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Ameliorative potential of *Tamarindus indica* seed coat against arsenic-induced hepatotoxicity in wistar rats

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Abstract
The aim of this study was to ascertain the toxic effect of sodium arsenite in rat and its amelioration by supplementation with *Tamarindus indica* seed coat powder (TISCP). Eighteen albino rats were randomly assigned to three groups. All animals received standard feed and water. The first group served as healthy control. The second and third group received sodium arsenite (2mg/kg b.wt) through drinking water. The third group also received TISCP (200 mg/kg body weight) daily for a period of 45 days. Biological samples were analysed for biochemical parameters and oxidative biomarkers for the determination of hepatotoxic effect in arsenic intoxication. Significant (P<0.05) increase activity of serum AST, ALT and ALP whereas decreased total protein and albumin level was recorded as a marker of hepatic damage. Decrease antioxidant status of animals was recorded with increased lipid peroxidation levels. Co-administration of TISCP during exposure to arsenic through drinking water restored serum hepatic and oxidative markers. It is concluded that TISCP has an ameliorative potential to protect the liver from arsenic induced damage.

Keywords: Rats, arsenic, toxicity, hepatic, tamarind

1. Introduction
Exposure to heavy metals has become a common problem throughout the world due to contaminated drinking water, food and air .Arsenic is one of most important metalloid persists in organic, inorganic and elemental form in nature and is ranked topmost in a list of 20 hazardous substances by the Agency for Toxic Substances and Disease Registry and the United States Environmental Protection Agency [1]. Trivalent arsenic species are most toxic than pentavalent arsenic compounds [2]. Long-term exposure to arsenic causes a wide range of adverse effects on health, including weight loss, skin lesions, cancer, cardiovascular disease (CVD), diabetes, liver disorders, immunotoxicity etc [3]. Arsenic mainly effects liver and kidney (Roy *et al*., 2008) and causes damage to them. Heavy metals induce over production of reactive oxygen species (ROS) and consequently enhance lipid peroxidation. Arsenic toxicity results from its ability to interact with sulfhydryl groups of enzymes and disrupt enzymes involved in cellular respiration that leads to inhibition of glycolysis and Krebs cycle and substitute phosphorus in a variety of biochemical reactions [4]. Recent findings suggested that exposure to arsenic causes oxidative stress through increased generation of Reactive Oxygen Species and inhibition of antioxidants in the body [5]. Generally arsenic undergoes hepatic biomethylation to monomethylarsonic acid and dimethylarsinic acids and are potent inhibitors if GSH reductase and causes hepatotoxicity in human and animals [6]. Arsenic toxicity is a serious problem worldwide, as there is no specific and efficacious therapeutic treatment of arsenicosis. The need for an effective therapy for arsenicosis is therefore obvious [7]. Due to rich biodiversity of India, a large number of plant species are available for treatment of various toxicities. Use of medicinal plants possessing potent antioxidant property can help to reduce oxidative stress and hepatotoxicity caused by metals.
Tamarindus indica Linn (Caesalpiniaceae) is commonly known as tamarind, (*Hindi: Imli*) [8]. It grows as a large tree and is found all over India. *T indica* was found to be used in jaundice and other liver complaints in folk medicine [9]. Tamarind fruit contains high amount of ascorbic acid and β- carotene, which are proved to be potent antioxidant and hepatoprotective [10].

The aqueous extract of leaves contain ascorbic acid, β-carotene and are proved to be anti-lipoperoxidant, stops the peroxidation of tissue lipid and antihepatotoxic (*in vitro*) [11]. Pharmacological studies of the plant revealed that tamarind possess antibacterial, antidiabetic, antifungal, antiinflammatory, antimalarial and antioxidant activities [12]. Keeping this in view the present study was designed to evaluate the activity of *Tamarindus indica* seed coat powder (TISCP) against sodium arsenite induced oxidative stress and hepatic dysfunction against arsenic induced toxicity in wistar albino rats.

2. Materials and methods

Animals and experimental design

2.1 Plant material:

Fresh matured tamarind fruits were procured from local supplier of Durg district of Chhattisgarh state. The pulp of the fruits was hand-scraped from the seeds. Seeds were cleaned and dried under shade at an ambient temperature of about 28 °C. Seed shell was separated carefully were milled into fine flour (*Tamarindus indica* seed coat powder, TISCP) and stored in airtight plastic containers at 4 °C until further use.

