

Changes in lipid profile of rat plasma after chronic administration of Laghobanondo Rosh (LNR) - an ayurvedic formulation

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Abstract

In this study, the lipid profile of rats' plasma was measured after chronic administration of LNR usually used in the treatment of pandu (anaemia). The animal used for this research work was albino rats (*Rattus norvegicus*: Sprague-Dawley strains) and LNR was administered per oral route at a dose of 100mg/kg body weight, once daily, up to 45 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. LNR remarkably decreased plasma triglycerides in both male and female rats and it was statistically very highly significant ($p=0.001^{***}$). Similar trend of result was noticed in case of total cholesterol in both sexes of the animal but it was not statistically significant (male, $p=0.296$, female, $p=0.511$). On the other hand, a reverse trend in the result was observed in case of VLDL, LDL and HDL. In case of LDL, the increase in male rats was statistically significant ($p=0.047^*$) but in female rats it was statistically insignificant ($p=0.506$). The increase in VLDL and HDL was statistically insignificant in the both male and female rats.

Keywords: Lipid profile; Ayurvedic formulation; LNR; Laghobanondo Rosh; Rat plasma.

Introduction

Ayurveda is a traditional medical system used by a majority of India's population. It is also used worldwide by the South Asian diasporas including Bangladesh and others (Gogtay et al., 2002). Ayurvedic medicines are divided into 2 major types: herbal-only and *rasa shastra*. Rasa shastra is an ancient practice of deliberately combining of herbs with metals (eg, mercury, lead, iron, zinc), minerals (eg, mica), and gems (eg, pearl) (Satpute, 2003; Shastri, 1979). The experts of Rasa shastra claim that these medicines, if properly prepared and administered, are safe and therapeutic (Satpute, 2003).

Laghobanondo Rosh (LNR) is an herbal metallic preparation used in the treatment of pandu (anaemia) in Bangladesh. Four important medicinal plants and five roasted and non-roasted metal have been used in this Ayurvedic formulation (Table 1). These metals (roasted and non-roasted) have the chelating ability with organic-liquids which help these metals easily assimilable, eliminating harmful effect and improving the biocompatibility (Kumar et al., 2006). Among the metal used in this preparation, Iron and Copper is reported as hematinic as well as hepato-protective (Sareker et al., 2007; Tripathi and Singh, 1996).

The fruits of *Piper nigrum* Linn belongs to the family Piperaceae constitute the Black pepper, which is a popular spice. It is

used in several ways as a spice in preparation of intercontinental food and in the folk medicine as a carminative, stimulant and stomachic in dyspepsia and flatulence. The herbal physicians prescribe Black pepper against the treatment of cholera, malaria, bacterial infection, paraplegia and arthritic diseases. As a tonic, it is used to treat weakness following fevers, vertigo and coma. Externally, it is used as a rubefacient and as a local application for treatment of sore-throat, piles and skin diseases (Davis and Granner, 1996; Sofowora, 1993). *Eclipta alba* belongs to the family Compositae commonly called as the trailing Eclipta. It is phytochemically rich in wedelolactone, β - amyryn, stigmasterol and luteolin-7-glucoside (Asolkar et al., 1992). The hepatoprotective (Singh et al., 2001), analgesic (Sawant et al., 2004), immunomodulatory (Jayathirtha and Mishra, 2004) and free radical scavenging action (Bhattacharya et al., 1997) has also been demonstrated. Traditionally, it has been used to produce beneficial neuropsychiatric alterations.

The plant *Solena amplexicaulis* belonging to Cucurbitaceae family are widely distributed in Indian, Srilanka, China, and Taiwan. The tubers, leaves and seeds of the plant are extensively used in traditional system for various ailments like hepatosplenomegaly, spermatorrhoea, thermogenic, appetizer, cardiogenic, diuretics, haemorrhoids and

invigorating (Kritchevsky, 1978). The root tubers of *Aconitum ferox* belonging to the family Ranunculaceae are used for various medical conditions in India, either as such or after processing by Ayurvedic method. It has wide variety of therapeutic indications including fever, neuralgia and rheumatism (Hanuman and Kartz, 1993). Isolation of one known diterpenoid alkaloid vakognavine, and nine known nor diterpenoid alkaloids, chasmaconitine, crassicauline-A, falconericine, bikhaconine, pseudaconine, neoline, senbusine-A, isotalatizidine and columbianine has been reported from root tubers of Ayurvedic-processed and unprocessed *Aconitum ferox* (Hanuman and Katz, 1994).

