

RESEARCH ARTICLE

Evaluation of the “Antidotal” Potential of *Mangifera indica* L. leaves extract on sodium arsenate exposed male Wistar rats using some biochemical markers

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Abstract: In order to evaluate the antidotal potential of *Mangifera indica* L leaves extract on sodium arsenate exposed male Wistar rats using some biochemical markers, forty-two apparently healthy male Wistar rats (weight range 120-160 g) were used in the study. The animals were randomly separated into six groups. Other than groups “A” (non-exposed control) and “B” (exposed control), groups; C, D, E, and F respectively were treated with different dosages of *Mangifera indica* L extract viz., 100 mg/kg and 200 mg/kg extract. Volumes of extract administered did not exceed 0.2 ml regardless of the body weight of the animal respectively. Some biochemical parameters assessed were: serum protein, albumin, conjugated bilirubin, unconjugated bilirubin (ICB), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), urea, creatinine, creatine kinase (CK), lactate dehydrogenase (LDH), acid phosphatase, prostatic phosphatase, serum lipid profile, that is total cholesterol, low density lipoprotein cholesterol (LDL), high density lipoprotein cholesterol (HDL), and the hormones, testosterone and luteinizing hormone levels. Group “B” had significantly ($P < 0.05$) higher activities for AST, GGT, CK, LDH and higher cholesterol concentration when compared to *Mangifera indica* treated groups and to the non-exposed control. Testosterone and LH were significantly ($P < 0.05$) lower in group “B” unlike the *Mangifera indica* treated groups and group “A”. This observation could be attributed to adverse effect of toxicosis on exposure to animals in group “B”. Antidotal property of the extract, due to one or more of its phytochemicals such as flavonoids, tannins alkaloid and anthraquinones could be the most probable reason for potential therapeutic potential. Conclusively, this observation gives credence to its cytoprotective and antidotal properties.

Keywords: Rats, arsenic, *Mangifera indica* and toxicity.

INTRODUCTION

Arsenic is among the most toxic heavy metals in the environment (ATSDR, 2005). It causes wide range of toxicity in man and animals (Garg, 2008). Among the sources of arsenic poisoning in our environments, the most frequent sources of arsenic are herbicides, insecticides and food preservatives (ATSDR, 2005). Arsenic compounds could be thiol-reactive, which inhibit enzymes by altering proteins by reacting with proteinaceous thiol groups. Also, pentavalent arsenate un-couples mitochondrial oxidative phosphorylation by a mechanism associated to competitive substitution of arsenate for inorganic phosphate in the formation of adenosine triphosphate (NRC, 2001). Some researchers also found out its increased risk of predisposing to diabetes mellitus, immunotoxicity and haematotoxicity respectively (ATSDR, 2005; Navas-Acien *et al.*, 2006). In addition, it also causes genotoxicity with an unclear mechanism. But recent work associated its toxicity to carcinogenicity due to its metabolites causing production of oxidants and oxidative DNA damage. There is an alteration in DNA methylation status and genomic distortion in replication that could lead to DNA damage repair, enhanced cell proliferation interference with germ cell production (NRC, 2001; Rossman, 2003). In the tropics, especially in the Sub-Saharan Africa, environment protection enforcement agencies are not strictly enforcing environmental laws. Currently, refuse and sewage disposal are very poor and often seep into water bodies (Abatan, 2012). These, coupled with the extensive system of management of animals predispose arsenic poisoning; which when eaten

by man could pose as a potential hazard to health.

In this study, the ameliorative potential of crude extract of *Mangifera indica* L. was evaluated in rats exposed to sodium arsenate using some biochemical markers. The phytochemical analysis of the crude plant extract was also carried out to scientifically evaluate its medicinal claim.

MATERIALS AND METHODS

Experimental animal welfare and ethics

Forty-two male apparently healthy Wistar rats of weight range 120-160 g were experimented upon. Animals were acclimatized for 2 weeks before commencement of the experiment and were kept in standard laboratory cages. The animals were fed on a standard diet (Grand[®] cereals, Jos, Nigeria) and provided with water *ad libitum*. Ethical permission was sought at the proposal presentation of the research and permission was granted by the Department of Veterinary Physiology, Biochemistry and Pharmacology. Subsequent monitoring was carried out to ensure strict adherence to international standards of animal welfare during the experimentation at the University of Ibadan, Ibadan, Nigeria.

Experimental design

Animals in group "A" were administered 0.2 ml of corn oil orally as a placebo substance to ensure rats in this group were given a highly absorbed lipid based substance with no deleterious effect, while animals in group "B" were given 0.2 ml (0.05 M NaAsO₂, Sigma-Aldrich, USA was diluted with distilled water to concentrations of 2.5 mg/kg body weight corresponding to 1/10th of the oral LD₅₀) of sodium arsenite orally. Groups "C" and "D" were given 100 mg/kg and 200 mg/kg extract, those in group E were given 200 mg/kg extract and sodium arsenite in 1 hour intervals, group "F" were given 100 mg/kg extract and sodium arsenite in 1 hour intervals. In order to achieve exposure of the arsenite and to evaluate the ameliorative effect of the extract, the animals were orally administered for 2 weeks at an arsenite dose of 2.5 mg/kg body weight corresponding to 1/10th of the oral LD₅₀). This

would enable us to study the exposure at sub-chronic rate.