2.2 Experimental animal

Eighteen Wistar albino rats of either sex weighing between 150-200 g were obtained from Small Animal laboratory, College of Veterinary Science & A.H., Anjora, Durg (Chhattisgarh). The animals were housed under standard environmental conditions (temperature of 22±1 °C with an alternating 12 hrs. light- dark cycle and relative humidity of 60±5%), one week before the start and also during the experiment as per the rules and regulations of the Institutional Ethical Committee.

2.3 Experimental design

Rats were divided into three groups with 6 animals in each group .Group 1 were considered healthy control group received standard feed and distilled water. In Group II and III animals were treated with sodium arsenite @2 mg/kg/day; through gavage for the period of 45 days. Group III animals along with sodium arsenite also received the treatment of TISCP @ 200mg/kg B.wt in feed for the complete duration of experiment.

2.4 Biological sample collection

The animals were anesthetized by light chloroform anaesthesia for the collection of about 2ml blood by puncturing the retro-orbital plexus on day 45th for the estimation of hepatic and oxidative markers. At the end of the experiment, the animals were sacrificed, liver samples were collected and subjected to chemical analyses.

2.5 Estimation of hepatic biomarkers

The serum samples extracted was stored at -20^{0} C were utilized for the estimation of Aspartate amino transferase (U/L), Alanine amino transferase (U/L), Alkaline phosphatase (U/L), Total protein(g/dl) using using semiautomated Biochemistry Analyzer (Systronics India Limited), as per the standard method and procedures given in commercial kits supplied by ARKRAY Healthcare Pvt. Ltd.

2.6 Preparation of liver homogenate

About 0.5 gm of each liver was homogenized by ultrasonic homogenizer in 5 ml ice-cold phosphate buffered saline (PBS) to obtain ultimately10% (w/v) whole liver homogenate

2.7 Estimationof oxidative markers

Determination of hepatic lipid peroxide (Malondialdehyde) (nmol/g tissue) It was determined colorimetrically according to Ohkawa, *et al*., (1979) [13]. Determination of hepatic reduced glutathione (mg/g tissue) was based on the reduction of 5, 5' dithiobis (2- nitrobenzoic acid) (DTNB) with glutathione (GSH) to produce a yellow compound. The reduced chromogen directly proportional to GSH concentration and its absorbance can be measured at 405 nm [14]. Hepatic superoxide dismutase activity was assessed by the method described by Marklund and Marklund (1974) [15]. The concentration of total protein in tissue homogenate was determined by Lowry *et al*. (1951) [16].

2.8 Estimation of arsenic

The liver tissue samples were wet digested and concentration of arsenic in the wet digested samples (Nitric, sulphuric and perchloric acid) was measured using a hydride generation atomic absorption spectrophotometer (AAS, ECIL-4141, India) at 193.7 nm wave length and 10 mA current and the values were expressed in µg/mL .

2.9 Statistical analysis

The data was analyzed statistically by using ANOVA using one way analysis of variance followed by DMRT (Duncan`s multiple range test) as per the methods suggested by Snedecor and Cochran (1994) [17].

3. Results and Discussion

Serum biochemical parameters are the earlier indicators of any pathophysiological state**.** In this study hepatic and oxidative stress biomarkers were assesses the effect of arsenic on liver tissue

3.1 Hepatic markers

Arsenic undergoes metabolism in the liver where it specifically binds with the thiol group of proteins and enzymes, thereby, alters the integrity of hepatocyte plasma membrane results in the leakage of hepatic marker enzymes AST, ALT and ALP in serum [18]. Activities of AST, ALT and ALP in the arsenic treated group II were statistically elevated ($P<0.05$) along with significant decrease in the total protein and albumin as compared to the healthy control group I (Fig. 1&2). Similar results were also recorded by Dua *et al*., (2015) [19]. *Tamaridus indica* treated group results in the restoration of serum enzymes and had significantly reduced ($P<0.05$) hepatic enzymes (AST, ALT and ALP) with increase in total protein levels. Further, AST, ALT, ALP and total protein values appeared more near to normal having no significant difference with Group III (Sodium arsenite+*Tamarindus indica)* and Group I (Fig. 3, 4 & 5).

