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Role of nutmeg in minimizing hepatotoxicity due to arsenic in albino rats

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Abstract- Arsenic is pervasive element in the environment and cause serious health problem in most of the countries. In India west Bengal, Bihar and Jharkhand found arsenic contamination in underground water. It causes serious health problem in human such as cancer in liver, kidney skin etc. It became severe in low-income nation such as Bangladesh. Arsenic out-turn into liver injury. In present study was conducted to evaluate the protective role of nutmeg (Myristica fragrans) against arsenic induced toxicity in albino rats. Albino rats were divided into three groups. Group A were control rat, group B received an acute dose of arsenic (8mg/kg bw) orally, group C received an acute dose of arsenic followed by daily administration of nutmeg (300mg/kg bw) orally. Arsenic treatment leads to increase in the liver injury. Biochemical analyses of treatment group show Arsenic induced rats had significantly (p\0.0001) found the changed serum levels of Serum Glutamic-Pyruvic Transaminase (SGPT), Serum Glutamic-Oxaloacetic Transaminase (SGOT), Alkaline Phosphatase (ALP), total bilirubin, urea, uric acid, creatinine and albumin and its degenerative effect; But, after the administration of M. fragrans extract, there was significant ($p<0.0001$) restoration observed in these liver and kidney function. Nutmeg become significant protection in the palliation of arsenic induced liver injury. The aim of the present review is to introduce *Myristica fragrans* as a potent medicinal property by highlighting its pharmacological and clinical application.

Key words: Arsenic, Hepato-Renal, *Myristica fragrans*, Medicinal Property

INTRODUCTION

Arsenic is a highly toxic metalloid, which is distributed in the natural and anthropogenic sources.[1] It became an important health concern. Arsenic in the bedrock or soil readily dissolves in surrounding and ground water and a high concentration of arsenic i.e above the WHO guideline level of 10µg L-1. Diseases associated with arsenic were pandemic in many countries.[2] Skin, liver, lung, and bladder cancers are caused by chronic exposure to arsenic-contaminated water and food.[3-7]

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Due to growing human activities such as mining, smelting, and pesticide manufacture, the risk of arsenic compounds has been elevated, resulting in arsenic accumulation in the soil.[8] Arsenic induces lipid peroxidation and thus act as a prooxidant and bring about protein and enzymeoxidation,glutathione depletion, DNA oxidation and DNA adducts.[9] Arsenic become serious problem to human life.

Plants and its products are being used as medicine since long year. There are large number of phytochemical property is found in plant food product.[10] *Myristica fragrans* is commonly known as "nutmeg". It produces

two spiece: mace and nutmeg. It belongs to the family myristicacea in the order magonliales. It possesses various therapeutic properties. For a long time *M.fragrans* has been used as a folklore medicine for treating diarrhea, mouth, sore and insomnia.[11] Nutmeg crude extract has been shown to have chemopreventive and anti-Helicobacter pylori properties.[12] It also shows hepatoprotective activities. Nutmeg contains numerous constituents, such as essential oil (monoterpenes, phenylpropanoids, myristicin, elemicin, safrole, eugenol, methyl isoeugenol), lignans and neolignans, diphenylalkanes, phenylpropanediols, steroids and cyclobutanones.[13] Cytotoxic and apotoxic effects of Myristicine have been reported such that cell viability was reduced by exposure to Myristicine in a dose dependent manner.[14]

The liver is an intra-abdominal organ (belonging to the gastrointestinal tract) that plays an important role in the detoxification and excretion of many endogenous and exogenous substances. The liver is a natural chemical factory that helps metabolism and detoxifies complex molecules. It neutralizes toxins and produces bile which helps in the digestion of fats and the elimination of toxins from the intestines.[15] The liver plays an important role in breaking down fat, converting glucose into glycogen, and maintaining proper blood sugar levels. Nutmeg is commonly used at home for cooking, flavouring, and in medicine, which is believed to have intermediate histological effects also on the kidney distorting the cyto-architecture of the renal corpuscle[16] at higher dose (500mg/kg) as aphrodisiac dose and psychoactive agent in male Wister rats. Nutmeg allergies are extremely rare, and it is a very safe spice.

The aim of the present study is to introduce *M. fragrans* as a potent medicinal plant by highlighting its traditional applications as well as the recent findings for novel pharmacological and clinical applications. Nutmeg is often used as a spice in a variety of meals, as well as in teas and soft drinks, as well as in milk and alcohol. the possible effects of nutmeg on the microscopic architecture of the liver would be assessed in the present studies.

