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Research Article

Evaluation of antihyperlipidemic activity of ethanolic root extract of *Glycyrrhiza glabra* Linn.

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ABSTRACT

Glycyrrhiza glabra Linn. is an Indian medicinal plant demonstrated to exert multiple health cures. This plant grows naturally in tropical, subtropical and temperate regions. It is traditionally used as anti-acne, anti-arthritis, anti-convulsant, anti-diabetic, anti-microbial, anti-inflammatory, wound healing, anti-oxidant, anti-platelet, anti-stress and nootropic, anti-viral, diuretic and gastroprotective. The antihyperlipidemic effect of ethanolic root extracts of *Glycyrrhiza glabra* was studied in wistar rats using High Fat Diet (FD) induced hyperlipidemic model, at the doses of 400 mg/kg body weight. The efficacy of extract was compared with standard drug simvastatin. The ethanolic extracts, significantly decreased the serum lipid profile level in a dose dependent manner in wistar rats. Ethanolic extracts at 400 mg/kg have shown significant antihyperlipidemic action. These results support the fact that this plant is used traditionally as antihyperlipidemic. The study will help in exploring new plant source as an antihyperlipidemic agent which can minimize the risk and side effects as compared to that of conventional medicine.

Keywords: *Glycyrrhiza glabra*, Antihyperlipidemic Activity, Simvastatin, High Fat Diet (FD) induced hyperlipidemic model.

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1. INTRODUCTION

Hyperlipidaemia has been found to be associated with the alteration of lipid and lipoproteins metabolism in the onset of diabetes mellitus¹. Several researches have reported the positive correlation of metabolic disorders in lipid and lipoproteins metabolism in individuals suffering from diabetes to arteriosclerosis^{2, 3}

Figure 1: Hypercholesterolemia

Hyperlipidemia may be a disorder of lipoprotein metabolism manifested as hypertriglyceridemia. Hyperlipidemic is a risk factor for gall stone, pancreatitis

and xanthomas, or coronary artery disease (CAD), myocardial infarction (MI), hypertension. CAD could be considered as the most common cause of death globally, including India, by 2020⁴. Till 1990's there has been 8 % increase in CAD's prevalence rate since 1960's in urban areas this increase has been found to be 2 %⁵. Hyperlipidemic, being one of a major complication of CAD inflammatory disorder resulting from excessive inflammatory response to various forms of injurious stimuli to the artery wall⁶. Medicinal plants play a major role in hypolipidemic activity and inhibition of hepatic cholesterol biosynthesis and reduction of lipid absorption in the intestine⁷.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Plant Materials and Authentication

Roots of *Glycyrrhiza glabra* Linn, (2kg) were collected from local area supplier of Delhi, during the month of March 2017. Sample of plant material was sent to Department of Botany National Institute of Science Communication and Information Resources, (NISCAIR) Delhi-110011, for identification and taxonomic authentication.

2.2.2 Experimental Animals

Wistar rat either sex, weighing 150-250g purchased from All India Institute of Medical Sciences animal house, New Delhi. All test animals are allowed free access to food and water ad libitum, both being withdrawn just prior to experimentation.

2.2 Methods

2.2.1. Preparation of plant extract of *Glycyrrhiza glabra*^{3, 6}

The roots of plant *Glycyrrhiza glabra* were collected from Delhi and were authenticated. The roots of the plant were collected and subjected to shade drying. The size were reduced and made to coarse powder and then further passed through the appropriate sieve no. to obtain uniform particle size. The powdered root part extracted with ethanol and water by soxhlet apparatus. The root extracts were filtered and collected, and concentrated by using Rotatory Flash Evaporator. The extracts were used for the further experimental models.

Figure 2: Root of *Glycyrrhiza glabra*

2.2.2. Determination of Body Weight

The weight of individual rat of each group was measured 'only' before and after administration of extract.

2.2.3 Animal grouping, feeding and extract administration

2.2.3.1. Experimental design:

Animals were fasted for 24 hours before the experiment with free access to water.

2.2.3.2. Preparation of drugs

The ethanolic root extract of *Glycyrrhiza glabra* will be suspended in 2% Tween80 and used for oral administration. Each time fresh preparations of the extracts were prepared.

2.2.3.3. Assessment of Antihyperlipidemic Activity^{3, 6}

Model- High Fat Diet (FD) induced hyperlipidemic model used for study.

