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Effect of *Magnifera indica* Leaf Extract on Paracetamol-induced Hepatic Toxicity in Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author EM conceptualized and designed the work, wrote the protocol and the first draft manuscript and participated in data acquisition and interpretation of results. Author OY participated in the design of the work, interpretation of results and did the critical revision of the draft article for suitability and intellectual content. Author EI was involved in data acquisition, statistical analysis, interpretation of result and critical revision of the manuscript. Author EB managed the literature searches and participated in data acquisition. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: This study evaluated the effect of methanolic leaf extract of *Magnifera indica* on paracetamol-induced hepatic toxicity in rats.
Study Design: Hepatic toxicity in rats was induced by oral administration of paracetamol followed by treatment with the leaf extract and evaluation of liver function parameters, lipid peroxidation activity and hematology.
Place and Duration of Study: Department of Veterinary Physiology, Pharmacology and Biochemistry, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria/ One year.
Methodology: Hepatic injury was achieved by oral administration of 2000mg/kg of paracetamol to rats. Three test doses (100, 200 and 300mg/kg) of *Magnifera indica* leaf extract (MILE) and a standard reference drug, silymarin (100mg/kg) were administered to the rats orally for ten days through gastric gavage. At the end of the treatment, blood was

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collected from the rats for liver function tests, hematology, malondiadehyde (MDA) levels and catalase activities. The effect of the extract was compared with silymarin and distilled water controls.
Results: Liver function test showed that the extract and reference drug caused various levels of significant (P=0.02 to P=0.001) reduction of Aspartate aminotransferase (AST), Alanine aminotransferase (AST), Alkaline phosphatase (ALP) and total bilirubin when compared to negative control. Hematological analysis indicated various levels of significant increase in packed cell volume, hemoglobin concentration, Red blood cell count and White blood cell count (P=0.05-P<0.001). The MDA was also significantly (P=0.043) reduced by silymarin and MILE at the doses of 200 and 300mg/kg while there was no significant (P=0.24) changes in catalase activities of both treated and control rats.
Conclusion: This study showed that *Magnifera indica* leaf extract ameliorated paracetamol-induced liver toxicity with optimum effect at 300mg/kg.

Keywords: Magnifera indica; paracetamol; aminotransferase; malondiadehyde, hematology.

1. INTRODUCTION

The liver is the largest organ in the body and it is involved in intense metabolism and excretion. It is involved in almost all biochemical pathways to growth, fight against diseases, nutrient supply and energy provision. The liver also helps in several vital functions such as metabolism, secretion, storage and also detoxification of a variety of drugs and xenobiotics [1].

Liver diseases have become one of the major causes of morbidity and mortality in man and animals all over the world [2]. Diseases of the liver such as hepatitis, jaundice, cirrhosis and fatty liver are very common and constitute a large public health problem with jaundice and hepatitis being the two major hepatic disorders that account for high death rate [3].

The liver plays a central role in haematopoiesis and synthesis of coagulation proteins and liver disease is associated with a broad range of hematological abnormalities [4].

For treatment of hepatic disorders, orthodox medicine has little to offer and the use of natural remedies for the treatment of liver diseases has a long history. Also medicinal plants and their derivatives are still employed all over the world as they contain active principles against a variety of diseases as well as the management of liver diseases [5].

Magnifera indica commonly known as mango is one of the medicinal plants used in Nigerian folklore medicine for the management of many diseases including liver disorders. *M. Indica* belongs to the family Anacardiaceae and grows up to 10-45m in height; dome shaped with dense foliage and typically heavily branched from a stout tree. It is found in most countries of Africa, Asia and other warm regions of the world [6]. *Magnifera indica* have been used traditionally as antibacterial; for chronic dysentery and heart diseases among others. The plant also possess antimalarial, antioxidant, anti-inflammatory, anti-diabetic, gastroprotective, antitumor, antidiarrheal, antiparasitic and immunodulatory properties e.t.c [7-10].

This study was therefore undertaken to evaluate the effect of *Magnifera indica* on paracetamol-induced liver injury with a view to validating its therapeutic use in folkloric medicine.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Materials

The fresh leaves of *Mangifera indica* were collected behind the administrative block of Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria and identified by Dr I. Dike of department of Forestry of the same University. A voucher specimen number VPP/HB/045/2013 was deposited in the department of Veterinary Physiology, Pharmacology and Biochemistry.

