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Effect of Asmarihara kasaya curna (ASM)-an ayurvedic formulation on lipid profile after chronic administration.

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Abstract – Asmarihara Kasaya Curna (ASM), a classical Ayurvedic preparation which is widely used in Urolithiasis .The research was carried out in order to observe the effect of this formulation on lipid profile reveals that it possesses *hypotriglyceridemic* property after chronic administration for 45 consecutive days. In the male rats there was a statistically very highly significant decrease in the Triglycerides (p=0.001***) content in the plasma. On the contrary an increase in the Total Cholesterol (p=0.285^{NS}), VLDL (p=0.173 ^{NS}) and LDL (p=0.023*) content in the plasma was noted. There was an insignificant decrease (p=0.771 ^{NS}) in HDL. In the female rats there was a statistically very highly significant decrease in the Triglycerides, content (p=0.001***) in the plasma. Interestingly none of the other changes in case of the total Cholesterol (p=0.924 ^{NS}), VLDL (p=0.329 ^{NS}), LDL (p=0.881 ^{NS}) and HDL (p=0.983 ^{NS}) were significantly different from their corresponding control values. But in male rats, the content of the LDL in plasma a statistically significant increase was noted which was different from female rats.

Keywords – Asmarihara Kasaya Curna, lipid profile, lithiasis, Ayurvedic formulation.

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1. Introduction

Ayurvedic medicine (also called Ayurveda) is one of the world's oldest medical systems native to India. Current practices derived from Ayurvedic medicine are regarded as part of CAM. According to some sources Up to 80% of people in India used to use some form of traditional medicines, a category which includes Ayurveda. It is also practiced in Bangladesh, Sri Lanka and outside South Asia[1]- [2].

Asmarihara Kasaya Curna (ASM), a classical Ayurvedic preparation that is included in the Bangladesh National Formulary of Ayurvedic Medicine 1992. (Approved by the Government of Bangladesh vide Ministry of Health and Family). It is indicated in the treatment of Asmari (lithiasis) a disease caused by the formation or lodging of a stone in the urinary passage. It is also called Mutrasmari. Renal stones occur when solutes come out from the solution either because they are present in excessive quantity (urine is over concentrated) or because of lack of inhibitors of crystallization. The majority are composed of calcium, magnesium, phosphate mixtures or urate. Nephrolithiasis is one of the most painful and common disorders of the urinary tract (Chandhoke, 2002) [3]-[9].

Actually, it is a preparation in which fifteen important medicinal plants are used in particular amount (Table1). It includes *Tribulus terrestris* is one of the most commonly utilized herbs in Ayurvedic medicine for renal dysfunction. It is proposed to be effective through diuretic, analgesic, and litholytic properties and is also reported to have hemostatic properties. It has been traditionally used as treatment for cystitis and renal calculi and as a diuretic (Nadkarni, 1976) [10]. *Aerva lanata* (L.), commonly known as Pasana bheda, is a plant of the family Saxifragaceae.It is a perennial herb with stout rootstock. The leaves are variable, coarsely hairy, sparsely hairy to glabrous, It is given to dissolve kidney stones and also as a diuretic [11]-[12]. *Tectona grandis* L.F., commonly known as sagauna, is a plant of the family Verbenaceae. Useful parts are leaves, seeds, bark, and root. It is used in Ayurvedic medicine as inflammation, diabetes, hemorrhages, urinary retention, kidney diseases, urinary calculi and arthritis. *Carica papaya* L, its Sanskrit name is Erandakarkati & commonly known as Papaya, is a plant of the family Passifloraceae .This species is native to Tropical America, now widely cultivated in most parts of India. It is used to treat indigestion, inflammations, urinary retention, constipation, dysmenorrhea, amenorrhea and general debility [13]. *Asparagus racemosus* , commonly known as satavari , is a plant of the family Liliaceae. It grows one to two metres tall and prefers to take root in gravelly, rocky soils high up in piedmont plains. [14]. It is recommended in Ayurvedic texts for the prevention and treatment of gastric ulcers, dyspepsia and as a galactogogue [15]. It is used as anti-diarrhoeatic, night blindness and kidney troubles. *Crataeva nurvala* (family: Capparidaceae) is commonly known as *barna* and *varuna* [16]. Leaves are deciduous three foliolate; petioles 3.8–7.6 cm long; leaflets 5–15 ovate, obovate, acute or acuminate,entire, glabrous on both surfaces.It is usually cultivated in Central India, Bengal, and Assam. Its bark is hot, tasting bitter at first and then sharp sweet, easy to digest, stomachic, laxative, antilithic, anthelmintic, expectorant, and antipyretic [17]-[18]. Kusa *(Desmostachya bipinnata* STAPF), Kasa *(Saccharum spontaneum L*) & Sali (*Oryza sativa* L.) are plants of the family Poaceae. *Desmostachya bipinnata* is perennial herb found in sandy desert areas [19]-[20]. *Desmostachya bipinnata* is sweet,cooling, oleaginous, diuretic; useful in the diseases of blood, biliousness, thirst, asthma, jaundice, strangury, diabetes,vesical calculi, diseases

