**Toxicological Evaluation of Aqueous Extract of *Mangifera Indica* Linn (Mango)Stem Bark on the Activities of Some Enzymes in Albino Rats**

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**Abstract:** Toxicological evaluation of aqueous extract of *Mangifera indica* Linn (Mango) stem bark on the activities of some enzymes in Albino Rats (80-140g) were critically examined. The albino rats (12) were randomly assigned into three (A-B) groups each of which contains four rats. They were acclimatised for a week and *Mangifera indica* stem bark extract was administered for three weeks after which they were sacrificed. Group A (Control) received equivalent volume of distilled water while group B and C received 25 mg/kg and 75 mg/kg bwt of the extract respectively. The specific activities of ALP and ACP in liver and kidney at all doses of the extract was significantly (p<0.05) decreased with a corresponding significant (p<0.05) increase in the serum compared with the control. The specific activities of AST and LDH in serum and liver at all doses shows no significant (p>0.05) difference compared with the control while the activity of LDH in kidney at the dose of 75 mg/kg body weight shows a significant (p<0.05) increase compared with the control. Also, the specific activity of ALT in liver, LDH in heart at the dose of 25 mg/kg bwt shows significant (p<0.05) decrease compared with the control group. Therefore, the aqueous extract of *Mangifera indica* stem bark at the dose of 25 mg/kg and 75 mg/kg body weight have brought about alterations in the concentration of the enzymes studied and may not be completely safe at the doses used in this study.

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**Key words:** *Mangifera Indica*;Stem bark; Medicinal plants; Toxicity; Biochemical indices

**1.0 Introduction**

A medicinal plant is any plant in which one or more of its organs, contains substances that can be used for therapeutic purpose of which are precursors for the synthesis of useful drugs. The crude extracts or purified form of plant has been used as medicines and cosmetics (Sofowora, 1993). The medicinal value of these plants lies in bioactive phytochemical constituents that produce definite physiological action on the human body (Akinmoladun *et al.,* 2007). Some of the most important bioactive phytochemical constituents are alkaloids, essential oils, flavonoids, tannins, terpenoid, saponins, phenolic compounds and many more (Edeoga *et al.,* 2005). These natural compounds formed the foundations of modern prescription drugs as we know today (Goh *et al.,* 1995).

*Mangifera indica* L. belongs to the family Anacardiaceae; the tree is a native of tropical Asia, though it is now completely naturalized in many parts of the tropics and subtropics (Ross, 1999). Other *Mangifera species* include *M*. f*oetida* (Horse Mango) which are grown in more localized areas but *Mangifera indica* - the common mango or Indian Mango - is the only mango species cultivated in many tropical and subtropical regions and its fruits are distributed worldwide. *M indica* is used as medicine to treat several ailments such as asthma, cough, diarrhoea, dysentery, jaundice, pain, malaria, anaemia and diabetes (Madunagu *et al*., 1990; Ogbe *et al.,* 2010; Ojewole, 2005).

Despite the therapeutic uses of medicinal plant, effective dosage of most medicinal plants has not been worked out. Therefore, this research is then carried out to determine the toxic and safety effect of aqueous extract of *Mangifera indica* stem bark.

**2.0 Materials and Methods**

**2.1 Experimental Animals**

Wistar male and female adult albino rats (12) weighing between 80-140g were obtained from the Animal Breeding Unit of the Faculty of Science, University of Ilorin, Ilorin, Kwara state, Nigeria. The animals were housed in standard plastic cages and were acclimatized for a week. They were maintained under standard conditions of 12hours light and dark cycle and were fed on standard pelleted feed and distilled water.

**2.2 Plant identification and preparation of extract**

The stems of the plant was obtained from Faculty of Agriculture, University of Ilorin, Ilorin, Nigeria and was authenticated in the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria, where a voucher specimen was deposited at the Departmental Herbarium.