2.2 Plant Extraction

The plant materials were dried under mild sunlight. They were pulverized into a coarse powder of about 1mm in diameter using a hammer mill. The plant material was extracted by cold maceration method for 48h in 80% methanol with intermittent shaking at 2h intervals. The extract was then filtered using Whatman filter papers (No.1). The filtrate was concentrated in electric oven at 40ºC. The extract was stored in a refrigerator at 4ºC as *Magnifera indica* leaf extract (MILE) until time of use [11].

2.3 Experimental Animals

Adult albino Wistar rats of both sexes weighing 150–180g, obtained from the laboratory animal unit of the faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria were used for the experiments. The animals were housed in stainless still cages at room temperature of between 25 and 30ºC and 12h light daily. The animals were fed *ad libitum* with growers rat feed (Vital feed®, Nigeria) and clean drinking water provided ad libitum also. They were allowed two weeks acclimatization before use and ethical conditions governing the conduct of experiments with life animals were strictly observed as stipulated by Ward and Elsea (1997) [12]. Also the experimental protocol was approved by the institution's ethical committee.

2.4 Acute Toxicity Study

The method of Lorke [13] was used for this study. Twenty five (25) mature albino rats of both sexes were used. They were randomly grouped into six groups (1-6) of 5 rats per group. Groups 1-5 rats received 100, 500, 1000, 2000 and 4000 mg/kg of *M. indica* extract respectively by oral gavage while group 6 rats received distilled water (10ml/kg). The rats were allowed free access to feed and drinking water and were observed for 48h for signs of toxicity and death.

2.5 Experimental Protocol

Paracetamol-induced hepatotoxicity model was used for this study [2]. Hepatotoxicity was induced in all thirty (30) albino Wistar rats by oral administration of paracetamol at the dose of 2000mg/kg. Twenty four hours later the rats were randomly divided into five groups of 6 rats per group and treated as follows for 10 days:

Group 1 (negative control group) rats received 10ml/kg of distilled water, group 2 rats (positive control group), received 100mg/kg sylimarin while groups 3, 4 and 5 rats received 100, 200 and 300mg/kg of *M. indica* extract, respectively all by gastric gavage. At the end of the 10 days treatment period, blood was

collected from the media canthus of the rats' eyes and the serum separated for serum biochemical analysis and whole blood used for hematology.

2.6 Biochemical Analysis

2.6.1 Liver function tests

Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) were evaluated using the method of Reitman and Frankel as described by Randox laboratories, United Kingdom using Randox kits [14]. Alkaline phosphatase (ALP) was assayed based on the methods of Kind and King [15]. Total protein in serum was assayed using direct biuret method while total bilirubin was determined spectrophotometrically using Randox kits.

2.6.2 Estimation of malondiadehyde

Lipid peroxidation was determined spectrophotometrically by measuring the level of the lipid peroxidation product, malondialdehyde (MDA) as described by Wallin et al. [16] using the principle that malondialdehyde reacts with thiobarbituric acid to form a red or pink coloured complex which in acid solution absorbs maximally at 532nm.

2.6.3 Catalase activity

This was done according to the method of Aebi [17]. The following were pipette into a test tube: phosphate buffer (2.5ml)+2ml H_2O_2 + 0.5ml of sample. To 1 ml portion of the mixture, 2 ml of dichromate acetic acid reagent was added. The absorbance was read at 240nm at a minute interval into 4 places.

Catalase activity was calculated using the following equation:

$$\text{Catalase concentration (IU/L)} = 0.23 \times \frac{\log \frac{Abs\ 1}{Abs\ 2}}{0.00693}$$

2.7 Hematology

2.7.1 Haemoglobin estimation

This was carried out using the standard hematology method as described by Coles [18]. Blood sample (0.2ml) was mixed with 4ml of Drabkins solution in a test tube and allowed to stand for 15 minutes at room temperature. The absorbance of the mixture was determined using E_1 calorimeter set at 540nm. The haemoglobin concentration was calculated using a standard graph.

2.7.2 Red blood cell (RBC) count

Blood sample was diluted 1:20 with 10% $NaCO_3$. The diluted sample was loaded into the Neubaer counting chamber with the aid of a Pasteur pipette. The RBC was counted from appropriate squares on the chamber under an electronic microscope [18].

2.7.3 Total white blood cell (WBC) count

The white blood cell count was determined following standard technique [19]. The blood sample was diluted 1:20 with Turks solution ((2% glacial acetic acid). The diluted sample was loaded into a Neubaer counting chamber with the aid of Pasteur pipette. The WBC was

calculated by counting the required number of squares on the counting chamber under a microscope.

