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Changes in lipid profile of rat plasma after chronic administration of Mallasindura (MSL) - an Ayurvedic metallic preparation

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Abstract:
Mallasindura (MSL) which is a classical Ayurvedic preparation of arsenical products was studied to observe its toxicological effects. This Ayurvedic drug is used in the treatment of bronchial asthma. The changes in the lipid profile parameters of rat plasma were examined after chronic administration of this drug. The animal used were albino rats (*Rattus novergicus*: Sprague-Dawley strains) and the drug was administered per oral route at a dose of 100 mg/kg body weight, once daily, up to 90 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, statistically a very highly significant decrease (p=0.001) in the triglyceride content in the plasma was noted. There was also a decrease in the total cholesterol, LDL, HDL and VLDL content (with p values of 0.682, 0.374, 0.796 and 0.328 respectively) of the plasma was observed. None of these values were statistically significant. In case of female rats, a similar declined trend was observed for triglycerides which was statistically very highly significant (p=0.001). And for total cholesterol, LDL, HDL and VLDL content in female rats, there was a similar trend like male rats with p values of (0.701, 0.395, 0.902 and 0.587 respectively) which were not statistically significant.

Key words:
Lipid profile, rat plasma, MSL, Ayurvedic preparation.

INTRODUCTION:

In recent years, Ayurveda (the life of science) as an alternative and complementary medicine is increasingly getting acknowledgement all over the world [8]. A branch of Ayurveda known as Rasa shastra (Vedic chemistry) is associated with the use of metals, gems, minerals and even poisons for manufacturing special formulations for treatment of chronic and complicated diseases [9]. Gold, silver, copper, lead, zinc, tin, iron and some of their alloys are usually used for the preparation of ayurvedic drugs. Mercury and sulphur are accounted for the conversion of these metals into Bhasma which is involved with metal extraction following the conversion of purified metal or its alloy into nontoxic form [10]. Literal meaning of Bhasma is ash, which involves different processing steps. The complete process is called *Bhasmikarana* which includes the sequential heating of metal with natural precursors (herbal juices, decoctions, and powders, etc) of medicinal importance [13]. *Bhasmikarana* converts the free metal into metal oxide or sulfide resulting an insoluble compound that has no or a little interaction with human tissues and body fluids. Thus mercury, gold, diamond, iron, and silver are transformed from their free state into potent medicines. They are usually very effective at low doses (few mg) [9]. Hence, the conversion of zero valent metal state to its higher oxidation state enables the complete elimination of the toxic nature of metal while incorporating the medicinal properties into it [16].

Mallasindura is a classical ayurvedic preparation (Table 1) which is used to treat bronchial asthma. It is a combination of rasa –mercury, ras karpura-mercurial compound, Bali – purified and processed sulphur and Malla-purified arsenic oxide with herbal juices [2]. Asthma is an inflammatory condition with recurrent reversible airways obstruction in response to irritant stimuli. These stimuli are too weak to affect non-asthmatic subjects. Symptoms of asthma include intermittent attacks of wheezing, shortness of breath with difficulty especially in breathing out and sometimes cough [4]. Heavy toxic metal such as mercury used in traditional medicine is presumed to act as a catalyst and can stimulate activity by their presence in the intestines. However, they do not reach the blood stream [6].

Arsenic is derived from a Greek word called Arsenikon which means ‘potent’. It is consumed as a poison as well as a therapeutic agent and it was explained as a metallic poison before 2000B.C. A transitional element or metalloid arsenic (atomic number 33, atomic wt. 75) has three different valance states: elemental arsenic with zero oxidation state, trivalent and pentavalent arsenic. It can form alloys with metals which can further reacts with carbon, oxygen and hydrogen through covalent bonds. It is commonly used in its trivalent and pentavalent state. The

toxicity of arsenicals depends on their valance state, physical state, absorption and elimination. In Ayurvedic preparations three commonly used arsenicals are Haritala (orpiment), manashila (realgar) and gouripasana (white arsenic). Malla sindura is a derivative of white arsenic (arsenic trioxide) [8, 11]. Metabolism of arsenic involves a sequential process of reduction of penta valent arsenic to its trivalent state with the help of a catalyst called arsenic methyltransferase which is then able to back to its pentavalent state by oxidative methylation. The most toxic arsenic compounds remain in the trivalent oxidation state and they show their toxicity while reacting with sulfur containing compounds with the formation of a reactive oxygen species (ROS) [5]. All arsenicals which are used in ayurvedic preparations are absorbed in gastrointestinal tract. Arsenic trioxide is highly water soluble and has better oral absorption and better bioavailabilability [8].