Plant authentication and extraction

Mangifera indica leaves were procured from University of Ibadan and authenticated at the Department of Botany, Faculty of Science, University of Ibadan, Ibadan, Nigeria (Voucher number: UIH-22463). Leaves of *Mangifera indica* were cleaned, and dried at room temperature. The dried leaves were ground into a fine powder. Cold extraction was done by soaking the ground leaves in N-hexane for 48 hours and then the same shaft soaked in 96% ethanol for 72 hours. Both the N-hexane fraction and ethanol fraction of the extract were collected and concentrated using rotary evaporator at 40°C as previously described by Olukunle *et al.* (2014).

Phytochemical analysis

The presence of alkaloids, cardenolides, tannins, anthraquinones, terpenoids and flavonoids was analyzed by simple qualitative chemical tests according to Ajagbonna *et al.* (2005).

Blood collection and serum biochemical parameters

The rats were anaesthetized using diethyl ether in a jar and blood samples were collected by venous puncture into sterile lithium heparinized sample tubes. Plasma and serum was centrifuged at 4000 rpm for 10 minutes. Serum was collected and analyzed using Randox[®] specific kits for activities of the CK, LDH, acid phosphatase, prostatic phosphatase, ALP, AST, ALT, and GGT. Total, conjugated, unconjugated bilirubin, urea and creatinine concentrations were also determined. Total protein and albumin concentrations were determined by spectrophotometric methods using the Sigma Diagnostic Kits while globulin was obtained from the difference of total protein and albumin. Serum lipid profile (total cholesterol, LDL-cholesterol, HDL-cholesterol, total triglycerides) were determined according to the method of (Chauhan and Agarwal, 2006). Serum testosterone and luteinizing hormone concentrations were determined using Sigma Diagnostics Kits.

Statistical Analysis

The data obtained were analyzed using the graph pad prism software. Analysis of variance was

used to compare means of various groups' parameters. $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

The results showed a significantly higher ($P < 0.05$) AST and GGT in the group "B" (the group given 0.2 ml sodium arsenite orally 0.05 M NaAsO₂, Sigma–Aldrich, USA was diluted with distilled water to concentrations of 2.5 mg/kg body weight) than the other groups. This is due to hepatotoxic effect due to the production of reactive oxygen species (ROS), nitrogen oxygen species (NOS) and the decrease in the endogenous antioxidants enzymatic and non-enzymatic cellular protectants. This eventually causes the release of enzymes into serum milieu that would increase the levels of the enzymes AST and GGT in the blood. Kindanemariam *et al.* (2015) also observed a similar increase in the levels of liver enzymes in rats. That indicates the degenerative changes due to the toxicosis induced by arsenic. While the *Mangifera* treated groups were having significantly ($P < 0.05$) lower AST and GGT and in the same vein, in group B, the total cholesterol and LDL cholesterol were significantly ($P < 0.05$) higher than other groups as shown in Table 2. It is possible that the arsenite must have induced fatty degenerative changes in the parenchymatous tissues. This must have resulted to hypercholesterolaemic and hyperlipidaemic effect in this study. This study is in line with the previous findings of Al-Forkan *et al.* (2016) that also observed a significant increase in liver enzymes sequel to exposure of rats to arsenic compound. The same level of significance ($P < 0.05$) in hepatic enzymes, cholesterol and LDL in group "B" was also manifested in Table 3 in CK and LDH parameters. This might be due to myolysis of the cardiac muscle and other skeletal muscles induced by toxicosis caused by arsenite in the exposed, untreated group. LDH on the other hand is an ubiquitous enzyme due to the fact it has multiple isoforms in different organs. Hence, the particular organs involved due to the degenerative effect sequel to arsenite exposure and quantifying the extent of adverse effect in various tissues cannot be categorically be pinned down to one particular organ. We therefore speculate that hepatotoxicity, myolysis and genotoxicity may be the cause of high level of LDH. This assumption is in concordance with the previous observations of Garg (2008) and Al-Forkan *et al.* (2016). Testosterone and LH was

significantly ($P < 0.05$) lower in group "B" when compared to other treated groups C to E and the control group A in Table 4. This could be associated with the cytotoxic effect of arsenite to the testicular tissues and Leydig cells in particular. The result of the phytochemical analysis in Table 5 shows that *Mangifera indica* was positive for tannins which must have interfered with the absorption of arsenic. This minimized the effect of arsenate toxicosis. This coupled with the antioxidative effect of the flavonoids and inhibition of pro-inflammatory endogenously produced substances may also decrease the adverse inflammatory degenerative effect of toxicosis induced by arsenite. In our study the extract was negative for terpenoids, which in contrast with the findings of Shailajan *et al.* (2016), who found terpenoids as one of the active principles in a previous study. The reason could be due to the method of phytochemical analysis and the disparity in the geographical location, which may determine the edaphic factors, season of procurement of leaves and *Mangifera indica* strain, the factors that may determine phytochemical constituents in plants. One or more of this constituents in tandem would have confer and prevent the adverse effect of arsenic poisoning.

