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Research Article

Ameliorative Effect of *Murraya Koenigii* on Arsenic Induced Toxicity in Swiss Albino Mice

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Abstract

Arsenic is a metalloid that can be toxic to humans and other living organisms, occurs naturally and anthropogenically throughout the world at varying concentrations, including concentrations of concern in soil or groundwater. Presently, in Bihar (India) 18 districts are affected with arsenic poisoning in ground water causing lots of health hazards among the population. This arsenic intoxication has caused lots of health related problems in the population. The present study has been designed, to study the ameliorative effect of Murraya Koenigii on sodium arsenite induced toxicity in Swiss albino mice. The treatment groups received sodium arseniteorally at the dose of 3mgkg-1 body weight daily for 4 weeks followed by administration of *Murraya Koenigii* 350mgkg-1 body weight daily by gavage method for 4& 6 weeks. Their biochemical levels like liver and kidney function tests were assayed and were observed with elevated levels. Furthermore, their free radical assessment like lipid peroxidation levels were assayed which was found to be many folds higher. But, after administration of aqueous extract of *Murraya Koenigii*, there was significant amelioration in the biochemical and lipid peroxidation levels. Therefore, it is evident from the present study that *Murraya Koenigii* possesses antidote effects and acts effectively against arsenic induced toxicity.

Keywords: Arsenic; Toxicity; Antidote effect; Swiss albino mice

Introduction

Arsenic is a metalloid that can be toxic to humans and other living organisms, occurs naturally and anthropogenically throughout the world at varying concentrations, including concentrations of concern in soil or groundwater. Acute arsenic exposure harms human health in many ways including the development of malignancies, severe gastrointestinal toxicities, diabetes, cardiac arrhythmias, cancer and death [1]. Natural sources such as volcanic action, erosion of rocks, and forest fires introduce arsenic into the environment (EPA, 2001). Anthropogenic sources include arsenic added to the soil plant system as insecticides, herbicides, pesticides, livestock dips and wood preservatives. It is estimated that more than 200 million people worldwide are chronically exposed to dangerous levels of arsenic which leads to several diseases, including various types of cancer [2]. Arsenical exposure through drinking water is common in many areas of the world [3]. Metabolic disorders, hypertrophy of adrenal glands [4] and anaemia [5], inhibition of the activity of steroidogenic enzymes [6] and reduction in the weight of the testis and accessory sex organs [7] are associated with exposure to arsenicals. Presently, in Bihar, 18 districts are highly affected with arsenic poisoning. The groundwater contamination by arsenic is maximum 1928ppb in Buxar district of Bihar [8,9].

Traditional medicines include herbal medicines composed of herbs, herbal materials, herbal preparations, and finished herbal products, that contain as active ingredients parts of plants, or other plant materials, or combinations thereof. Since last two decades, the phytoremediation of various heavy metals borne diseases has gained special attention to researchers.

In Sidhha system of medicine it's known as Karuveppilai and used as herb in Ayurvedic medicine. The leaves of *Murraya Koenigii* are also used as herb in Ayurvedic medicine. Their properties include much value as an antidiabetic, antioxidant, antimicrobial, anti-inflammatory hepatoprotective, anti- hyper cholesterol lemic, and etc [10,11]. Curry leaves are also known to be good for hair, for keeping it healthy and long as it also contains iron. Although, most commonly used in curries leaves from the curry tree can be used in many other dishes to add spice. The essential oil from leaves of *Murraya Koenigii* exhibit strong antibacterial as well as antifungal activity [12-14].

Present study aims to illustrate the ameliorative effect *Murraya Koenigii* on arsenic induced toxicity in mice.

Materials and Methods

Animals

Swiss albino mice (Mus musculus), weighing 30g to 35g 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. 2015/3D-16/12/15. Food and water to mice were provided ad libitum (prepared mixed formulated food by the laboratory itself). The experimental animals were housed in conventional polypropylene cages in small groups (2 each). The mice were randomly assigned to control and treatment groups. The temperature in the experimental animal room was maintained at 22±2^{0}C with 12h light/dark cycle.

