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MANGIFERIN PROTECTS FREE RADICAL DAMAGE -AN *IN VIVO* MODEL STUDY

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ABSTRACT

Arsenic is a heavy metal of considerable environmental concern that causes liver damage. Excessive generation of reactive oxygen species (ROS) induced by arsenic has a major role in arsenic induced hepatotoxicity. This study examined an eco-healthy approach to find out impact of mangiferin administration against arsenic induced damage on hepatic tissue lipids and oxidative stress biochemical parameters of rats. Mangiferin, a xanthone glycoside found in *Mangifera indica*, reported to have a wide range of pharmacological activities. It was shown that arsenic exposure induced significant increases in the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), lipid peroxidation levels exposed groups of rats compared to control group while the antioxidant enzymes catalase (CAT) and glutathione peroxidase (GPx) were significantly decreased. These biochemical changes were confirmed by histopathological changes. But supplementation with mangiferin however normalized these alterations and preserved normal histological architecture. In conclusion mangiferin prevent arsenic induced hepatotoxicity and oxidative damage by scavenging free radicals and improving intracellular antioxidant defense status.

KEYWORDS: Arsenic Trioxide, Mangiferin, Free Radical Damage, Hepatic Toxicity, Antioxidant Status

The occupational and environmental exposure of arsenic leads to health problem (Chen et al., 2012). It is more potent toxicant in its inorganic form (Flanagan et al., 2012). Arsenic can enter the body through food, drinking water, and skin contact. The largest threat from arsenic originates through contaminated groundwater usage. Human exposure to arsenic leads to cancer, organ injury, genotoxicity and immunotoxicity (Park, 2003). Bashir et al (2006) stated the role of reactive oxygen species (ROS) in arsenic toxicity. The harmful side effect of arsenic is primarily due to the alterations in redox homeostasis (Mathews et al., 2012). Oxidative stress may, therefore, be one of the reasons for arsenic induced tissue damages. Arsenic can disrupt biological systems by altering the molecular interactions, cell signaling, and ultimately cellular function (Rani et al., 2017).

Considering the relationship between arsenic exposure and oxidative stress, it is reasonable that administration of some antioxidant should be an important approach in arsenic intoxication. Antioxidant supplementation enhances the endogenous antioxidant status and prevents oxidative stress (Halliwell, 2006). Recently, attention has been focused on phytochemicals and polyphenols such as the flavanoids, alkaloids and xanthones in the prevention and management of environmental contaminant related side effects. Mangiferin, obtained from *Mangifera indica* possess extensive pharmacological actions (Sinha et al., 2013). It is a natural C-glycoside ($C_{19}H_{18}O_{11}$) xanthone. Several previous studies have shown that mangiferin has antioxidant, anticancer, anti-inflammatory, anti-apoptotic, and anti-diabetic effects (Pal et al., 2013; Masud Parvez, 2016). In our previous study, we analyzed the potential nature of mangiferin over the cardiac antioxidant system (Binu et al., 2016).

The liver is the primary vital organ involved in the detoxification process. Estimation of thiobarbituric acid reactive substances (TBARS) reveals the membrane peroxidation rate induced by ROS. The enzymatic antioxidants GPx and CAT are the first line of defense for cells to protect from oxidative damage and the serum markers AST and ALT reflect the function of the liver. Histopathological examination helps to reveal the changes occurring in the hepatic tissue. Hence the present study planned to unearth the protective capability of mangiferin on arsenic induced toxicity in liver by accessing the above mentioned parameters.

MATERIALS AND METHODS

Animal Models

Male Wistar rats (body weight 180-250g) were used in the present study. The animal use protocol had been approved by Institutional Animal Ethical committee (IAEC), School of Biosciences, Mahatma Gandhi University, Kottayam, Kerala, India. Experiments were conducted as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India (Reg. No: B29122014/3).

