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Changes in lipid profile of rat plasma after chronic administration of Astavarga Kvatha Curna (AST) - an Ayurvedic formulation

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Abstract
The aim of the present study was to observe changes in the lipid profile parameters of rat plasma after chronic administration of Astavarga Kvatha Curna (AST) which is used in the treatment of *vata roga* (neurological diseases) in Bangladesh. The animals used for this research work were albino rats (*Rattus novergicus*: Sprague-Dawley strain) and AST was administered per oral route at a dose of 100ml/kg body weight, once daily, up to 41 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 case of male rats, administration of AST decreased all the lipid profile parameters (triglycerides, total cholesterol, LDL and HDL) except VLDL with p values of p=0.892, p=0.787, p=0.754, p=0.682 respectively. Similar trend in the result was observed in case of female rats. Triglycerides (p=0.605), total cholesterol (p=0.953), LDL (p=0.811) and HDL (p=0.836) were decreased in comparison with the control group. On the contrary, VLDL was increased with p value of p=0.739. The observed alteration in lipid profile parameters in both male and female rats was not statistically significant.

Keywords: Lipid profile; rat plasma; AST; ayurvedic formulation.

Introduction

Ayurveda remains one of the most ancient and yet living traditions that is practised widely in India, Sri Lanka and other countries and has a sound philosophical and experiential basis (Dahanukar et al., 2000; Chopra et al., 2002). Astavarga Kvatha Curna is an Ayurvedic formulation that is included in the Bangladesh National Formulary (BNF) of Ayurvedic Medicine 1992. It is widely used in the treatment of *vata roga* (neurological diseases). Actually, it is a preparation of eight important medicinal plants that were used in equal amount (Table 1).

Sida cordifolia Linn belongs to the family Malvaceae is found in the Indian system of medicine (Ayurveda) and is known as bala (Dhalwal et al., 2005). The aqueous extract of whole plant of *S. cordifolia* was reported to possess hepatoprotective activity against carbon tetrachloride, paracetamol, and rifampicin-induced hepatotoxicity (Kotoky and Das, 2000). The compound sitoindoside X, an acylsterylglycoside, isolated form the roots of *Sida cordifolia* has been proved as an adaptogenic and immunostimulant (Ghosal et al., 1988).

Barleria prionitis Linn belonging to the family Acanthaceae and is commonly known as Vajradanti. Different parts of *B. prionitis* is used in different diseased as juice of the leaf is used in cataract and fever. The dried bark is used in cough treatment and the leaves chewed to relieve toothache (Gupta et al., 2000).

Ricinus communis Linn belongs to the family Euphorbiaceae is a soft-wooded small tree widespread throughout tropics and warm temperature regions of the world (Ivan, 1998). In India *R. communis* is cultivated widely throughout India for the production of castor oil that is extracted from its seeds. The root of the plant is also useful as an ingredient of various prescriptions for nervous system diseases and rheumatic affections such as lumbago, pleurodynia and sciatica (Nadkarni, 1954).

Zingiber officinale commonly known as ginger, belonging to the family Zingiberaceae is a familiar dietary spice attributed with several medicinal properties. It is called Mahaoushadha, the 'great medicament', because the dried rhizome of *Z. officinale* forms an essential ingredient in several Ayurvedic formulations. It is used in the treatment of several ailments including nausea, respiratory disorders, cardiovascular health and rheumatic disorders (Polasa et al., 2003). Besides, it is reported to inhibit various inflammatory mediators such as prostaglandins and proinflammatory cytokines (Grzanna et al., 2005; Sharma et al., 1994).

Pluchea lanceolata belongs to the family Compositae. It is widely used in the treatment of rheumatoid arthritis in the

indigenous systems of medicine. In Ayurvedic texts this plant is reported for its therapeutic usefulness in diseases similar to rheumatoid arthritis and other afflictions of joints. Tribally, a poultice of leaves is applied to the inflamed areas of the body. The Ayurvedic practitioners use this drug for treating pain and swelling of the body joint (Vaidya, 1970; Prasad et al., 1965) .The leaves are aperient and used as a laxative, analgesic, and antipyretic. The isolation of quercetin and isorhamnetin has been reported from the air-dried leaves of *P. lanceolata* (Chawla et al., 1991; Dixit et al., 1991).

Vitex negundo Linn belongs to the family Verbenaceae has been used for various medicinal purposes in the Ayurvedic and Unani systems of medicine. Almost all the parts are employed, but the leaves and the roots are important as drugs. It is reported that the petroleum ether extract of *V. negundo* leaves possess significant analgesic activity (Gupta et al., 1997). At the same time, dried leaves powder of this plant has been reported to provide anti-arthritic activity in rats.

The plant *Cedrus deodara* (Roxb.) Loud belonging to the family Pinaceae grows extensively on the slopes of the Himalayas. Deodar forests are common from Kashmir (alt. 1500–3000 m), especially Krishnaganga, Kishtwar and Jhelum, to Garhwal (Gulati, 1977). It has been reported that the wood of *C. deodara* has been used since ancient days in Ayurvedic medical practice for the treatment of inflammations and rheumatoid arthritis (Kirtikar and Basu, 1933).