2.7.4 Packed cell volume (PCV)

Blood samples were collected into PCV tubes (heparinized) using capillary action. One end of the tubes was sealed with plasticine and then centrifuged using the haematocrit centrifuge for 5 minutes at 2500rpm. The test was read using a PCV hematocrit reader [19].

2.8 Statistical Analysis

The results were presented as mean±standard error of mean (S.E.M) and analyzed using one way analysis of variance (ANOVA) and the differences between the means were tested using Post Hoc LSD and values of $P<0.05$ to $P<0.001$ were considered statistically significant.

3. RESULTS

3.1 Plant Extraction

The yield of the methanolic extract of *M. indica* was 3.46% w/w dry mater.

3.2 Acute Toxicity Test

Administration of the extract even at 4000mg/kg produced no signs of toxicity or death after 48h.

3.3 Liver Function Parameters

The result of the liver function tests is presented in Table1.

The result showed that *M. indica* extract caused a significant (P=0.02) reduction of the AST while the reference drug, silymarin and the extract at 300mg/kg also caused a significant (P=0.001) decrease in AST when compared to the negative control. Also, the ALT, ALP and the total bilirubin were significantly (P=0.02) reduced by silymarin and *M. indica* extract at the dose of 300mg.kg when compared to the negative control group. However, there were no significant variations in the serum total protein of both the treated and control rats.

3.4 Malondiadehyde

(Fig. 1) shows the result of the effect of *M. indica* extract on the malondiadehyde (MDA) levels of paracetamol-induced hepatic injured rats. The result showed that silymarin (100mg/kg) and *M. indica* extract at the doses of 200 and 300mg/kg significantly (P=0.04) reduced the MDA levels of treated rats when compared to the negative control rats. The extract at the dose of 100mg/kg caused an insignificant reduction of the MDA level of treated rats.

3.5 Catalase

The result of the catalase activities of paracetamol-induced liver injured rats is presented in (Fig. 2). The result showed that there were no significant changes in the catalase activities of both treated and untreated control rats (P=0.24).

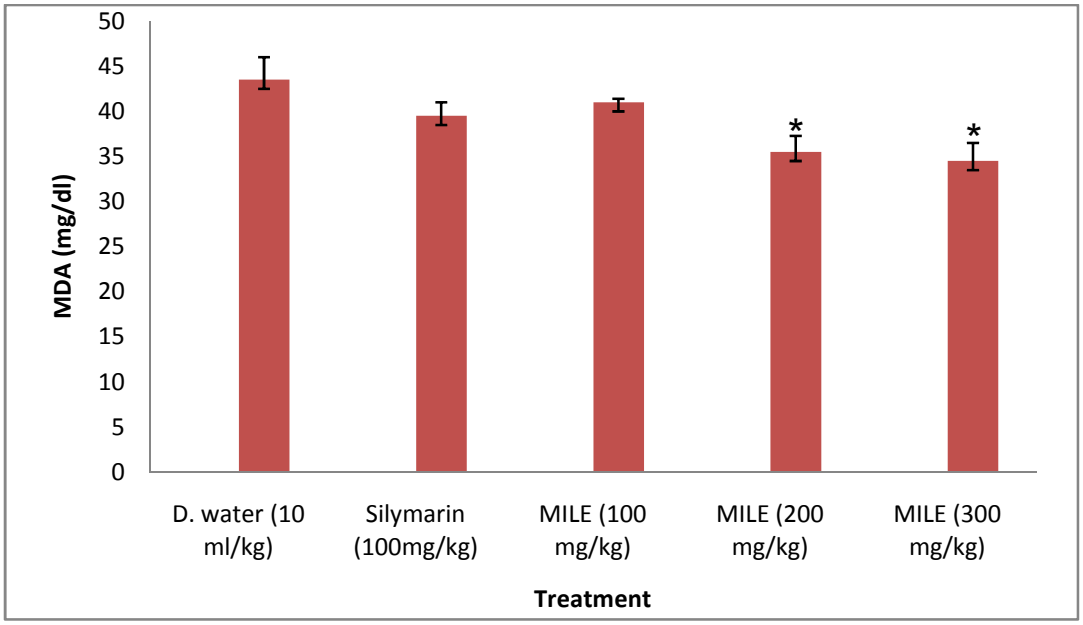

Fig. 1. MDA levels of paracetamol-induced liver injured rats
*Test extract: Significant from distilled water control, *P= 0.04*