Preparation of Feed

Normal animal food pellets was crushed in mortar and pestle to crush into small pieces and then grinded into fine powder in mixer grinder. The other ingredients i.e. cholesterol 2% , Cholic acid 1% , sucrose 40% , and butter 10% were added in the mixer grinder in an ascending order of their quantity and mixed well. This dried powder was then mixed with same quantity of water every time to make small balls of feed and later this was stored in self sealing plastic covers in refrigerator at 2°C to 8°C. The feed for normal group was prepared by grinding only the normal food pellets and then mixing with water without the other excipients. This preparation of feed was done once in three days for all the animals. 30 Wistar rats were randomly divided into 5 groups each group containing six animals. The chronic experimental hyperlipidemia was produced by feeding the above prepared food for 21 days. The rats were then given ethanolic and aqueous root extract of (400 mg/kg, oral) and Simvastatin (10 mg/kg, oral) once daily in the morning. During these days, all the groups also received fat diet in the same dose as given earlier. The hyperlipidemic control i.e., group II animals received the hyperlipidemic diet and the vehicle. The control group animals received the normal laboratory diet and vehicle.

Table 1: Classification of groups according to their doses

Group	N	Treatment	Dose
Group I	6	Normal saline	10ml/kg body wt (Normal Saline)
Group II	6	High Cholesterol diet	
Group III	6	High Cholesterol diet+ Simvastatin	10mg/kg body weight
Group IV	6	<i>Glycyrrhiza glabra</i> ethanolic root extract of low dose + HFD	200mg/kgbody weight
Group V	6	<i>Glycyrrhiza glabra</i> ethanolic root extract of high dose + HFD	400mg/kgbody weight

N= Number of animals in each group

HFD= High fat diet

On day 21, animals were anaesthetized with chloroform and blood was collected by tail vein. The blood samples were centrifuged for 15 min at 2500 rpm to obtain serum. The collected serum was analyzed for serum Total Cholesterol, Triglycerides, High Density Lipoprotein Cholesterol, Low Density Lipoprotein Cholesterol and Very Low Density Lipoprotein Cholesterol.

2.3 Statistical Analysis

The mean $\pm$ S.E.M. values were calculated for each group. The data were analyzed using one way ANOVA followed by Dunnet's test. *p $\leq$ 0.05 was consider to be statistically significant ***P <0.001was very significant, ****P<0.0001 was extremely significant.

3. RESULTS

Antihyperlipidemic activity of ethanolic root extracts of *Glycyrrhiza glabra*

Table 2: Effect of ethanolic extracts of roots of *Glycyrrhiza glabra* on body weight HCD induced hyperlipidemic rats.

Day	Mean Body weight (gm) (% change in body weight)				
	Normal	HCD	STD	Eth. 200 mg/kg	Eth. 400 mg/kg
0 th day	138.66	144.44	146.33	144.44	144.83
5 th day	161.33	164.66	165.33	166.50	167.44
10 th day	169.10	179.66	180.00	180.00	181.00
15 th day	178.33	185.33	187.33	185.33	188.00
20 th day	183.66	190.33	192.33	190.33	199.33

Values are Mean $\pm$ S.E.M. (n=6); Significance vs. Control group: ***P < 0.001, **P < 0.01 and *P < 0.05, using one- way ANOVA followed by Dunnett's t test.

Figure 3: Effect of the ethanolic root extract of *Glycyrrhiza glabra* on serum lipid profile levels (mg/dL) at 0th, 5th, 10th, 15th and 20th Day in HCD induced wistar rats.

Table 3: Various parameters of antihyperlipidemic activity in wistar rats, using ethanolic root extracts of *Glycyrrhiza glabra*

Group No	Treatment	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	VLDL (mg/dl)	LDL (mg/dl)
I	Normal	77.77 $\pm$ 4.05	70.15 $\pm$ 6.16	34.81 $\pm$ 1.58	14.03 $\pm$ 1.23	27.08 $\pm$ 2.91
II	HCD	183.33 $\pm$ 6.67	148.92 $\pm$ 6.53	18.51 $\pm$ 1.36	29.77 $\pm$ 1.30	135.04 $\pm$ 5.47
III	HCD + Simva. (10mg/kg)	103.33 $\pm$ 7.1*	97.22 $\pm$ 5.28*	31.47 $\pm$ 2.54*	19.60 $\pm$ 1.0*	51.80 $\pm$ 3.7*
IV	HCD + Eth.(200mg/kg)	150.0 $\pm$ 10.3*	121.54 $\pm$ 3.2*	25.92 $\pm$ 1.58*	24.41 $\pm$ 0.7*	99.77 $\pm$ 9.85*
V	HCD + Eth.(400mg/kg)	128.33 $\pm$ 6.0*	112.74 $\pm$ 5.5*	28.10 $\pm$ 2.19*	22.51 $\pm$ 1.1*	77.67 $\pm$ 3.97*

All values were expressed as Mean $\pm$ SEM, n.s. (non significant) as compared to vehicle control group.