The stem bark was dried at room temperature and pulverized. 30g of the powder was turned into 200ml of distilled water and boiled on steam water bath at 450C for 30 minutes, it was then filtered with Whatman No.1 filter paper. The filtrate was therefore concentrated under pressure using steam water bath at 450C, thereby generating the crude extract. The generated crude extract was used to prepare the various desired concentrations (25mg/kg bwt and 75mg/kg bwt) to be administered. The reconstituted aqueous extract was administered orally using cannula to all animals in different groups (Yakubu *et al.,* 2005).

**2.3 Animal grouping and extract administration**

Wistar male and female adult albino Rats (12)were randomly assigned into three groups (A-C), each group containing 4 rats. The control (group A) was orally administered distilled water only. The extract was dissolved in 58ml of distilled water of varying concentrations of 25 and 75mg/kg body weight of extract and was orally administered to rats in groups B and C respectively for twenty one days.

**2.4 Preparation of serum and organ homogenates**

The rats were placed under diethyl ether anaesthesia; the neck area was shaved to expose the jugular veins. The veins after being slightly displaced (to avoid contamination with interstitial fluid) were then sharply cut with a sterile scalpel blade. Blood collected into clean and dry centrifuge tubes was allowed to clot for 30 minutes. This was then centrifuged at 33.5 g for 15 minutes using a Uniscope Laboratory Centrifuge (model SM800B). The sera were aspirated with Pasteur pipettes and stored frozen overnight at -200C before being used for the biochemical analyses. The animals were quickly dissected and the liver, kidney and heart were removed, blotted with clean tissue paper, weighed, and homogenized in 0.25M sucrose solution (1:5 w/v). The homogenates were kept frozen for 24 hours before being used for the analyses (Yakubu *et al.*, 2005).

**2.5 Determination of biochemical parameters**

The protein content of homogenates was determined using the Biuret method of Henry *et al.* (1974). Activities of aspartate transaminase (AST) (EC 2.6.1.1) and alanine transaminase (ALT) (EC 2.6.1.2) were determined based on the method described by Schmidt and Schmidt (1963), Lactate Dehydrogenase (LDH) (EC 1.1.1.27) Activity was determined as described by Wrobleski La Due (1955), alkaline phosphatise (ALP) (EC 3.1.3.1) and acidic phosphatise (ACP) activity was determined as described by Wright *et al*. (1972). All measurements were done using Spectronic 21 spectrophotometer (Bausch and Lomb, NY).

**2.6 Statistical analysis**

The data were expressed as mean ± standard error of mean (SEM). Statistical analysis was performed using analysis of variance (ANOVA) and Duncan multiple range test at 5% level of confidence (p<0.05).

**3.0 Results**

Table 1 shows the specific activity of alkaline phosphatase (ALP) in serum, liver and kidney of rats administered with aqueous extract of *Mangifera indica* stem bark. The activity of ALP in the serum of rats administered with 25 and 75 mg/kg body weight of the extract was significantly (p<0.05) increased compared with the control. In the liver and kidney of rats administered with 25 and 75 mg/kg body weight of the extract, there was a significant (p<0.05) decrease in the activity of ALP compared with the control. Furthermore, there was no significant (p>0.05) difference in the activity of ALP in the serum, liver and kidney at the dose of 25 and 75 mg/kg body weight of the extract (Table 1).

Table 2 shows the specific activity of acid phosphatase (ACP) in serum, liver and kidney of rats administered with aqueous extract of *Mangifera indica* stem bark. The activity of ACP in the serum of rats administered with 25 and 75 mg/kg body weight of the extract was significantly (p<0.05) increased compared with the control. In the liver and kidney of rats administered with 25 and 75 mg/kg body weight of the extract, there was a significant (p<0.05) decrease in the activity of ACP compared with the control. Furthermore, there was a significant (p<0.05) difference in the activity of ACP in the serum, liver and kidney at the dose of 25 and 75 mg/kg body weight of the extract (Table 2).

Table 3 shows the specific activity of aspartate transaminase (AST) in serum, liver and heart of experimental rats. The specific activity of serum AST, liver AST and heart AST within the groups (25 and 75mg/kg bwt) show no significant (p>0.05) difference compared with the control group. But there was a significant increase in the activity of AST in the serum at the dose of 25mg/kg compared with the dose of 75mg/kg (Table 3).