Allium sativum Linn belongs to the family Liliaceae has been ascribed with many therapeutic values. There are several reports that *A. sativum* exerts hypolipidemic and hypocholesterolemic effects (Augusti and Mathew, 1973; Sodimu et al., 1984). Anti-oxidant properties have also been attributed to garlic principles (Torok et al., 1994; Augusti and Sheela, 1996). Its use as a traditional medicine to prevent thrombosis, inflammation and cellular oxidative stress has also been well-documented (Yamasaki et al., 1994).

Our research group is reporting the changes in lipid profile (Ullah et al., 2010), liver and kidney function parameters (Kaiser et al., 2010) of rat plasma after chronic administration of different Ayurvedic formulations of Bangladesh. The aim of this research is the continuation of our effort to observe the changes in lipid profile of rat plasma after chronic administration of AST, an Ayurvedic formulation.

Materials and Methods

Chemicals and reagents

All the 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 Astavarga Kvatha Curna (AST), it was collected from Sree Kundshawri Aushadhalaya Ltd, Chittagong.

Dose and route of administration

The liquid AST was administered to the animals 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 40 ml/kg body weight. For all the studies, the drug was administered orally [per oral (p.o.) route]. Ketamine was administered intra-peritoneally (500 mg/kg i.p.).

Experimental animals and their 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 this 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 the 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 groups.

Preparation of the plasma for intended test
At the due of the 41-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 heparinized 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 both male and female rats, the changes in the tested lipid profile parameters of rat plasma were not statistically significant. In the male rats, all the tested lipid profile parameters (triglycerides, total cholesterol, LDL and HDL) were decreased except VLDL with p values of p=0.892, p=0.787, p=0.754, p=0.682 respectively (graph 1). Similar trend in the result was observed in the female rats. Triglycerides (p=0.605), Total Cholesterol (p=0.953), LDL (p=0.811) and HDL (p=0.836) were decreased in comparison with the control group. But the amount of VLDL was increased with p value of p=0.739 and it was not statistically significant (graph 2).

Discussion
The liver and some other tissues of the body participate in the uptake, oxidation and metabolic conversion of free fatty acids, synthesis of cholesterol and phospholipids and secretion of specific classes of plasma lipoproteins. Thus lowering of serum lipid levels through dietary or drug therapy seems to be associated with a decrease in the risk of vascular disease and related complications (Brown et al., 1993). Many herbs and plant products have been shown to have hypolipidemic properties (Karunanayake et al., 1993).

The underlying mechanism by which AST exerts its cholesterol lowering effect may be due to 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). AST may 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).

The decrease of serum TG level is another important finding of this experiment. Recent studies also show that triglycerides are independently related to coronary heart disease (El-Hazmi and Warsy, 2001). 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 tissue 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.

Conclusion
The result of present study suggests that AST alters the lipid profile parameters of rat plasma after chronic administration. However, the level of alteration was not statistically significant. So, it can be inferred that this formulation can be used in the treatment of *vata roga* without significant alteration in the lipid profile parameters.

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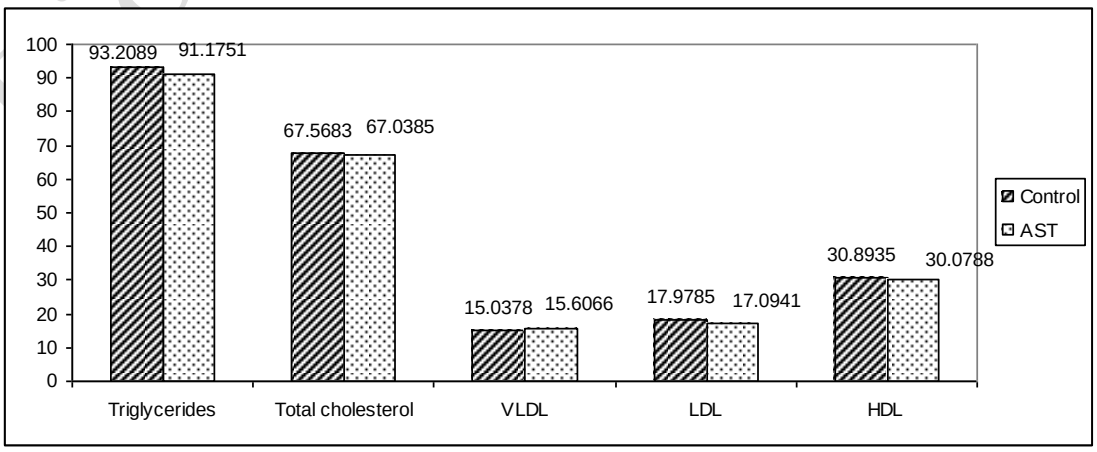
Table 1: Formulary of Astavarga Kvatha Curna (AST).

Ayurvedic Name	Parts Used	Botanical Name	Amount Used
**Bala	Root	*Sida cordifolia*	1 part
****Sahacara	Pulp	*Barleria prionitis* Linn	1 part
****Eranda	Root	*Ricinus communis* Linn	1 part
*Sunthi	Rhizome	*Zingiber officinale* Roxb.	1 part
***Rasna	Root / leaf	*Pluchea lanceolata*	1 part
**Suradruma (kastha sara)	Heart wood	*Cedrus deodara*	1 part
******Sinduvara mula. (nirgundi)	Root	*Vitex negundo*	1 part
****Lasuna	Bulb	*Allium sativum* Linn	1 part

Graph 1: Effect of AST on lipid profile parameters of male rats' plasma.



Graph 2: Effect of AST on lipid profile parameters of female rats' plasma.