of bladder; sedative to pregnant uterus; causes "kapha"(Ayurveda) [21]-[22]. Kasa *at al* erect perennial herb. The root according to ayurveda roots are sweet, astringent, emollient, diuretic, lithotriptic, and useful in treatment of dyspepsia. The stems (culm) are useful in vitiated conditions of pitta and vata burning sensation, renal and vesicol calculi. Sali a small annual herb grows up to 1 meter in height. Leaves simple, long, narrow, scrubby surfaces. This species is native to India and Indo-China and is cultivated throughout warmer parts of the world. Useful parts are root, grains Plant pacifies vitiated vata, pitta, urinary retention, diarrhea, colic and general weakness [23-[25]. *Boerhaavia diffusa Linn,* commonly known as Punarnava.Useful part is Whole plant. It is used as laxative,cardiac disorders, urinary infection, vesical stone. *Tinospora cordifolia*, commonly known as Guduchi is an herbaceous vine of the family Menispermaceae [26]. The plant is a glabrous climbing shrub found throughout to the tropical areas of India, Myanmar and Sri Lanka. It is useful in fever, urinary disorders, and dyspepsia and kidney troubles. *Achyranthes aspera Linn*, its Sanskrit name is apamarga Useful parts are whole plant, branches and roots .Plant is used in painful inflammations, dysentery. *Cucumis sativus* L., its Sanskrit name is trapusa, is an herbaceous vine of the family Cucurbitaceae . An annual trailer herb, Leaves simple, hairy on both surfaces; Useful parts are roots, leaves. it is used in kidney diseases, burning sensation, jaundice, urinary retention constipation, calculi. *Nardostachys jatamansi*, its Sanskrit name is Jatamamsi,An erect perennial herb.Useful part is Rhizomes. Used in kidney diseases hypertension. *Hyoscyamus niger* , its Sanskrit name is Parasika Yavani is an herbaceous vine , of the family Solanaceae. An erect annual or bie -nnial herb grows up to 1.5 meters in height. Useful parts are leaves, seeds. Plant is used in piles, skin eruptions, dysentery, rheumatism, scabies, and bronchial affections and in leprosy [27]-[28]. The present study was undertaken to explore the effect of this formulation on the lipid profile so that it could be presumed whether it can be used in the treatment of lipid disorders or not.

2. Materials and Methods

2.1. 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 Asmarihara Kasaya Curna (ASM) was collected from Sri Kundeswari AushadhalayaLtd, Chittagong,Bangladesh.

Table 1. Formulary of ASM [29]

