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## ASSESSMENT OF HEPATOPROTECTIVE ACTIVITY OF *MUSA PARADISICA* LINN. WHOLE PLANT EXTRACT AGAINST CARBON TETRACHLORIDE INDUCED HEPATOTOXICITY IN WISTAR RATS

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#### Keywords:

Carbon tetrachloride, *Musa paradisica*, Silymarin, Histopathology

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ABSTRACT: Ethonolic whole plant extract of Musa paradisica has a multiple pharmacological activities including antimicrobial, antiviral, antiinflammatory, anticancer effects. The present study aims to determine the effect of *Musa paradisica* on serum and tissue superoxide dismutase (SOD) levels and the histology in carbon tetrachloride (CCl<sub>4</sub>)-induced liver injury. Wister rats aged were injected Intraperitoneally with 50% CCl<sub>4</sub> in olive oil. Musa paradisica was orally administered before or after CCl4 treatment in various groups. Twenty-four hours after CCl<sub>4</sub> injection, serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities, serum and liver superoxide dismutase (SOD) activities were measured and Histological changes of liver were examined by microscopy. Serum ALT and AST activities significantly decreased in a dose-dependent manner in both pre-treatment and post-treatment groups with ethonolic whole plant extract of Musa paradisica. The present study demonstrates that Musa paradisica possesses hepatoprotective effects against CCl<sub>4</sub>- induced hepatotoxicity and that the effects are both preventive and curative. Musa paradisica should have potential for developing a new drug to treat liver toxicity.

**INTRODUCTION:** Banana (*Musa paradisica*) is one of the most popular fruits in the world. A member of the genus Musa (part of the family Musaceae), it is considered to be derived from the wild species *Musa acuminata* and *Musa balbisiana*, Bananas are rich sources of carbohydrates and potassium while they are low in protein <sup>1</sup>.

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In India dried fruits, flowers and roots are employed orally for diabetes. The roots are used as anthelmimtic, aphrodisiac and laxative. The fresh fruit is used for peptic and duodenal ulcers<sup>2</sup>. Banana contains completely different amino acids like essential amino acid, tryptamine, tryptophan, flavonoids and sterols<sup>3</sup>. Until date completely different elements of genus *Musa sapientum* have been studied for antiulcerogenic<sup>4, 5, 6</sup>, hypoglycaemic<sup>7, 8</sup>, hypolipidemic<sup>9</sup>, antimicrobial<sup>10</sup>, antihypertensive, wound healing, antacid, diuretic and antiestrogenic activities<sup>11</sup>. The pill extract of banana was found to enclose analgesic Property<sup>12</sup>. Antidiarrhoeal activity <sup>13</sup> inflammation, pain & snakebite<sup>14</sup>. The Ayurvedic Pharmacopoeia of India recommends the fresh rhizomein dvsuria. polyuria (in females) and menstrual disorders; the flower in asthma, bleeding disorders, vaginal discharges and leucorrhoea (API. VOL-IV) but proof in the literature there's no for Hepatoprotective activity of whole plant of Musa paradisica Linn. Liver ailments stay one in every Of the intense health issues <sup>15</sup>. Present medicines have very little to offer for alleviation of hepatic diseases and it's primarily the plant based preparations that are used for the treatment of liver disorders <sup>16</sup>. The chemical composition of banana fibre is cellulose (50-60%), hemicelluloses (25-30%), pectin (3-5%), lignin (12-18%), water soluble materials (2-3%), fat and wax (3-5%) and ash (1-1.5%)<sup>17</sup>. Traditionally Musa paradisiaca is used in abscess, alopecia (female), burns, cancer, cataplasm, diarrhoea dysentery, dog bites, snake bite, dyspepsia, fracture, gangrene, hematuria, hemiplegia, haemoptysis, haemorrhage, lizard bites, migraine, ringworm, shingles, smallpox, syphilis, tuberculosis, tumour, uraemia, psoriasis, urticaria, warts and wounds <sup>18</sup>.

