Hepatoprotective effect of edible Oyster mushroom *Pleurotus cornucopiae* against Sodium Arsenite induced hepatotoxicity in rats

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**Abstract**

Arsenic poisoning in ground water had been major challenge for the mankind in arsenic hit areas. In India, this problem is a big alarm in the entire Gangetic belt. The arsenic poisoning through drinking water has caused lots of health hazards to humans especially related hepato-biliary. This present study was carried out to investigate the therapeutic role of the ethanolic extract of *Pleurotus cornucopiae* on Sodium arsenite induced hepatotoxicity in rats. Sodium arsenite at the dose of 8mg/kg body weight orally caused liver damage in rats as manifested by the significant rise in serum levels of Serum Glutamic Pyruvate Transaminase (SGPT), Serum Glutamate Oxalate Transaminase (SGOT), Alkaline Phosphatase (ALP) and Bilirubin levels compared with control. Oral administration of extracts of *P. cornucopiae* 400 mg/kg body weight for 30 days to Sodium arsenite treated rats showed significant amelioration in these levels. Thus, the entire study reveals that ethanolic extract of *P. cornucopiae* at the dose of 400 mg/kg body weight shows hepatoprotective effect against arsenic induced toxicity.

**Keywords:** Pleurotus cornucopiae, Sodium arsenite, SGPT, SGOT, ALP, Bilirubin.

**Introduction**

Liver disease is considered as one of the serious health related problems, as it is one of the important organ responsible for detoxification and deposition of endogenous and exogenous substances. Steroids, vaccines and antiviral drugs which have been employed as a therapy for liver diseases, have potential side effects especially when administered for long duration. Hence, hepatoprotective drugs from plant sources seem to be attractive alternative. Ayurveda is a traditional system of medicine being protected in Indian sub-continent for over 5000 years. In ayurveda several herbal drugs have been prescribed as ‘liver tonics’ to reduce the toxicity due to ingested xenobiotics. It gives an elaborate amount of medical plants, their uses in herbal therapeutics based upon traditional wisdom and knowledge. Some 400mg mentioned in ayurveda are highly reputed for their potential benefits in the treatment of liver disorders[1]. Arsenic is a naturally occurring metalloid (atomic number 33), located on group V of the periodic table. Exposure to high levels of arsenic through drinking water has been recognized for many decades in some regions of the world, i.e. China, India, and some countries in Central and South America. A huge population is at risk of cancer and other diseases because of chronic arsenic exposure[2,3]. Environmental exposure to arsenic can cause a variety of cancers, most commonly non-melanoma skin cancers, and chronic toxicity may manifest as diffuse symptoms not easily recognizable as chronic heavy metal toxicity. General adverse health effects associated with human exposure to arsenicals include cardiovascular diseases, neurologic and neurobehavioral disorders, developmental abnormalities, hearing loss, diabetes, fibrosis of the liver and lung, haematological disorders and blackfoot disease [4-6]. In humans, arsenic is known to cause cancer of the skin [7] lung, bladder, liver and kidney [4,5,8]. Arsenic is methylated by alternating reduction of pentavalent arsenic to trivalent and addition of a methyl group from S-adenosylmethionine [9]. Glutathione and possibly other thiols, serve as reducing agents [10,11]. Liver is the most important site of arsenic methylation [9,12] but most organs show methylating activity. The end metabolites are methylarsenic acid (MMA) and dimethyl arsenic acid (DMAA). These compounds are readily excreted in urine. However, reactive intermediates may be formed. Arsenite is known to bind to cellular sulfhydryl, particularly vicinal ones, accounting for its ability to interfere with energy generation [13]. Once in the tissues, arsenic exerts its toxic effects through several mechanisms, the most significant of which is, the reversible combination with sulfhydryls groups. Arsenic also inhibits numerous other cellular enzymes, especially those involved in cellular glucose uptake, gluconeogenesis, fatty acid oxidation and product-ion of glutathione through sulfhydryl group binding [14]. A second major form of toxicity is termed as “arsenolysis”. Pentavalent arsenate can substitute competitively for phosphate in biochemical reactions, where ADP normally phosphorylates to form ATP. In the presence of arsenic,ADP arsenate is the end product and high energy phosphate bonds are not formed. The unstable ADP-arsenate decomposes spontaneously and irreversibly resulting in loss of energy by the cell. ROSs is capable of damaging a wide variety of cellular macromolecules including DNA, lipids and proteins. Finally, cellular signal transduction can be altered (e.g. activation of Trans factors, changes of gene