Ayurvedic Name of plants	Botanical Name	Family	Amount Used
Pasana bheda (Rt.)	Saxifraga ligulata	Saxifragaceae	1 Part
Sagauna (sakka) (Sd.)	Tectona grandis L.F.	Verbenaceae	1 Part
Papita (eranda karkati) (Rt.)	Carica papaya L.	Passifloraceae	1 Part
Satavara (satavari) (Rt.)	Asparagus Racemosus	Liliaceae	1 Part
Gokharu (goksuru) (Fr.)	Tribulus Terrestr	Zygophyllaceae	1 Part
Baruna (varuna) (St.Bk.)	crataeva Nurvala	Capparaceae	1 Part
Kusa (Rt.)	Desmostachya bipinnata STAPF	Poaceae	1 Part
Kasa (Kasa) (Rt.)	Saccharum spontaneum L.	Poaceae	1 Part
Sali (Rt.)	Oryza sativa L.	Poaceae	1 Part
Punarnava(sveta punarnava) (Rt.)	Boerhaavia diffusa Linn.	Nyctaginaceae	1 Part
Giloya (guduci) (St.)	Tinospora Cordifolia	Menispermaceae	1 Part
Ciracida (apamarga) (Rt.)	Achyranthes aspera Linn.	Amaranthaceae	1 Part
Khira (trapusa) (Sd.)	Cucumis sativus L.	Cucurbitaceae	1 Part
Jatamamsi (Rt.)	Nardostachys jatamansi	Valerianaceae	1 Part
Khurasani Ajavayana(Sd./Lf.) (Parasika Yavani)	Hyoscyamus niger	Solanaceae	1 Part

2.2 Dose and route of administration

The liquid Asmarihara Kasaya Curna 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 Intraperitoneally (500 mg/kgi.p.).

2.3 Experimental animals & their Management

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 70 - 90 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 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 according to sex. Thus ten rats were taken for each group for both control and the experimental group.

2.4. Preparation of the Plasma for intended Test

At the due of the 45-days 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.

2.5 Determination of Lipid profile

Triglycerides and total cholesterol concentration as well as protein content were evaluated according to the instruction of manufacturer of assay kits (purchased from Sigma Chemical Co, St Louis, MO, USA). Serum total cholesterol and high-density lipoprotein cholesterol were determined using Randox Laboratory kit reagents. Serum triacylglycerol level was estimated using Randox Laboratory test kit and VLDL-cholesterol was calculated using formula TG/2.2 mmol/l. (Friedewald *et al.*, 1972).LDL was determined by differential substraction of the sum of the cholesterol fractions from the total cholesterol then [LDL-chol] = [Total chol] - [HDL-chol] – [(TG/2.2)] [30]-[32].

2.6 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

3. Results and discussion

In the male rats there was a statistically very highly significant decrease in the Triglycerides (p=0.001)***content in the plasma. On the contrary there was not notable decrease in the total Cholesterol (p=0.285), VLDL (p=0.173) content in the plasma but only in the case of the content of the LDL (p=0.023)* in plasma a statistically significant increase was noted. There was an insignificant decrease (p=0.771) in HDL. In the female rats there was a statistically very highly significant decrease in the Triglycerides content (p=0.001***) in the plasma. Interestingly none of the other changes in case of the total Cholesterol (p=0.924)), VLDL (p=0.329), LDL (p=0.881) and HDL (p=0.983) were significantly different from their corresponding control values. But in male rats, the content of the LDL in plasma a statistically significant increase was noted which was different from female rats. (Table 2 & Figures 1 & 2) The observed hypotriglyceridemic effect of this formulation both in male and female rats 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 cetyl-CoA carboxylase (Mc Carty, 2001) and production of triglycerides precursors such acetyl-CoA and glycerol phosphate. The increase the level of HDL by increasing the activity of LCAT, which may contribute to the regulation of blood lipids. LCAT play a key role in lipoprotein metabolism and most of the lipoprotein changes are the outcome of primary abnormality owing o the liver diseases (Seidel and Wall, 1983). It also incorporates free cholesterol into HDL and transferring back to VLDL or LDL, which is taken back by the liver cells (Rajlakshmi and Sharma, 2004).