The liver demonstrates a major role in the metabolism of xenobiotics by regulating the synthesis, secretion and metabolism of xenobiotics. Various physiochemical functions of the body, including oxidation, reduction, hydroxylation, hydrolysis, conjugation, sulfation, acetylation etc. are well balanced by the liver alone. Injury to liver and damage to the hepatic parenchyma are always proved to be associated with distortion of different metabolic functions of the liver <sup>19, 20</sup>.

# **MATERIALS AND METHODS:**

**Collection of Plant Material:** The source of freshly collected whole plant in CSIR-NBRI, Lucknow. The plant material was identified and authenticated by a botanist, Department of Botany, NBRI, Lucknow.

**Preparation of Plant Extract:** The 4 kg whole plant part (leaf, fruit, root, stem and flower) was collected, cut into as small pieces and shade-dried, after that it was tray dried under controlled conditions and powdered. The powdered plant material 1000 g was macerated with 50% ethanol. The process of extraction was repeated for four times, filtered, concentrated on rotavapour (Buchi, USA) and then freeze-dried (Freezone 4.5, Labconco, USA) under reduced pressure to obtain 73 g of solid residue (yield 7.3 % w/w). The extract obtained was further subjected to toxicological and pharmacological investigations.

Experimental Animals: Wistar rats weighing 160-200g from our own breeding colony (Animal House-holding NBRI, Lucknow), were kept in cages with free access to foods and water, in a room with controlled temperature (22-24<sup>o</sup>C) and relative humidity 44-56%, light/dark cycles of 12 hours respectively for one week before and during the experiments. Animals were provided with a standard rodent pellet diet (Amrut, India) and the food was withdrawn 18-24 h before the experiment though water was allowed ad libitum. The composition of diet is 10% protein, 4% arachis oil, 1% fiber, 1% calcium, 1000 IU/gm vitamin A and 500 IU/gm Vitamin D. All experiments were performed in the morning in accordance with the current guidelines for the care of laboratory animals and the ethical guidelines for investigations of experimental pain in conscious animals. The protocol for this study has been approved by the Institutional Animal Ethics Committee as per the guidance of the Committee for the Purpose of Control and Supervision of Experiments on Animals-CPCSEA, New Delhi, with number (IAEC CPCSEA/07/2014).

**Determination of Effective Dose:** The MPE (*Musa paradisica* extract) was administered at different doses of 100, 150, 300, 600 and 1200 mg/kg/day orally for 4 days of five groups of rats (five in each group) and the animals were observed for mortality during the course of treatment or on the 5<sup>th</sup> day were tested again at lower dose levels and dose showed no mortality in rats and was selected as effective dose.

**CCl<sub>4</sub> Induced Hepatotoxicity:** Rats were divided into five groups of six animals in each group. Group I (control) animals were administered a single daily dose of liquid paraffin (1 ml/kg body weight, p.o.). Group II (toxic control) received carbon tetrachloride (1 ml/kg b.w., i.p.). Test groups (Groups III–IV) were administered orally 200 and 400 mg/kg b.w. 50% ethanolic extract, respectively, in the form of aqueous suspension

daily once a day. Group V received silymarin, the hepatoprotective compound known (Sigma Chemicals Company, USA), at a dose of 100 mg/kg, p.o., along with carbon tetrachloride. The Test drug was given simultaneously with carbon tetrachloride. Treatment duration was 14 days. Carbon tetrachloride was administered as a 30% solution in liquid paraffin for every 72 h. Animals were sacrificed 48 h after the last dose and blood was collected, allowed clotting and the serum separated. Liver was isolated for further biochemical investigations<sup>21, 22</sup>.

**Biochemical Investigations:** Serum biochemical parameters such as alanine transaminases (ALT), asparate transaminases (AST), total bilirubin (TB) and alkaline phosphatase (ALP) were assayed as per the methods <sup>21, 22</sup>. Total protein and albumin were evaluated by the method <sup>20</sup> and enzymatic parameters like- lipid peroxidation (LPO), catalase superoxide dismutase (SOD) by the methods <sup>23, 24, 25</sup>.

