Research Article

Study of Combined Tulsi extract and Cinnamon oil to reduce hypercholesterolemia in dyslipidemic rats

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Abstract

Objectives: The studies were intended to investigate the possible antihyperlipidemic effect of tulsi and cinnamon oil in Triton induced hyperlipidemic rats. **Materials and methods:** Tulsi and cinnamon oil were evaluated for antihyperlipidemic activity by using Triton induced hyperlipidemia model in male Wistar albino rats (200-250g), A comparison was also made between the action of tulsi (0.5 mg/kg b.w) and cinnamon oil extracts(1.8mg/200g b.w) with antihyperlipidemic drug Atorvastatin (0.18mg/200g). Parameters assessed were body weight, total cholesterol, triglyceride, HDL-C, LDL-C. The results of the study were suggested by mean ± SEM. Statistical significance of data was assessed by one way analysis of variance (ANOVA) followed by a comparison between different groups using "Tukey - Kramer" test. **Results:** Oral administration of 0.5 mg/kg of tulsi with dist. water and Cinnamon oil of 1.8 mg/200g suspended in tween80 solution exhibited significant reduction ($P < 0.01$) in serum biochemical parameters, the Serum parameters such as total Cholesterol, triglycerides, low density lipoprotein (LDL), very low density lipoprotein (VLDL) levels were decreased and an increase in high density lipoprotein (HDL) levels in hyperlipidemic rats of triton model was observed as compared to hyperlipidemic positive control. The results are statistically significant. **Conclusion:** The results demonstrated that tulsi and cinnamon oil extracts possessed significant antihyperlipidemic activity.

Keywords: Tulsi oil, Cinnamon oil, Atorvastatin, Triton x-100, Hyperlipidemia

Introduction

Hyperlipidemia is the major risk factor for CAD in patients suffering with cardio vascular disorders. Hyperlipidemia is deeply involved in the etiology of atherosclerosis (Phogat et al., 2010). Hyperlipidemia in many cases in the modern age is caused by over-consumption of alcohol or fatty foods; Attention is also being paid to treatment of patients with hyperlipidemia using strict management and appropriate exercise (Reiner et al., 2006). Currently available antihyperlipidemic drugs have been associated with a number of side effects (Sirisha and Shivani, 2014), naturally available herbal drugs have lesser side effects and are easily available. The literature survey reveals that Ayurvedic drugs are able to reduce the lipid content of blood (Pushpangadhan et al., 1995), The safety of herbal medicines has become an issue for the regulatory authorities, as many serious unwanted effects have been observed with the use of herbal medicines such as hepatotoxicity, renal failure and allergic reactions. Most of the time such unwanted effects are observed due to poor quality of herbal material, incorrect or misidentified herbs, incorrect processing methods, supply of adulterated or contaminated herbs or products. The genus, species and part of the plant are listed somewhere on the product or packaging the crude material. Unfortunately a botanically correct label does not necessarily confirm that the product contains what is listed on the label.

Ocimum sanctum (Tulsi) is known as holy basil and historically used in ayurvedic medicine. That has various pharmacological activities (Prakash and Gupta, 2005). Fixed oil of *Ocimum sanctum* in rats fed with high fat diet indicates that treatment with fixed oil of OS decreased the high serum lipid profile and expressed antiatherogenic and

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cardioprotective actions against hyperlipidemia (Suanarunsawal et al., 2010). Linoleic acid and linolenic acid present in *Ocimum sanctum*as fixed oil were possibly responsible for both lipid lowering and cardiac protective action against hyperlipidemia (Singh et al., 2011).

The Cinnamon is commercially known as Indian cassia and has been used in traditional medicine as an astringent, stimulant, diuretic, carminative and in cardiac disorders (Kar et al., 2003). Cinnamate, a phenolic compound found in inner bark of cinnamon (Rahman et al., 2013), lowers cholesterol level in high fat-fed rats by inhibiting hepatic HMG-CoA reductase activity (Dhulasavant et al., 2010). Hence the present study is designed to evaluate the antihyperlipidemic activity using marketed herbal extracts.