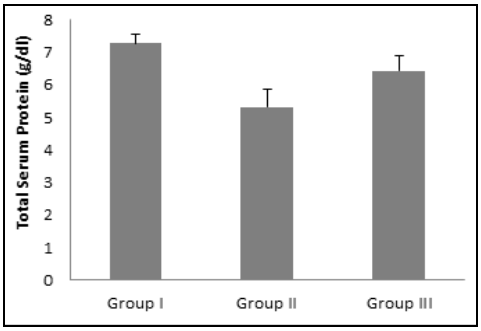

Fig 1: Effect of TISCP on Total protein concentration in arsenic induced toxicity in rats

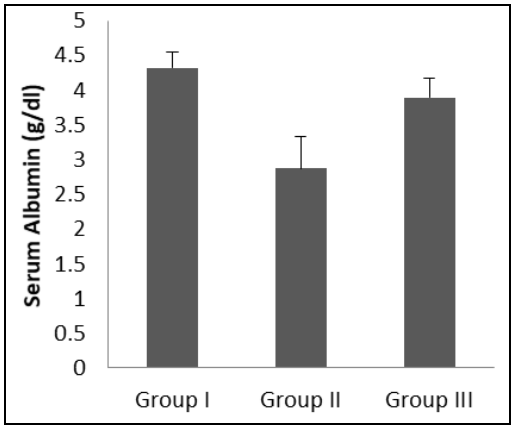

Fig 2: Effect of TISCP on serum albumin levels in arsenic induced toxicity in rats

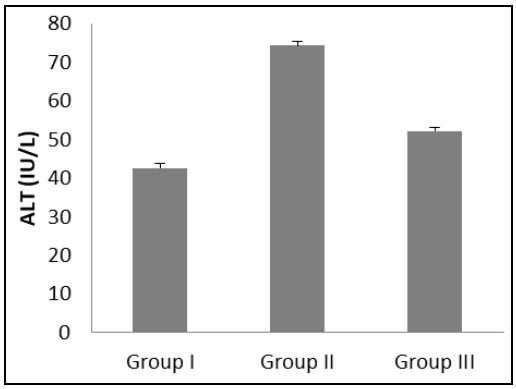

Fig 3: Effect of TISCP on serum ALT level in arsenic induced hepatotoxicity in rats

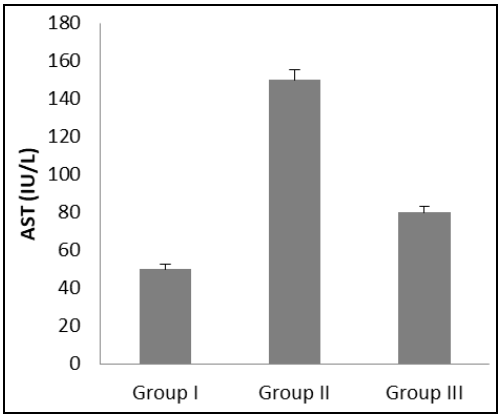

Fig 4: Effect of TISCP on serum AST level in arsenic induced hepatotoxicity in rats

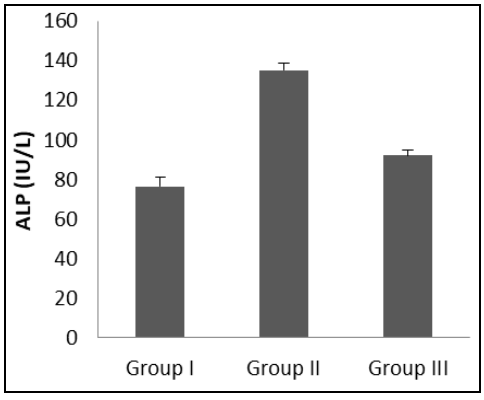

Fig 5: Effect of TISCP on serum ALP level in arsenic induced hepatotoxicity in rats