METHOD & METHODOLOGY

Chemical :- Chemical Arsenic was used as sodium (meta) arsenite (90%) manufactured by Sigma-Aldrich, USA (CAS Number: 7784-46-5; S7400-100G), Lot# SLBH5736V, P Code 1001683292 was purchased from the licensed and approved scientific store of Patna, Bihar, India.

Preparation of *Myristica fragrans* seed Ethanolic Extract :- In the present study, the nutmeg from the nearby local market of sufficient amount were bought, washed with distilled water to remove dirt and soil, dried in shade and finally crushed to powder. The powdered material was soaked with absolute ethanol approximately 200 ml ethanol. The material was then left for 48 hrs, the material was put in the water bath at 65^0C and pressure was 3000-4000 psion a rotary evaporater. Now ethanolic extract was stored in fresh container. For administration 10 ml dose was prepared before administration.

Animal :- Twenty-four female healthy Charles Foster rats, were randomly selected of 8 weeks old weighing 160 g to 180 g for this study. The animals were procured from the animal house of Mahavir Cancer Sansthan and Research Centre, Patna, India (CPCSEA Reg-No. 1129/bc/07/CPCSEA). The experimental work was approved by the Institutional Animal Ethics Committee (IAEC) with IAEC No. 2020/1B-27/08/20. All animal trials followed the rules set forth by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), based in New Delhi, India.

Experimental Design :- Rats were randomly distributed into control and treated groups:

Group I: (n = 6) Control. Group II: (Arsenic treated)-18 rats were orally induced with arsenic at the dose of 8 mg/kg body weight/day for 90 days. After 90 days of treatment with arsenic, group II rats were further divided into three sub groups (n = 6 each).

Subgroup I: (Arsenic-treated rats)-Rats were euthanized to measure the level of arsenic toxicity.

SubgroupII: (Arsenic pretreated control subgroup)-Rats were left untreated for the next 90 days to allow for auto recovery.

Subgroup III: (*M.fragrans* administered sub group)-Rats were orally administered with *M.fragrans* ethanolic rhizome extract at the dose of 300 mg/kg body weight/day for further next 90 days.

Biochemical Assay :-Biochemical analysis was performed through the serum by standard kit process (Coral crest) on (UV- Vis) spectrophotometer (UV-10, Thermo Scientific, USA). In liver function tests (LFT), Serum glutamate pyruvate transaminase (SGPT) and

serum glutamate oxaloacetate transaminase (SGOT) were measured using the Reitman and Frankel method, alkaline phosphatase (ALP) using the the Kind and King method, and total bilirubin activity using the Jendrassik and Grofs method.

RESULT

When compared to the control group, the arsenic-treated group had a substantial (p0.0001) rise in serum levels of SGPT, SGOT, ALP, and total bilirubin. The presence of an elevated hepatic serum marker implies a problem with liver function.

However, when arsenic-treated rats were left without medication for another 90 days for auto repair, serum levels of SGPT, SGOT, total bilirubin, and ALP were significantly lower (p0.0001) than when arsenic-treated rats were left without treatment. After the *M.fragrans* administration upon arsenic treated rats, there were significant (p\0.0001) decrease in the serum levels of SGPT, SGOT, ALP and total bilirubin in comparison to the arsenic treated control rats, denoting the protective effect of the plant extract against arsenic induced hepatotoxicity (Figs. 1, 2, and 3).

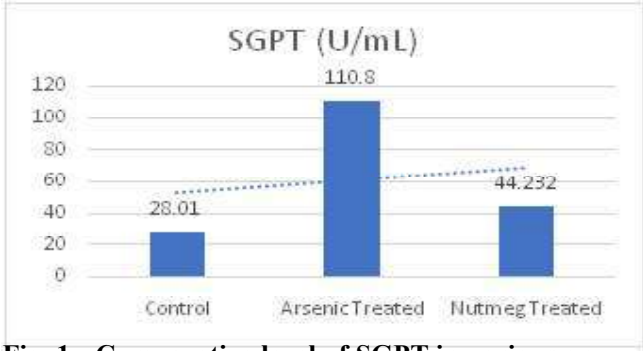


Fig. 1 - Comparative level of SGPT in various groups of rats(n=6)