Our research group is working on the Ayurvedic formulations of Bangladesh. One of the dimensions of our work is to observe their effect on various biochemical parameters of rat plasma after chronic administration. The aim of the present study was to observe the change in lipid profile of rats' plasma after chronic administration of LNR at the same to interpret whether it could be used in the treatment of patients who are prone in the development of cardiovascular disease.

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 Laghobanondo Rosh (LNR), it was collected from Sree Kundshawri Aushadhalaya Ltd, Chittagong.

Dose and route of administration

The liquid Laghobanondo Rosh (LNR) 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 norvegicus* : 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 end of the 45-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 analysis. All analysis was 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 was expressed as Mean \pm 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

Similar trend of changes in the lipid profile parameters were observed in both male and female rats during the course of research. The decrement of plasma triglycerides in both sexes of animals was noteworthy and it was statistically very highly significant ($p=0.001^{***}$). In case of total cholesterol, a decreasing trend was observed in both male ($p=0.296$) and female rats ($p=0.511$). On the contrary the concentration of VLDL, LDL and HDL cholesterol was increased in both male and female rats and the increase of LDL in male rats was statistically significant ($p=0.047^*$). The increase in VLDL and HDL in both male and female rats was not statistically significant (Table 2, Graph 1 and 2).

Discussion

Atherogenicity with subsequent cardiovascular manifestations is one of the major causes of death and morbidity in the world (Raju and Binda, 2005). Various studies indicate that high serum cholesterol levels are strongly related to coronary atherosclerosis and increased risk of cardiovascular diseases. Clinical studies have also shown that lowering levels of serum cholesterol using diet or drugs decreases the incidence of coronary heart disease (Steiner and Li, 2000; Treasure et al., 1995). The levels of serum TC and TG were found to be significantly reduced in the LNR treated animals. This might be due to the reduced hepatic triglyceride synthesis and or reduced lipolysis that might be due to the increase in serum insulin levels in the LNR treated rats. (Sivaraj et al., 2009). The observed hypotriglyceridemic effect may be due to a decrease of fatty acids synthesis (Bopanna et al., 1997), enhanced catabolism of LDL, activation of LCAT and tissues lipases (Khanna et al., 2002) and/or inhibition of acetyl-CoA carboxylase (McCarty, 2001) and production of triglycerides precursors such acetyl-CoA and glycerol phosphate.

The underlying mechanism by which LNR exerts its cholesterol lowering effect seems to be a decrease in cholesterol absorption from the intestine, by binding with

bile acids within the intestine and increasing bile acids excretion (Kritchevsky, 1978; Kelly and Tsai, 1978). LNR can also act by decreasing the cholesterol biosynthesis especially by decreasing the 3-hydroxy-3-methyl-glutaryl coenzyme A reductase (HMGCoA reductase) activity, a key enzyme of cholesterol biosynthesis (Kedar and Chakrabarti, 1982; Sharma et al., 2003) and/or by reducing the NADPH required for fatty acids and cholesterol synthesis (Chi, 1982). HDL cholesterol is an independent negative risk factor for coronary artery disease, at present representing the only protective factor against atherosclerosis. The HDL increasing effect of LNR is largely attributed to its central function in the reverse cholesterol transport, a process whereby excess cell cholesterol is taken up and processed by HDL particles for further delivery to the liver for metabolism (Martinez et al., 2004).

Conclusion

LNR is a popular Ayurvedic formulation used in the treatment of pandu (anemia) in Bangladesh. In the present study, the changes caused by LNR was not congruent incase of all the studied parameters of lipid profile particularly incase of LDL and VLDL. So it necessitates further investigation for using LNR in the patients who are at risk in the development of cardiovascular disease.

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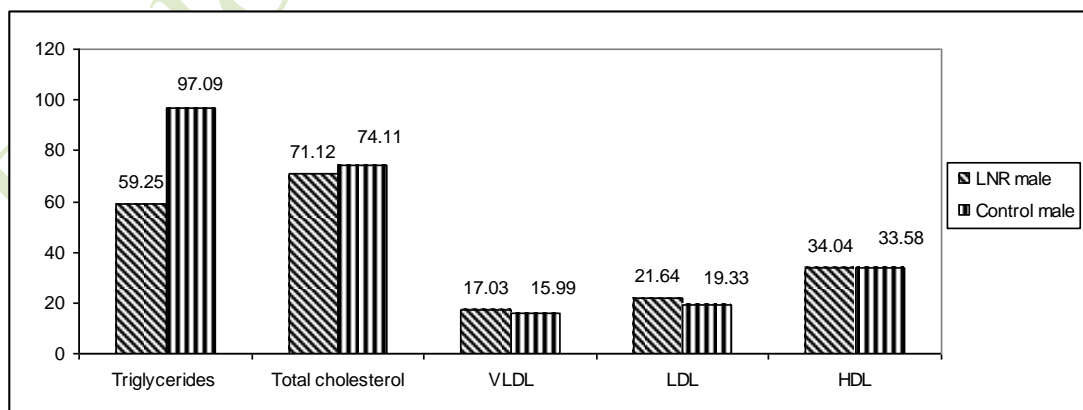
Table 1: Formulary of LNR.

