**IJPSR (2015), Vol. 6, Issue 5** **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 10 September, 2014; received in revised form, 13 November, 2014; accepted, 06 January, 2015; published 01 May, 2015

# TOXICOLOGICAL EFFECT OF MALLASINDURA (MSL) ON LIVER FUNCTION PARAMETERS OF RAT PLASMA AFTER CHRONIC ADMINISTRATION

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**Keywords:**

Liver function parameters, rat plasma, Mallasindura (MSL), Ayurvedic preparation.

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**ABSTRACT:** Mallasindura (MSL), a widely used ayurvedic preparation is used for the treatment of bronchial asthma. It contains rasa –mercury, Rasakarpura -mercurial compound, Bali –purified and processed sulphur and Malla-purified arsenic oxide with herbal juices. The liver function parameters of rat plasma were studied after chronic administration of MSL. The animals used to conduct this experiment were albino rats (*Rattusnovergicus*: Sprague-Dawley strains) and the drug was administered per oral route. The administered dose of the drug was 100mg/kg body weight, once daily up to 90 days for the whole study. There were forty rats for four randomized groups, equally of both sexes. Among them one male and one female group were used as test. In case of male rats, there was increase in total protein and albumin content, which was statistically highly significant with a p value of 0.001. Along with this, a statistically highly significant declining trend was followed for bilirubin, sGPT, sGOT activities (p values are 0.001 and 0.009 respectively). In case of female rats, a statistically highly significant increase was observed for both total protein and albumin with a p value of 0.001. Changes in billlirubin, sGPT and sGOT activities were also reduced and was statistically highly significant (p =0.001). The change observed for ALP content of plasma was statistically insignificant for both of male and female rats

**INTRODUCTION:** A classical Ayurvedic preparation, mallasindura is used for the treatment of bronchial asthma. It contains four compounds, rasa –mercury, rasa karpura-mercurial compound, Bali –purified and processed sulphur and Malla-purified arsenic oxide with herbal juices [1]. (**Table1**) Asthma is a broad concept about respiratory system disorder and can cause various episodes of difficulty of breathing [2]. A chronic inflammatory disease of airways is known as asthma which can be characterized by activation of mast cells, infiltration of eosinophils and T helper 2 lymphocytes. Airway smooth muscle contraction contributed to the most of the symptoms of asthma [3].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.6(5).1958-64

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.6(5).1958-64

The specific underlying cause of asthma associated with hyperactivity of lung to one or more stimuli. Possibly, it can be triggered by a number of factors such as allergens (pollen, moulds as well as dander, saliva and urine of animals), industrial chemicals (isocyanate containing paints, epoxy resins, aluminium, hair sprays, penicillins and cimetidine , drugs (aspirin, ibuprofen and other prostaglandin synthetase inhibitors, β-blockers), foods (nuts, fish, seafood, dairy products, tartrazine, benzoic acid and sodium metasulphite), industrial triggers – grain dust, cotton dust, grain weevils and mites) environmental pollutants (cigarette smoke and sulphur dioxide) and miscellaneous (cold air, exercise, hyperventilation, viral respiratory tract infections, emotion or stress) [2].

Pathogenesis of asthma is associated with exposure to allergens of genetically disposed individuals that can cause activation of Th2 lymphocytes and cytokine generation which in turn promotes differentiation and activation of eosinophils.

Further release and production of IgE as well as expression of IgE receptors on mast cells and eosinophils can be caused by those allergens. Leukotriene B4 and cysteinyl leukotrienes (C4 and D4); interleukins IL-4, IL-5, IL-13; and tissue-damaging eosinophil proteins are among the important mediators of asthma. Measurement of forced expiratory volume in 1second (FEV1) or peak expiratory flow rate is important for monitoring the treatment [4].