Study Design

The rats were randomized into 4 groups comprising 6 animals each. No experimental treatment was performed on the control group. One group received 4

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mg/kg b.wt of arsenic trioxide in distilled water. Next group had only 100 mg/kg b.wt mangiferin in 0.1 % DMSO. Arsenic plus betulinic acid group received 4 mg/kg b.wt arsenic trioxide and 100 mg/kg b.wt mangiferin. All experimental treatments lasted for 30 days. At the end of the experimental period, animals were decapitated, blood was collected and centrifuged at 3000 rpm for 20 minutes; the clear serum obtained was taken out and used for the determination of liver marker enzymes. The hepatic tissue was dissected out and washed in ice-cold saline. Tissue was minced and homogenized (10%, w/v) in Tris–HCl buffer (pH 7.4). The homogenate was centrifuged (3000× *g* for 15 min at 4^{o} C) and the clear supernatant was used for the analysis of lipid peroxidation and antioxidant properties.

Biochemical Analysis

Serum aspartate amino transaminase (AST) and alanine amino transferase (ALT) were detected (Agappe Diagnostic Ltd., Ernakulam, Kerala, India). Thiobarbituric acid reactive substances (TBARS) activity was estimated by the method of Beuge and Aust (1978). Catalase (CAT) activity in the sample was measured according to the method of Aebi (1974).The activity of glutathione peroxidase (GPx) was determined by the method of Rotruck et al (1973).

Histopathology

Small sections of liver were fixed in 10 % buffered formalin and processed for embedding in paraffin. Sections of 5-6 µm were stained with hematoxylin and eosin and examined for histopathological changes under the microscope (Olympus Microscopes).

Statistical Analysis

The data are reported as mean ± SD. Statistical analysis was performed by one-way analysis of variance (ANOVA) and post hoc comparisons (Tukey Test) using Statistical Package for Social Sciences 20.0 software (SPSS Inc.; Chicago, IL, USA). $P < 0.05$ values were considered statistically significant.

RESULTS

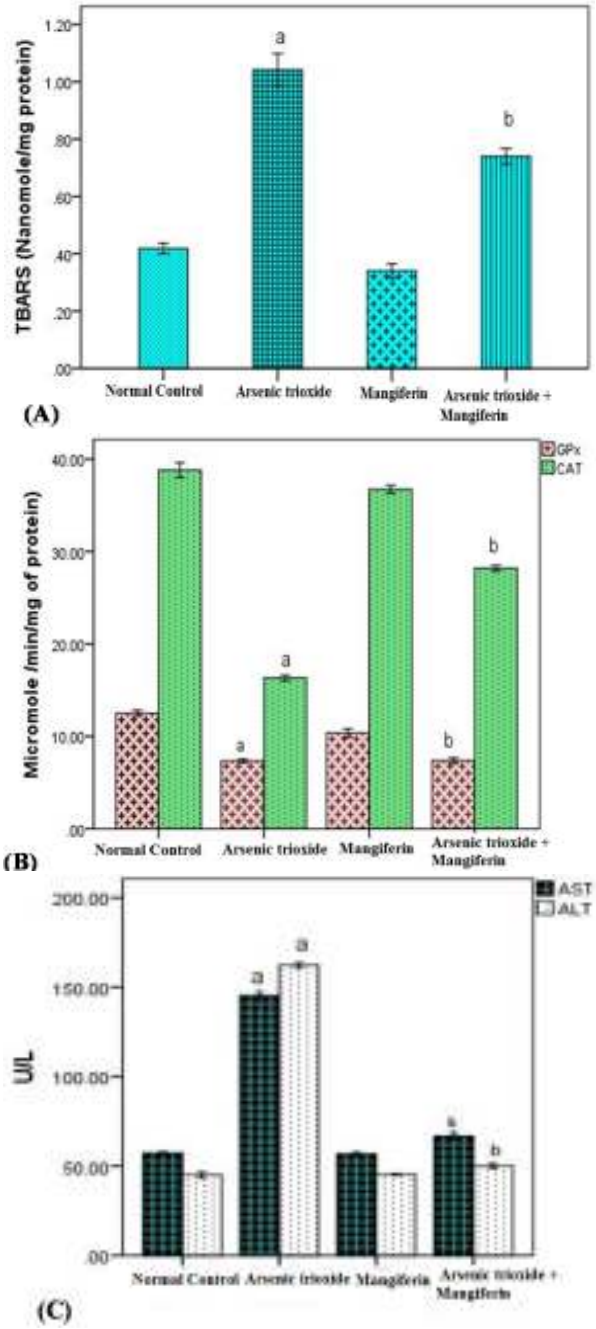

Figure 1: Effect of mangiferin on hepatic (a) lipid peroxidation (b) GPx and CAT activity (c) Serum AST and ALT level. Data represented as mean ± SD, n=6. ^{a}P <0.05 compared to normal control, ^{b}P <0.05 compared to Arsenic trioxide +Mangiferin.

