eISSN: 09748369, www.biolmedonline.com

Effect of Rohitakarista (RHT), an ayurvedic formulation, on the lipid profile of rat plasma after chronic administration

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Abstract
Rohitakarista (RHT), a popular Ayurvedic formulation, is the preparation of *Tecomella undulate* with other medicinal plants. In this study, the lipid profile of rats' plasma was studied after chronic administration of RHT usually used in the disease of spleen, abdomen and localized abdominal swelling or tumor. The animal was albino rats (*Rattus novergicus*: Sprague-Dawley strains) and RHT was administered per oral route at a dose of 100mg/kg body weight, once daily, up to 46 days for all the experiments. Forty rats, equally of both sexes, were randomly grouped into four where one male and one female group were used as control and other groups were used as test. In both of the male and female rats there was a statistically very high significant decrease in the Triglycerides (p=0.001***). On the contrary, a statistically very highly significant increase in the total cholesterol (p=0.001***), VLDL (p=0.001***) and HDL (p=0.001***) was noted. Similar results were observed in case of female rats. Also the increase in LDL (p=0.028*) was statistically significant for both of the sexes of RHT treated rats.

Keywords: Lipid profile; Rohitakarista; RHT; Ayurvedic formulation; Rat plasma.

Introduction

Ayurvedic drugs remains as one of the most ancient and yet living traditions practiced widely in various parts of the world including India, Sri Lanka and other countries and has a sound philosophical and experiential basis (Dahanukar and Thatte, 2000; Chopra and Doiphode, 2003). Ayurvedic practice offers an integrated approach for the prevention and healing through a system of lifestyle interventions and natural therapies.

"Rohitakarista" is an OTC drug in Ayurvedic treatment and used traditionally for the treatment of pliha (disease of spleen), udara (disease of abdomen), gulma (localized abdominal swelling or tumour). Actually, it is the preparation of *Tecomella undulate* stem and bark with some other medicinal plants in small amount (Ullah *et al.,* 2008). *Tecomella undulata* (Smith) Seem commonly known as `Rajasthan Teaka is one of the codominant species in the desert of western Rajasthan (Arya *et al.,* 1992). Isolation of different chemical constituents including two flavones namely cirsimaritin and cirsilineol (Azam and Ghanim 2000) have been reported from *Tecomella undulate.* Among the other plants of this formulation the oil of *Cinnamomum zeylanicum* has shown various therapeutic actions (Lawless, 2002) which includes antimicrobial, antioxidant (Baratta *et al.,* 1998a) and fungicidal activity against anthracnose and crown rot pathogens (Ranasinghe *et al.,* 2002). *Elettaria cardamomum* (Linn.) which is an herbaceous perennial of the ginger family (Zingiberaceae) has been traditionally used to treat skin condition and in digestion (Nair et al, 1998). *Piper longum* Linn. is a member of the family piperaceae used traditionally for the treatment of intestinal ailments (Satayvati *et al.,* 1987). *Terminalia bellerica* belongs to the family Combretaceae and routinely used as traditional medicine to get remedies from several ailments such as fever, cough, diarrhea, skin diseases and oral thrush (Rastogi *et al.,* 1999). *Emblica officinalis* is a principal constituent of many Ayurvedic preparations (Scartezzini and Speroni, 2000). *E. officinalis* which is a gastroprotective (Al-Rehaily *et al.,* 2002), cytoprotective, and immunomodulating (Sai Ram *et al.,* 2002) possess radiation protective activity (Scartezzini and Speroni, 2000), antidiabetic activity (Sabu and Kuttan, 2002), inhibits clastogenicity of benzopyrene and cyclophosphamide (Sharma *et al.,* 2000; Haque *et al.,* 2001), *Terminalia chebula* (Family, Combretaceae), is commonly known as Black Myroblans in English and Harad in Hindi, indigenous in Pakistan and India among many Asian and African countries, is a popular folk medicine used for the treatment of cancers (Hartwell, 1982). In the present study,

the investigation for the effect of this formulation on lipid profile will help us to develop a new traditional medicine for the hyperlipidemic patient especially with diabetic complication.

Materials and Methods

Chemicals and Reagents
All other reagents and chemicals that were used in this work were of analytical grade and were prepared in all glass-distilled water. To evaluate the lipid profile of Rohitakarista (RHT), it was collected from Sree Kundshawri Aushadhalaya Ltd, Chittagong.

Dose and route of administration
The liquid Rohitakarista was administered at a volume such that it would permit optimal dosage accuracy without contributing much to the total increase in the body fluid. For investigating the lipid profile, the drugs were administered per oral route at a dose of 100mg/kg body weight. For all the studies, the drug was administered orally [per oral (p.o.) route]. Ketamine were administered intra-peritoneally (500 mg/kg i.p.).