**TABLE1: FORMULARY OF MALLASINDURA:**

| Ayurvedic Name | Chemical name | Amount used |
|---|---|---|
| ShuddhaParada | Purified and processed Mercury | 108 g |
| Rasakarpura | Mecrurial compound | 108 g |
| ShuddhaGandhaka | Purified and processed Sulphur | 66 g |
| ShuddhaMalla | Purified Arsenic oxide | 54 g |

A Sanskrit word ayurveda means the "Scripture for longitivity" which represents an ancient system of traditional medicine practiced mostly in India and in other South Asian countries [5]. Ayurveda has been found as the Indian traditional system of medicine in many scientific journals which is also associated with pharmaceutical science.

It was the most ancient practice of medical science in India since 5000 years B.C. The ingredients used in these medicinal preparations were derived from plant, animal, metal and mineral sources. Ayurvedic classics which were written before 8$^{th}$ century AD such as Charaka Samhita and Susruta Samhita described the use of metals and minerals in the formulations of therapeutics [6].

A specialized branch of Ayurveda is Rasashaastra, literal meaning of which is science of mercury. Actually, it can be defined as the science of study of mineral and metallic substances including their various processing steps to be used in therapeutic formulations. 'Rasa' is a synonym of mercury. An inorganic form of mercury known as mercuric sulphide (HgS) is widely used in ayurvedics and mercurial chlorides are used rarely due to its toxicity. However, complex and intensive processing is required for their utilization in medicine and alchemy. As negligible amount of mercurial sulphide compounds are absorbed from G.I. tract during the use of ayurvedic medicine they become non-toxic [7].

Ayurvedic metallic preparations are generally obtained from Bhasmas which is a processed form of raw metals and herbs such as Swarna Bhasma, a therapeutic form of gold with a crystalline size of 28 to 35 nm and was made from pure gold [8]. Bashma cannot be metabolized in the body. Therefore, it is not able to produce any harmful product. Moreover, this process is important for (i) alleviating harmful acids from body which can lead to illness, (ii) maintaining an optimum alkalinity for good health [9]. The complete process of preparing metallic bashma involves with metal extractions and by sequential heating of medicinally important natural precursors such as herbal juices, decoctions, and powders. The name of this process is *Bhasmikarana* [10].

This process helps to prepare specially desired chemical compound with essential medicinal benefit by eliminating the toxic nature of the metal[11].Bhasma mostly contains nanosized particles although micronized particles are also present. The interaction of these particles with biological system depends on various factors such as cell type, environment, and characteristics of particles (size, shape and composition) [12].

Absorption and assimilation of Ayurvedic medicine into the body may be increased due to the reduction of particle size (1-2µ) during the processing of bashma [9]. Metallic Bhasma which is prepared by treating mercury with different substances are of superior quality [7]. As bashma is highly potent they are rarely used as an individual therapeutic agent. Therefore, for dose facilitation and for avoidance of unwanted effect these drugs are used in combination with other herbal preparations [13].

Arsenic is a metalloid or transitional element which has an atomic number of 33 and atomic wt.75 and can exist in three different valence state: elemental, trivalent and pentavalent arsenic. Among them trivalent arsenic is the most commonly used one. Mallasindura is a formulation of arsenic trioxide[14].For the metabolism of arsenicals possibly there are two proposed pathway: (i) oxidative

methylation and (ii) glutathione conjugation. It was proposed that arsenite (iii) is sequentially converted to monomethylarsenoic acid and dimethyl arsenoic acid in both human and laboratory animals by oxidative methylation.

The glutathione conjugation mechanism was supported by the fact that intracellular arsenicals were mostly trivalent which were excreted from the cells as GSH conjugates [15]. Although arsenicals are known as poison, in 1970 Chinese physician revived the arsenicals anticancer therapeutic property and in 2000 arsenic trioxide is approved by FDA for the treatment of acute promyelocytic leukemia [16].

Mallasindura is used by Bangladeshi people for a long time. Therefore, the aim of the present study was to observe the toxicological effect of this preparation on liver function after chronic administration.

## MATERIALS AND METHODS:

### Chemicals and Reagents:

This experiment was done by using all analytical grade reagents and chemicals and solutions were prepared by using glass – distilled water. To evaluate the effect of mallasindura (MLS) on liver function parameters of rat plasma, it was collected from Sree Kundeshawri Aushadhalaya Ltd, Chittagong.

