diabetes and hypertension (Tan et al., 2005). The liver and some other tissues participate in the uptake, oxidation and metabolic conversion of free fatty acid, synthesis of cholesterol and phospholipids and secretion of specific classes of plasma lipoprotein. Lowering of serum lipid level through dietary or drug therapy seems to be associated with a decrease in the risk of vascular disease and related complications. Though there was a large class of hypolipidemic drugs used in the treatment, none of the existing ones available worldwide is fully effective, absolutely safe and free from side effect (Betteridge, 1997). Hence, efforts are being made to find out safe and effective agents that may be beneficial in correcting the lipid metabolism and preventing cardiac diseases. Among natural materials, medical plants have been shown to have anti-hyperlipidemic properties (Sitohy et al., 1991)

Result of this study reveals that oral administration of

G. glabra root extract at three different doses to the animals for 30 days caused a significant reduction in serum total cholesterol and triglyceride as shown in Table 1. This is similar to that reported by others (Waltner-Law et al., 2002; Shalaby et al., 2004). The authors of previously mentioned studies attributed the hypocholesteremic effect of *G. glabra* to the presence of certain isoflavones, which act as anti-oxidants via inhibition of LDL-cholesterol oxidation and which inhibit the local mechanism of atherosclerosis. Moreover, it was reported that the glycosides of *G. glabra* prevent accumulation of cholesterol in cells as well as human blood serum (Nikitina et al., 1995).

The repeated administration of GG ethanolic extract for a period of 30 days resulted in a significant increase in HDL-c, when compared with untreated animals. It is well documented that while low level of HDL-c is indicative of high risk for coronary artery disease, an increase in HDL level is considered beneficial. Epidemiological studies have also shown that high HDL-cholesterol levels could potentially contribute to anti-atherogenesis, including inhibition of LDL oxidation to protect the endothelial cells from the cytotoxic effects of oxidized LDL (Assmann and Nofer, 2003).

The presented result on LDL-cholesterol and VLDL-cholesterol, showed a significant decrease as shown in Table 1. A significant decline in plasma LDL-cholesterol in treated group could be correlated with saponin content of GG root; saponin enhances the hepatic LDL-receptor levels, increase hepatic uptake of LDL-cholesterol and aids its catabolism to bile acid (Venkatesan et al., 2003).

Saponin is known to lower triglyceride by inhibiting pancreatic lipase activity. Furthermore, the decline in VLDL cholesterol levels in treated group could be directly correlated to decline in triglyceride levels of these groups, as it is well established that VLDL particles are the main transporters of triglyceride in plasma (Hertog et al., 1993). Thus, a simultaneous decline in both triglyceride and VLDL-cholesterol in treated group indicates the possible effect of saponins, and on the other hand, the effect of phytosterol content of the root on triglyceride metabolism through a decreased absorption of dietary cholesterol (Hertog et al., 1993; Fuhrman and Aviram, 2001).

The presence of phytosterols and saponins in GG root could be important in cholesterol elimination. Phytosterols are reported to displace intestinal cholesterol and reduce cholesterol absorption from intestine (Ikeda and Sugano, 1998). Saponins are capable of precipitating cholesterol from micelles and interfere with enterohepatic circulation of bile acids, making it unavailable for intestinal absorption (Fuhrman et al., 2002). Thus, the presently noted reduced cholesterol level in dyslipaemic animals administered ethanolic extract and its fractions could be due to both phytoesterol and saponin content of GG root.

The beneficial effect of dietary flavonoid derived from

the ethanolic extract of licorice root against atherosclerotic lesion development in association with inhibitor of LDL oxidative atherosclerotic mice has been demonstrated (Fuhrman et al., 2002). Investigation of the relationship between excretion and liver dysfunction is important for predicting the pharmacokinetic in patient with liver dysfunction to avoid drug adverse reaction. Some of the constituent's plants of the herbal mixture namely *G. glabra* are traditionally used and scientifically proven for the treatment of the liver disorder (Roche and Samuel, 2008).

There was significant reduction in the levels of liver enzymes (GGT and ALP) and pancreatic enzyme amylase and also glucose concentration. This was observed after treatment of animals with GG extract in comparison with the untreated animals at all tested doses. Our results reveal that GG reduced significantly the level of hepatic enzymes in serum of animals. This can be explained by hepatoprotective effect of GG by inhibitory effect on immunomediated cytotoxicity against the hepatocyte. It has been demonstrated that the root of

GG is a traditional medicine used mainly for the treatment of peptic ulcer, hepatitis, pulmonary and skin disease, although the clinical studies suggest that it has several other useful pharmacological properties like anti-inflammatory, anti-viral, hepatoprotective and cardio protective effects. Glycyrrhizic acid, the major component of licorice shows hepatoprotective effect by preventing changes in cell membrane permeability, and increasing survival rate of hepatocyte (Maurya et al, 2009).