Chemicals

Sodium Arsenite (98.5%) manufactured by Sigma-Aldrich, USA

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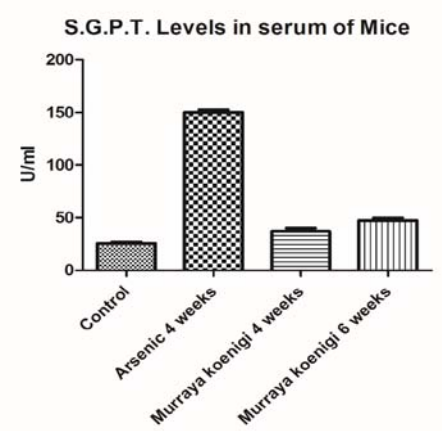
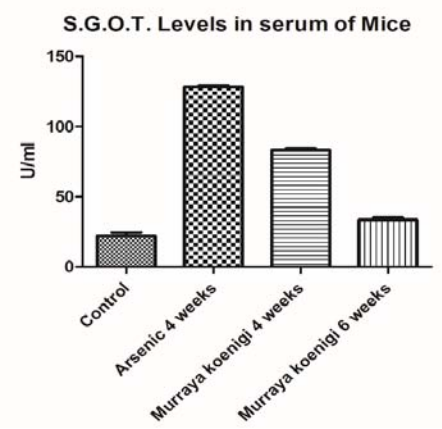


Graph Figure 1: Effect of *Murraya Koenigii* on Arsenic induced toxicity showing SGPT activity (n=6, values are mean± S.D).



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

(CAS Number: 7784-46-5), was obtained from the Scientific store of Patna of Bihar India.

Preparation of *Murraya Koenigii* aqueous extract: In the present study, leaves of *Murraya Koenigii* were procured locally from Patna, Bihar, India The identity of the medicinal plant was confirmed by Dr. Ramakant Pandey (Botanist), Department of Biochemistry, Patna University, Patna, Bihar, India. The collected leaves of *Murraya Koenigii* were shade dried and were grinded to fine powder. The aqueous extract dose was calculated after LD50 estimation which was found to be 4800mgkg^{-1} body weight and the final dose was fixed to 350mgkg^{-1} body weight.

Study groups & sampling

The control group of 6 mice received distilled water as drinking water. The treatment groups (n= 18) received Sodium arsenite daily at the dose of 3mgkg^{-1} body weight for 4 weeks orally (after estimation of LD50 value which was found to be 8mgkg^{-1} body weight) followed by administration of *Murraya Koenigii* 350mgkg^{-1} body weight daily by gavage method for 4and 6 weeks. Mice were sacrificed after completion of their treatment and their blood were collected and serum were extracted for biochemical assays and lipid peroxidation estimation.

Biochemical evaluation

The Liver Function Test (LFT) were assayed by methods as Serum Glutamate Pyruvate Transaminase (SGPT), Serum Glutamate Oxalate Transaminase (SGOT) [15], Alkaline Phosphatase ALP [16], Bilirubin [17].The Kidney Function Test (KFT) were assayed by methods as Urea by [18,19] Uric acid by [20] and Creatinine by [21].

Lipid Peroxidation (LPO)