Arsenic has been referred as a potent poison due to its discrete nature. However, it has also been used as an ailment for certain diseases. Therapeutic uses of arsenic have been documented for more than 2,400 years [1]. Information about the use of arsenicals (orpiment and realgar) in external uses of skin diseases was found in Charak Samhita (400B.C). Gouripasana (white arsenic) is used as a first line chemotherapeutic agent against certain hematopoietic cancer in western medicine in recent days [8]. Often risk of cancer in case of low level exposure of arsenic trioxide (< or = 60 ppb of arsenic) is lower compared to its unexposed counterpart [14]. By producing conduction delay and increasing triangulation arsenic was found to be able to make significant prolongation of cardiac action potential duration at several stages of repolarization [8]. A potassium bicarbonate-based solution of arsenic trioxide (As_2O_3) which is known as Fowler's solution was used to treat a variety of disorders such as leucocythamia in 1878 , syphilis and trypanosomiasis in 1910 [1]. It is contraindicated for patients with electrolyte imbalance. It was also associated with the increase of atherosclerosis and platelet aggregation as well as for reducing fibronolysis [8]. Therefore, the present study was conducted to observe whether the benefit of using this Ayurvedic metal preparation can outweigh the associated risk or not.

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 effect of mallasindura (MLS) on lipid profile of rat plasma, it was collected from Sree Kundeshawri Aushadhalaya Ltd, Chittagong.

Dose and route of administration:

The liquid "Mallasindura" 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 drug was administered per oral route at a dose of 100 mg/kg body weight. For all the studies, the drug was administered orally. Ketamine were 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 whole experiment period. 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 group.

Preparation of Plasma for the Test:

At the due date of the 90 days treatment period, the animals were fasted for 18 hours and also 24 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 4,000rpm 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 [3], 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.10) was applied for the

analysis of data. Differences between groups were considered significant at $p < 0.05$, 0.01 and 0.001.

RESULTS:

There was a similar trend of changes in tested lipid profile parameters of rat plasma in comparison with control group for both sexes of rats. The only change for triglycerides was statistically significant. In male rats there was a statistically very highly significant decrease with p=0.001 in the triglycerides content in the plasma. Also a negligible decrease in the total cholesterol, HDL, LDL and VLDL content (with p value of 0.682, 0.796, 0.374, and 0.328 respectively) in the plasma was noted; none of the decreases were statistically significant. (Table 2, graph 1).

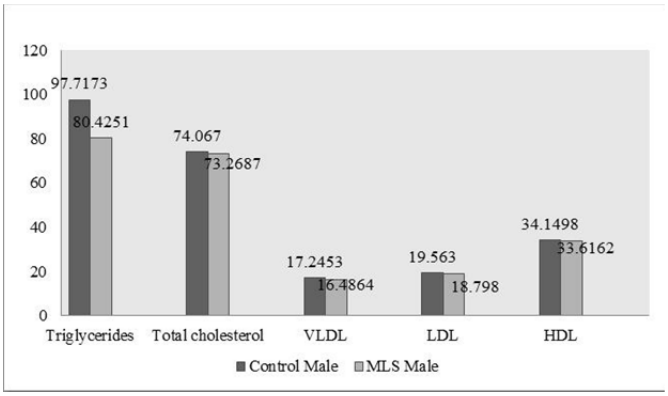
In case of female rats, a similar declining trend was noted for triglycerides which was statistically very highly significant (p=0.001). And for total cholesterol, HDL, LDL and VLDL content in female rats, there was a similar pattern of results with p values of (0.701, 0.902, 0.395 and 0.587 respectively). None of these changes were statistically significant (Table 2, graph 2).