In hyperglycemia, free amino groups of proteins react slowly with the carbonyl groups of reducing sugars such as glucose, to yield a Schiff's-base intermediate (Bucala, 1999). Such alterations in blood glucose level could be due to the stress of the diabetic injury and this is in agreement with the reports of others (Fuhrman et al., 2002; Powell et al., 2005) which shows that diabetes is one of the metabolic causes of steatosis (the presence of fat droplets within the hepatocytes). In addition, Kleiner et al. (2005) emphasized that steatosis could take one of two forms either as multiple small vesicles (micro-vesicular) or a single large vesicle that may cause ballooning of the hepatocyte (macro-vesicular) so that it resembles a mature adipocyte.

Our results also showed a significant decrease in the concentration of urea, uric acid and creatinine after oral administration of GG extract. This is in agreement with the reports of others (Fukai et al., 1998) as it has been reported that anti-nephritis activity of glabradin, a pyranis of laven isolated from GG, was evaluated after its oral administration to mice with glomerular disease, by measuring urinary protein excretion, blood urea nitrogen and serum creatinine level, which reduced the amount of the earlier parameters significantly. Glycyrrhizic acid exhibit anti-inflammatory activity by inhibitory glucocorticoid metabolism (Sitohy et al., 1991; Fukai et al., 1998).

Hyperuricemia is a metabolic disorder which plays an important role in the development of gout and several oxidative stress diseases such as cancer and cardiovascular diseases. Elevated levels of monosodium urate or uric acid crystals, are deposited on the cartilage of a specific joint, tendons and surrounding tissues. This in turn causes an inflammation of these tissues that is very painful and sensitive. Today, there are a growing number of scientific studies to support traditional and natural remedies. Nitric oxide also has been implicated in both osteoarthritis and rheumatoid arthritis, while studies show that anti-oxidant scavenge this oxidant and potentially aid in the treatment or prevention of symptoms of arthritis (Strazzullo and Puig, 2007).

Conclusion

This study reveals that GG had various effect on mice in the reduction of serum lipid profile, kidney function and glucose concentration and has been shown to have significant free radical quenching effect and potent anti-oxidant agents against cardiovascular, kidney and liver diseases.