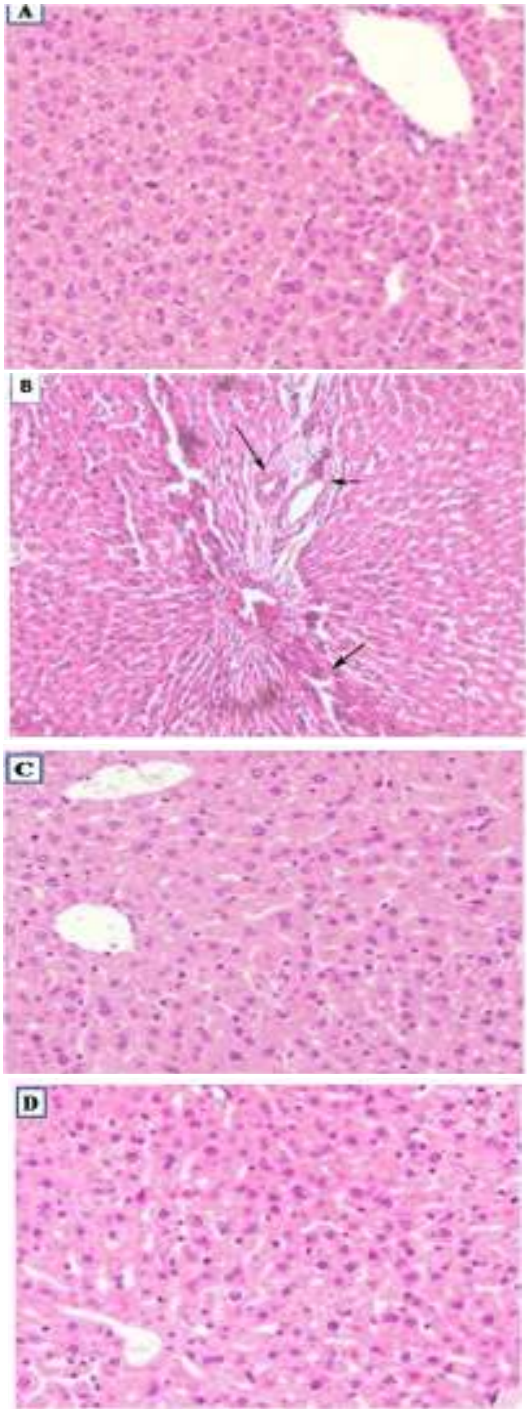

Figure 2: Effect of mangiferin on arsenic induced liver damage in rats (A) normal control rat's liver showing normal hepatic morphology (B) Arsenic treated rat's hepatic tissue showing appearance of Hemorrhage (↷), and Focal Necrosis (↙) (C) Mangiferin administered rats showed normal hepatic structure and morphology (D) Combination therapy of Mangiferin with arsenic trioxide showing normal structure without any hepatic parenchymal degeneration and vascular lesions. Hematoxylin and eosin (magnification 100×).

Effect of mangiferin on hepatic lipid peroxidation level and antioxidant system

The lipid peroxidation product TBARS level was increased, where as enzymatic antioxidants GPx and CAT activities were decreased in arsenic exposed group. Co-administration with mangiferin reduced lipid peroxidation and retain the CAT and GPx activities in the liver ($p<0.05$) (Figure 1A & B).

Effect of mangiferin on arsenic induced changes in serum marker enzymes

A significant increase ($p<0.05$) in the level of AST and ALT in serum was observed in arsenic treated rats when compared with normal control rats. Administration of mangiferin along with arsenic significantly ($p<0.05$) restored the levels of AST and ALT (Figure 1C).

Effect of mangiferin on arsenic induced in the liver histopathology

Treatment with arsenic caused marked changes such as hemorrhage and necrosis in hepatic tissue (Fig. 2B). However, cotreatment with mangiferin ameliorated these alterations (Fig.2D). No histopathological alterations were observed in rats treated with mangiferin alone (Fig.2C) and in control rats (Fig. 2A).