Table 4 shows the specific activity of alanine transaminase (ALT) in serum, liver and heart of rats administered with aqueous extract of *Mangifera indica* stem bark. In the serum and heart of rats administered with 25 and 75 kgmg/kg bwt of the extract, there was no significant difference in the activity of ALT when compared with the control group while the liver ALT of the 25 mg/kg bwt group shows significant decrease, the 75 mg/kg bwt group shows no significant difference (Table 4).

Table 5 shows the specific activity of lactate dehydrogenase (LDH) in serum, kidney, heart and liver of rats administered with aqueous extract of *Mangifera indica* stem bark. The activity of LDH in the serum and liver of rats administered with 25 and 75 mg/kg of the extract shows no significant (p>0.05) difference when compared with that of the control group. In the kidney of rats administered with 75 mg/kg bwt of the extract, there is a significant (P<0.05) increase in the activity of LDH when compared with the control group. In the heart of rats administered with 25 mg/kg bwt of the extract, there was a significant decrease in the activity of LDH when compared with the control group (Table5).

**Table 1: Activity of Alkaline Phosphatase (ALP) in serum, liver and kidney of rats administered with aqueous extract of *Mangifera indica* stem bark**

|  |  |  |  |
| --- | --- | --- | --- |
|  | Specific activity (µmol/min/mg protein) | | |
| Groups | Serum | Liver | Kidney |
| Control | 23.03±1.03a | 22.99±0.13a | 54.20±2.11a |
| 25 mg/kg bwt | 27.47±1.02b | 15.75±0.08b | 49.20±1.24b |
| 75 mg/kg bwt | 27.02±1.15b | 18.55±0.10b | 47.50±1.14b |

Values are expressed as Mean ± SEM (n = 4). Values in each column with different superscript are significantly different (P<0.05).

**Table 2: Activity of Acid Phosphatase (ACP) in serum, liver and kidney of rats administered with aqueous extract of *Mangifera indica* stem bark**

|  |  |  |  |
| --- | --- | --- | --- |
|  | Specific activity (µmol/min/mg protein) | | |
| Groups | Serum | Liver | Kidney |
| Control | 44.16±2.06a | 83.76±0.94a | 143.91±1.07a |
| 25 mg/kg bwt | 53.42±3.89b | 79.77±1.20b | 127.79±2.76c |
| 75 mg/kg bwt | 61.75±2.26c | 74.34±1.26c | 138.64±2.40b |

Values are expressed as Mean ± SEM (n = 4). Values in each column with different superscript are significantly different (P<0.05).

**Table 3: Activity of Aspartate Transaminase (AST) in serum, liver and heart of rats administered with aqueous extract of *Mangifera indica* stem bark**

|  |  |  |  |
| --- | --- | --- | --- |
|  | Specific activity (µmol/min/mg protein) | | |
| Groups | Serum | Liver | Heart |
| Control | 0.017±0.001ab | 0.018±0.004a | 0.018±0.004a |
| 25 mg/kg bwt | 0.021±0.000b | 0.016±0.001a | 0.017±0.001a |
| 75 mg/kg bwt | 0.016±0.002a | 0.018±0.004a | 0.017±0.001a |

Values are expressed as Mean ± SEM (n = 4). Values in each column with different superscript are significantly different (P<0.05).

**Table 4: Activity of Alanime Transaminase (ALT) in serum, liver and heart of rats administered with aqueous extract of *Mangifera indica* stem bark**

|  |  |  |  |
| --- | --- | --- | --- |
|  | Specific activity (µmol/min/mg protein) | | |
| Groups | Serum | Liver | Heart |
| Control | 0.060±0.000a | 0.047±0.009a | 0.030±0.004a |
| 25 mg/kg bwt | 0.150±0.070b | 0.015±0.005c | 0.028±0.005a |
| 75 mg/kg bwt | 0.055±0.005a | 0.035±0.010b | 0.023±0.005b |

Values are expressed as Mean ± SEM (n = 4). Values in each column with different superscript are significantly different (P<0.05).