**Histopathological Assessment:** Sections taken from the liver, fixed in 10% formalin, dehydrated with ethanol (50-100%), cleared in xylene, and embedded in paraffin. Sections (4-5 mm thick) were prepared and then stained with hematoxylin and eosin (H&E) dye for photo microscopic observation, including cell necrosis, fatty change, hyaline degeneration, ballooning degeneration, infiltration of Kupfer cells and lymphocytes. **Statistical Analysis:** All the statistical comparison between the groups were made by means of One Way Analysis of Variance (ANOVA) and followed by Student-Newman-Keuls test. The p<0.05regarded as significant using, Graph Pad Prism 5.03 Software (CA, USA). The data expressed are Mean± standard error of mean (S.E.M.).

## **RESULTS:**

TABLE 1: EFFECT OF TEST DRUG ON E	BODY
WEIGHT AND LIVER WEIGHT IN CONTROL	AND
TOXIC GROUP.	

Treatment/dose	Body weight	Liver weight
Control	$189.00 \pm 4.53$	$6.40 \pm 0.06$
CCl4	$180.9\pm3.98$	$7.69 \pm 0.29z$
MP-200	$182.36 \pm 3.94$	$7.11\pm0.19$
MP-400	$1.81 \pm 4.11$	$6.75 \pm 0.15c$
SYL-100	$185.66\pm4.39$	$6.45\pm0.09c$

Note: All values expressed as g, in form of mean $\pm$ SEM, where n=6. If zp< 0.05 when compared to respective control and cp<0.05 when compared to respective CCl4 control.

50% ethanolic whole plant extract of *Musa paradisica* at a dose of 200 mg and 400 mg/kg once daily for 14 days and standard drug Silymarin at a dose of 100mg/kg were subjected for studying the body weight and liver weight in hepatotoxic rats. The study showed that the body weights were significantly decreased in CCl<sub>4</sub> groups. However, 50% ethanolic whole plant extract of *Musa paradisica* showed a dose dependent protection in body weight. The result of the high dose (400 mg/kg) was compared with standard drug Silymarin (100 mg/kg) (**Table 1**).

TABLE 2: EFFECT OF TEST DRUG ON BIOCHEMICAL PARAMETERS IN CCl<sub>4</sub> INDUCED HEPATOTOXICITY IN RATS.

Treatment	ALT(IU/L)	AST (IU/L)	ALP (IU/L)	Total	Albumin	TBL
				Protein(g/dl)	(g/dl)	(mg/dl)
Control	$68.5 \pm 7.745$	$108.25 \pm 9.25$	$83.59 \pm 7.52$	$6.59\pm0.72$	$4.213 \pm 0.440$	$0.59 \pm 0.05$
$CCl_4$	$178.91 \pm 9.54$	$228.70 \pm 17.66$	140.27±7.90	$2.18\pm0.56$	$1.080 \pm 0.230$	$1.78\pm0.08$
MP-200	93.76±5.73**	159.52 ± 9.03**	109.09±8.29*	$6.80 \pm 0.40 **$	3.310±0.629*	1.08±0.18**
MP-400	79.09±4.89**	129.78±11.80**	95.60±6.85**	$8.44 \pm 0.28 **$	4.676±0.094**	0.69±0.18**
SYL-100	75.82±6.25**	135.60± 3.89**	89.46±6.78**	$7.25 \pm 0.40 **$	5.030±0.130**	1.09±0.14**

Note: All values expressed as g/dl and mg/gdl in form of mean  $\pm$  SEM, where n=6. If \*p < 0.05, \*\*p < 0.05 when compared with respective CCl<sub>4</sub> treated group. TP: total protein, TB: total bilirubin, SEM: standard error of mean.

TABLE 3: EFFECT OF TEST DRUG ON ANTIOXIDANT AND LIPID PER-OXIDATION IN LIVER HOM	IOGENATE OF
CCL4INDUCED HEPATOTOXICITY IN RATS.	