### Dose and route of administration:

For optimal dosage accuracy mallasindura was administered in such a volume so that it could not cause a much increase of total volume of body fluids. The drug was administered per oral route at a dose of 100 mg/kg body weight to observe the effect on liver function parameters of rat plasma.

### Experimental animals and their Management:

Forty eight-week old albino rats (*Rattusnovergicus*: Sprague-Dawley strain) of both sexes, were used in this experiment. These animals were bred and maintained at the Animal House of the Department of Pharmacy, Jahangirnagar University, were apparently healthy and weighed 50 to 70 g. The animals were housed in a well-ventilated hygienic experimental animal house.

Constant environmental and adequate nutritional conditions were maintained for the animals throughout the whole experimental period. Plastic cages having dimensions of 30 x 20 x 13cm were used to keep the rats 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 a natural day night cycle. They were fed with "mouse chow" (prepared according to the formula developed at BCSIR, Dhaka). Absolute compliance as well as ethical guide for care and use of laboratory animals was followed to carry out all experiment on rats. Before starting the experiment, the animals were carefully marked on different parts on their body which was later used as identification mark for a particular animal. Therefore, the response of a particular rat prior to and after the administration was noted separately.

A group of equal number of rats were used for drug treated group and control group. Distilled water was administered to the animals 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. At the early stage of the experiment the animals were randomly divided into four groups of 10 animals per sex. Therefore, ten rats were taken for both control and 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 4,000 rpm 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 hours of sample collection.

### Determination of the liver function parameters:

Biochemical analysis was carried out to determine the state of the liver. The studies involved analysis of parameters such as total protein, serum albumin, liver enzymes such as serum glutamic oxaloacetic

transaminase (SGOT), serum glutamic pyrubic transaminase (SGPT) and alkaline phosphatase (ALP). Biuret method (Plummer 1971) was used to determine the total protein content. [17] Serum bilirubin concentration was analyzed by using Evelyn and Malloy (1938) method [18].

The method described by kind and king (1954) was used to determine the alkaline phosphates activities [19]. UV- visible spectrophotometer (Model No: UV-1601 PC) was used to determine the absorbance for the whole test.

The results of the group were presented as the Mean±SEM (Standard error of the mean).Unpaired "t' tests were performed and Statistical package for social sciences (SPSS) and WINDOWS (Ver.10) were used for the analysis of those data. The level of significance were determined at $p < 0.05$, 0.01 and 0.001

**RESULTS:**

A similar trend of observation for the biochemical parameters of rat plasma was noted for both sexes while comparing with control groups.

In the male rats, there was increase in total protein and albumin content and it was statistically very highly significant (p=0.001) for total protein and significant for albumin (p=0.013). (**Table 2, Graph 1**) Along with this, there was an increase in the ALP content (**Table2, Graph 3**) but it was not statistically significant (p= 0.685). Moreover, there was decrease in bilirubin, sGPT and sGOT activities in which changes in bilirubin and sGOT activities were statistically highly significant with a p value of 0.001 and 0.009 respectively (**Table 2, graph 2** and **3**).

**TABLE 2: EFFECT OF MALLASINDURA (MSL) ON LIVER FUNCTION PARAMETERS OF RAT PLASMA**

| Parameters | Control Male | MSL male | p-Values | Control Female | MSL Female | p- values |
|---|---|---|---|---|---|---|
| Total protein | 5668.099 ± 113.6431 | 6330.400 ± 97.8394 | (p=0.001)*** | 5587.5451 ± 109.1316 | 6471.1070 ± 68.0745 | (p=0.001)*** |
| Albumin | 4566.4226 ± 106.8903 | 5918.4895 ± 58.0898 | (p=0.013)* | 4467.8174 ± 82.9280 | 4991.7906 ± 39.3837 | (p=0.009)** |
| Bilirubin | 0.1323 ± 0.003957 | 0.08835 ± 0.003308 | (p=0.001)*** | 0.11512 ± 0.004082 | 0.05814 ± 0.003392 | (p=0.001)*** |
| sGPT | 61.9538 ± 0.1363 | 59.5953 ± 0.1946 | (p=0.019)* | 58.5861 ± 0.1401 | 55.6263 ± 0.1901 | (p=0.001)*** |
| sGOT | 111.345 ± 0.2824 | 107.2960 ± 0.677 | (p=0.009)** | 104.935 ± 0.2296 | 103.1971 ± 0.3407 | (p=0.058) |
| ALP | 44.9107 ± 0.1065 | 45.9378 ± 0.1113 | (p=0.685) | 42.2093 ± 0.1105 | 43.4886 ± 0.128 | (p=0.593) |