Materials and methods

Tulsi oil was procured from Vedic Tulsi Amrit, New Delhi and it is made from pure extract of 5 different types of tulsi leaves. Tulsi leaves contain Rama tulsi, Shyama tulsi, Rosary tulsi, Vana tulsi, Shukla tulsi (Each 50mg/ml). Cinnamon oil was purchased from local ayurvedic drug supplier.

Triton x-100 purchased from Sigma Aldrich USA, Atorvastatin (Zydus Healthcare) and Diagnostic kits for estimation were purchased from pathozyme diagnostic kits Kolhapur. All other chemicals were of analytical grade.

Animals

Male Wistar albino rats weighing between 200-250 g were procured from Central animal house, M.R.Medical College, Kalaburagi. The animals were acclimatized for one week in the laboratory condition. They were maintained in pathogen free environment in a standard well aerated condition at temperature 25 ± 2°C and relative humidity of 30-70% with a 12:12 light-dark cycle, and fed with standard pellet supplied by Hindustan lever. Co. Mumbai with water *ad libitum* throughout the course of study. The animals were fasted for 18 h prior to the experiment. All the animal experiments were conducted according to the ethical norms approved by CPCSEA, Government of India and ethical clearance was granted by Institutional Animal Ethics Committee (HKESCOP/IAEC/58/ 2013-14). The present study was conducted in Department of Pharmacology H.K.E.S MTRIPS, Kalaburagi after taking permission of I.A.E.C (142/1999 CPCSEA.5th July 1999).

Preparation of dose (Ghosh, 2001)

Tulsi oil: (0.5 mg/200 g b.w p.o)

Tulsi oil was taken and mixed in distilled water. The solution was freshly prepared every day before dosing the animals.

Cinnamon oil: (1.8 mg/200 g b.w p.o)

Cinnamon oil was taken and suspended with 2% Tween 80 solution. The solution was freshly prepared every day before dosing the animals.

Triton x-100: (100mg/kg)

Triton x-100 was freshly prepared with 2% Tween 80 solution

Preparation of standard

Atorvastatin 0.18mg/200g b.w p.o was used as the reference standard drug for evaluating the antihyperlipidemic activity which was made into suspension in distilled water using Tween-80 as a suspending agent.

Experimental Design

Male Wistar albino rats weighing between 200-250 g were divided into 5 groups of 6 animals in each group. Group I served as normal control and this group did not receive triton x-100 except regular standard pellet diet. Group II was hyperlipidemic control (positive control) and this group did not receive any treatment except triton-x-100 for 7 days. Group III received triton x-100 (100 mg/kg) after 72 hours of induction of hyperlipidemia Tulsi oil (0.5 mg/kg/day p.o) extract was administered for seven days. Group IV received triton x-100 (100 mg/kg) after 72 hours of induction of hyperlipidemia Cinnamon oil (1.8mg/day p.o) extract was administered for seven days. Group V was administered with standard antihyperlipidemic drug atorvastatin (0.18mg/200g b.w/day p.o) for seven days after 72hours of triton-x-100.

Collection of blood

The blood was collected by orbital plexus of rat under ether anaesthesia and centrifuged at 2000 rpm for 30 minutes to get serum.

Biochemical analysis

The serum was assayed for total cholesterol, triglycerides, high-density lipoproteins (HDL), low-density lipoproteins (LDL) and very low density lipoproteins (VLDL) using kits. Cholesterol, triglyceride were estimated by CHOD-PAP method and high density lipoprotein by GPO-PAP method and low density lipoproteins were calculated using Friedewald formula and VLDL:TG/5 (Reddy et al., 2014; Lin et al., 1998).

Statistical analysis

Results were expressed as mean ± SEM. Statistical significance of data was assessed by one way analysis of variance (ANOVA) followed by a comparison between different groups using “Tukey - Kramer” test. A value of $P<0.05$ was considered to be statistically significant. The Triton model group was compared with normal control group and all other treatment groups were compared with

Triton model group.