Treatment/Dose	Catalase (U/mg)	SOD (U/mg)	LPO (U/mg)
Control	$24.14 \pm 2.11$	$112.47 \pm 2.41$	$0.4 \pm 0.05$
$\mathrm{CCl}_4$	$7.09 \pm 0.41$	$54.36\pm3.52$	$4.50\pm0.41$
MP-200	$15.19 \pm 1.05*$	$78.21 \pm 4.15*$	$3.29 \pm 0.39$ *
MP-400	$20.39 \pm 0.74$ **	$94.05 \pm 2.72$ **	$0.98 \pm 0.08$ **
SYL-100	$22.38 \pm 0.79$ **	$103.80 \pm 2.80$ **	$0.87 \pm 0.08$ **

Note: All values expressed as U/mg in form of mean  $\pm$  SEM, where n=6. If \*p < 0.05, \*\*p < 0.05 when compared with respective CCl<sub>4</sub> treated group. SOD: superoxide dismutase; LPO: lipid peroxidation, SEM: standard error of mean.

#### **Pharmacological studies:**

Effect of Test drug on ALT, AST, ALP, Total Protein, Albumin and Total Bilirubin actions: Table 2 that CCl<sub>4</sub> caused a significant elevation of liver serum markers. In the CCl<sub>4</sub> treated group, the level of ALT, AST, ALP, Total Protein, Albumin and Total Bilirubin (TBL) were significantly raised. In contrast, the groups treated with 50% ethanolic whole plant extract of *Musa paradisica* in doses of (200 and 400 mg/kg) once daily for 14 days prohibited the hepatotoxicity in a dose dependent manner. Effect of Test drug on CAT, SOD and LPO actions: Table 3 illustrated the lipid peroxidation and the enzymatic and non-enzymatic antioxidant level in the liver of experimental animals. Administration of CCl<sub>4</sub> led to elevation in the levels of LPO and drop in enzymatic scavenger viz. CAT, SOD levels in the liver homogenate. Treatment of rats with 50% ethanolic whole plant extract of *Musa paradisica* in doses of (200 and 400 mg/kg) noticeably prohibited the CCl<sub>4</sub> induced alterations of various parameters LPO, CAT, SOD.



FIG.1: HISTOPATHOLOGICAL ANALYSIS OF LIVER

- (a) Liver sections of normal control rats showing: normal hepatic cells with well preserved cytoplasm; well brought out central vein; prominent nucleous and nucleolus in Group-I.
- (b) Liver section of CCl<sub>4</sub> (1 ml/kg, i.p.) treated rats showing: massive fatty changes, necrosis, ballooning degeneration, and broad in filtration of the lymphocytes and kupffer cells around the central vein and the loss of cellular boundaries in Group-II.
- (c) Liver section of rats treated with CCl<sub>4</sub> (1 ml/kg, i.p.)+ MPE (200 mg/kg, p.o.)×14 days, showing: well brought out central vein, hepatic cell with well preserved cytoplasm, prominent nucleus and nucleolus in Group-III.
- (d) Liver section of rats treated with CCl<sub>4</sub> (1 ml kg, i.p.) + MPE (400 mg/kg, p.o.)×14 days, showing: well brought out central vein, hepatic cell with well preserved cytoplasm, prominent nucleus and nucleolus in Group IV.
- (e) Liver section of rats treated with  $CCl_4$  (1 ml kg, i.p.) + silymarin (100 mg/kg, p.o.)×14 days, showing: well brought out central vein, hepatic cell with well preserved cytoplasm, prominent nucleus and nucleolus in Group-V.

**DISCUSSION:** The world health organization surveyindicates that a total of about 70-80% of the world population rely on non commercial medicine mainly the herbal sources, in the primary health care units <sup>26</sup>. The results of the present study clearly indicated hepatoprotective effects of the ethanolic extract of *Musa paradisica* against CCl<sub>4</sub>-induced hepatic damage in rats. Hepatotoxicity induced by CCl<sub>4</sub> is one of the best characterized systems of xenobiotic-induced hepatotoxicity in experimental animals.