The liver is known as one of the most important organs in the body for its ability to metabolize nutrients, detoxify harmful substances and perform many other vital functions. The liver enlargement caused by arsenic poisoning in this study might have linkage with liver dysfunction. We, therefore, measured the levels of the enzymes e.g. ALP and ALT in serum as the elevated activity of these enzymes is known to have an association with liver as well as some other organ dysfunction. Assessment of liver damage is usually made by determination of serum enzyme levels of ALT, AST and ALP [20]. Necrosis results in the release of these enzymes into circulation; therefore, it can be measured in serum. High levels of AST indicate liver damage, ALT catalyses the conversion of alanine to pyruvate and glutamate and is released in similar manner [21]. The results demonstrated that TISCP caused significant decrease in serum ALT and AST levels. Serum ALP levels are related to the function of hepatic cells. Increase in serum ALP in arsenic induced toxicity was also observed by Barai *et al*., (2017) [22]. The results of the study indicated that the TISCP significantly lowered the serum enzymatic hepatic markers level. Effective restoration of ALT, AST and ALP activity points towards an early improvement in the secretary mechanism of hepatic cells.

3.2 Oxidative stress markers

The inexorable generation of free radicals due to As-intoxication could be associated with increased levels of lipid peroxidation principally involved in oxidative modifications of structural lipids and proteins of cell membrane causing damage to cell membrane [19].

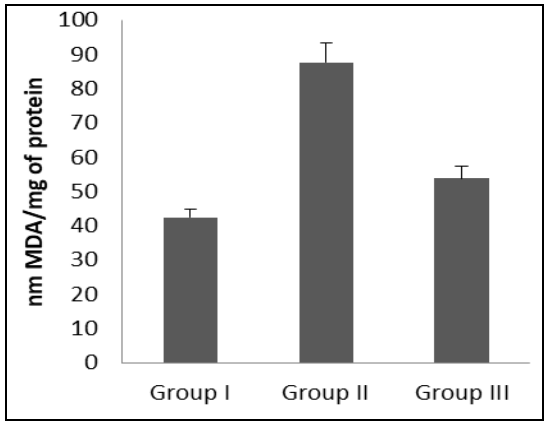

Fig 6: Effect of TISCP on liver tissue MDA level in arsenic induced toxicity in rats

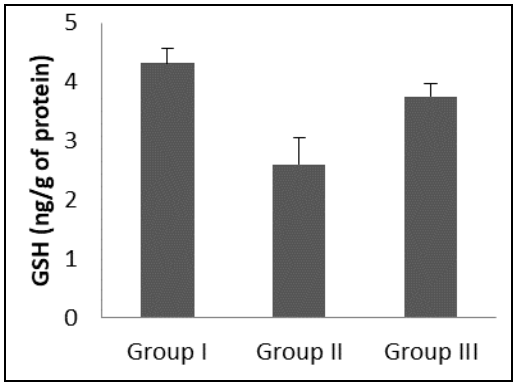

Fig 7: Effect of TISCP on liver tissue GSH level in arsenic induced toxicity in rats

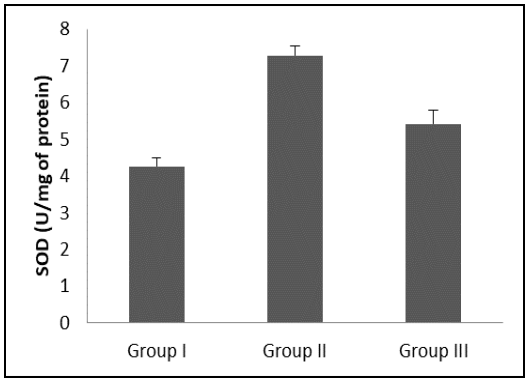

Fig 8: Effect of TISCP on liver tissue SOD activity in arsenic induced toxicity in rats

There was a significant increase in LPO activity in group II rats as compared to healthy control group and group III rats given Tamiridus indica along with sodium arsenite. Tamaridus indica treatment significantly reduced (P<0.05) lipid peroxidation in the studied samples of group III. However, LPO values appeared more near to normal with *Tamarindus indica* treatment (Group III) (Fig. 6). Present study corroborated with the findings of Yaser and Ghader, (2019) [23].

Adequate levels of the cellular GSH pool is required not only for maintaining the cellular redox status by keeping sulfhydryl groups of cytosolic proteins in their reduced form but also because numerous toxic or potentially toxic compounds, including some metals, are either taken up by or removed from the cells by GSH-mediated pathways [24]. Administration of sodium arsenite caused significant decrease (P<0.05) in the GSH level in erythrocytes (Fig. 7). However, GSH values appeared more near to normal with *Tamarindus indica* treatment (Group III). Cytosolic GSH is continuously cycled in and out of mitochondria as it is a major antioxidant and detoxifying agent [25]. Increased ROS and depleted levels of reduced glutathione (GSH) following arsenic exposure in the present study demonstrate an image in which energy metabolism and antioxidant system endure negative transformations.

