Morphological and Biochemical Effects of Crude Aqueous Extract of Mangifera indica L. (Mango) Stem Bark on the Liver in Wistar Rats

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Abstract: The objective of this study was to investigate the effects of Mangifera indica L. (mango) stem bark crude Aqueous Extract (AE) on the morphology and biochemical functions of the liver in wistar rats. Adult wistar rats used in the study were divided into 2 groups: Group 1 rats were untreated and served as control and Group 2 experimental rats were orally given 1 mL (100 mg) daily of aqueous extract for a period of 14 days. The body weight changes and the weight of the liver were measured and the serum aspartate aminotransferase (AST),Alanine Transaminase (ALT), Alkaline Phosphatase (ALP), Total Bilirubin (TB) and Conjugated Bilirubin (CB) levels were determined. There was no significant difference (p<0.05) in body weight gain between the two groups at the end of the experiment. The treated group had a significant decrease in liver weight (p<0.05) when compared with control. The treated group also had a significant increase in AST when compared with control. There were no significant increases in ALT, ALP and total bilirubin when compared with the control. The study suggests derangement of liver function and possible damage to the hepatocytes by the crude AE at this dose and duration.

Key words: Aqueous extract, liver, Mangifera indica, toxicity, wistar rats

INTRODUCTION

Mangifera indica L. (Anacardiaceae) is one of the most important tropical plants marketed in the world (Ross, 1999). It is grown widely in different parts of Africa, especially in the southern part of Nigeria, where it is valued for its edible fruit (Nwinuka et al., 2008). There are many traditional medicinal uses for the bark, roots and leaves of M. indica throughout the globe (Ross, 1999). Mangifera indica is used medicinally to treat ailments such as asthma, cough, diarrhea, dysentery, leucorrhoea, jaundice, pains, malaria (Madunagu et al., 1990; Gilles, 1992) and diabetes (Ojewole et al., 2005; Muruganandan et al., 2005; Perpetuo and Salgado 2003; Mahabir and Gulliford, 1997).

Phytochemical research from different parts of M. indica has demonstrated the presence of phenolic constituents, triterpenes, flavonoids, phytosterols, and polyphenols (Singh et al., 2004; Selles et al., 2002; Anjaneyulu et al., 1994; Kharn et al., 1994; Saleh and El-Ansari, 1975). This species is purported to possess numerous therapeutic uses including analgesic, anti-inflammatory (Garrido et al., 2001), immunostimulant (Makare et al., 2001; Garcia et al., 2002, 2003a), antioxidant (Martinez et al., 2000; Sanchez et al., 2000, 2003), spasmylytic, anti diarrhoea (Sairam et al., 2003), dyslipidemic (Anila and Vijayalakshmi, 2002), antidiabetic (Aderibigbe et al., 1999, 2001), antiamebic (Tona et al., 2000), anthelmintic, antiallergic (Garcia et al., 2003b) and antibacterial applications (Bairy et al., 2002).

The liver is the largest solid organ in the body. It is the centre of all metabolic activities in the body. Drugs and other foreign substances are metabolized and inactivated in the liver and is therefore susceptible to the toxicity from these agents. Certain medicinal agents when taken in overdoses and sometimes even when introduced within therapeutic ranges may injure the liver.

Millions of people in various traditional systems, including Nigeria, have resorted to the use of medicinal plants to treat their ailments; this could be as a result of the high cost of orthodox health care, or lack of faith in it, or maybe as a result of the global shift towards the use of natural, rather than synthetic products (Omonkhua and...
Onoagbe, 2008). While the craze for natural products has its merits, care must be taken not to consume plants or plant extracts that could have deleterious effects on the body, either on the short term or on the long term (Omonkhua and Onoagbe, 2008). There is therefore the need to study these plants for their biochemical/toxicological effects.

Reports regarding the effects of crude Aqueous Extract (AE) of *M. indica* stem bark on the morphology and the biochemical functions of the liver/liver are scanty in existing literatures. Hence, the present study was undertaken to investigate the effects of the crude AE of the stem bark of this tree on the morphology and biochemical functions of the liver in wistar rats.

**MATERIALS AND METHODS**

**Location and duration of study:** This study was conducted at the histology laboratory of the College of Medicine, Ambrose Alli University, Ekpoma, Edo State, Nigeria. The preliminary studies, animal acclimatization, drug procurement, actual animal experiment and evaluation of results, lasted for a period of one month (April, 2010). However, the actual administration of the drug to the test animals lasted for two weeks (2nd to 15th April, 2010).

