AMELIORATED ACTIVITY OF MURRAYA KOENIGII LEAVES CHLOROFORM EXTRACT (MKCE) AGAINST LEAD INDUCED HEPATIC DYSFUNCTIONS IN MICE

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ABSTRACT: Lead is a well-known hepatotoxic and all-pervading heavy metal. Ingestion of lead induces structural dysfunctions, physiological distortion and biochemical impairment in the liver. The present study was aimed to ascertain the ameliorated effect of chloroform extract of Murraya leaves against the deleterious effects of lead on hepatic function parameters. Murraya koenigii Chloroform Extract (MKCE) was prepared by maceration. Male albino Swiss mice were divided into three groups of six each. Group I (Control group) served as normal diet and water ad libitum as control group, Group II (lead treated group) received intraperitoneal injection of lead acetate (15mg/kg) daily once whereas Group III (MKCE with lead-treated group) received MKCE (50 mg/kg) and injection of lead acetate intraperitoneally (15mg/kg) daily once time. Experimental study continued for consecutive 7 days. On the 8th day, liver were desiccated from all animals; homogenized to collect supernatant into vials. Serum ALT, AST, ALP, TB, TP and ALB levels were estimated. Significant rise in levels of ALT, AST, ALP, TB were observed along with decreased level of TP and ALB in lead-intoxicated mice. Hepatic enzyme levels were significantly reduced (p<0.001) and augmented levels of protein and albumin in group III. Results in the study revealed significant hepatoprotective activity of MKCE against lead intoxication in mice by restoring all hepatic function markers to near normal level.

INTRODUCTION: Liver is a major organ in the body and plays a vital role in the physiological functions, secretion of bile, metabolism and detoxification of xenobiotics and/or drugs 1. Xenobiotics like carbon tetrachloride 2, fat soluble chemical as industrial pollutant, drugs like acetaminophen, isoniazid, rifampicin 3, and ingestion of heavy metals in drinking water like lead, arsenic, mercury, copper and cadmium 4 are serious environmental pollutants and also examples of hepatotoxic agents. In the metabolic pathway, transformation reactions (oxidation, reduction or hydrolysis) followed by conjugation converts xenobiotics into suitable forms for elimination from the body. However it requires a multiple transformation reactions for eliminating xenobiotics, hepatotoxic drugs, heavy metals but their intermediates induce hepatotoxicity also. Therefore liver is most influenced by xenobiotics and becomes susceptible to xenobiotic-induced injury/toxicity 3.

A large number of medicinal plants and naturally occurring products like silymarin, resveratrol, curcumin and gingko with high efficacies and either low or no toxicity 3 have been reported as potent therapeutics for exerting hepatoprotection in liver damage. One of these medicinal plants, Murraya koenigii L. (Rutaceae) is also reported as antioxidant 5 and hepatoprotective agent 6.
It is a small shrub, about 2.5 meters in height widely used as a spice having its characteristic flavour and aroma. Besides it has been reported for various pharmacological activities like antimicrobial, antidiabetic, anti-inflammatory and antinociceptive, anticancer and caring effect in haematological indices. The present study was aimed to ascertain the ameliorated effect of chloroform extract of *Murraya koenigii* leaves against the deleterious effects of lead on hepatic function parameters.

**MATERIAL AND METHODS:**

**Collection of *Murraya koenigii* leaves:** Fresh *Murraya koenigii* were purchased from the local market in the City of Karad (Western Maharashtra) in the month of March 2015 and was validated/authenticated them from the Department of Botany, Yashwantrao Chavan College of Sciences, Karad.

**Preparation of chloroform extract of *Murraya koenigii* leaves:** Chloroform extract of *Murraya koenigii* leaves was macerated by adding about 70 g of shade dried leaves of *Murraya koenigii* in 2.5 l of chloroform. The collected extract was obtained 6% of yield from the crude leaves and stored in suitable container as *Murraya koenigii* Chloroform Extract (MKCE). According to earlier report, the LD50 value for the aqueous extract of leaves of *Murraya koenigii* was found to be 150mg/kg i.p. in rats. Doses of lead acetate (15 mg/kg i.p) and MKCE (50 mg/kg p.o) were used in our previous study.

**Experimental Animals:** Male Swiss albino mice (n=6 in each group) weighing between 25-30 g were used in the study. Animals were obtained from the Animal House, Krishna Institute of Medical Sciences, Karad, India. The animals were maintained under standard husbandry conditions at room temperature, light: dark cycle for an acclimatization period of 15 days. Experiment was compiled with the guidelines of Committee for the Purpose of Control And Supervision of Experiments on Animals (CPCSEA) for animal experimentation of laboratory and Institutional Animal Ethics Committee (Reg. No. 255/PO/ 2000/ bc/CPCSEA) KIMS, Karad approved for the study.

**Experimental Design:** Male Swiss albino mice were randomly divided into three groups with each consisting of six animals and was continued at once daily for the consecutive seven days.

**Group-I (Normal):** Mice received normal diet and water only *ad libitum*

**Group-II (Pb treated):** Mice received Lead acetate (15 mg/kg i.p)

**Group-III (Pb + MKCE treated):** Mice received MKCE (50 mg/kg p.o) + lead acetate (15 mg/kg i.p))

On 8th day, mice were anaesthetized under ether and sacrificed by cervical decapitation.

Liver tissues were collected with vials containing saline and stored at 4°C for further analyzing hepatic function parameters.

**Blood lead analysis:** Blood lead levels in mice were analyzed by atomic absorption spectrometry in accordance to our previous study.