Results

Triton induced hyperlipidemic model

In Triton-x-100 induced hyperlipidemic model results showed serum lipid lowering potentials of tulsi and cinnamon oil extracts selected for the study. The results represent 7 days study (Figure 1) these results are comparable to standard drug atorvastatin. Both extracts showed significant serum lipid lowering effect in hyperlipidemic rats. Cinnamon oil showed significant effect which brought down total cholesterol 298.4 ±1.12 to 242.3 ±1.2, triglycerides161.4 ± 2.1 to136.1±0.8, LDL-C 236.8 ± to 178.4±1.5and increased level of HDL-C 30.2 ± 0.8 to 36.6±0.8 after 7th day. Standard antihyperlipidemic agent atorvastatin (0.18 mg/200g b.w) also able to reduce the elevated serum lipide level towards the normal (Table 1).

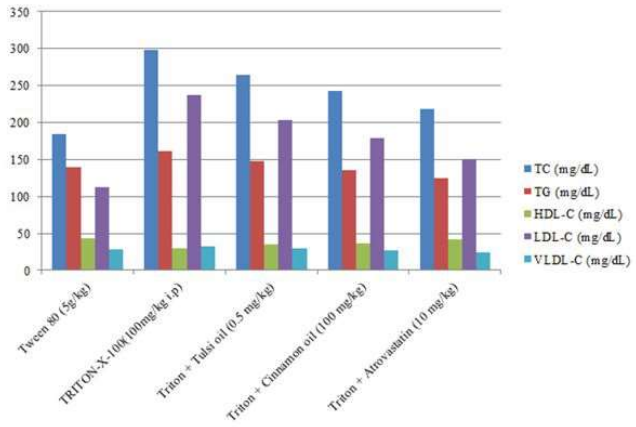

Figure 1. Average percentage change in selected serum biochemical parameters in Triton induced hyperlipidemia in rats

Discussion

There was marked increase in the level of serum total cholesterol, triglycerides, LDL,VLDL and decrease in the level of good cholesterol carrier HDL-C in the animals treated with Triton-x-100. Elevated levels of blood cholesterol especially LDL-C was the major risk factor for the coronary heart disease. Treatment with tulsi oil extract (0.5 mg/200g) and cinnamon oil (1.8 mg/200 g) significantly decreased the levels of serum total cholesterol, triglycerides, LDL-C, VLDL-C as compared to hyperlipidemic control. Several preclinical models have been used to explore antihyperlipidemic effects of novel compounds on hyperlipidemia, however, the male rat or mice model (for avoiding influence of estrogen on lipid metabolites) are prepared. This drug may also enhance the synthesis of LDL apoproteins (Apo B) as well as receptor proteins to accelerate the turnover of cholesterol. The present investigation with Triton-x-100 hyperlipidemic animals shows the stimulation of LDL catabolism.

Triton acts as a surfactant, suppresses the action of lipases and blocks the uptake of lipoproteins by extra hepatic tissues, thus resulting in increase in the levels of circulatory lipids and it is probable that the extracts might have interfered with clustering lipoproteins coated with triton. In this way, lipoprotein may get freely catabolized by these enzymes. The extracts evaluated in this study may inhibit oxidative modification of LDL and thus accelerate the turnover of LDL cholesterol in liver. Tulsi oil well known for its therapeutic potential and as medicinal plant is widely used for management of diseases. In this study it shows the effect as antihyperlipidemic. Eugenol is major constitute in tulsi leaves but not much acknowledged for the mechanism. Cinnamate, a phenolic compound found in inner bark of cinnamon.

Table 1. Basal levels of all selected serum biochemical parameters in rats treated with Triton, Tulsi and Cinnamon oil

Groups	TC (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL-C (mg/dL)
Normal control	184.8 ± 0.9	139.6±1.6	43.8±0.9	113.0±0.8	27.92±0.7
Triton-x-100 (100 mg/kg i.p)	298.4 ±1.12	161.4±2.1	30.2±0.8**	236.8±1.7*	32.25±0.1***
Triton+Tulsi oil (0.5 mg/200 gm b.w p.o)	264.5 ±0.6	147.7±1.1	34.8±0.3*	202.9±1.4**	29.55±0.5
Triton+Cinnamon oil (100 mg/kg p.o)	242.3 ±1.2*	136.1±0.8*	36.6±0.8	178.4±1.5	27.20±1.2**
Triton+Atrovastatin (10 mg/kg p.o)	218.6±1.3**	124.9±2.7**	41.8±1.2	150.4±1.8***	24.9±0.6

Values indicate mean ± SEM for 6 animals in each group.