Name of the plants/ ingredients	Used Parts	Botanical / Scientific Name	Family	Amount Used
Parada		Mercury		1 part
Gandhaka		Sulphur		1 part
Lauha (bhasma)		Roasted Iron		1 part
Visa (vatsanabha)	Root	<i>Aconitum ferox</i>	Ranunculaceae	1 part
Abhraka (bhasma)		Mica Ash		1 part
Marica	Fruit	<i>Piper nigrum Linn</i>	Piperaceae	8 parts
Tankana (bhasma)		Roasted Sodium bicarbonate		4 parts
Bhrngaraja rasa	Pulp	<i>Eclipta alba</i>	Compositae	Q.S. (for bhavana) seven days
Amlavetasa rasa	Fruit	<i>Solena amplexicaulis</i>	Cucurbitaceae	Q.S. (for bhavana) seven days

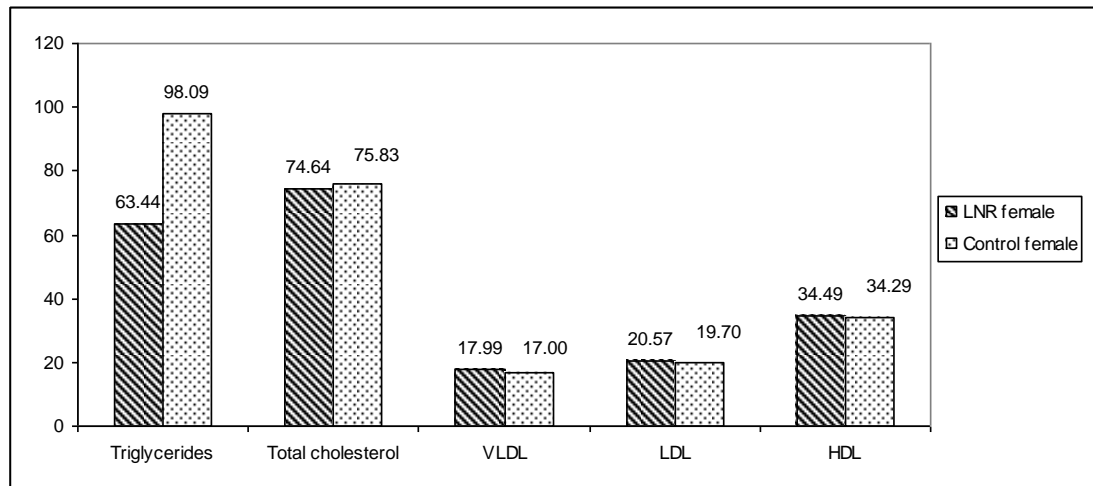
Table 2: Changes in lipid profile after chronic administration (100mg/kg body weight) of LNR.

Parameters	Male rats			Female rats		
	Control (n=10)	LNR (n=10)	P value	Control (n=10)	LNR (n=10)	P value
Triglycerides	97.0937 ± 1.6715	59.2511 ± 1.5071	p=0.001***	98.09145 ± 3.2554	63.4442 ± 2.1146	p=0.001***
Total cholesterol	74.1098 ± 2.8489	71.124 ± 1.6426	p=0.296	75.8331 ± 1.6594	74.6375 ± 1.3029	p=0.511
VLDL	15.9976 ± 0.6657	17.0293 ± 0.3716	p=0.208	17.0045 ± 0.5728	17.994 ± 0.5659	p=0.236
LDL	19.3315 ± 0.7475	21.6366 ± 0.6106	p=0.047*	19.7019 ± 0.6879	20.5689 ± 0.6642	p=0.506
HDL	33.5799 ± 0.9018	34.0395 ± 0.7671	p=0.791	34.286 ± 1.0708	34.4918 ± 1.0013	p=0.915

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



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



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