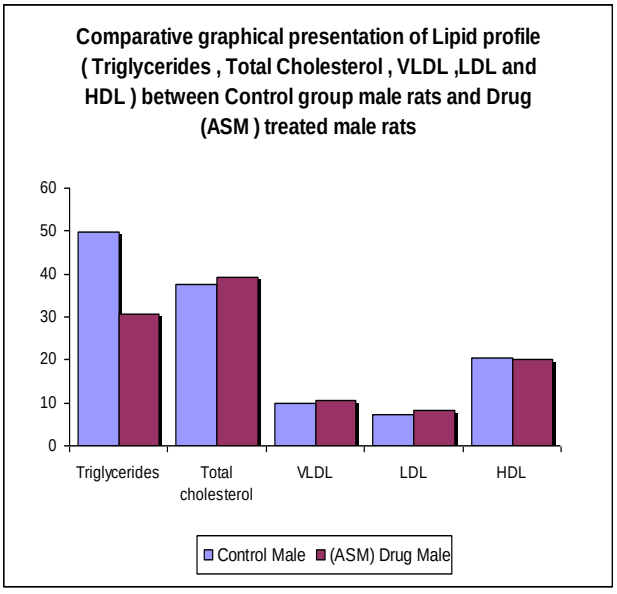

Figure 1. Comparative graphical presentation of Lipid profile between Control group male rats and Drug (ASM) treated male rats.

Table 1. Effect of ASM on lipid profile

Parameters	Male			Female		
	Control (n= 10)	ASM (n= 10)	P values	Control (n= 10)	ASM (n= 10)	P values
Triglyce-rides	49.8602 ± 4.6468	30.4951 ± 0.7485	p=0.001***	62.8226 ± 8.2458	40.0316 ± 1.3968	p=0.001)***
Total choleste-rol	37.4927 ± 4.0334	39.0799 ± 1.5436	p=0.285 NS	50.3236 ± 1.2016	49.5208 ± 0.8652	p=0.924) NS
VLDL	9.9729 ± 0.9291	10.6091 ± 0.2204	p=0.173 NS	12.5647 ± 1.6493	11.4793 ± 0.3936	p=0.329) NS
LDL	7.2304 ± 1.2526	8.3518 ± 0.2208	p=0.023*	15.2669 ± 1.9932	15.9434 ± 0.5102	p=0.881) NS
HDL	20.2901 ± 1.8523	20.2071 ± 0.4325	p=0.771 NS	22.4932 ± 1.3675	22.3663 ± 0.6908	p=0.983) NS

Note: *p<0.05, **p<0.01, ***p<0.001,NS=Not Significant

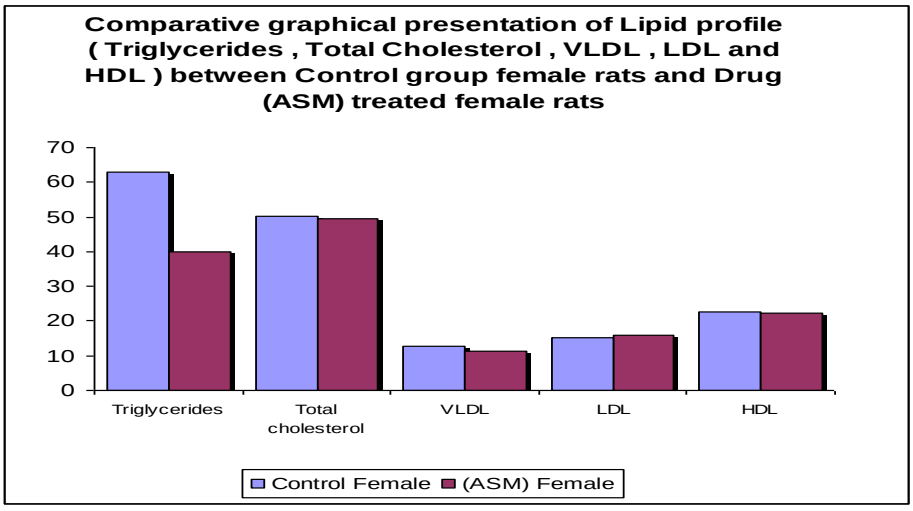

Figure 2. Comparative graphical presentation of Lipid profile Between Control group female rats and Drug (ASM) treated Female rats.

The decrease in cholesterol in the both sexes of the animal may be due to the decrease in absorption from the intestine, by binding with bile acids within the intestine and increasing bile acids excretion (Kritchevsky, 1978; Kelly and Tsai, 1978).

4. Conclusion

The Ayurvedic formulation Asmarihara Kasaya Curna (ASM) may be useful in the treatment of hypertriglycerdemia. The effect of this formulation on LDL is different in male and female rats. It necessitates precise study of this preparation on this parameter to find out.

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