**Table 5: Activity of Lactate Dehydrogenase (LDH) in serum, kidney, heart and liver of rats administered with aqueous extract of *Mangifera indica* stem bark**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Specific activity (µmol/min/mg protein) | | | |
| Groups | Serum | Kidney | Heart | Liver |
| Control | 0.022±0.004a | 0.048±0.013a | 0.079±0.010b | 0.038±0.010a |
| 25 mg/kg bwt | 0.022±0.004a | 0.048±0.013a | 0.043±0.011a | 0.036±0.005a |
| 75 mg/kg bwt | 0.040±0.014a | 0.170±0.010b | 0.075±0.006b | 0.069±0.047a |

Values are expressed as Mean ± SEM (n = 4). Values in each column with different superscript are significantly different (P<0.05).

**4.0 Discussion**

Measurement of the activities of ‘marker’ enzymes in tissues and body fluids can be used in assessing the degree of assault and the toxicity of a chemical compound on organs/tissues (Malomo, 2000; Yakubu *et al.,* 2003). Such measurement can also be used to indicate tissue cellular damage caused by a chemical compound long before it is revealed by histological techniques (Akanji, 1986).

Alkaline phosphatase (ALP) is a ‘marker’ enzyme of damage for the plasma membrane and endoplasmic reticulum (Wright and Plummer, 1974; Shahjahan *et al.,* 2004). It is frequently used to assess the integrity of the plasma membrane (Akanji *et al.,* 1993). Enzymes from diseased or damaged tissues may become recognizable in the serum presumably by leakage through altered cell membrane of the rat organs (Akanji and Ngaha, 1989). The significant reduction in the activity of alkaline phosphatase at all doses of the extract in the liver and kidney that was accompanied by a corresponding increase in the serum is an indication that the enzyme have leaked from the organs through the disrupted plasma membrane of the hepatic cells and nephrons into the serum. This is suggestive of permeability changes due to the chemical components in the extract. The loss in ALP activity in the liver and kidney of the animals may adversely affect the transfer of metabolites or required ions across the cell membrane and consequently insufficient ions and metabolites to these organs (Akanji *et al.,* 1993) and might also adversely affect other metabolic processes where the enzyme is involved such as the synthesis of nuclear proteins, nucleic acids and phospholipids as well as in the cleavage of phosphate esters (Yakubu, 2006). A previous report had shown that the plant extract contains saponins (Ijeh and Ukweni, 2007). Saponins are known for their wide range of biological activities which include disruption of biological membranes resulting in escape of large quantities of metabolites including enzymes and generation of free radicals (Francis *et al*., 2002; Nadi *et al.,* 2004; Sparge *et al*., 2004). Therefore, the reduction in ALP activity accompanied by increased serum enzyme may suggest saponin-induced toxicity (Salau *et al.,* 2012).

Acid phosphatase (ACP) is an indicator of damage to the lysosomal membrane (Collins and Lewis, 1971). The serum ACP activities also showed a similar trend with serum ALP activities in rats administered aqueous extract of *M. indica* stem bark suggesting that the integrity of the lysosomal membrane in the cells of the various tissues has been compromised. This is because ACP is a lysosomal enzyme, which is often used to monitor prostatic cancer (Burtis and Ashwood, 1999).

The aminotransferases (ALT and AST) are ‘markers’ of liver damage and can thus be used to assess liver cytolysis with ALT being a more sensitive biomarker of hepatotoxicity than AST (Pramyothin *et al*., 2006). The serum ALT activities in rats administered with aqueous extract of *Mangifera indica* stem barkdid not show significant increase compared with control, which suggests that the integrity of the cell membranes in the various tissues studied has not been adversely affected. The increase in the liver AST at the dose of 25mg/kg body weight of the extract suggests that stimulation of the enzyme activities by the extract which could be due to stress imposed on the tissues by the extract (Umezawa and Hooper, 1982; Malomo *et al.,* 1993, 1995). While decrease at the dose of 75mg/kg body weight of the extract may suggest inhibition of the enzyme activity at the cellular/molecular level. Also, the decrease in liver ALT at the dose of 25mg/kg body weight suggests that administration of *M. indica* may have led to inhibition of the enzyme activity at the cellular/molecular level (Akanji *et al.,* 1993). This shows that the administration of the extract may alter protein metabolism, amongst others, at the subcellular level and this may predispose to impairment of the function of the liver.