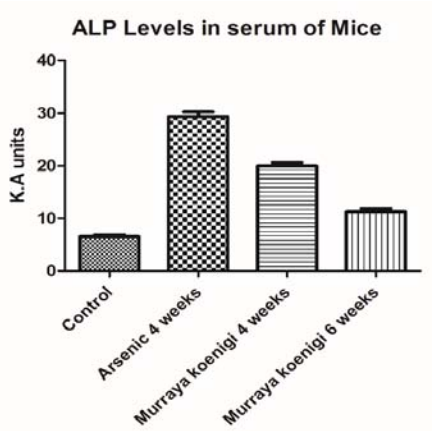
Thiobarbituric acid reactive substances (TBARS), as a marker for LPO, were determined by the double heating method [22]. The principle of the method was a spectrophotometric measurement of the color produced during the reaction to thiobarbituric acid (TBA) with malondialdehyde (MDA). For this purpose, 2.5ml of 100g/l trichloroacetic acid (TCA) solution was added to 0.5ml serum in a centrifuge tube and incubated for 15min at 90^{0}C. After cooling in tap water, the mixture was centrifuged at 3000g for 10min, and 2ml of the supernatant was added to 1ml of 6.7g/l TBA solution in a test tube and again incubated for 15min at 90^{0}C. The solution was then cooled in tap water and its absorbance was measured using Thermo Scientific UV-10 (UV –Vis) spectrophotometer (USA) at 532nm.

Statistical analysis

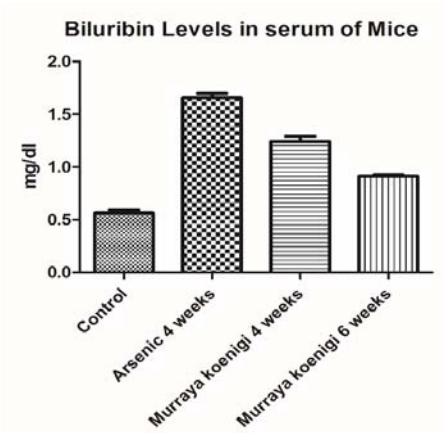
Results are presented as mean ±SD and total variation present in a set of data was analysed through one way analysis of variance (ANOVA). Difference among mean values has been analysed by applying Dunnet's t-test. Calculations were performed with the Graph Pad Prism Program (Graph Pad software, Inc., San Diego, U.S.A.). The criterion for statistical significance was set at P< 0.05.

Results

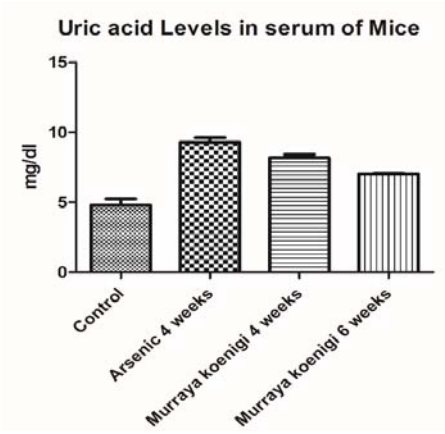
The liver function test parameters - SGPT levels in control mice was 25.67 ± 1.20 U/ml while in arsenic four weeks treated group it was 150.3 ± 2.33 U/ml (Graph Figure 1). In *Murraya Koenigii* administered group of mice SGPT levels was 124.33 ± 2.33 U/ml after 4 weeks and 47.33 ± 1.90 U/ml after 6 weeks. SGOT levels in control mice was 22.00 ± 2.64 U/ml while in arsenic four weeks treated group it was 128.7 ± 2.60 U/ml (Graph Figure 2). In *Murraya Koenigii* administered group of mice SGOT levels was 83.67 ± 2.40 U/ml after 4 weeks and 33.67 ± 2.87 U/ml after 6 weeks. ALP levels in control mice was 6.567 ± 0.35 K.A units, while in arsenic four weeks treated



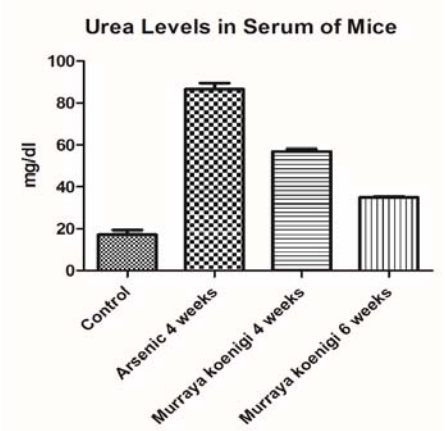
Graph Figure 3: Effect of *Murraya Koenigii* on Arsenic induced toxicity showing ALP activity (n=6, values are mean± S.D).