Experimental animals and Management
Forty eight-week old albino rats (*Rattus novergicus* : Sprague-Dawley strain,) of both sexes, bred and maintained at the Animal House of the Department of Pharmacy, Jahangirnagar University, were used in the toxicological experiment. These animals were apparently healthy and weighed 50-70 g. The animals were housed in a well ventilated hygienic experimental animal house under constant environmental and adequate nutritional conditions throughout the period of the experiment. All of the rats were kept in plastic cages having dimensions of 30 x 20 x 13 cm and soft wood shavings were employed as bedding in the cages. Feeding of animals was done *ad libitum*, along with drinking water and maintained at natural day night cycle. They were fed with “mouse chow” (prepared according to the formula developed at BCSIR, Dhaka). All experiments on rats were carried out in absolute compliance with the ethical guide for care and use of laboratory animals.

Before starting an experiment the animals were carefully marked on different parts of their body, which was later used as identification mark for a particular animal, so that the response of a particular rat prior to and after the administration could be noted separately. A group of equal number of rat as the drug treated group was simultaneously employed in the experiment. They were administered with distilled water as placebo as par the same volume as the drug treated group for the same number of days and this group served as the control. Prior to the experiment, they were randomly divided into 4 groups of 10 animals / sex. Thus ten rats were taken for each group for both control and the experimental group.

Preparation of the Plasma for intended Test
At the due of the 90-day treatment period, the animals were fasted for 18 hours and also twenty-four hours after the last administration, the animals were anaesthetized using i.p. Ketamine (500 mg/kg i.p.). Blood samples were collected from post vena cava and transferred into heparinised tubes immediately. Blood was then centrifuged at 4,000 *g* for 10 min using bench top centrifuge (MSE Minor, England) to remove red blood cells and recover plasma. Plasma samples were separated and were collected using dry Pasteur pipette and stored in the refrigerator for analyses. All analyses were completed within 24 h of sample collection.

Determination of Lipid profile
Triglycerides, Total Cholesterol and HDL concentration were evaluated according to the instruction of manufacturer of assay kits (purchased from Sigma Chemical Co, St Louis, MO, USA). According to Friedewald’s formula (Friedewald et al., 1972), VLDL and LDL were calculated as: VLDL cholesterol = TG/5 and LDL cholesterol = TC – (VLDL+HDL cholesterol).

Statistical Analysis
The group data are expressed as Mean ± SEM (Standard Error of the Mean). Unpaired "t" tests were done for statistical significance tests. SPSS (Statistical Package for Social Science) for WINDOWS (Ver. 11) was applied for the analysis of data. Differences between groups were considered significant at $p < 0.05$, 0.01 and 0.001.

Results

In the male rats there was a statistically significant decrease in the Triglycerides (p=0.001***). On the contrary a statistically very highly significant increase in the total cholesterol (p=0.001***), VLDL (p=0.001***) and HDL (p=0.001***) was noted. Also the increase in LDL (p=0.028*) was statistically significant. Like male, the decrease of Triglycerides was statistically very high significant (p=0.001***) for female rats. Similar

results were found for total cholesterol (p=0.001***), VLDL (p=0.001***) and HDL (p=0.001***) which were statistically very high significant. For female, the increase in LDL (p=0.034*) was statistically significant (Table 1, Figure 1 and Figure 2).

Discussion

During the study, for both of male and female rats, similar trend of changes of parameters of lipid profile was found. For both of the sexes there was a statistically significant decrease in the Triglycerides level. On the contrary, a statistically very highly significant increase in the total cholesterol, VLDL and HDL was noted in case of male and female. Irrespective of sexes, also the increase in LDL was statistically significant. The increase in plasma cholesterol, triglycerides, LDL and VLDL and decrease in HDL is related with blood glucose level (Mitra *et al.,* 1995) where hormone-sensitive lipase (HSL) enhance release of free fatty acids from adipose tissue (Al-Shamaony *et al.,* 1994). The excess fatty acids in the plasma converted to phospholipids and cholesterol in the liver which later release into blood in the form of lipoproteins (Bopanna *et al.,* 1997). There are some other factors also related to the increase of lipid components in plasma. There is no clear correlation among the changes of lipid components as TG is decreased but other component like TC, LDL and VLDL is increased which is unusual. RHT reduced TG, like other plants constituents (Lee *et al.,* 2000), which could be interpreted that RHT increased lipase acivity which hydrolysed TG. However, the increase in TC, LDL and VLDL contribute in the development of hyperlipidemia (Ross, 1999), demands further study for exact interpretation of the result. Increase of these components are a risk factor for coronary heart disease (Mironova et al, 2000) may have relation with blood glucose level (Pushparaj et al, 2000; Pepato et al, 2003) although it was not studied. RHT may potentiate the activity of hydroxyl-methyl-glutaryl-CoA reductase is the main factor for cholesterol biosynthesis. Although the highly significant increase in HDL is positive, the changing pattern of HDL is not usual with the changes of TC, LDL and VLDL as they were also increased high significantly.

Conclusion

The result of triglycerides is not congruent with the results of other lipid components. Further study may help to interpret the result precisely. More investigations are necessary to come to a decision for the use of this formulation in diabetic complications.