**Animals:** Wistar rats weighing 150 g each were obtained from the Experimental Animal Unit of College of Medicine, Ambrose Alli University, Ekpoma, Edo State, Nigeria. Rats were acclimatized to the experimental room having temperature 19±1°C, controlled humidity conditions (65%) and 12:12 h light: Dark cycle. The experimental animals were housed in standard plastic cages, fed with standard diet, and water ad libitum. All experimental procedures were approved by the Animal Care and Use Committee of College Medicine, Ambrose Alli University, Ekpoma, Edo State, Nigeria.

**Collection of medicinal plant and Preparation of aqueous extract:** *Mangifera indica* stem bark was obtained freshly from a farm in Ekpoma in Esan West LGA, Edo state of Nigeria. The plant was identified and authenticated at the Botany department of Ambrose Alli University, Ekpoma. The stem bark of *Mangifera indica* was cut into smaller pieces and sun-dried for two weeks. The dried sample was pulverized using mortar and pestle. The resulting powder material was used in the extraction process. Extraction was carried out using the method described elsewhere (Harboone, 1972; Ekpe et al., 1990; Uhegbu et al., 2005; Nwinuka et al., 2008) and modified in our laboratory. Briefly, 20 g of powdered sample of the herb was extracted by soaking in 200 mL of distilled water in a beaker, stirred for about 6 min and left overnight. Thereafter, the solution was filtered using filter paper (Whatman No. A-1) to remove cellulose fibers and extract stored in a refrigerator at 4°C.

**Experimental design:** After acclimatization period, rats were weighed and divided into two groups comprising five animals in each group as follows:

- **Group 1:** Rats were untreated and served as control.
- **Group 2:** Experimental animals were orally given 1 mL (100 mg) daily of aqueous extract for a period of 14 days.

The 1 mL of crude AE used in this experiment was based on the previous work done with this plant (Nwinuka et al., 2008).

**Sample collection:** At the end of experimental period, rats were weighed and anesthetized with chloroform. Blood samples were collected by cardiac puncture in non-heparinized tubes, centrifuged at 2000 rpm for 20 min and blood sera were then collected and stored at 4°C prior immediate determination of Alanine Transaminase (ALT), Aspartate Transaminase (AST), Alkaline Phosphatase (ALP), total and conjugated bilirubin (TB and CB). The liver from both control and test animals were removed and weighed to the nearest 0.01 g.

**Biochemical assay:** Estimation of AST activities and ALT activities were done using Reitman-Frankel method (Widmann, 1980). Estimation of ALP activities using King and King (1954) method. Bilirubin (Total and conjugated) were determined using Malloy and Evelyn (1932) method.

**Statistical analysis:** The data of liver and body weights and biochemical analysis were analyzed using the Statistical Package for Social Sciences (SPSS for windows, version 12.0). Comparison were made between control and experimental groups using student’s t-test. Values of less than 0.05 were regarded as statistically significant.

**RESULTS**

**Gross morphology:** During the following 14 days after the administration of the crude AE, one treated animal died on the 12th day (weight-175 g), no death was seen in control group. There was significant alteration in water and food consumption; the treated group rats consuming and drinking far less than the control group rats. The rats in the treated group appeared emaciated and inactive compared with the control group rats, which appeared well nourished and active. The livers obtained from the control group showed no difference in their normal gross anatomical features, that is, size, colour, consistency etc but the test groups decreased markedly in size.
Effect of crude AE of Mangifera indica on body and liver weights (Table 1): The control group showed a mark increase in body weight whereas the treated group had a slight increase in body weight. The increase in body weight of control group was not statistically significant (p<0.05) compared with the treated group. There was however a significant decrease in the weight of the liver in the treated group (p<0.05) when compared with the control.

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial</th>
<th>Final</th>
<th>Increase (%)</th>
<th>Liver weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>150.00</td>
<td>225.00±50.00</td>
<td>50</td>
<td>7.92±0.77</td>
</tr>
<tr>
<td>Treated</td>
<td>150.00</td>
<td>166.67±14.43**</td>
<td>11</td>
<td>5.52±0.21*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ±SD.

*: Significantly different statistically from the control at p<0.05, t-test.

**: Significantly different statistically from the control at p>0.10, t-test

Effect of crude AE of Mangifera indica on serum TB, ALT, AST and ALP (Table 2): There was a slight increase in the levels of total bilirubin concentrations and ALT and ALP activities in the treated group when compared with control. The treated group showed a significant increase in serum AST activity (p<0.05) when compared with the control.

