ISSN- 2230-7346
Journal of Global Trends in Pharmaceutical Sciences

EFFICACY OF GLYCYRRHIZA GLABRA MOTHER TINCTURE IN HYPERLIPIDEMIA PATIENTS: A DOUBLE BLIND RANDOMIZED CONTROLLED TRIAL

Dr. Parveen Kumar Sharma[1], Dr. Siva Rami Reddy E[2*]

[1]Director Academic & Research, Tantia University, Sri Ganganagar, Rajasthan.
[2]Faculty of Homoeopathy, Tantia University, Sri Ganganagar, Rajasthan.

*Corresponding author E-mail: dr.sivaramireddy@gmail.com

ARTICLE INFO

Key words:

RCT, hyperlipidemia, glycyrrhiza glabra

Access this article online
Website: https://www.jgtps.com/
Quick Response Code:

ABSTRACT

Aim: Glycyrrhiza glabra has anti toxidant, anti inflammatory and anti diabetic activities. In the present randomized controlled study is that efficacy of glycyrrhiza glabra mother tincture in hyperlipidemia. Hyperlipidemia patients meeting the inclusion criteria were randomly assigned in to the treatment and placebo. **Methods**: 30 all randomized patients in treatment group took glycyrrhiza glabra mother tincture; whereas the patients in placebo group took placebo mother tincture for 12 weeks. The lipid profile, blood pressure, body mass index and liver enzymes of the patients were evaluated at baseline, and after 6 and 12 weeks of the clinical trial. **Results:** The final results showed that glycyrrhiza glabra mother tincture significantly p value 0.05 improved LDL, HDL, cholesterol, compared to the placebo group. It had no effect on systolic or diastolic blood pressure was not significant (P > 0.05) compared to placebo. The glycyrrhiza glabra showed an inhibitory effect on of hepatic enzymes and possible liver toxicity. No serious side effect was reported for glycyrrhiza glabra mother tincture administration. Therefore, glycyrrhiza glabra mother tincture could be considered as a supplement for treatment of dyslipidemia.

INTRODUCTION

Glycyrrhiza glabra is one of the most popular medicinal plants belonging to the Fabaceae family (also known as Leguminosae), and its members are now commonly used as feed and food. The genusg Glycyrrhiza is derived from the Greek words glykos (sweet) and rhiza (root)[1]. It is also called licorice, liquorice, glycyrrhiza, sweet wood, and Liquiritiae radix (in English); süssholz, lakritzenwurzel (in German); reglisse and bios doux (in French); shirin bayan, mak (in Persian); liquirizia and regaliz (in Italian and Spanish, respectively). This species is a native of Mediterranean areas, but it is now also present in India, Russia, and China[2,3]. The extracts are currently used in pharmaceutical and food industries, as well as in the manufacture of functional foods and food supplements[4-7]. Hyperlipidemia is a medical condition characterized by an increase in one or more of the plasma lipids, including triglycerides, cholesterol, cholesterol esters, phospholipids and or plasma lipoproteins including very low density lipoprotein and low-density lipoprotein along with reduced high density lipoprotein levels[8,9]. Hypercholesterolemia and hypertriglyceridemia

are the main causeof atherosclerosis which is strongly related to ischemic heart disease (IHD). There is a strong relation between IHD and the high mortality rate. Furthermore elevated plasma cholesterol levels cause more than four million deaths in a year[10]. Atherosclerosis is a process of arteries hardening due to deposition of cholesterol in the arterial wall which causes narrowing of the arteries. Atherosclerosis and atherosclerosis associated disorders like coronary, cerebrovascular and peripheral vascular diseases are accelerated by the presence of hyperlipidemia[11].

Classification of Apolipoprotein:

- Chylomicrons – Triglyceride rich bearer of dietary fats.
- Very Low Density Lipoprotein (VLDL) – Triglyceride rich bearer of hepatic blended triglycerides (TG)
- Intermediate and Low Density Lipoprotein (IDL & LDL) – Cholesterol rich leftover particles got from lipolysis of triglycerides in VLDL
- High Density Lipoprotein (HDL) – Cholesterol rich molecule that transports cholesterol to liver for removal or reusing.