Lactate dehydrogenase (LDH) is an index of myocardial infarction, red blood cell disease like haemolytic anaemia, kidney disease including kidney transplantation, rejection, and testicular tumour; lung disease such as pneumonia and congestive heart failure, as well as lymphomas (Pagana, 1998). The observed increase in lactate dehydrogenase (LDH) activity of kidney of rats administered at the dose of 75 mg/kg body weight of the extract suggests an enhancement of the activities of the existing enzymes by the extract. The increase may also be as a result of stress imposed on the tissue by the extract, which may lead to loss of the enzyme molecule through leakage into extracellular fluid, which has not been significantly noticed in the serum In a bid to offset this stress, the tissue may increase the *de novo* synthesis of the enzyme, thus accounting for the increase in LDH activities in these tissues (Umezawa and Hooper, 1982; Malomo *et al.,* 1993, 1995).

**5.0 Conclusion**

The administration of the aqueous extract of *Mangifera indica* stem bark to rats has resulted in alteration of all the enzymes assayed for in some of the tissues at all doses (25 mg/kg and 75 mg/kg body weight). These alterations may adversely affect the integrity of the tissues investigated. Therefore, the aqueous extract of *Mangifera indica* stem bark may not be completely safe as oral remedy at the dose of 25 and 75 mg/kg body weight investigated in this study.