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expression), cell growth, proliferation and differentiation can be promoted and apoptosis leading to cell death or cancer developments can be induced [15,16]. Mushrooms are nutritionally functional food and a source of physiologically beneficial and nontoxic medicines [17]. They have been used in folk medicine throughout the world since ancient times. Attempts have been made in many parts of the world to explore the use of mushrooms and their metabolites for the treatment of a variety of human ailments. The significant medicinal effect of mushrooms and their metabolites are attention of the public [18]. Pleurotus species are commonly called Oyster mushrooms. There are approximately 40 species of this mushroom. They are distributed worldwide, both in temperate and tropical parts of the world. Oyster mushrooms now rank second among the important cultivated mushrooms in the world [19]. The significant pharmacological effects and physiological properties of mushrooms are bioregulation (immune enhancement), regulation of biorhythm and maintenance of homeostasis and cure of various diseases and prevention and improvement from life threatening diseases such as cancer, heart diseases and cerebral stroke. Mushrooms are well known to have effective substances for antifungal, anti-inflammatory, antiviral, antidiabetic, hypolipidemic, antitumor, antibacterial, hepatoprotective, antithrombotic and hypotensive activities [17, 20]. Hence, search for new anthepatotoxic substances from mushrooms has been a matter of great importance.

Materials and Methods

Experimental animals

Charles Foster rats (24 females), weighing 160g to 180g of 8 weeks old, were obtained from animal house of Mahavir Cancer Institute and Research Centre, Patna, India (CPCSEA Regd-No. 1129/bc/07/CPCSEA). The research work was approved by the IAEC (Institutional Animal Ethics Committee) with IAEC No. IAEC/2012/12/04. Food and water to rats were provided ad libitum.

Preparation of ethanolic extract of Pleurotus cornucopiae

The medicinal plant used for the experiment was Pleurotus cornucopiae (Oyster Mushroom). Fresh mushrooms of cornucopiae genera were purchased from Gitanjali farm, Pusa, Samastipur, Bihar, India. The mushroom was identified by Dr. Ramakant Pandey (Botanist), Department of Biochemistry, Patna University, Patna. The mushroom was washed in tap water and left for air dry. Then it was kept in incubator at 37-40°C for 24-48 hours until it dries and crushed in mortar and pestle to make it into powder form. Weight of the powder was taken and diluted for 5 times in 70% of alcohol and were left for 24 hours to soak. Ethanolic extract was taken by using Soxhlet. Residual solid part was again dried and crushed to make fine powder. Then it was filtered and 400mg of P. cornucopiae was diluted in 5% of ethanol (400 mg dissolved in 10 ml of the ethanol as stock solution) and administered to rats.

Experimental Design

The animals were divided into 5 groups of 6 rats each. Group 1: Control rats given physiological saline solution 10 ml/kg body weight Group 2: Rats given Sodium arsenite (8 mg/kg body weight) for 45 days orally once daily using an intragastric tube. Group 3: 45 days Sodium Arsenite pre-intoxicated rats then administered with P. cornucopiae 400 mg/kg body weight for 30 days orally once daily using an intragastric tube. Group 4: Rats administered Sodium Arsenite 8mg/kg body weight + P. cornucopiae 400 mg/kg body weight administrated orally. Group 5: 45 days Sodium Arsenite 8 mg/kg body weight intoxicated rats left for 30 days on normal diet. At the end of the experimental period in 24 h after last treatment the animals were sacrificed by anesthetizing with diethyl ether. Blood was collected without anticoagulant for the separation of serum. The liver tissues were excised immediately and washed with chilled physiological saline and fixed in 10% neutral formalin for light microscopy study. For the light microscopic study the Haemotoxylin-Eosin stained slides were prepared and the sections were viewed under light microscope.

Biochemical analysis

Blood samples were taken into centrifuge tube with rubber caps, labeled and centrifuged at 3000 rpm for 15 minutes. The Liver Function Test (LFT) as Serum Glutamic Pyruvate Transaminase (SGPT) and Serum Glutamic Oxaloacetate Transaminase (SGOT) were measured according to method [21], Alkaline Phosphate (ALP) by method [22] while total bilirubin activity by method [23].