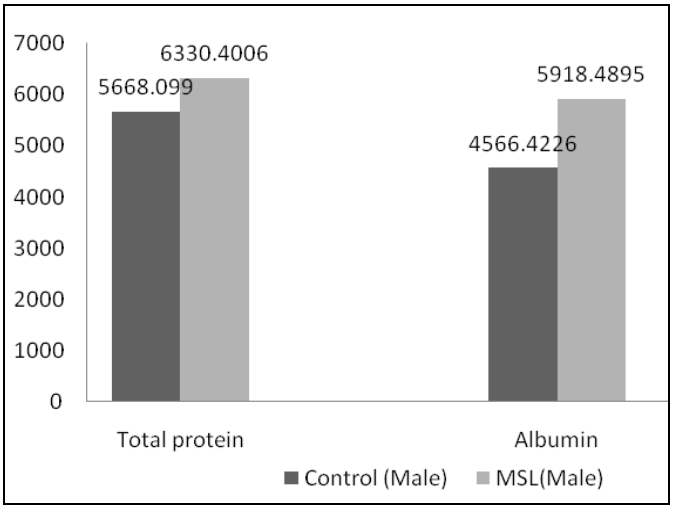
In case of female rats, a similar trend was noticed for total protein and albumin content and it was statistically very highly significant with a p value of 0.001 and 0.009 respectively (**Table2, Graph 4**). The increase in ALP content of female rats was not statistically significant (**Table2, Graph 6**). Simultaneously, there was a decrease in billirubin, sGPT and sGOT activities in which the results for both billirubin and sGPT was statistically very highly significant with a p value of 0.001 in each case (**Table 2, Graph 5** and **6**).

**DISCUSSION:** The synthetic ability of liver can be detected by assessing plasma protein especially serum albumin. Serum bilirubin and alkaline phosphatase can be used to predict hepatic excretory function [20]. Bilirubin concentration in the blood is a useful indicator for the diagnosis of liver disease. Bilirubin can be leaked from liver into the blood due to impaired liver function or blocked bile secretion in a disease condition called jaundice in which skin and eyeballs becomes yellowish [21].

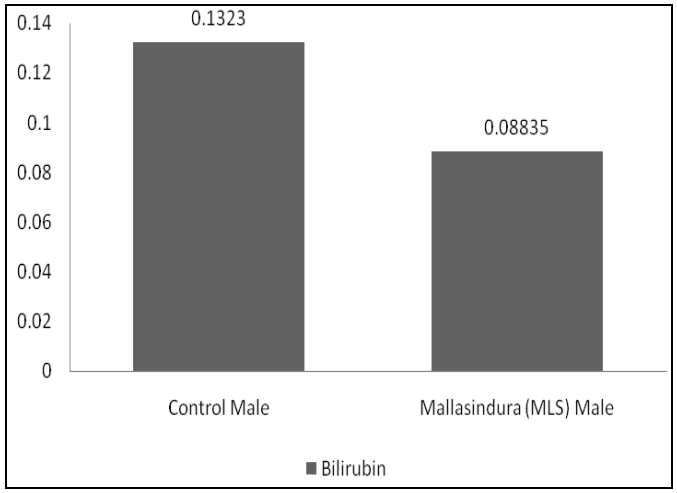
Unconjugated bilirubin has a greatest importance to the practitioner as it is the potentially toxic form of bilirubin. From the breakdown of hemoglobin, bilirubin is produced and then transported to the liver after binding with albumin. However, a small amount of bilirubin remains unbound or free in the plasma. Hyperbilirubinemia may be contributed due to defective hepatic uptake, impaired conjugation or impaired hepatic excretion of bilirubin [22]. As there was a greater reduction in bilirubin content while consuming mallasindura it means an improved liver function.