**Tissue homogenate:** Ten percent liver homogenate in 0.15 M KCl was homogenized in glass mortar pestle and centrifuged in cold (0-4 °C) at 12000 rpm for 30 min. The obtained supernatant was added into eppendorf tubes, labeled and stored at -20 °C and assayed all parameters.

**Assessment of liver function:**

**Asparate Pyurvate (AST) and Alanine Pyurvate (ALT):** It was tested by a combined method of Mohun and Cook (1957) and Reitman and Frankel (1957).

**Alkaline Phosphatase (ALP):** It was analyzed by method of Kind and King (1954).

**Total Bilirubin (TB):** It was determined by method of Mallay and Evelyn (1937).

**Total Protein (TP):** It was estimated by method of Lowry *et al.* (1951).

**Albumin (ALB):** It was measured in the method by Kingsley and Frankel (1939).
Statistical analysis: Data were expressed as the mean ± S.E.M (n=6). Statistical analysis was done using analysis of one way Analysis of Variance (ANOVA) followed by Dunnett’s test and all readings were considered significant at p < 0.05.

RESULTS: The current study was estimated the hepatoprotection ability of MKCE either to reduce the deleterious effects of lead or to preserve the hepatic physiologic mechanisms distorted by lead. All these examinations indicate a hepatoprotective potential of the extract. Elevation of levels in hepatic markers is indicated liver damage as shown in Table 1.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal</th>
<th>Pb treated</th>
<th>MKCE + Pb treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (U/l)</td>
<td>90.81 ± 0.72</td>
<td>170.11 ± 4.86 **</td>
<td>105.31 ± 1.91 **</td>
</tr>
<tr>
<td>ALT (U/l)</td>
<td>48.78 ± 1.05</td>
<td>81.45 ± 3.18 **</td>
<td>62.45 ± 2.00 **</td>
</tr>
<tr>
<td>ALP (U/l)</td>
<td>101.88 ± 0.84</td>
<td>300.22 ± 1.82 **</td>
<td>158.58 ± 9.62 **</td>
</tr>
<tr>
<td>TB (mg %)</td>
<td>0.049 ± 0.002</td>
<td>0.43 ± 0.06 **</td>
<td>0.043 ± 0.01 NS</td>
</tr>
<tr>
<td>TP (mg %)</td>
<td>6.44 ± 0.21</td>
<td>0.63 ± 0.16 **</td>
<td>5.67 ± 0.26</td>
</tr>
<tr>
<td>ALB (mg %)</td>
<td>2.12 ± 0.02</td>
<td>0.45 ± 0.06 **</td>
<td>1.32 ± 0.15 **</td>
</tr>
</tbody>
</table>

Data represents the mean ± SEM of six mice. AST: aspartate pyruvate, ALT: alanine pyruvate, ALP: alkaline phosphatase, BIL: total bilirubin, TP: total protein; ALB: albumin, *P<0.05 compared to control, **P<0.001 compared to control, #P<0.001 compared to control, NS: non-significant.

A significant rise of ALT, AST, ALP levels is shown in lead-intoxicated group II. Treatment with chloroform extract of *Murraya koenigii* L. has shown attenuated levels of hepatic enzyme (p<0.001) in group III. Total protein and albumin levels were significantly decreased in group II (p<0.001) while increased levels of TP (p < 0.05) and ALB (p < 0.001) in MKCE treated group when compared to control group. It is indicated the hepatoprotective activity of MKCE against lead induced hepatic damage.

DISCUSSION: Lead is a ubiquitous heavy metal and well-known hepatotoxic agent. Ingestion of lead induces structural dysfunctions, physiological distortion and biochemical impairment in the liver. In the assessment of liver damage by lead, the determination of hepatic enzyme like AST, ALT, and ALP levels is mostly used. Generally necrosis or membrane damage of tissue caused by diseases like viral hepatitis, cardiac infarction, injury to muscle releases a high level of hepatic enzyme into circulation. Elevated level of serum hepatic enzymes is an indicative of liver injury due to cellular leakage and loss of functional integrity of cell membrane of liver. Hepatic enzyme AST plays vital role in catalyzing conversion of alanine into pyruvate and glutamate. Therefore ALT parameter is more specific to the liver and for detecting liver injury. Our study is also in agreement with other studies regarding to elevated levels of AST, ALT, and ALP in lead treated group and reversed to some degree by use of some plant extracts such as *Ocimum sanctum*, oil of *Sesamum indicum*, *Moringa oleifera*, *Pongamia Pinnata*, *Coriandrum sativum*, and natural antioxidants like Curcumin, Hesperetin.

Decrease in protein content in mice treated with lead might be related to decrease hepatic DNA and RNA in hepatocytes so it may result decreased capacity to synthesize protein. The beneficial effects of MKCE may offer protection by stabilizing the cell membrane in hepatic damage induced by lead which is in conformity with study by Zongping *et al.* (2013).

Although the exact mechanism of lead toxicity is not completely cleared but cumulative data showed that oxidative stress plays an essential role in its toxicity. Thus in further studies, many antioxidant enzyme parameters like superoxide dismutase, catalase, reduced glutathione, glutathione peroxidase, glutathione-s-transferase, total antioxidant capacity, thiobarbituric acid reactive substances should be investigated to elaborate free radical scavenging effects of MKCE in mice due to its presence of flavonoids and phenolics.
CONCLUSION: Results of the study indicated that MKCE exerted significant hepatoprotective activity against lead intoxication in mice by restoring all possible hepatic function markers to near normal level. Further phytochemical investigations are needed to characterize the active hepatoprotective principle of MKCE.

CONFLICT OF INTEREST: None declared.

ETHICAL APPROVAL: Institutional Animal Ethics Committee.

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