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**References**

1. Akanji MA. A comparative biochemical study of the interaction of some 18 trypanocides with rat tissue cellular system. 1986; Ph.D thesis, University of Ife, Ile-Ife.
2. Akanji MA, Ngaha EO. Effect of repeated administration of berenil on urinary excretion with corresponding tissue pattern in rats Pharmacol. & Toxicol. 1989; 64: 272
3. Akanji MA, Olagoke OA, Oloyede OB. Effect of chronic consumption of metabisulphite on the integrity of the rat kidney cellular system. Toxicol. 1993;81: 173-179.
4. Akinmoladun AC, Ibukun EO, Afor E, Obuotor EM, Farombi EO. (2007). Phytochemical constituent and antioxidant activity of extract from the leaves of *Ocimum gratissimum.* Sci. Res. Essay. 2007; 2: 163-166.
5. Burtis CA, Ashwood ER. (1999). Tietz’s Textbook of Clinical Chemistry (3rd Ed). W.B.Saunders Company, Pennnsylvannia, U.S.A. 2&3. 1999; 634-713, 1168.
6. Collins AJ, Lewis DA. Lysosomal enzyme level in blood of arthritic rats. Biochem. Pharmacology. 1971; 28:251-253.
7. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical Constiuents of some Nigerian medicinal plants*.* Afri. J. Biotechnol. 2005; 4 (7): 685-688.
8. Francis G, Kerem Z, Makkar HPS, Becker K. The biological action of saponins in animal systems: a review. Br J Nutr. 2002; 88(6): 587-605.
9. Goh SH, Chuah CH, Mok JSL, Soepadmo E. Malaysian Medicinal Plants for the Treatment of Cardiovascular Diseases. Selangor Darul Ehsan: Pelanduk Publication. Kaula Lumpur, Malaysia. 1995
10. Henry RJ, Cannon DC, Winkleman JW. Clinical Chemistry, Principles and Techniques. Harper and Row, 2nd ed. 1974
11. Ijeh II, Ukweni AI. Acute effect of administration of ethanol extracts of *Ficus exasperata* vahl on kidney function in albino rats. J Med Plant Res. 2007; 1(2):27-29
12. Madunagu BE, Ebana RUB, Ekpe ED. West African Journal of Biology and Applied Chemistry. 1990; 35, 25 –30.
13. Malomo SO, Ale OO, Adedoyin AM. Effect of chloroquine on some leukocyte enzymes during protein energy malnutrition- an in vitro study. Biosci. Res. Commun. 1993; 5: 53-55.
14. Malomo SO, Daramola AS, Balogun EA. Some serum and tissue enzyme changes in mice infected with *Plasmodium yoelii nigeriensis* before and after administration of halofantrine hydrochloride. Niger. J. Biochem. Mol. Biol. 1995; 10:71-77.
15. Malomo SO. Toxicological Implication of Ceftriaxone Administration in Rats. Nig. J. Biochem. & Mol. Biol. 2000; 15(1): 33-38.
16. Nandi B, Roy S, Bhattacharya S, Babu SPS. Free radicals mediated membrane damage by the saponins acaciaside A and acaciaside B. Phytotherapy Res. 2004; 18 (3): 191-194.
17. Nakanishi M, Goto K. Inhibitory effect of anti-inflammatory drugs on enzyme release from rabbit polymorphonucleus leukocytes lysosomes. Biochem. Pharmacol. 1975; 24: 421-424.
18. Ogbe RJ, Adoga GI, Abu AH. J. Med. Plants Res. 2010; 4, 8, 680 – 684.
19. Ojewole J. Method of Experimental Chemical Pharmacology. 2005; 27, 547 – 554.
20. Pagana KD. Manual of diagnostic and laboratory tests. St. Louis; Mosby, Inc. p. 44. Proc. Soc. Exp. Biol. Med. 1998; 90, 210–213.
21. Pramyothin P, Samosorn P, Poungshompoo S, Chaichantipyuth C. The protective effects of *Phyllanthus emblica* Linn. Extract on ethanol induced rat hepatic injury. J Ethnopharmacol. 2006; 107(3): 361–364.
22. Ramalingam V, Vimaladevi V. Effect of mercuric chloride on membrane-bound enzymes in rat testis. Asian J. Androl. 2002; 4: 309-311.
23. Ross JA. Medicinal Plants of the World, Human Press Inc, New Jersey. 1999; 199.
24. Salau AK, Yakubu MT, Oluleye DS, Oloyede OB, Akanji MA. Toxicological evaluation of aqueous leaf extract of *Ficus exasperata* on selected tissues of normal Wistar rats. Centrepoint Journal (Science Edition). 2012; Volume 18, No. 1, pages 55 – 66
25. Schmidt E, Schmidt FW. Determination of serum GOT and GPT activities. Enzyme Biology Chemistry. 1963; 3:1-5.
26. Shahjahan M, Sabitha KE, Malbika J, Shyamala-Devis CS. Effect of *Solanum trilobatum* against carbon tetrachloride induced hepatic damage in albino rats. Indian. J. Med. Res. 2004; 120: 194-198.
27. Sparge SG, Light ME, Van Staden J. Biological activities and distribution of plant saponins. J Ethnopharmacol. 2004; 94(2-3): 219-243.
28. Umezawa H, Hooper IR. Aminoglycoside antibiotics. Spinger-Verky, Berlin, Hadelberg. 1982
29. Wright PJ, Leathwood AO, Plummer DT. Enzymes in rat urine: Alkaline Phosphatase. Enzymologia. 1972; 42: 317 – 327.
30. Wright PJ, Plummer DT. The use of urinary enzyme measurement to detect renal damages caused by nephrotic compounds. Biochem. Pharmacol. 1974; 12: 65.
31. Wroblewski F, La Due JS. Lactate dehydrogenase activity in the blood. Proceedings Society Experimental Biomedical. 1955; 90: 210-215.
32. Yakubu MT, Bilbis LS, Lawal M, Akanji MA. Effect of Repeated Administration of sildenafl citrate on selected enzyme activities of liver and kidney of male albino rats. Nig. J. Pure & Appl. Sci. 2003; 18: 1395-400.
33. Yakubu MT, Akanji MA, Oladiji AT. Aphrodisiac potentials of aqueous extract of Fadogia agrestis (Schweinf. Ex Heirn) stem in male albino rats. Asian J Androl. 2005; 7:399–404.
34. Yakubu MT. Aphrodisiac potentials and toxicological evaluation of aqueous extract of *Fadogia agrestis* (Schweinf. Ex Hiern) stem in male rats. Ph. D Thesis, University of Ilorin, Ilorin, Nigeria.2006.

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