These lipoproteins move into the circulation system where they got hydrolyzed by endothelial lipoprotein lipase which hydrolyzes the triglyceride into glycerol and non esterifies unsaturated fats. After which the chylomicron remainders are invested in the liver and bundled with cholesterol, cholesterol esters and ApoB100 to shape VLDL. After the arrival of VLDL into the circulation system it will be changed over into IDL by the activity of lipoprotein lipase and hepatic lipase, where phospholipids and apolipoproteins moved back to HDL. Besides, after the hydrolysis by hepatic lipase, IDL will be changed over to LDL and misfortune more apolipoproteins. Fringe cholesterol is come back to the liver by invert cholesterol transport pathway utilizing HDLs which are initially orchestrated by the liver what's more, discharged into the blood. In the blood, HDL cholesterol is esterifies by LCAT to cholesterol ester what's more, moved to VLDL and chylomicrons to return to the liver through LDL receptor. Cholesterol ester are moved to LDL particles by CETP and afterward exposed to LDL-receptors interceded endocytosis. At long last, cholesterol esters are hydrolyzed to cholesterol and removed from the body as bile corrosive[12].

Material and methods:

Preparation of glycyrrhiza glabra mother tincture

Glycyrrhiza glabra mother tincture was obtained from Ganganagar Pharmacy, Sri Ganganagar, Rajasthan, India. The glycyrrhiza glabra mother tincture was kept at room temperature in darkness until use.

Preparation of mother tincture bottles:

Extraction of glycyrrhiza glabra mother tincture was purchased and stored as mentioned above. Glycyrrhiza glabra mother tincture and placebo were prepared into bottle in Department of homoeopathy pharmacy, Sri Ganganagar Homoeopathic Medical College, Hospital and Research Center, Sri Ganganagar. The glycyrrhiza glabra mother tincture bottle was identified at the department of homoeopathy Pharmacy, Sri Ganganagar Homoeopathic Medical College, Hospital and Research Center, Sri Ganganagar. The placebo mother tincture bottle was filled with neutral and inert additive substance whereas; each glycyrrhiza glabra mother tincture bottle was filled with 200µl/kg body weight mother tincture (recommended dose given for rats in 20 µl/100 g body weight) & per orally in de ionized water (180 µl) as vehicle for administration[13]. There were no in clinical studies on the anti hyperlipidemic effect of glycyrrhiza glabra mother tincture. In the abovementioned works, administration of glycyrrhiza glabra mother tincture was safe and effective.

Study design:

The study was fully conducted in accordance with the Ethical Committee of the Sri Ganganagar Homoeopathic Medical College, Hospital and Research Center, Sri Ganganagar and written informed consent was obtained from all patients before their inclusion in the randomized controlled study. The study was a randomized, double-blind, placebo controlled, three months, clinical trial which was carried

out on 30 hyperlipidemic outpatients of Sri Ganganagar Homoeopathic Medical College, Hospital and Research Center, Sri Ganganagar. The authors applied inclusion and exclusion criteria for patients to improve the quality of the results in this study.

Inclusion criteria: Male and female outpatients aged 25 to 65 years; incidence of hyperlipidemia with at least one of the following factors: cholesterol level >200 mg/dl or TG level higher than 150 mg/dl or LDL-C level higher than 130mg/dL or HDL-C level <40 mg/dl.

Exclusion criteria: The patients who had a history of chronic or metabolic diseases such as diabetes, Ischemic heart disease, hypertension, tachycardia, peripheral vascular disease, coronary artery disease, thyroid dysfunction, hospitalized, cannot follow therapeutic lifestyle modification and pregnancy. In addition, the exclusion criterion was a recent change in dosage of antilipemic agents such as hydroxymethylglutaryl coenzyme A (HMG-COA) reductive inhibitor, or adding hypoglycemic agents such as first and second generation sulfonylureas or supplements or drugs known to affect the blood lipids, presence of side effects and unwillingness to participate in study.

Other exclusion criteria were: LDL level more than 190 in patients who need medical treatment (for healthy people or with one risk factor); LDL ≥ 160 in patients who need drug treatment (for those with two or more risk factors of the following:

- Smoking.
- Hypertension.
- Low HDL level (less than 40).
- History of coronary artery disease at an early age in the household (less than 55 years in males and in females under age 65 years old), 5. Age above 65 years old).

Sample size

To have a power of 90%, a two sided test was used, with a significance level of 0.05, and a 20% minimum detectable mean difference changes for LDL-C and SD 20.5% between treatment and placebo group. Finally, minimum sample size of 30 patients for each arm was calculated. Because of expected dropout, we considered 15 patients in each group.

The patients were randomly divided into the treatment (15 patients) treatment group and the placebo (15 patients) groups. Finally, 30 patients successfully completed the randomized controlled study.

Interventions

Participants were randomized to 2 intervention groups of 15 patients. The patients in the treatment group were taking glycyrrhiza glabra mother tincture, for 12 weeks; whereas the patients in placebo group were taking placebo (mother tincture) for 12 weeks. Participants did not receive any other hypocholesterolemic drugs during the randomized controlled study. The patient's compliance and medication adherence were confirmed through checking with the patient and his/her caregiver along with a mother tincture count at each visit.