DISCUSSION

Previous studies (Ghosh et al., 2009; Rashid et al., 2013), explained the ROS mediated cellular damage by heavy metals. The aim of the present study was to investigate the preventive role of mangiferin against arsenic-induced liver injury. We observed that arsenic intoxication caused hepatic damage by disturbing its redox homeostasis potential. Co-treatment with mangiferin prevented or reduced arsenic-related hepatic oxidative impairment. Liu and Jan (2000) showed a strong connection between the formation of ROS and arsenic mediated oxidative stress. In the current investigation, the arsenic-induced high lipid peroxidation rate in the hepatic tissue of experimental rats. Arsenic has been reported to cause ROS mediated lipid peroxidation (Ahmad et al., 2000). Mittal et al (2007) reported that arsenic induces ROS mediated free radical damage in the liver. Free radicals attack lead to destabilization of the cell membrane (Renugadevi and Miltonprabhu, 2010). In rats, which received mangiferin and arsenic simultaneously reduce

lipid peroxidation (TBARS) level when compared to rats treated with arsenic alone. This may be due to antioxidant potential of polyphenolic compound mangiferin. It has the ability to quench free radicals thereby significantly reducing lipid peroxidation (Sinha et al., 2013).

To further evaluate the arsenic-induced oxidative damage, the activities of enzymatic antioxidants CAT and GPx were studied. Sener et al (2005) reported that, ROS generated in tissues and normally scavenged by enzymatic and non-enzymatic antioxidants. Arsenic reduced CAT and GPx activity because of super oxide radical production (Manna et al., 2008). CAT and GPx are the important cellular antioxidants which mainly participate in the scavenging of hydrogen peroxide and lipid peroxy radicals. Depleted levels of antioxidants represent an excess free radical formation by arsenic. Arsenic-treated rats showed a significant reduction in the antioxidant enzymes activity when compared to normal control whereas, mangiferin co-treated group GPx and CAT activity was significantly maintained when compared to arsenic alone treated rats. Restoration of these enzymatic antioxidants in the liver of arsenic along with mangiferin treated rats could be related to its antioxidant stimulating activity (Das et al., 2012). Mangiferin may enhance the radical scavenging activity of antioxidant enzymes and then prevent peroxidation of membranes.

Oxidation of membrane by ROS produces a large amount of lipid peroxide or peroxy radicals which may alter the permeability of the membrane. Tissue injury due to arsenic intoxication could be assessed by measuring serum hepatic markers which are the biochemical analyzers of hepatic damage. A significant increase in the activities of intracellular enzymes AST and ALT in the serum of arsenic-treated rats indicate the cellular leakage and loss of the membrane integrity. These enzymes are proved considerably valuable in the diagnosis of hepatotoxicity or damage and are located in the cell cytoplasm and are emptied into the circulation once the cellular membrane is damaged (Lin et al., 2000). A similar result was reported by Mathews et al (2014). Significantly decreased activities of AST and ALT were observed in arsenic and mangiferin co-treated rats compared to the arsenic group, which proves the protective effect of mangiferin. The hepatic defensive nature of mangiferin is further confirmed by the histological findings. The histological observation in arsenic treated rats showed tubular necrosis and hemorrhage in the liver might be accumulation of free radicals.

The increased production of lipid peroxides and associated ROS leads to the damage in membrane structure and other pathological changes in the liver tissue of arsenic intoxicated rats. Administration of mangiferin reduced the histological alterations induced by arsenic. It can be attributed to the antioxidant properties or metal chelating efficacy of mangiferin, which significantly reduced oxidative stress and leading to the reduction of histological alterations and restoration of a normal physiological status of rats. Moreover, membrane stabilizing properties of mangiferin might be helpful to decrease histopathological changes caused by arsenic in the liver tissues of rats.

Therefore, this study showed the potentiality of mangiferin to reduce arsenic toxicity by preventing lipidperoxidation and enhancing hepatic antioxidant status. Based on these findings, the study proposes scientific validation to explore the possible mechanism of protection by mangiferin in arsenic exposure.

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