□ ** p<0.01, when compared with normal control group (i.e., group I), ANOVA followed by Dunnett's t-test.

□ *p<0.05, when compared with normal control group (i.e., group I), ANOVA followed by Dunnett's t-test.

□ p>0.05, ns (not significant) when compared with the group I.

Figure 4: Effect of Ethanolic extract of *Glycyrrhiza glabra* on concentrations of TC, TG, HDL, VLDL, and LDL, of rats fed high fat containing diet. The values are mean $\pm$ S.E.M. for six rats. Groups' I with II and III, IV, V, VI, VII are compared with Group II, ** p<0.01, * p<0.05.

4. DISCUSSION

Plants have formed the basis of sophisticated traditional medicine system and natural products make excellent leads for new drug development. Approximately 80% of the world inhabitants rely on traditional medicine for their primary health care and play an important role in the remaining health care system of the remaining 20% of the population. The World Health Organization (WHO) is encouraging, promoting and facilitating the effective use of herbal medicine in developing countries for health problems. Plants have been used as medicines for thousands of years and are used today in their natural form as well as in other forms. The importance of plants has been forgotten by modern man as a result of his dependence on the quick result of allopathic medicines. Plant drugs are being rediscovered because of growing awareness of unwanted side effects of modern drugs. The plant has been chosen for this study as it was easily available and the antihyperlipidemic activity has not been reported earlier. *Glycyrrhiza glabra*, belongs to family *Fabaceae*. Roots of *Glycyrrhiza glabra* have been suggested in the Indian system of medicine for a number of diseases.

Glycyrrhiza glabra is used as mild laxative, anti-arthritis, anti-inflammatory, anti-biotic, anti-viral, anti-ulcer, memory stimulant (being MAO inhibitor), anti-tussive, aphrodisiac, anti-mycotic, estrogenic, antioxidant, anti-caries agent, anti-neoplastic, anti-cholinergic, anti-diuretic, hypolipidemic agent. In the present study wistar rats were chosen for the antihyperlipidemic activity.

Hyperlipidemia is a well-known risk factor for cardiovascular disease. Atherosclerotic coronary artery disease (CAD) is one of the major causes of premature death globally and it is expected to be the most important cause of mortality. It has been established that nutrition plays an important role in the etiology of hyperlipidemias and atherosclerosis. Several animal and human studies have confirmed the hypercholesterolemic properties of saturated fatty acids and cholesterol which include increasing total cholesterol and altering lipoprotein pattern and whose mechanisms remain under study. Cholesterol feeding has been often used to elevate serum or tissue cholesterol levels to assess hypercholesterolemia-related metabolic disturbances in different animal models. Rats fed with a diet supplemented with 100g cholesterol and 50gm Cholic acid in coconut oil with egg for 21 days served as the experimental model. The mechanism of action of cholic acid is twofold: an increase in cholesterol absorption and a concomitant suppression of cholesterol 7 α -hydroxylase activity that results in decreased cholesterol excretion. Cholic acid improves cholesterol absorption by its emulsifying property. 24 Wistar rats were randomly divided into 4 groups of six each. The chronic experimental hyperlipidemia was produced by feeding the above prepared food for 21 days. The rats were then given test plant extracts i.e., *Glycyrrhiza glabra* ethanolic root extracts (400 mg/kg, oral) and Simvastatin (10 mg/kg, oral) once daily in the morning.

The results have revealed that keeping the animal on HFD significantly increased the TC, TG, LDL-C level in serum ($P < 0.05$) as compared to rats on normal diet. When HFD was coadministered with *Glycyrrhiza glabra* extracts, the elevated levels of TC, TG and LDL-C condition have been shown considerably. It was noted that TC, TG and LDL-C lowering activity of ethanolic root extract (400mg/kg) of *Glycyrrhiza glabra* was more significant. There was significant elevation in plasma HDL-C in *Glycyrrhiza glabra* treated rats as compared to HFD rats, the efficacy of