There was a significant increase in SOD activity in group II rats as compared to animals of healthy control group. *Tamarindus indica* treatment significantly reduced (P<0.05) superoxide dismutase in the studied samples and SOD values appeared more near to normal in group II animals (Fig. 8). Similar observations were recorded by Patlolla *et al*., (2009) [4]. SOD an antioxidant enzyme have protective role against free radical induced toxicity. Increase SOD quenches the free radicals produced as a response of arsenic toxicity and suggests an adaptive response to increased formation of ROS [26].

The potential toxicity of arsenic associated with ROS generation can therefore be attributed to exerts direct toxic effect on lipid, protein, and DNA of the tissues and leads to apoptotic cell death [27]. In this study, a significant increase in the accumulation of intercellular ROS was observed with an increase in LPO content and activities of the antioxidant enzyme SOD whereas reduced GSH level was recorded in animals exposed to arsenic treatment. Yaser and Ghader (2019) [23] reported a significant decrease in values of glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT) in serum (P<0.05) with significant increase in MDA levels in rats given sodium arsinite.

It has been reported that *T. indica* contains flavonoids, ascorbic acid and β carotene [9]. A number of scientific reports indicated that flavonoids, ascorbic acid and β carotene have protective effect on liver due to their antioxidant properties [28]. Presence of those compounds in *T. indica* may be responsible for its protective effect on arsenic-induced liver damage in rats. Roy *et al*. (2018) [12] also observed free radical scavenging effect of tamarind pulp in toxicity studies.

Based on the results of the present study, it can be concluded that the TISCP suppresses arsenic-induced cell damage. However, co-administration of TISCP along with sodium arsenite leading to quenching of free radical and helps in the restoration of liver damage due to arsenic.

3.3 Arsenic concentration

Blood arsenic concentrations in the arsenic experimental animals are presented in Fig.9 Significantly (P<0.05) increased concentration of arsenic was observed in blood of rats administered alone with sodium arsenite followed by Group II and group I respectively. *Tamarindusindica* treatment was found to be effective in significant (P<0.05) reduction of arsenic levels in blood samples (Fig. 9).

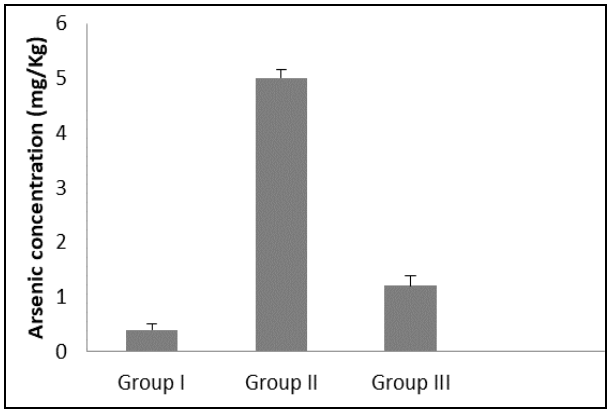

Fig 9: Effect of TISCP on arsenic body burden in arsenic induced toxicity in rats.

Bioaccumulation of arsenic in tissues is the major cause of pathogenesis. Increase arsenic burden in tissue is directly proportional to liver damage due to increased free radical generation. [29]. Arsenic concentration was found to be increased in blood due to arsenic exposure. TISCP treatment restores the normal arsenic values in blood. Presence of substantial quantities of antioxidants like flavanoids, and phenolic acids and saponins in TISCP [30] could be accountable for clearance of arsenic body burden.

4. Conclusion

In a nutshell, our results are strongly suggestive of the protective role of TISCP in restoring hepatic enzymes and prevent the arsenic-induced alterations in the oxidative stress markers value. Treatment with TISCP reduced the peroxidation rate and restore the body's antioxidant capacity and hepatotoxicity induced by sodium arsenite. Co-administration of *T. indica* also reduces the arsenic increased concentration levels in blood samples.

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