



International Journal of
Experimental Pharmacology

www.ijepjournal.com

ANTI-HYPERLIPIDAEMIC EFFECT OF *TINOSPORA SINENSIS* FLOWERS ON HYDROGENATED GROUNDNUT OIL INDUCED HYPERCHOLESTEROLEMIA IN ALBINO RATS

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ABSTRACT

The present study was attempted to evaluate the anti-hyperlipidemic activity of ethanol extract of *Tinospora sinensis* flowers in Hydrogenated Groundnut Oil induced hypercholesterolemia in albino rats. Hydrogenated groundnut oil induced a significant increase in total cholesterol, phospholipids, Low density lipoproteins, Triglycerides and decrease in high density lipoproteins. Reduction in the activity of lipoprotein lipase was observed. Hydrogenated groundnut oil also decreased the levels of SOD, CAT and reduced glutathione with associated increase in lipid peroxidation. Treatment with *Tinospora sinensis* flowers (200 mg./kg b.wt./day) altered the improper metabolic profile and it was leads to hypolipidemia.

Keywords: Lipid profile, antioxidant, *Tinospora sinensis* flowers, Hydrogenated Groundnut Oil.

INTRODUCTION

Cardiovascular diseases are the most common cause of death worldwide. Abnormalities in plasma lipoprotein and derangement in lipid metabolism rank as the most firmly established and best under-stood risk factor for atherosclerosis and cardiovascular complication. Approximately 10% of the global population is affected by dyslipidemia. The search for new drugs able to reduce and/or to regulate serum cholesterol and triacylglycerol levels has gained importance over the years, resulting in numerous reports on significant activities of natural agents [1, 2].

Tinospora sinensis (syn: *Tinospora malabarica*) is a plant that grows almost throughout India and other South East Asian countries and belongs to the family Menispermaceae. The stem of this plant has great therapeutic value traditionally in treating debility, dyspepsia, fever, inflammation, syphilis, ulcer, bronchitis,

jaundice, urinary disease, skin disease [11] and liver disease and known for its adaptogenic and immunomodulatory properties. The ethanol and alcoholic extracts of this species are reported to have many biological potential, such as anti-inflammatory, anti-diabetic, hepatoprotective, and immunomodulatory, and adaptogenic. Previous phytochemical investigations have discovered that this species contains steroids, flavonoides and alkaloids. In India, cassava is used for the treatment of ringworm, tumor, conjunctivitis, sores and abscesses [3].

MATERIALS AND METHODS

Preliminary Phytochemical Screening

In order to detect the various constituents present in the ethanol extract of *Tinospora sinensis* flowers it was subjected to the tests as per.

Animals

Male wistar rats weighing about 150-200 g were procured and maintained to laboratory condition. The animals were fed with standard pellet diet (Kamathenu Agencies, Bangalore, India) and clean water ad libitum and

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routinely housed in controlled conditions with temperature of 25-26 ° C, relative humidity of 60-80% and 12-h light/dark cycle and animals were cared for in accordance with the principles and guidelines of Indian National Law on Animal care and use. The animals were acclimatized for 2 weeks before experimentation.

Experimental Design

Four groups of rats, six in each received the following treatment schedule.

Group I : Normal diet and water

Group II : Cholesterol 1% + Hydrogenated Groundnut oil (orally)

Group III : Cholesterol 1% + Hydrogenated Groundnut oil (HGO) and ethanol extract of *Tinospora sinensis* flowers (200mg/kg Bw/day)

Group IV : Cholesterol 1% + Hydrogenated Groundnut oil and standard drug (Atorvastatin 3mg/kg Bw/day)

Collection of Blood

The experiment was continued for 30 days and the animals were sacrificed on the 31st day by cervical decapitation. The blood was collected and liver were removed quickly. The latter were washed with ice-cold saline and stored at 20°C for further analysis. Liver was homogenized (10% W/V) in cold 100 mm phosphate buffer, pH 7.2 and used for the assay of lipoprotein lipase (LPL) activity.