Glycyrrhiza glabra extract in preventing the elevation seen in various components of lipid profile under experimentally induced hyperlipidemia. Flavonoids are reported to increase HDL-C concentration and decrease in LDL and VLDL levels in hypercholesteremic rats. The presence of flavonoids and polyphenols found in *Glycyrrhiza glabra* extracts could therefore be considered responsible for increasing HDL and decreasing LDL and VLDL in *Glycyrrhiza glabra* treated rats. Atorvastatin which was used as positive control in this study is a HMG-CoA reductase inhibitor. HMG-CoA reduces serum triglyceride levels through the modulation of apolipoprotein C-III and lipoprotein lipase. Studies have shown that increased formation of free radical reactive oxygen species contribute to the progression of cardiovascular disease, reactive oxygen species induced cardiac dysfunction and cardiac apoptosis.

The roots extract of *Glycyrrhiza glabra* were subjected to Soxhlet extraction with ethanol extract 14.7 % w/w and water extract 11.8 % w/w yielded respectively. Phytochemical analysis of the plant extract showed different phytoconstituents such as glycyrrhizin, glycyrrhizinic acid, glabrin A&B, glycyrrhetol, glabrolide, isoglabrolide, isoflavones, coumarins, triterpene sterols.

High fat diet chosen in the present study contains the common ingredients in our daily food. The high fat diet used in this study contains saturated fatty acids which increases the activity of HMG CoA reductase, the rate determining enzyme in cholesterol biosynthesis. This may be due to higher availability of acetyl CoA, which stimulated the cholesterologenesis rate. Simvastatin (10mg/kg oral) and the *Glycyrrhiza glabra* ethanolic extracts (400mg/kg) showed better Antihyperlipidemic activity.

5. CONCLUSION

The yield of ethanolic roots extract of *Glycyrrhiza glabra* was found to be 14.7 % w/w and 11.8 % w/w respectively. Phytochemical analysis of the plant extracts showed the presence of different phytoconstituents viz. triterpene, saponin, flavonoids, polysaccharides, pectins, simple sugars, amino acids, mineral salts, asparagines, bitters, essential oil, fat, female hormone estrogen, gums, mucilage (Rhizome), protein, resins, starches (30%), sterols, volatile oils, tannins, glycosides, and various other substances^{8,9}. Glycyrrhizin, a triterpenoid compound, accounts for the sweet taste of licorice root. Several phytoconstituents like glycyrrhizin, glycyrrhizinic acid, glabrin A&B, glycyrrhetol, glabrolide, isoglabrolide, isoflavones, coumarins, triterpene sterols and flavonoids are known to have antihyperlipidemic properties. This study has revealed that the ethanolic root extract of *Glycyrrhiza glabra* has significant antihyperlipidemic activity. The result of the present study suggests that the roots of *Glycyrrhiza glabra* can be used as antihyperlipidemic agent for the prevention and treatment of CVD diseases. It was observed that keeping the animals on HFD significantly increased the TC, TG, LDL-C level in serum ($P < 0.05$) as compared to animals on normal diet. When HFD was coadministered with *Glycyrrhiza glabra* extracts, the elevated levels of TC, TG and LDL-C condition have shown considerable decline. It was noted that TC, TG and LDL-C lowering activity of ethanolic root extracts of *Glycyrrhiza glabra* was more significant. There was significant elevation in plasma HDL-C in *Glycyrrhiza glabra* treated rats as compared to HFD rats. In the present study, the pharmacological screening has led to the conclusion that, ethanolic root extract of *Glycyrrhiza glabra* has significant antihyperlipidemic activity. Hence it can be

exploited as an antihyperlipidemic therapeutic agent or adjuvant in existing therapy for the treatment of hyperlipidemia.

This study reveals that tested root extract of *Glycyrrhiza glabra* has significant free radical scavenging activity. The result of the present study suggests that the roots of *Glycyrrhiza glabra* can be used as a source for the management of different diseases.

The broad spectrum antihyperlipidemic activities of the plant extract may be due to its active constituents like

glycyrrhizin, glycyrrhizinic acid, glabrin A&B, glycyrrhetol, glabrolide, isoglabrolide, isoflavones, coumarins, triterpene sterols and flavonoids. This study has demonstrated the antihyperlipidemic activities of root extract from *Glycyrrhiza glabra* plant. The antihyperlipidemic activity of the plant extract of *Glycyrrhiza glabra* may play a significant role in the prevention and treatment of atherosclerosis and other cardiovascular diseases. Thus, *Glycyrrhiza glabra* plant can be considered as an easily accessible source of natural antihyperlipidemic.

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