Statistical analysis

Results are presented as mean ± S.E and total variation present in a set of data was analyzed through one-way analysis of variance (ANOVA). Difference among means has been analyzed by applying Dunnet’t test at 99.9% (p < 0.001) confidence level. Calculations were performed with the Graphpad Prism Program (GraphPad Software, Inc., San Diego, USA).

Results

Morbidity and mortality

The rats after arsenic exposure (8mg kg⁻¹ body weight) for 45 days had shown symptoms of toxicity such as nose bleeding, nausea, lack of body co-ordination (11 percent of rats showed paralysis like symptoms), blackening of tongue and foot and general body weakness.

Biochemical changes

Arsenic intoxicated group showed a significant increase in serum SGPT, SGOT, ALP and Bilirubin content as compared to control after acute treatments (p<0.05). When Group1, Group2, Group3,
Group 4 and Group 5 were compared, a significant (p<0.05) difference was obtained [Graph Figure 1-4].

Figure 1: Effect of *Pleurotus cornucopiae* on Arsenic induced toxicity showing SGPT activity (n=6, values are mean± S.D).

Figure 2: Effect of *Pleurotus cornucopiae* on Arsenic induced toxicity showing SGOT activity (n=6, values are mean± S.D).

Figure 3: Effect of *Pleurotus cornucopiae* on Arsenic induced toxicity showing ALP activity (n=6, values are mean± S.D).

Figure 4: Effect of *Pleurotus cornucopiae* on Arsenic induced toxicity showing Bilirubin activity (n=6, values are mean± S.D).
Discussion

Liver is an important organ actively involved in metabolic functions and is a frequent target of number of toxicants. The main function of the liver is detoxification of xenobiotics and toxins [24]. Because liver performs many vital functions in the human body, damage of liver causes unbearable problems [25]. SGPT and SGOT are the most often used and most specific indicators of hepatic injury and represent markers of hepatocellular necrosis [26]. Arsenic is known to produce damages in liver function [27]. SGPT and SGOT are reliable determinants of liver parenchymal injury [28]. Activities of SGPT and SGOT are significantly increased in arsenic treated rats indicating liver dysfunction. Assay of serum ALP activity has been recognized as a suitable marker of skeletal and hepatobiliary disorder. Moreover, an elevated serum level of ALP activity is frequently associated with various pathological conditions [29-31]. Alkaline phosphatase is a non-specific tissue enzyme widely spread, mainly in the osteoblasts, liver and biliary canaliculi [32]. Bilirubin is the conventional indicator of liver diseases [33]. Hyperbilirubinemia is a very sensitive test to substantiate the functional integrity of the liver and severity of necrosis which increases the binding, conjugating and excretory capacity of hepatocytes that is proportional to the erythrocyte degeneration rate [34]. In the present study, administration of Sodium arsenite treated rats showed an increase in the levels of SGPT, SGOT and ALP activities when compared with control rats. Oral administration of P. cornucopiae (400 mg/kg body weight) to sodium arsenite treated rats showed an inhibition in the elevated levels of serum SGPT, SGOT, ALP and Bilirubin than sodium arsenite alone treated rats. Similar changes noticed that oral administration of P. cornucopiae and sodium arsenite together treated rats showed SGPT, SGOT, ALP and bilirubin levels were decreased. Yet, no study reports the therapeutic effect of Pleurotus cornucopiae against arsenic induced toxicity in in-vivo system. Thus, Pleurotus cornucopiae is the novel medicinal plant possessing the hepato-protective effect against arsenic induced toxicity.

Conclusion

In the present times, the study on search for antidote against arsenic induced toxicity is very few. The present study is indeed a novel work which deciphers for the first time as the antidote against arsenic induced toxicity. Oral administration of Pleurotus cornucopiae (400 mg/kg body weight) on sodium arsenite pre-treated rats showed significant amelioration. These properties of P. cornucopiae make it a suitable and novel antidote for arsenic toxicity in rodents and possibly in human subjects.

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Conflict of Interest

The authors declare no conflict of interest.

References

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