Outcome measures

Lipid profile (Cholesterol, TG, HDL and LDL), blood pressure (SBP and DBP), BMI index and liver enzymes (ALT, AST, ALP) were measured at baseline, 6 weeks and 3 months after intervention in treatment and placebo group.

Masking

The enrolled participants were assigned using a stratified randomization and all of them received glycyrrhiza glabra mother tincture or placebo mother tincture, which were prepared in the same way. For randomization, a randomized code number was obtained from Microsoft Excel for each pillbox (treatment and control groups). All mother tincture bottles had similar colour, shape, size, texture and odour. The mother tincture bottles were stored in a dark container and coded by a pharmacist. The participants and those assessing outcomes were blinded until all participants finished the protocol.

Safety

The patients were requested to inform investigators about any adverse events or complaints for all illnesses, and hospitalizations that occurred during the trial. The symptoms were checked and recorded at the beginning and at each visit by general physician, cardiologist. Also, possible side effects were checked and recorded via telephone call every week and the general physician/homoeopathy

physician was responsible for continuing or discontinuing the drugs.

Statistical analysis

Baseline characteristics were analyzed using independent t-test or $\chi 2$ tests. The significant differences at various time points were assessed by repeated measures of ANOVA. The variables were reported as mean and standard deviation (Mean ± SD). P value less than 0.05 was considered statistically significant.

RESULTS

Among 30 type 2 hyperlipidemia patients with mean ± SD, age group cases were observed 0.33±0.72 in control group and 0.46±0.74 in a test group, P_{value} showed 0.29. Patient years mean ± SD were 0.6 ±0.73 in control group and test group was 0.6 ±0.73, P_{value} showed 0.5 (not significant). The Male 30 hyperlipidemia patients were 0.66 ±0.72 in control group and 0.4 ±0.5 in test group, P_{value} is 0.102 and female patients were 0.4 ±0.63 in control group and 0.53 ±0.6 in test groups, P_{value} was 0.305. Marital status of married patients mean ± SD were 0.46 ±0.51 in control group and 0.4 ±0.63 in test group, P_{value} showed 0.39. In single patients values were 0.33 ±0.48 in control group and 0.71 ±0.67 in test group, P_{value} showed 0.03. Level of education in under graduate mean and SD values were 0.46 ±0.74 under control group, 0.26 ±0.593 under test group, P_{value} showed 0.212, post graduate mean and SD values were 0.13 ±0.35 under control group, 0.26 ±0.45 under test group, P_{value} showed 0.216 and doctor of philosophy mean and SD values were 0.26 ±0.59 under control group, 0.6 ±0.91 under test group, P_{value} showed 0.156. In former and current mean and SD were 0.53 ±0.63, 0.33 ± 0.62 and 0.53 ±0.74 and 0.6 ± 0.82, P_{values} were 0.212 in control group and 0.617 in test group (Table 1). In 30 hyperlipidemia patient mean ±SD of cholesterol is 209.9 ±0.2 in control group, 210.8 ±0.3 in test group, P_{value} is 0.167 at baseline. After 6 weeks of the cholesterol mean and standard deviation values are 209.4 ±0.5 in control group, 179.8 ±1.1 in test group, p_{value} is 0.001. After 12 weeks mean ±SD values 209.5 ±0.9 in control group, 140.0 ±0.7 in test group, P_{value} is 0.0001. P value is very signification in cholesterol variable. Triglycerides base line mean ±SD values are 159.6 ±0.82 in control group, 159.8 ±0.4 in test group, P_{value} is 0.22. After 6 weeks mean ± SD values were 158.9 ±1.53 in control group, 130.8 ±4.8 in test group, P_{value} is 0.001. After 12 weeks mean ± SD values were 158.2 ±1.5 in control group, 97.2 ±1.0 in test group, P_{value} is 0.001. In low density lipoprotein base line values were 149.6±0.82 in control group, 140.93±0.4 in test group, P_{value} is 0.108. After 6 weeks mean ± SD values were 148.6 ± 1.0 in control group, 130.4 ± 1.9 in test group, P_{values} 0.001. After 12 weeks mean ± SD values were 148.2 ± 1.0 in control group, 100.2 ± 0.7 in test group, P_{value} is 0.0001. HDL base line mean ± SD values were 32.8 ±1.7 in control group, 33.06 ± 2.2 in test group, P_{value} 0.34. After 6 weeks mean ± SD values were 32.6±1.6 in control group, 39.8±2.3 in test group, P_{value} is 0.001. After 12 weeks mean ± SD value is 32.4 ± 1.8 in control group, 50.86 ± 2.2 in test group, P_{value} is 0.0001 (Table 2). Results of systolic blood pressure (mm of Hg) mean ± SD values were 139.3 ± 0.61 in control group, 138.9 ± 2.0 in control group, P value is 0.21. After 6 weeks mean ± SD values were 139.1 ±0.5 in control group, 129.4 ± 1.0 in test group, P value is 0.001. After 12 weeks mean ± SD values were 138.2 ± 0.5 in control group, 120.0 ± 0.2 in test group, P_{values} is 0.0001. In Diastolic blood pressure mean ± SD values were 89.6 ±0.8 in control group, 89.7 ±0.7 in test group, P_{value} is 0.29. After 6 weeks mean ± SD values were 86.5±0.2 in control group, 83.0 ±0.5 in test group, P_{values} 0.001. After 12 weeks mean ± SD values were 85.0 ±0.2 in control group, 80.1 ±0.2 in test group, P_{values} is 0.0001 (Table 3).