REFERENCES

- Al Qarawi A, Rahman HA, Mougy SE (2001). Hepatoprotective activity of Licorice in rat liver injury models. *J. Herb. Sp. Med.*, 8: 7-14.
- Arystanova T , Irismetov M, Sophekova A (2001). Chromatographic determination of glycyrrhizinic acid in *Glycyrrhiza glabra*. *Preparation. Chem. Nat. Com.*, 37: 89-91.
- Assmann G, Nofer J (2003). Atheroprotective effects of high-density lipoproteins. *Annu. Rev. Med.*, 54: 321-341.
- Belinky PA, Aviram M, Fuhrman B, Rosenblat M, Vaya J (1998). The antioxidative effects of the isoflavan glabridin on endogenous constituents of LDL during its oxidation. *Atherosclerosis*, 137: 49-61.
- Betteridge J (1997). Lipid Disorders in Diabetes Mellitus. In: *Text Book of Diabetes*, Pickup JC, Williams G (Eds.). Blackwell .Science. London. pp. 1-35.
- Bucala R (1999). Advanced glycosylation end products and diabetic vascular diseases. In: *Oxidative stress and vascular disease*, Keaney Jr. JF Ed. Kluwer Acad. Pub. Dord., pp. 287-303.
- Chong PH, Bachenheimer BS (2000). Current, new and future treatments in dyslipidaemia and atherosclerosis. *Drugs*, 60: 55-93.
- Fuhrman B, Aviram M (2001). Flavonoids protect LDL from oxidation and attenuate atherosclerosis. *Curr. Opin. Lipidol.*, 12: 41-48.
- Fuhrman B, Volkova N, Kaplan M, Presser D, Attias J, Hayek T, Aviram M (2002). Antiatherosclerotic effect of licorice extract supplementation on hypercholesterolemic patients: Increased resistance of LDL to atherogenic modifications reduced plasma lipid levels and decreased systolic blood pressure. *Nutrition*, 18: 268-273.
- Fukai T, Baosheng C, Maruno K, Migakawa Y, Konoshi M (1998). An isoprenylated flavonone from *Glycyrrhiza glabra* and re-assay of licorice phenols. *Phytochemistry*, 49: 2005-2013.
- Hertog MG, Feskens EJ, Hollman PC, Katan MB, Kromhout D (1993). Dietary antioxidant flavonoids and risk of coronary heart disease the Zutphen. *Lancet.*, 342: 1007-1011.
- Ikeda I, Sugano M (1998). Inhibition of cholesterol absorption by plant sterols for mass intervention. *Curr. Opin. Lipidol.* 9: 527-531.
- Khatta KF, Simpson TJ (2010). Effect of gamma irradiation on the antimicrobial and free radical scavenging activities of *glycyrrhiza glabra* root. *Radiat. Phys. Chem.*, 79: 507-512.
- Kleiner DE, Brunt EM, Natta MV, Behling C, Contos MJ, Cummings OW, Ferrell LD, Liu Y C, Torbenson MS, Unalp-Arida A, Yeh M, McCullough AJ, Sanyal AJ (2005). Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology*, 41: 1313-1321.
- Lyra R, Oliveira M, Lins D, Cavalcanti N (2006). Prevention of type 2 diabetes mellitus. *Arq. Bras. Endocrinol. Metabol.*, 50: 239-249.
- Maurya SK, Raj K, Srivastava AK (2009). Antidyslipidaemic activity of *glycyrrhiza glabra* in high fructose diet induced dyslipidaemic Syrian golden hamsters. *Indian J. Clin. Biochem.* 24: 404-409.
- Nikitina NA, Khalilov EM, Torkhovskaia TI, Tertov VV, Orekhov AN (1995). Decrease in atherogenicity of blood serum *in vitro* under the effect of polyunsaturated phosphatidylcholine micelles (In Russian). *Biull. Eksp. Biol. Med.*, 119: 497-501.
- Olukoga A, Donaldson, D (1998). Historical perspectives on health, the history of Licorice: The plant, its extract, cultivation. *J. R. Sco. Health*, 118: 300-304.
- Powell EE, Jonsson JR, Clouston AD (2005). Steatosis: Co-factor in other liver diseases. *Hepatology*, 42: 5-13.
- Rice-Evans CA, Miller NJ, Paganga G, (1996). Structure antioxidant activity relationship of flavonoids and phenolic acids. *Free radical. Biol. Med.*, 20: 933-956.
- Roche B, Samuel D (2008). Liver transplantation in viral hepatitis: Prevention of recurrence. *Best pract. Res. Clin. Gastroen.*, 22: 1153-1169.
- Ross IA (2001). *Glycyrrhiza glabra*. Medicinal plants of the world. Chemical constituents, traditional and modern medicinal uses, Humana Press, Totowa, N. J., 2: 191-240.
- Shalaby MA, Ibrahim HS, Mahmoud EM, Mahmoud AF (2004). Some effects of *glycyrrhiza glabra* (licorice) roots extract on male rats. *Egyptian J. Nat. Toxins.*, 1: 83-94.
- Sitohy MZ, El Massry RA, El Saadany SS, Labib SM (1991). Metabolic effect of licorice root (*glycyrrhiza glabra*) on lipid distribution pattern liver and renal functions of albino rats. *Food Nahrung.* 35: 799-806.
- Strazzullo P, Puig JG (2007). Uric acid and oxidative stress: Relative impact on cardiovascular risk. *Nutr. Metab. Cardiovasc. Dis.*, 17: 409-414.
- Tan BK, Tan CH, Pushparaj PN (2005). Anti-diabetic activity of the semi-purified fractions of *Averrhoa bilimbi* in high fat diet fed-streptozotocin-induced diabetic rats. *Life Sci.*, 76: 2827-2839.
- Trivedi N, Rawal UM (2000). Hepatoprotective and toxicological evaluation of *Andrographis paniculata* on severe liver damage. *Ind. J. Pharmacol.*, 32: 288-293.
- Vaya J, Belinky PA, Aviram M (1997). Antioxidant constituents from licorice roots: Isolation, structure elucidation and anti oxidative capacity toward LDL oxidation. *Free radical Biol. Med.*, 23: 302-313.
- Venkatesan N, Devaraj SN, Devaraj H (2003). Increased binding of LDL and VLDL to apo B, E receptors of hepatic plasma membrane of rats treated with Fibernat. *Eur. J. Nutr.*, 42: 262-271.
- Waltner-Law ME, Wang XL, Law BK, Hall RK, Nawano M, Granner DK (2002). Epigallocatechingallate, a constituent of green tea, represses hepatic glucose production. *J. Biol. Chem.*, 277: 34933-34940.
- Yoshinari O, Sato H, Igarashi K (2009). Antidiabetic effect of pumpkin and its components, trigonelline and nicotinic acid, on Goto-Kakizaki rats. *Biosci. Biotechnol. Biochem.*, 73: 1033-1041.
- Zhan C, Yang J, (2006). Protective effects of isoliquiritigenin in transient middle cerebral artery occlusion-induced focal cerebral ischemia in rats. *Pharmacol. Res.*, 53: 303-309.