**$P < 0.01$ significant when compared to group-1 with group-2

***$P < 0.01$ significant when compared to group-2 with group-3, 4, 5.

*$P < 0.01$, **$P < 0.05$ significant when compared group-5 with group 3, 4.

Ext-Extract.; n = 6

Conclusion

The results show that tulsi (P<0.01) and cinnamon oil (P<0.001) possess antihyperlipidemic activity. Among them cinnamon oil possesses more activity; the results are statically significant compared to Atorvastatin (P<0.05).

References

Dhulasavant V, Shinde S, Pawar M, Naikwade N. 2010 Antihyperlipidemic activity of *Cinnamomum tamala* nees on High cholesterol diet induced hyperlipidemia. International Journal of Pharm Tech Research, 2(4): 2518-21.

Ghosh MN. 2001. Fundamentals of Experimental Pharmacology, 4th Edition Hilton and Company, Calcutta. pp. 192.

Kar A, Chaudhary BK, Bandopadhay NG. 2013. Comparative evaluation of hypoglycaemic activity of some Indian medicinal plants in alloxan diabetic rats. Journal of Ethnopharmacology, 84: 105-08.

Krishnakumari S, Priya K. 2006. Hypolipidemiac efficacy of *Achyranthus aspera* on lipid profile in seasome oil fed rats. Ancient Science of Life, 3: 49-56.

Lin CC, Yen MH, Lo TS, Lin JM. 1998. Evaluation of the hepatoprotective and antioxidant activity of *Boehmeria nivea* var. nivea and *B. nivea* var. Tenacissima. Journal of Ethnopharmacology, 60 (1): 9-17.

Phogat P, Deep A, Sharma PC, Mittal SK, Kakkar S, Goyal R, Thakral K. 2010. Introduction to hyperlipidemia and its management. Pharmacologyonline, 2: 251-266.

Prakash P, Gupta N. 2005. Therapeutic uses of *Ocimum sanctum* Linn (*Ocimum sanctum*) with a Note on eugenol and its pharmacological actions: a short review. Indian Journal of Physiology and Pharmacology, 49(2): 125-31.

Pushpangadhan P, Damodar VK, Iyengar PK. 1995. Role of traditional medicine in primary health care. Science for Health. Published by State Committee on science. Government of Kerala.

Rahman S, Begum H, Rahman Z, Ara F, Iqbal J, Yousuf AKM. 2013. Effect of cinnamon (*Cinnamon cassia*) a lipid lowering agent on hypercholesterolemic rats. Journal of Medical College, 3(2): 94-98.

Reddy MV, Rajini A, Nikitha S, Thomas J, Hemamalini K. 2014. Antihyperlipidemic activity of *Trichopus zeylanicus*leaves against high fat diet and triton-x-100 induced hyperlipidemia. World Journal of Pharmacy and Pharmaceutical Sciences, 2(3): 1017-25.

Reiner Z, Tedeshi, Reiner E. 2006. Atherosclerosis a paradox of eastern countries. Atherosclerosis, 7: 461.

Singh V, Birendra VK, Suvaagiya V. 2011. A review of ethnomedical use of tulsi. International Research Journal of Pharmacy, 2(10): 1-3.

Sirisha K, Shivani J. 2014. Antihyperglycemic and antihyperlipidemic activities of new polyherbal formulations. International Journal of advances in Pharmacy, Biology and Chemistry, 3(1): 189-98.

Suanarunsawal T, Boonnak T, Ayutthaya W, Thirawarapn S. 2010. Antihyperlipidemic and Cardioprotective effects of *Ocimum sanctum L.* fixed oil in rats feed with high fat diet. Journal of Basic Clinical Physiology and Pharmacology, 21:387-400.