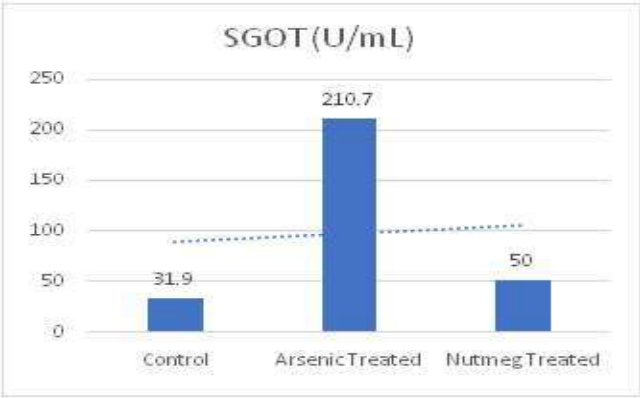


Fig. 2 - Comparative level of SGOT in various groups of rats (n=6)

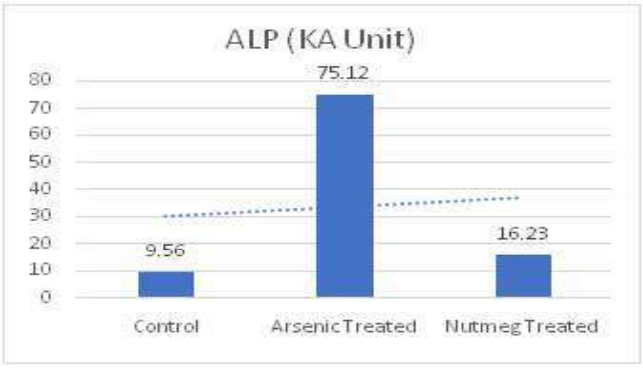


Fig. 3 - Comparative level of ALP in various groups of rats (n=6)

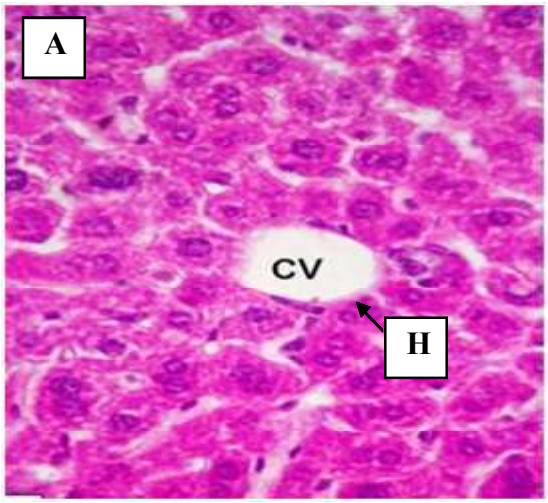
DISCUSSIONS

Now a day's no of people from different countries like India, Bangladesh and West Bengal suffers from chronic and acute toxicity. The chronic exposure to arsenic causes various types of carcinogenic and non-carcinogenic health effects. Arsenic has been found to affect a few downstream signalling cascades, as well as cell development, proliferation, and death. Prolonged consumption of arsenic contamination liquids such as flowle's solution has been known to cause similar hepatic lesions. In the present study, arsenic treated rats showed significantly increased in the levels of SGPT, SGOT, ALP and total Bilirubrin. It causes impairment in liver function and its property as the human subjected to long term consumption of arsenic contaminated water was needed for generation of hepatic fibrosis. After 6 months of arsenic exposure, there is elevation of hepatic arsenic, it confirms arsenic accumulation in the liver.