Hepatic enzymes are cytoplasmic in nature and therefore, alteration of membrane permeability occurs due to hepatocyte injury or liver damage. As a result, hepatic enzymes such as sGPT (ALT), sGOT(AST) are usually leaked into circulation [23]. Liver's protein synthesizing ability and excretory function can also be detected by using serum liver enzymes. Elevated serum enzyme is the early sign of liver injury. sGPT and sGOT can be raised parallely in most cases of liver damage and increase of those enzymes can be dramatic in case of acute hepatocellular injury, viral hepatitis, hypoxic or ischemic injury, and acute toxic injury or Reye syndrome [20].

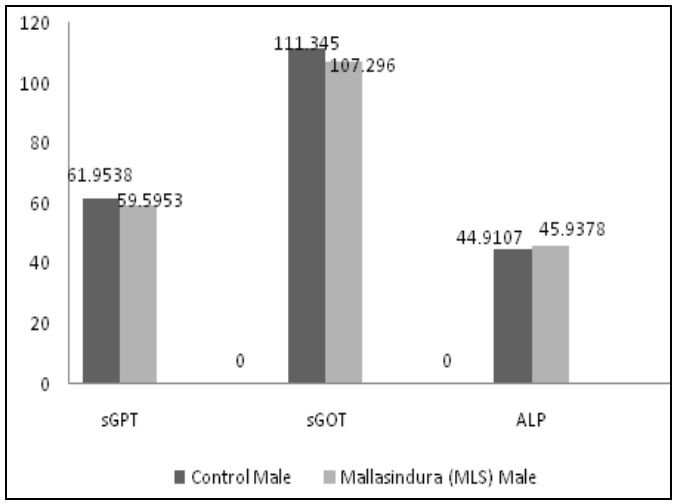
In the present study, a statistically significant reduction of sGOT and sGPT activities was observed which means that administration of MSL may have hepatoprotective effect.



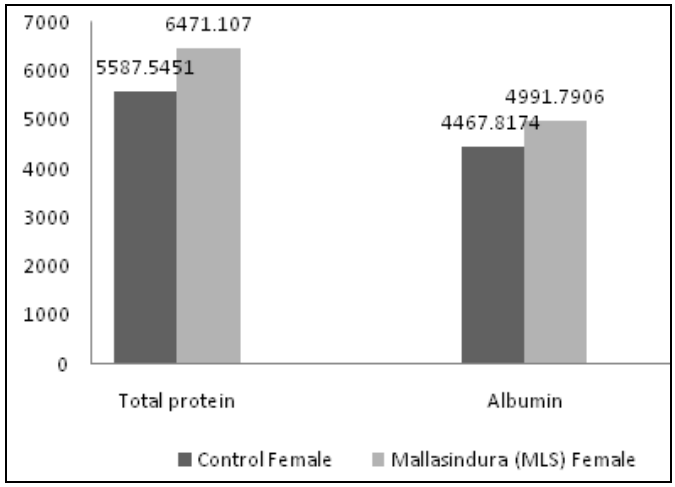

**GRAPH 1: COMPARATIVE GRAPHICAL PRESENTATION OF TOTAL PROTEIN AND ALBUMIN CONTENT OF RAT PLASMA BETWEEN CONTROL MALE AND MALLASINDURA MALE.**