Liver Lipid Extraction

Lipids were extracted from tissues by the method of using chloroform – methanol mixture (2:1 v/v). A known weight of tissue was homogenized in 7.0 ml of chloroform – methanol using potter Elvehjam homogenizer. The contents were filtered into a previously weighed side arm flask; residue on the filter paper was scrapped off and homogenized with 14 ml of chloroform – methanol mixture. This was again filtered into the side arm flask and the residue was successively homogenized in chloroform – methanol (2:1 v/v) and each time this extract was filtered [4].

Statistical Analysis

All the grouped data were evaluated statistically and significance of changes was determined using one-way analysis of variance followed by Dunnett's test. Results are presented as mean ± SEM among values of 6 rats from each group statistical significance was set at P<0.05.

Biochemical Analysis

The serum and liver were assayed for total cholesterol, triglycerides, phospholipids, high density lipoprotein (HDL), low density lipoprotein (LDL), lipoprotein lipase (LPL). The serum cholesterol levels were determined by Zak's method. The triglyceride, phospholipids, HDL, LDL and LPL, LPO, SOD, CAT, GSH was calculated by using standard method.

Table 1. Effect of *Tinospora sinensis* flowers on liver cholesterol, Triglycerides, phospholipids, LDL, HDL, LPL

Groups	TC mg/g	TG mg/g	PL mg/g	LDL mg/dL	HDL mg/dL	LPL (μ moles of free fatty acid released/h/mg protein)
	liver	liver	liver	Liver	Liver	liver
Group I	5.093±0.78	5.12±0.84	21.21±0.52	82.93±3.22	34.56±2.28	83.01±0.39
Group II	9.02±0.40	8.18±0.72	37.19±1.32	136.13±0.84	25.33±1.26	52.51±1.35
Group III	7.43±1.40	6.07±0.40	30.66±1.62	94.33±7.58	33.63±2.87	71.52±2.09
Group IV	7.1±0.28	5.1±0.39	26.1±0.77	87±1.26	34.3±1.82	73.18±2.40

Table 2. Effect of *Tinospora sinensis* flowers SOD, CAT, GSH, LPO

Groups	SOD (unit / min/mg protein)	CAT (μ m of H ₂ O ₂ /min/mg protein)	GSH (μm of GSH/mg protein)	LPO (μ m of MDA/min/mg protein)
	Liver	Liver	Liver	Liver
Group I	3.36±0.51 ^a	33.45±2.6 ^a	239.06±2.99 ^a	3.83±0.14 ^a
Group II	2.26±0.20 ^b	23.34±0.51 ^b	214.45±2.01 ^b	7.13±0.5 ^b
Group III	2.8±0.2 ^a	27.4±1.00 ^a	238.3±1.45 ^a	4.82±0.52 ^a
Group IV	2.7±0.1 ^a	30.5±0.21 ^a	244.9±2.24 ^a	4.28±0.33 ^a

Results were expressed as mean ± S.E. The data were statistically analysed by one way analysis of variance (ANOVA) and p values <0.05 were considered significant.

RESULTS AND DISCUSSION

Phytochemical screening of *Tinospora sinensis* flowers revealed the bioactive constituents such as alkaloids, flavonoids, tannins, steroids, saponins,

glycosides, carbohydrate, protein and amino acid. Phytochemical screening of *Tinospora sinensis* flowers plant compounds flavonoids steroids, are reported to

modulate lipid levels [5]. The presence of flavonoids and steroids in *Tinospora sinensis* flowers might have contributed in lipid lowering effect to *Tinospora sinensis* flowers in similar manner. Tannins are reported to increase in activity of the endothelium bound lipoprotein lipase activity, which hydrolyzes triglycerides as reported. The presence of tannins in *Tinospora sinensis* flowers might be involved in triglyceride lowering activity but this need to be invested by further studies. Feeding HGO caused hyperlipidemia and altered the lipid component of plasma lipoproteins. Simultaneous *Tinospora sinensis* flowers supplementation significantly lowered the serum levels of total cholesterol, LDL, TG, and PL and elevated HDL levels. Moreover *Tinospora sinensis* flowers supplementation reduced the accumulation of total cholesterol, TG and PL in the liver and other peripheral tissues emphasizing its potent hypolipidemic properties. Simultaneous administration of animals with *Tinospora sinensis* flowers and HGO caused increase in the level of LPL. *Tinospora sinensis* flowers also resulted in significant reduction the oxidative stress by lowering the serum and LPO. Elevated SOD, CAT and GSH activities, serum, levels on *Tinospora sinensis* flowers supplementation may be due to elimination of the reactive toxic intermediates formed as a consequence of HGO induced oxidative stress. Similarly *Tinospora sinensis* flowers supplementation significantly reduced the levels of LPO and elevated the activities of the antioxidant enzymes SOD, CAT, and GSH in liver.