This method usually used to evaluation hepatoprotective properties of many bioactive substances and medicinal plants <sup>27</sup>. Liver as a vital organ in the body playing a Central role in metabolic homeostasis and detoxification of a variety of drugs and xenobiotics is vulnerable to a wide range of toxic, microbial, metabolic, circulatory and neoplastic insults <sup>28, 29</sup>. CCl<sub>4</sub>-induced hepatic damage is widely used for hepatoprotective drug screening.

 $CCl_4$ Hepatotoxicity of involves its biotransformation into free radicals such as trichloromethyl free radical  $(CCl_3)$ and trichloroperoxyl radical (CCl<sub>3</sub>O<sub>2</sub>-), and increased lipid peroxidation <sup>20</sup>. Bilirubin is excreted by the liver, and any interference with the normal liver function affects its rate of conjugation and excretion  $^{31}$ .

Hepatocyte injury initiates the activation of Kupffer cells which secrete potent mediators of the early inflammatory response, such as reactive oxygen species (ROS), especially superoxide anions that accounted for the formation of peroxynitrites and hydrogen peroxides (H2O2) therefore oxidative stress can be occur <sup>32</sup>. The antioxidants could attenuate this oxidative damage caused by free radicals indirectly by enhancing natural defenses of cell and or directly by scavenging the free radicals <sup>33</sup>. Antioxidants such as superoxide dismutase (SOD) can scavenges the superoxide anions whereas the glutathione reduced (GSH) is responsible to remove H2O2 through the action of glutathione peroxidase <sup>34</sup> and also, H2O2 is consumed by the action of catalase  $^{35}$ .

Assessment of liver function can be made by estimating the activities of serum ALT, AST, ALP and bilirubin, which are enzymes originally present in higher concentrations in cytoplasm. When there is hepatopathy, these enzymes leak into the bloodstream in conformity with the extent of liver damage <sup>36</sup>. Total bilirubin, a byproduct of the breakdown of red blood cells in the liver, bilirubin is a good indicator of liver function. High levels will cause icterus and are indicative of damage to the liver and bile duct <sup>37</sup>. Silymarin used as a standard drug derived from the milk thistle Silybum marianum. This has been shown to reduce lipid peroxidation and inhibit fibrogenesis in rodent animal models <sup>38, 39</sup>. The histopathological studies are direct means of assessing the defensive effect of the drug. The groups received CCl<sub>4</sub> alone, the spoils of cells around the central vein were well evident. While, the amount of damage was found minor in the studies included pre-treatment of MPE. The results of the histopathological study supported and well associated with data obtained from an assessment of the biochemical parameters.

This study quantifies ethanolic whole plant extract of *Musa paradisica* flavonoids content and

investigates the in vivo antioxidant effects of this extract in a carbon tetrachloride (CCl<sub>4</sub>) -induced hepatotoxicity rat model as an alternative for improving and/or reversing liver damage.

**CONCLUSION:** The 50% ethanolic whole plant extract of *Musa paradisica* could efficiently manage the AST, ALT, ALP and TB levels and elevated the protein levels in the protective studies. The defensive effect of MPE may be ascribed due to the abridged lipid peroxidation and improved defense of the hepatocytes against the reactive oxygen species. The histopathological studies also authenticate the activity of the drug. Therefore the study scientifically supports the treatment of this plant in various Ayurvedic preparations and traditional medicine for treatment of liver disorders and as a tonic.

**CONFLICT OF INTEREST**: We have no conflict of interest to declare.

**CONTRIBUTIONS OF AUTHORS:** Pritt Verma: Literature search, study design, data collection, data analysis and data interpretation writing. Dr. Ch. V Rao: Data interpretation and manuscript review, data analysis. Shravan Kumar Paswan: Data Analysis, Histopathological study, Acute toxicity.

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