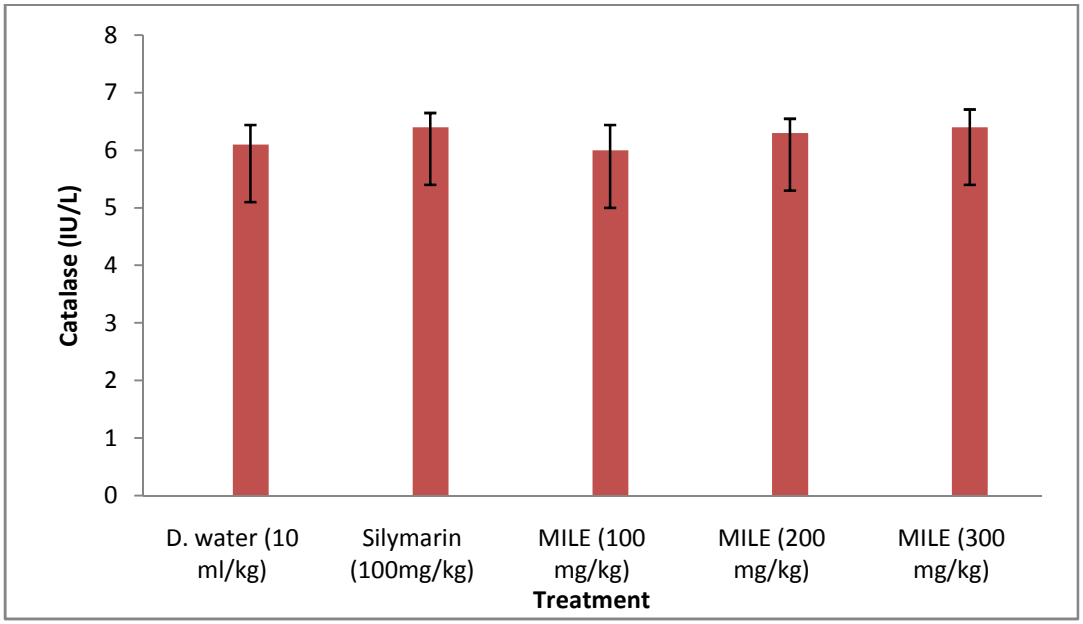

Fig. 2. Catalase activities of paracetamol-induced liver injured rats
Test extract: No significant difference from distilled water control, P=0.24

Table 1. Effect of MILE on the liver function parameters of paracetamol-induced liver injury in rats

Group	Treatment	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Total protein (g/dl)	Total bilirubin (mg/dl)
1	D. water (10ml/kg)	93.0±3.0	111.0±4.0	35.0±3.4	11.0±0.4	0.35±0.5
2	Silymarin (100mg/kg)	52.0±1.2**	82.0±3.5*	22.9±0.2*	10.7±0.1	0.23±0.2*
3	MILE (100mg/kg)	75.6±2.1*	100.1±3.4	35.0±1.2	10.6±0.4	0.35±1.2
4	MILE (200mg/kg)	61.3±1.3*	73.3±3.7	32.7±0.9	10.8±0.3	0.33±0.9
5	MILE (300mg/kg)	58.0±0.3**	72.0±1.5*	27.1±2.2*	10.9±0.3	0.27±0.6*

**P=0.02; **P<0.001 when compared to Distilled Water control (negative group)*

Table 2. Effect of *M. indica* extract on hematological parameters of paracetamol-induced liver injury in rats

Group	Treatment	PCV (%)	Hb (g/dl)	RBC ($x10^6$)	WBC ($x10^3$)
1	D. water (10ml/kg)	31.0(2)	9.3(0.8)	2.2(5)	4.2(2)
2	Silymarin (100mg/kg)	37.0(2.3)*	12.4(1.8)*	2.8(6.5)	13.9(2.3)***
3	MILE (100mg/kg)	37.0(3.5)*	12.5(0.2)*	2.7(7.6)	4.7(2.8)
4	MILE (200mg/kg)	44.5(1.5)**	13.7(0.4)*	3.4(3.5)*	7.7()7.4*
5	MILE (300mg/kg)	44.7(1.7)**	13.8(1.3)*	4.0(4.1)*	12.7(3)***

**P=0.05; **P=0.01; ***P=001 when compared to D. Water control (negative control)*

3.6 Hematology

The result of the effect of *M. indica* leaf extract on the haematology of paracetamol-induced liver injured rats is presented in Table 2 (above). The result indicated that there was a dose dependent and various levels of significant (P=0.05 and P=0.01) increase in the PCV and hemoglobin concentrations by both the reference drug and *M. indica* extract when compared to the untreated control. The RBC count was significantly (P=0.05) increased by MILE at the doses of 200 and 300mg/kg when compared to the negative control. Also there was a highly significant (P<0.001) increase in the WBC count by silymarin and the extract at 300 mg/kg body weight.