The present study shows that *Tinospora sinensis* flowers supplementation to HGO –fed rats has a profound effect on the levels of serum and liver, lipids and lipoproteins. Biological membranes are composed of PL and proteins. The lipid bilayer is highly impermeable to most polar molecules and ions but they are quite fluid in nature and interact with membrane proteins. The degree of fluidity of the plasma membrane was generally recognized to be important for appropriate functioning of cells [14]. Abnormalities in membrane fluidity can give rise to pathological conditions. The dietary content of cholesterol, a component of cell membranes, generally parallels that of fat and could also influence the membrane fluidity and thereby, its function. Feeding HGO as in our study may also contribute to changes in membrane cholesterol composition and function. Moreover, intervention studies showed that dietary lipids are significant determinants of plasma cholesterol concentration [6], and evidence from animal and human studies have documented the hypercholesterolemia effect of dietary saturated fatty acids. Thus, feeding a diet rich in highly saturated fatty acids and cholesterol is known to cause hyperlipidemia in most

animal and human studies. The high concentration of plasma cholesterol observed in HGO – fed rats as compared to the control rats in the present study agree with our previous findings [7] also showed that increased intake of dietary cholesterol and hydrogenated groundnut oil significantly elevates serum cholesterol levels.

LPL plays an important role in the metabolism of plasma lipoproteins and thus, the transport of lipids to peripheral tissues. Absence or low LPL activity causes marked lipemia and triglyceridemia. The activities of both Serum LPL are significantly lowered in HGO–fed rats. These results correlate with previous findings, which showed significantly reduced LPL activity on feeding a high-fat, high –cholesterol diet. A number of studies reported that plasma LPL, directly or indirectly, may promote or protect against atherosclerosis, [8] reported that increased LPL activity is antiatherogenic, and showed that a decrease in LPL activity is atherogenic. Significantly lowered LPL activity in HGO–fed rats can cause accumulation of cholesterol and VLDL. *Tinospora sinensis* flowers administration resulted in the optimum activity of plasma LPL. Thus comparatively low levels of VLDL and LDL found in the *Tinospora sinensis* flowers treated animals may be correlated with the optimal activity of plasma LPL observed in these animals.

Lipid peroxidation is complex processes that can occur in biological membrane are made up of molecular oxygen – reactant polyunsaturated fatty acid, leading to the production of lipid hydroperoxides and their metabolites. The lipid peroxidation can occur in various pathological conditions including atherosclerosis, rheumatoid, arthritis, angina, cancer and irritable bowel diseases (IBA). GSH is multifunctional intracellular non-enzymic antioxidants which scavenge hydroxyl radical and singlet oxygen directly and also detoxifies hydrogen peroxide and other lipid peroxide radicals by the catalytic action of offering protection to the leads from the deleterious effects of reactive oxygen species. Our results show that *Tinospora sinensis* flowers has a definite and important role of play in preserving erythrocyte membrane structure and function by suppressing oxidative stress, enhancing antioxidant profile, and thus countering the hypercholesterolemia included alterations in cell functions[9].

CONCLUSION

The results from our study reveals the lipid lowering efficacy of *Tinospora sinensis* flowers which act on a potential modulation of cellular lipid homeostasis and antioxidant status and may well from the basis for a model to develop an effective antihyperlipidemic drug that possess antioxidant capacity.

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