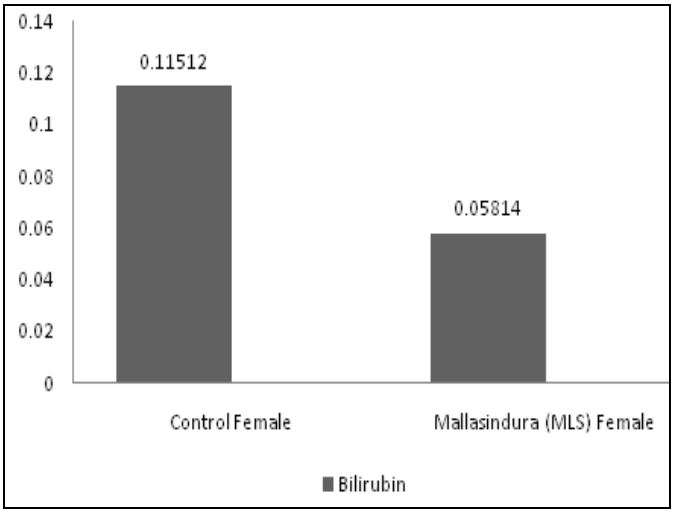

**GRAPH 2: COMPARATIVE GRAPHICAL PRESENTATION OF BILIRUBIN CONTENT OF RAT PLASMA BETWEEN CONTROL MALE AND MALLASINDURA MALE.**



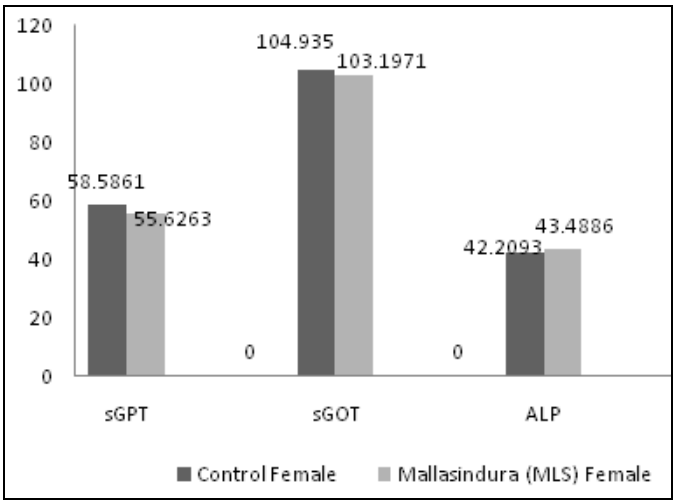

**GRAPH 3: COMPARATIVE GRAPHICAL PRESENTATION OF SGPT, SGOT AND ALP CONTENT OF RAT PLASMA BETWEEN CONTROL MALE AND MALLASINDURA (MSL) MALE.**




**GRAPH 4: COMPARATIVE GRAPHICAL PRESENTATION OF TOTAL PROTEIN AND ALBUMIN CONTENT OF RAT PLASMA BETWEEN CONTROL FEMALE AND MALLASINDURA FEMALE.**




**GRAPH 5: COMPARATIVE GRAPHICAL PRESENTATION OF BILIRUBIN CONTENT OF CONTROL FEMALE AND MALLASINDURA FEMALE.**




**GRAPH 6: COMPARATIVE GRAPHICAL PRESENTATION OF SGPT, SGOT AND ALP CONTENT OF RAT PLASMA BETWEEN CONTROL FEMALE AND MALLASINDURA FEMALE.**

**CONCLUSION:** Ayurvedic preparation Mallasindura (MSL) is used to treat asthma. Biochemical parameters of rat plasma were observed after chronic administration of mallasindura to predict the condition of the liver. No toxic effect was observed from the result of the present study. The greater increase of total protein and albumin and significant decrease of billirubin and enzymatic activity indicated the improved liver function. Therefore, it can be presumed that the ayurvedic drug Mallasindura can be used in the treatment of asthma for long term without any toxic effect on liver.

**ACKNOWLEDGEMENT:** Mr. Shafiqur Rahman is greeted thankfully by the authors for his contribution of technical assistance for the whole period of study.

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**How to cite this article**:
Begum G, Hamid K and Choudhuri MSK: Toxicological Effect of Mallasindura (MSL) On Liver Function Parameters of Rat Plasma after Chronic Administration. Int J Pharm Sci Res 2015; 6(5): 1958-64.doi: 10.13040/IJPSR.0975-8232.6(5).1958-64.