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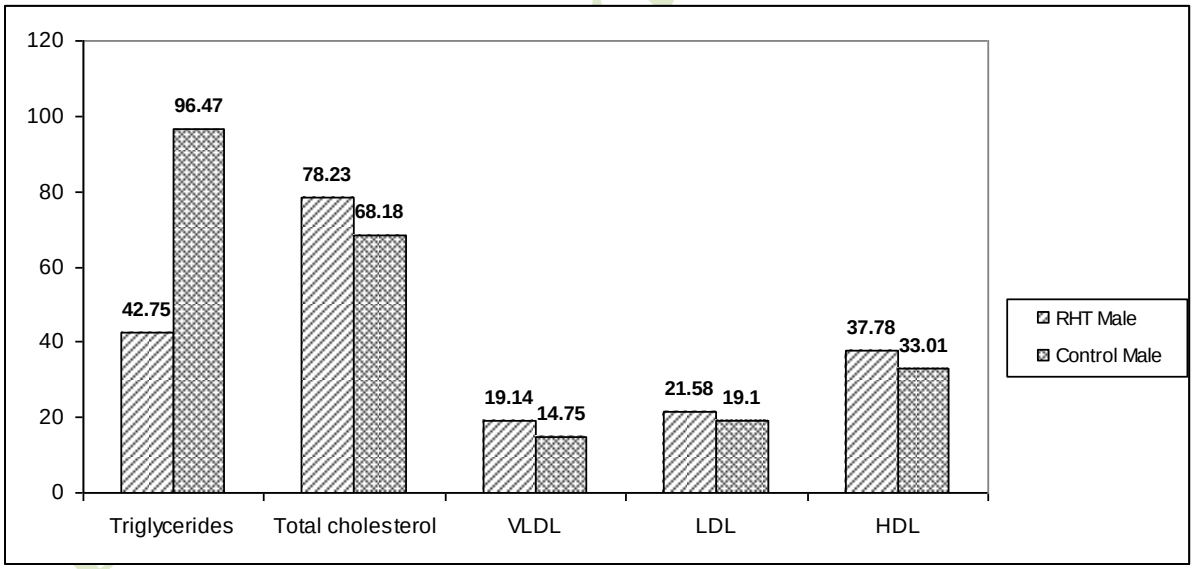
Table 1: The plants and ingredients used in the formulation of Rohitakarista (RHT).

Name of Plants / Ingredients	**Used Parts**	**Botanical Name**	**Family**	**Amount Used**
Rohitaka	Stem & Bark	*Tecomella undulata*	Bignoniacea	4.800 kg
Water for decoction				49.152 L
			reduced to	12.288 L
Guda (Molasses or Brawn sugar)				9.600 kg
Amalaki	Fruit powder	*Embelica officinalis*	Euphorbiaceae	48 g
Bibhitaka	Fruit powder	*Terminalia beleracia*	Combretaceae	48 g
Cavya	Stem	*Pipper retrofractum*	Piperaceae	48 g
Citraka	Root	*Plumbago zeylanica*	Plumbaginaceae	48 g
Dhataki	Flower	*Woodfordia fruticosa*	Lythraceae	768 g
Ela	Sod	*Elettaria cardamomum*	Scitaminaceae	48 g
Haritaki	Fruit powder	*Terminalia chebula*	Combretaceae	48 g
Pippali	Fruit	*Pipper longum*	Piperaceae	48 g
Pippali mula	Root	*Pipper longum*	Piperaceae	48 g
Rohitaka patra	Leaf	*Tecomella undulata*	Bignoriacea	48 g
Sunthi	Rhizome	*Zingiber officinale*	Zingiberaceae	48 g
Tvak	Stem & Bark	*Cinnamomum zeylanicum*	Lauraceae	48 g

Table 2: Changes in lipid profile after chronic administration (100 mg Kg^{-1}) RHT.

Parameters	Male Rats			Female Rats		
	Control (n= 10)	RHT (n= 10)	P values	Control (n= 10)	RHT (n= 10)	P values
Triglycerides	96.47 ± 1.1113	42.7500 ± 1.2076	0.001***	97.9667 ± 3.5946	70.3778 ± 1.3073	0.001***
Total cholesterol	68.18 ± 1.6964	78.2300 ± 1.6352	0.001***	75.2444 ± 1.6415	88.4889 ± 2.0884	0.001***
HDL	33.01 ± 0.8822	37.7800 ± 0.7223	0.001***	34.3778 ± 1.0118	42.1000 ± 1.3489	0.001***
LDL	19.1 ± 0.7734	21.5800 ± 0.6883	0.028*	19.6556 ± 0.6976	22.2556 ± 0.8686	0.034*
VLDL	14.75 ± 0.7606	19.1400 ± 0.7850	0.001***	17.7444 ± 0.4385	20.9778 ± 0.2278	0.001***

Graph 1: Comparative graphical representation of lipid profile between control and drug male rats.



Graph 2: Comparative graphical representation of lipid profile between control and drug female rats.