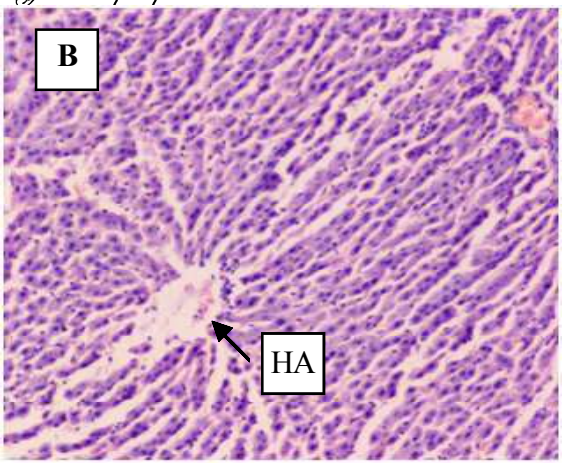
Gora *et al.* (2015)[17] evaluated that high dose of arsenic cause acute liver damage and hepatocyte necrosis, causing hepatocyte enzymes to leak into the blood. In addition, they observed that the degree of damage to hepatocytes was detected by the activity of antioxidant enzymes. In the present study it is observed that the relative weight of the rat liver was increased in arsenic treated in comparison to control rat. This observation was confirmed in the study by Shiguang and Beynem (2001)[18]., they also reported a significant increase in the liver weight in male rat feed 100mg/kg of arsenic for 2 week. Because the liver is a highly active metabolic site, it is a major site of arsenic poisoning and is involved in the methylation process using S-adenosylmethionine as the methyl donor by the arsenic methyl transferase enzymes GSH as an essential cofactor.[19]

Because the liver is the primary site of protein synthesis, arsenic-induced hepatic damage results in a decrease in the level of antioxidant enzymes with arsenic trioxide. Recent studies have clearly shown that arsenic compounds during metabolism in cell generate reactive oxygen species, hydroxy radical and hydrogen peroxide leading to oxidative stress. Liu *et al.* (2001)[20] suggested that enhanced production of free radicals and inhibition of antioxidant enzymes as possible to explain arsenic induced oxidative damage.

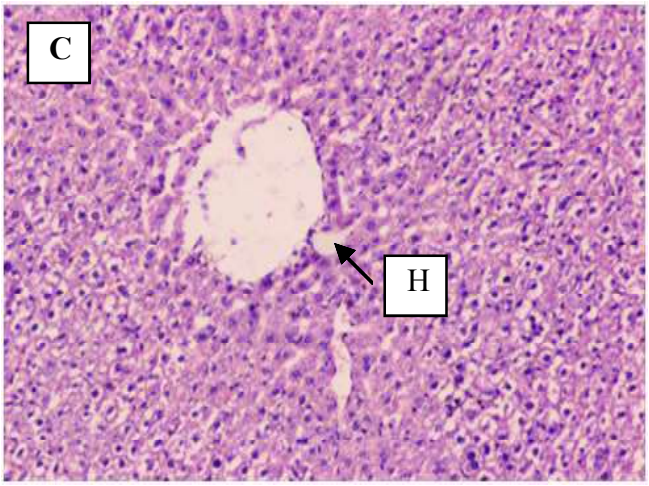
Arsenic induced toxicity arousing increased production of hydrogen peroxide. These effect cause formation of reactive oxygen species resulting in oxidative stress.[21] As shown in fig. 1, the activity of SGPT, SGOT, ALP, and total bilirubin in serum can be used to identify liver impairment. The increase in SGOT and SGPT in serum may be due to hepatocellular necrosis, which causes increase in the permeability of the cell membrane resulting in release of transaminases in the blood stream consequently, the hepatic serum biomarkers perturbations seem to be correlated with the liver histopathological study. The histological examination revealed severe abnormalities in the hepato-cellular architecture, including degraded hepatocytes, sinusoidal vacuolizations, and portal vein haemorrhages, which are consistent with prior studies.[22] Furthermore, serum levels of SGPT, SGOT, ALP, and total bilirubin were significantly reduced after *M.fragrans* was administered to arsenic-pretreated rats. *M.fragrans* also stabilised the hepatic membrane's functional integrity and histological alterations.



A: The normal architecture of the liver was visible in the control liver segment, with well-arranged H and CV X500.



B: Degeneration in the H, haemorrhages in the PV, and vacuolizations in the sinusoids X400 in an arsenic-treated liver segment.



C: The *M.fragrans* administration upon arsenic pretreated sub group liver sections showing significant restoration in the CV and H X500.

(CV- Central vein, H- hepatocytes, HA- Hepatic Artery)

Fig. 4 Microphotograph of liver sections stained with hematoxylin and eosin

CONCLUSION

The overall conclusion of the study is that arsenic intoxication causes severe liver damage. Furthermore, there was a considerable improvement in the liver's functioning after *M. fragrans* administration. Through suppression of lipid peroxidation, there was also considerable restoration at the cellular level. Hence, *M. fragrans* possesses phytoremedial properties against arsenic induced toxicity in rats, which in the future might be taken for the therapeutic development of antidote drug against arsenic poisoning in liver and kidney.

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