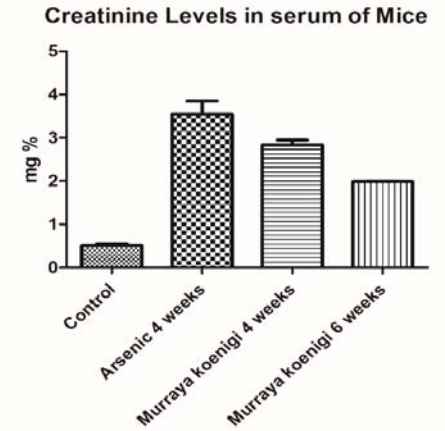
Graph Figure 4: Effect of *Murraya Koenigii* on Arsenic induced toxicity showing Bilirubin activity (n=6, values are mean± S.D).



Graph Figure 6: Effect of *Murraya Koenigii* on Arsenic induced toxicity showing Uric Acid activity (n=6, values are mean± S.D).



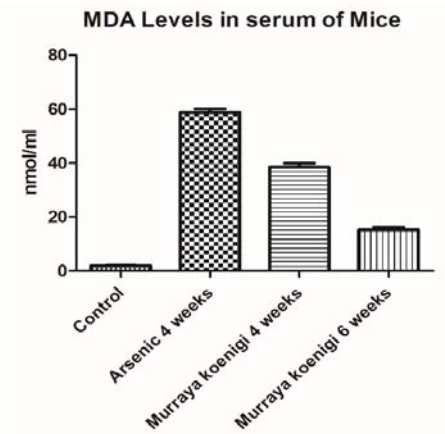
Graph Figure 5: Effect of *Murraya Koenigii* on Arsenic induced toxicity showing Urea activity (n=6, values are mean± S.D).



Graph Figure 7: Effect of *Murraya Koenigii* on Arsenic induced toxicity showing Creatinine activity (n=6, values are mean± S.D).

group it was 29.67 ± 1.76 K.A units (Graph Figure 3). In *Murraya Koenigii* administered group of mice ALP levels was 18.77 ± 0.52 K.A. units after 4 weeks and 11.77 ± 0.57 K.A. units after 6 weeks. Bilirubin levels in control mice was 0.5633 ± 0.029 mg/dl while in arsenic four weeks treated group it was 1.677 ± 0.06 mg/dl (Graph Figure 4). In *Murraya Koenigii* administered group of mice bilirubin levels was 1.2133 ± 0.02 mg/dl after 4 weeks and 0.9133 ± 0.08 mg/dl after 6 weeks.

The Kidney Function Tests - Urea levels in control mice was 17.33 ± 2.02 mg/dl while in arsenic four weeks treated group it was 86.67 ± 2.96 mg/dl (Graph Figure 5). In *Murraya Koenigii* administered group of mice urea levels was 57.16 ± 1.13 mg/dl after 4 weeks and 35.67 ± 1.24 mg/dl after 6 weeks. Uric acid levels in control mice was 4.800 ± 0.45 mg/dl while in arsenic our weeks treated group it was 9.733 ± 0.23 mg/dl (Graph Figure 6). In *Murraya Koenigii* administered group of mice uric acid levels was 8.201 ± 0.28 mg/dl after 4 weeks and 7.033 ± 0.17 mg/dl after 6 weeks. Creatinine levels in control mice was 0.5167 ± 0.03 mg% while in arsenic four weeks treated group it was 3.733 ± 0.23 mg% (Graph Figure 7 in *Murraya Koenigii* administered group of mice creatinine levels was 2.856 ± 0.17 mg% and after 4 weeks and 1.972 ± 0.24 mg% after 6 weeks.