DISCUSSION

According to our data and previous research does not have a serious side effect in therapeutic doses. Also, in this study we observed that the serum level of liver enzymes like ALT, AST and ALKP were P value significant in test group. Some studies showed that green leaf lettuce contains water soluble, antioxidant compounds such as phenolic acids, flavonoids, anthocyanins, lactucin, vitamins A and C. Santhosh kumar et al. investigated antidyslipidaemic activity of glycyrrhiza glabra in high fructose diet induced dsyslipidaemic syrian golden hamsters[14].

Table 1: Demographic data of the patients in both study groups (M±SD)

Variable	Control group	Test group	P_{value}
Age Years	0.33 ±0.72 0.6 ±0.73	0.46 ±0.74 0.6 ±0.73	0.29 0.5
Gender Female Male	 0.4±0.63 0.66±0.72	 0.53 ±0.6 0.4 ±0.5	 0.305 0.108
Marital status (n) Married Single	 0.46 ±0.51 0.33 ±0.48	 0.4 ±0.63 0.71 ±0.67	 0.39 0.03
Level of education (n) Under graduate Post graduate Doctor of Philosophy	 0.46±0.74 0.13±0.35 0.26 ±0.59	 0.26±0.593 0.26±0.45 0.6±0.91	 0.212 0.216 0.156
Smoking Former current	 0.53 ±0.63 0.53±0.74	 0.33 ±0.61 0.6 ±0.82	 0.212 0.617

Table 2: The measurements of lipid profile between two groups (M±SD)

Variable	Control group	Test group	P Value
Cholesterol			
At base line	209.9±0.2	210 ±0.3	0.167
After 6 weeks	209.4 ±0.5	179.8 ±1.1	0.001
After 12 weeks	209.5 ±0.9	140.0 ±0.7	0.0001
TG			
At base line	159.6 ±0.82	159.8±0.4	0.22
After 6 weeks	158.9±1.53	130.8±4.8	0.001
After 12 weeks	158.2±1.5.1	97.2 ±1.0	0.001
LDL			
At base line	149.6±0.82	140.93±37.4	0.108
After 6 weeks	148.6 ± 1.0	130.4±1.9	0.001
After 12 weeks	148.2 ± 1.0	100.2±0.7	0.0001
HDL			
At base line	32.8 ±1.7	33 ±2.2	0.34
After 6 weeks	32.6 ± 1.6	39.7 ±2.3	0.001
After 12 weeks	32.4 ± 1..8	50.86 ± 2.2	0.0001

Table 3: The results of blood pressure in both groups (M±SD)

Variable	Control group	Test group	P Value
SBP (mm of Hg)			
At base line	139.3 ±0.61	138.9±2.0	0.21
After 6 weeks	139.1 ±0.5	129.4 ± 1.0	0.001
After 12 weeks	138.2 ±0.5	120.0 ± 0.2	0.0001
DBP (mm of Hg)			
At base line	89.6±0.8	89.7±0.7	0.29
After 6 weeks	86.9±0.2	83.8±0.5	0.001
After 12 weeks	86.0±0.2	80.0±0.2	0.0001

Vaya elinky PA, Aviram M al., some sesquiterpene lactones which can be detected in glycyrrhiza are licochalcone, glabridin, isoliquiritigenin, licocoumarin and class chalcone flavonoid[15]. The above mentioned components may play a role in antihyperlipidemic activity of glycyrrhiza glabra mother tincture. Also, we hope that this study will help pave the way for other researchers to join in the attempt to bridge the gap between alternative medicine and evidence based medicine.