<table>
<thead>
<tr>
<th>Group</th>
<th>TB (µg/dL)</th>
<th>ALT (mU/L)</th>
<th>AST (mU/L)</th>
<th>ALP (mU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.56±0.05</td>
<td>9.00±1.00</td>
<td>9.33±0.58</td>
<td>32.67±2.01</td>
</tr>
<tr>
<td>Treated</td>
<td>0.59±0.02</td>
<td>9.00±1.41</td>
<td>11.00±1.00*</td>
<td>35.00±1.00</td>
</tr>
</tbody>
</table>

Values are expressed as mean ±SD. *: Significantly different statistically from the control at p<0.05 t-test

DISCUSSION

The observed decrease in the weight of the liver in this study indicates that crude AE of M. indica might have toxic effect on this organ at this dose. It has been reported that increase or decrease in either absolute or relative weight of an organ after administering a chemical or drugs is an indication of the toxic effect of that chemical (Simons et al., 1995).

Serum AST, ALT, ALP and bilirubin are the most sensitive markers employed in the diagnosis of hepatic damage because they are cytoplasmic enzymes released into circulation after cellular damage (Sallie et al., 1991). The significant increased activity of AST and the insignificant increased activities of ALT, ALP and the level of bilirubin in serum indicate M. indica - induced hepatocellular damage.

The AST and ALT enzymes are involved in amino acid metabolism and an increase in these enzymes in serum indicate tissue damage or toxic effects in liver (Klassen and Plaa, 1966; Worblewski and La Due, 1955; Okonkwo et al., 1997; Varley et al., 1991). Smith et al., (1998) reported an increase in ALT in oral acetaminophen-induced hepatotoxicity in rats indicating a biochemical evidence of significant liver damage (Smith et al., 1998). Mizutani and co-workers (1999) reported an increase in serum ALT activity in methimazole-induced hepatotoxicity in mice.

Several studies on different parts of M. indica have demonstrated the presence of phenolic constituents, triterpenes, flavonoids, phytosterols, and polyphenols (Singh et al., 2004; Selles et al., 2002; Anjaneeyulu et al., 1994; Kharn et al., 1994; Saleh and El-Ansari, 1975), which are known to possess antioxidant properties (Martinez et al., 2000; Sanchez et al., 2000, 2003). The role of antioxidants in preventing various human diseases by preventing oxidative stress and damage in biological tissues have been demonstrated in many experiments (Repetto et al., 2002).

Interestingly, phenolic compounds have the potential to function as antioxidants by scavenging the superoxide anion, hydroxyl radical and peroxyl radical or quenching singlet oxygen, thus inhibiting lipid peroxidation in biological systems (Severi et al., 2009). Moreover, studies have also revealed that polyphenols exhibit clear cytoprotective effect on rat or tumour hepatocytes injury system (Sugikami et al., 1999; Lima et al., 2006; Yao et al., 2007), and human hepatocytes against oxidative injury induced by hydrogen peroxide (H₂O₂) or carbon tetrachloride (CCl₄) in vitro (Zhao and Zhang, 2009).

In view of these reported beneficial effects of M. indica on the liver, the elevated levels of the serum enzymes and the decrease liver weight observed in this study rather suggest a physiological dysfunction arising from an overdosage. Nwinnuka et al. (2008) in their study on the effect of M. indica on the haematopoietic system reported an improvement on the haematological parameters with a similar dose and duration. Thus, while this dose might be beneficial to the haematopoietic system, it is however toxic to the liver.

There are reports that a vegetable Vernonia amygdalina (Ojiako and Nwanjo, 2006) and the plant Cissus populnea (Ojekale et al., 2007) are hepatoprotective at low doses but hepatotoxic at higher doses. Moreover, vitamin E an antioxidant has been reported to have prooxidative effects at high doses (Mukai, 1993; Thomas et al., 1996; Eder, 2002). There are reports also, that green tea polyphenols can in fact cause oxidative stress and liver toxicity in vivo at certain concentrations (Lambert et al., 2007). It can be suggested based on these reports that the dose used in this study is above the safe dose for rats. The dose might have been too toxic to the rats to have caused the death recorded in
the experimental group. Also, this dose may have been responsible for the markedly reduced food and water intake in the experimental group.

CONCLUSION

Our study suggests that crude AE of M. indica has no significant effect on somatic growth but causes a significant decrease in liver weight. The investigation also shows that the crude AE causes increases in liver enzymes, which suggest toxicity to the liver. It is therefore recommended that further studies be conducted to determine the safe dose of this crude AE and its indiscriminate consumption should be discouraged.

REFERENCES