Graph Figure 8: Effect of *Murraya Koenigii* on Arsenic induced toxicity showing Lipid peroxidation activity (n=6, values are mean± S.D).

Lipid peroxidation levels in control mice was 1.987 ± 0.06 nmol/ml while in arsenic four weeks treated group it was 58.73 ± 1.36 nmol/ml (Graph Figure 8). In *Murraya Koenigii* administered group of mice Lipid peroxidation levels was 38.27 ± 0.32 nmol/ml after 4 weeks and 14.43 ± 0.40 nmol/ml after 6 weeks.

Discussion

Liver is one of the important organ actively involved in metabolic functions and is in frequent target of number of toxicants. The main function of the liver is detoxification of toxins and xenobiotics [23]. Because liver performs many vital functions in the human body, damage of liver causes serious problems [24]. SGPT and SGOT are the most often used and most specific indicators of hepatic injury and represent markers of hepatocellular disease [25]. Arsenic is known to produce damages in liver function [26]. SGPT and SGOT are reliable determinants of liver parenchymal injury [27] and their activities are significantly increased in arsenic treated mice 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 [28,29]. Alkaline phosphate is a non-specific tissue enzyme widely spread, mainly in the osteoblasts, liver and biliary canaliculi [30,31]. Bilirubin is the conventional indicator of liver diseases [32]. 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 [33].

In the present study, administration of Sodium arsenite treated mice showed insignificant increase in the levels of SGPT, SGOT, ALP and bilirubin activities when compared with control mice. Oral administration of aqueous extract of *Murraya Koenigii* (350 mg/kg^{-1} body weight) to sodium arsenite treated mice showed an inhibition in the elevated levels of serum SGPT, SGOT, ALP and Bilirubin than sodium arsenite alone treated mice.

The protective nature of *M. Koenigii* leaves extract was well studied [34,35]. The effect attributed to the combined effect of carbazole alkaloids – Mahanimbine, Girinimbine, Isomahanimbine, murrayazoline, Murrayazolidine, Mahanine and ascorbic acid, α-tocopherol and mineral (Zn, Cu, Fe) contents of *M.Koenigii* leaves extract. This study proved *M. Koenigii* a promising and a rich source of free radical quenchers, which have been mediated through hepatocyte membrane stabilizing activity along with the reduction of fat metabolism [36]. The normal morphology of cell was maintained after ethanolic challenge when aqueous extract containing tannins and carbazole alkaloids of *M. Koenigii* was given.

In kidney, arsenic exerts its deleterious toxic effects through several mechanisms, the most significant of which is the reversible combination with suflydryl group of proteins present in glomerular filtration membrane [37]. In the present study also, there was decrease in the levels of kidney function tests especially the urea, uric acid and creatinine levels denotes the ameliorative effect. Aqueous extract of leaves produced a significant dose-dependent decrease in serum urea and creatinine levels [38]. Arsenic causes oxidative damage by producing reactive oxygen species [39,40] which damage proteins. Due to lipophilic in nature, arsenic also attaches to lipid, increases the lipid peroxidation [41] resulting in deposition of lipid droplets in the slit pores of glomerular filtration membrane. Both the reasons discussed above cause decreased Glomerular Filtration Rate (GFR), thereby causing accumulation of nitrogenous waste product in blood. In the present study also, there was immense increase in the levels of lipid peroxidation (MDA) levels denotes the similar effect but there was declination in the levels of the same by the ameliorative effect of aqueous extract of *Murraya Koenigii* denotes the antioxidant activity. The entire study therefore denotes the ameliorative effect of *Murraya Koenigii* [42-45].

Conclusion

It is evident from study that *Murraya Koenigii* acts effectively against arsenic induced toxicity. It restores the liver function test as well as kidney function test effectively. It also restores lipid peroxidation levels up to the normal levels, thus indicates that *Murraya Koenigii* possesses ameliorative effect against arsenic induced toxicity.

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