CONCLUSION

In this randomized controlled study, as evident from mentioned results, the hypolipidemic effect of glycyrrhiza glabra mother tincture could be related glycyrrhiza glabra extract (> 10% flavanoids content/ licochalcone, glabridin, isoliquiritigenin, licocoumarin and class chalcone flavonoid) as the main antioxidant constituents in glycyrrhiza glabra mother tincture. However, further researches are required to clarify the mechanism of this effect.

REFERENCES:

1. Ahn, J, Lee, H, Jang, J, Kim, S, & Ha T (2013). Anti obesity effects of glabridin rich supercritical carbon dioxide extract of licorice in high fat fed obese mice. *Food and Chemical Toxicology*, 51, 439–445.
2. Ahn, S.-J, Song, Y.-D, Mah, S.-J, Cho, E.-J, & Kook JK. (2014). Determination of optimal concentration of deglycyrrhizinated licorice root extract for preventing dental caries using a bacterial model system. *Journal of Dental Sciences*, 9(3), 214–220.
3. Albermann, M. E, Musshoff, F, Hagemeier, L. , & Madea B (2010). Determination of glycyrrhetic acid after consumption of liquorice and application to a fatality. *Forensic Science International*, 197(1), 35–39.
4. Asha, M. K, Debraj, D, Prashanth, D, Edwin, J R, Srikanth, H. S, Muruganantham, N, Agarwal, A. (2013). In vitro anti-Helicobacter pylori activity of a flavonoid rich extract of Glycyrrhiza glabra and its probable mechanisms of action. *Journal of Ethnopharmacology*, 145(2), 581–586.
5. Asl, M. N & Hosseinzadeh, H. (2008). Review of pharmacological effects of *Glycyrrhiza* sp. and its bioactive compounds. *Phytotherapy Research*, 22(6), 709–724.
6. Biondi, D. M, Rocco, C, & Ruberto, G. (2003). New dihydrostilbene derivatives from the leaves of *Glycyrrhiza glabra* and evaluation of their antioxidant activity. *Journal of Natural Products*, 66(4), 477–480.
7. Carmeli, E, & Fogelman, Y. (2009). Antioxidant effect of polyphenolic glabridin on LDL oxidation. *Toxicol Ind Health*, 25(4–5), 321–324.
8. Mishra, P. R., Panda, P. K., Apanna, K.C., Panigrahi, S (2011). Evaluation of acute hypolipidemic activity of different plant extracts in Triton WR-1339 induced hyperlipidemia in albino rats. *Pharmacologyonline.*; 3: 925-934.
9. Jeyabalan, S., Palayan, M.(2009).Antihyperlipidemic activity of Sapindusemarginatus in Triton WR-1339 induced albino rats.*Res. J. Pharm. Tech.*; 2(2):319-323.
10. Brouwers, M. C., Van Greevenbroek, M. M.,Stehouwer, C. D.,de Graaf, J.,Stalenhoef, A. F. (2012). Thegenetics of familial combined hyperlipidaemia. *Nat. Rev. Endocrinol*; 8(6): 352-62.
11. Wells, G. B.,Dipiro J, Schwinghammer T, Hamilton C. (2007). Phamacotherapy Handbook, 7thedn, USA, The Mcgraw Hill Companies; pp. 98-108.
12. Magnin D R, Biller, S. A.,Wetterau, J., Robl, J A.,Dickson, J. K.Jr, Taunk, P., Harrity,T. W.,Lawrence, R. M.,Sun, C.Q.,Wang, T.,Logan, J.,Fryszman, O.,Connolly, F.,Jolibois, K., Kunselman, L. (2013). Microsomal triglyceride transfer protein inhibitors: discovery and synthesis of alkyl phosphonates as potent MTP inhibitors and cholesterol lowering agents.*Bioorg. Med. Chem. Lett.*; 13(7): 1337-1340.
13. Surendr singh, Rohith K, Ritu K, P karla et. (2014). Evaluation of safety

profile of homoeopathic mother tinctures. *Ind J Reser Homooepathy*.; 8(2):81-86.

14. Santosh K M, Kanwal, Arvind (2009). antidyslipidaemic activity of glycyrrhiza glabra in high fructose diet induced dsyslipidaemic syrian golden hamsters. *Indian Journal of Clinical Biochemistry*, 24 (4) 404-409.
15. Vaya J, Belinky PA, Aviram M. (1997). Antioxidant Constituents from Licorice Roots: Isolation, Structure Elucidation and Antioxidative Capacity toward LDL Oxidation. *Free Radical Biol Med*; 23(2): 302-13.