4. DISCUSSION

The effect of *Magnifera indica* on hepatic injury was evaluated using experimental models of liver injury in rats induced by paracetamol. Paracetamol is a common analgesic and antipyretic drug but for screening of hepatoprotective agents, paracetamol-induced hepatotxicity has been used as a reliable method. Liver damage by paracetamol is due to its toxic metabolite, N-acetyl-p-benzoquinoneimine (NAPQI) which is activated by hepatic cytochrome P-450. In normal cases, the NAPQI generated overwhelms the detoxification process leading to liver damage [20].

In general, activities of some basic liver function enzymes, just like bilirubin and protein in plasma or serum are analyzed to indirectly asses the integrity of liver tissues after exposure to certain pharmacological agents or substances [21]. Administration of paracetamol caused significant elevation of liver enzymes such as AST, ALT, ALP and bilirubin levels as seen in the untreated control rats. These enzymes are cytoplasmic in location and are released into circulation after liver damage indicating hepatotoxicity.

Oral treatment with *M. indica* extract caused various levels of significant reduction of the elevated liver marker enzymes. The effect of the extract at 200 and 300mg/kg was comparable to silymarin. This reduction of the liver marker enzymes is an indication of plasma membrane stabilization as well as regeneration of damaged liver tissues which then prevents the leakage of the enzymes into the blood stream [3]. This is an indication of the usefulness of the plant in the treatment of liver disorders which is in line with the findings of Shardul and Gangdhar [22]. They reported that increase in liver marker enzymes by drug or extract is a sign of hepatoprotective activity.

Serum bilirubin is considered as an index for the assessment of hepatic function and increased serum level indicates hepatobilliary disease [22]. Paracetamol increased serum bilirubin but treatment with MILE at 300mg/kg significantly reduced it (Table 1). Previous works indicated reduction of serum bilirubin as a mechanism of hepatoprotective activity [2]. This reinforces the hepatoprotective activity of the extract.

There was no significant difference in the total protein levels of both treated and control rats and this may be attributed to the duration of the study.

Hepatic lipid peroxidation is measured by the level of MDA and is increased in paracetamol toxicity as seen in the untreated control rats in this study. Treatment with silymarin and MILE at 200 and 300mg/kg reduced the MDA levels of the rats (Fig 1). This result agrees with the

work of Parma et al. [2]. This may have been brought about by increased activity of glutathione peroxidase or by inactivation of lipid peroxidation by the extract [23].

Reduced catalase activities as seen in the negative group of rats in this study, is an indication of generation of free radicals and also a feature of hepatic damage due to paracetamol toxicities [24]. The scavenging enzyme, catalase, was increased though not significantly by treatment with MILE suggesting that *M. indica* may possess antioxidant activity.

Hepatic synthetic dysfunctions have adverse effects on both cellular and soluble components of blood and can lead to haemolytic anaemia [25]. Paracetamol toxicity caused decreased values of hematological parameters as seen in the negative control rats which was significantly and dose dependently increased on administration of *M. indica* extract and silymarin (Table 2). The reversal of the hematological parameters may be due to increased erythropoiesis induced by the extract or by prevention of the hemolysis of the acanthocytes (spur cells) which results from abnormal lipid composition of the red blood cell membrane following hepatic dysfunction [25].

5. CONCLUSION

Magnifera indica has demonstrated significant curative potential of hepatic injured rats as evidenced by reversal of various altered biochemical and hematological parameters which may be due to the extract's glutathione-like effects, membrane stabilization and restoration of damaged hepatic tissues or through antioxidant activities. However, more work is required to isolate the active compound(s) responsible for the antihepatotoxic activity and to determine the exact mechanism of action.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and therefore have been performed in accordance with the ethical standards laid down in the 1964 Declarations of Helsinki and Michael Okpara University of Agriculture, Umudike, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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