Table 1: Formulary of Mallasidura:

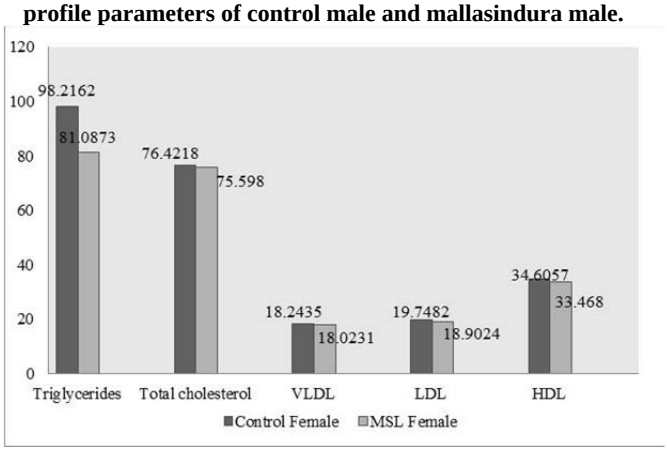
Ayurvedic Name	Chemical name	Amount used
Shuddha Parada	Purified and processed Mercury	108 g
Rasakarpura	Mecrurial compound	108 g
Shuddha Gandhaka	Purified and processed Sulphur	66 g
Shuddha Malla	Purified Arsenic oxide	54 g

Table 2: Effect of MSL on lipid profile parameters of male rats' and female rats plasma

Parameters	Male rats			Female rats		
	Control (n=10)	*MLS (n=10)*	*P values*	*Control (n=10)*	*MLS (n=10)*	*P values*
Triglycerides	97.7173 ± 2.2317	80.4251 ± 2.0151	(p=0.001)***	98.2162 ± 2.9163	81.0873 ± 1.8913	(p=0.001)***
Total cholesterol	74.067 ± 1.5887	73.2687 ± 1.1818	(p=0.682)	76.4218 ± 1.6773	75.598 ± 1.2871	(p=0.701)
VLDL	17.2453 ± 0.5708	16.4864 ± 0.3738	(p=0.328)	18.2435 ± 0.6932	18.0231 ± 0.373	(p=0.587)
LDL	19.563 ± 0.7216	18.798 ± 0.8943	(p=0.374)	19.7482 ± 0.6783	18.9024 ± 0.757	(p=0.395)
HDL	34.1498 ± 0.9213	33.6162 ± 1.0404	(p=0.796)	34.6057 ± 0.9908	33.468 ± 1.1672	(p=0.902)



Graph 1: Comparative graphical representation of lipid profile parameters of control male and mallasindura male.



Graph 2: Comparative graphical representation of lipid profile parameters of control female and mallasindura female.

DISCUSSION:

Breakdown of cholesterol produces bile acids (mainly triglyceride rich very low density lipoproteins) which are secreted from liver and an essential component of bile. Cholesterol is reproduced from bile acids in the liver. Bile acids binding resins such as cholestyramine and cholestipol are anion exchange resins and they bind with negatively charged bile acids and bile salts in the small intestine and therefore, prevent those returning to bile and reduce bile concentrations. This causes hepatocytes to convert more cholesterol to bile acids. Consequently, the intracellular cholesterol concentration is decreased and hence increased uptake of cholesterol by hepatocytes [7]. As Bhasma is a processed metal preparation and the heavy metal mercury is presumed to act as a catalyst and stimulates activity by their presence in the intestine [6]. Therefore, it can be assumed that mallasindura acts in the similar way of bile acid binding resins in case of its cholesterol (Triglycerides, VLDL and LDL) lowering effects.

In a prospective study based on western population moderate and highly significant association was observed between triglyceride values and coronary heart disease risk factor [12]. In another study, it was found that incidences of CHD and stroke per 1000 person-years were 9.59 and 7.45 respectively and myocardial and brain infections were 3.84 and 6.29 respectively. As CHD risk was linearly and continuously increased by triglycerides and LDL cholesterol, they were reported as the third potent risk factor for CHD and their combined effects might be additive [15].

From the present study, it is observe that mallasindura decreases triglycerides of rat plasma of both sexes which is statistically very significant (p=0.001). Apart from this, the present drug is able to add an extra benefit to treat bronchial asthma in patients with coronary heart disease (CHD).

CONCLUSION:

In the present study, no toxic effect of studied Ayurvedic drug was observed. Moreover, another beneficial effect of lowering plasma triglycerides was noticed. Therefore, it can be concluded that the ayurvedic drug Mallasindura may be used for the treatment of bronchial asthma without any toxic effect and it can be a positive addition to treat bronchial asthma in patients with CHD.

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