CONCLUSION

Mangifera indica due to its phytochemical constituents confer antioxidative and cytoprotective activities to prevent the adverse toxicokinetics pathway of arsenic toxicosis in the biological system. This would subsequently delay cellular degenerative effects that could be detrimental to the biological system.

Table 1: Effect of *Mangiferaindica* extract on serum biochemical parameters (Mean±SEM) of male Wistar rats exposed to sodium arsenite.

PARAMETER	GROUP A	GROUP B	GROUP C	GROUP D	GROUP E	GROUP F
Totalprotein (g/dL)	6.90±0.05	7.03±0.08	6.92±0.15	6.95±0.05	7.06±0.08	6.94±0.05
Albumin (g/dL)	3.76±0.09	3.92±0.05	3.80±0.11	3.95±0.15	4.03±0.09	3.90±0.05
Globulin (g/dL)	3.13±0.13	3.10±0.04	3.12±0.10	3.00±0.10	3.03±0.03	3.04±0.02
Totalbilirubin (mg/dL)	0.56±0.03	0.40±0.04	0.50±0.09	0.40±0.10	0.40±0.05	0.48±0.05
Conjugated bilirubin (mg/dL)	0.26±0.06	0.20±0.04	0.20±0.04	0.15±0.05	0.20±0.05	0.16±0.02
ICB (mg/dL)	0.30±0.05	0.20±0.07	0.30±0.07	0.25±0.05	0.20±0.05	0.32±0.037
AST (IU/l)	12.67±0.66 ^b	17.00±0.40 ^a	12.00±1.2 ^b	12.50±0.50 ^b	9.33±0.66 ^b	14.40±0.50 ^b
ALT (IU/l)	9.66±0.33	7.50±0.28	10.00±0.81	9.50±0.50	6.33±0.33	7.20±0.58
ALP (IU/l)	31.33±1.85	34.75±2.68	28.75±0.85	41.50±1.50	34.33±3.28	27.80±0.58
GGT (U/L)	8.33±0.33 ^b	15.50±0.28 ^a	7.25±1.25 ^b	6.00±1.00 ^b	9.66±0.33 ^b	12.00±0.70 ^b
Urea (mg/dL)	31.33±0.88	29.25±0.75	27.75±1.37	32.00±0.0	28.00±1.00	29.80±0.58
Creatinine (mg/dL)	0.73±0.03	0.67±0.02	0.67±0.04	0.70±0.0	0.66±0.06	0.74±0.02

Means bearing different superscripts ab along the same row differ significantly (P<0.05)

Table 2: Effects of *Mangiferaindica* extract on lipid profile (Mean±SEM) of male Wistar rats exposed to sodium arsenite.

PARAMETER	GROUP A	GROUP B	GROUP C	GROUP D	GROUP E	GROUP F
Total Cholesterol (mg/dL)	151.7±7.21 ^b	184.3±3.75 ^a	145.8±4.82 ^b	145.5±6.50 ^b	140.3±1.45 ^b	156.0±3.71 ^b
Total Triglyceride (mg/dL)	55.00±4.58	49.00±1.87	58.50±5.07	61.50±0.50	55.00±6.24	55.60±2.54
HDL-Cholesterol (mg/dL)	41.67±4.91	32.75±3.14	44.50±2.10	43.50±3.50	39.67±0.66	48.40±1.69
LDL-Cholesterol (mg/dL)	79.00±5.19 ^b	103.50±3.01 ^a	83.25±6.44 ^b	83.50±6.50 ^b	76.67±5.54 ^b	99.80±7.06 ^b

Means bearing different superscripts ab along the same row differ significantly (P<0.05)

Table 3: Effects of *Mangifera indica* extract on markers of oxidative stress in male Wistar rats exposed to sodium arsenite.

PARAMETERS	GROUP A	GROUP B	GROUP C	GROUP D	GROUP E	GROUP F
LDH(u/L)	22.33±0.88 ^b	25.50±1.84 ^a	23.00±2.12 ^b	23.50±0.50 ^b	23.00±3.05 ^b	19.80±1.24 ^b
CK(u/L)	15.00±1.15 ^b	22.75±1.75 ^a	16.25±1.11 ^b	13.00±1.00 ^b	13.00±1.15 ^b	13.00±0.31 ^b
Acid Phos (u/L)	1.96±0.23	1.45±0.13	1.60±0.08	1.80±0.10	2.13±0.08	1.82±0.07
Pros Phos	0.83±0.08	0.77±0.11	0.62±0.06	0.85±0.15	1.00±0.05	0.88±0.03

Means bearing different superscripts ab along the same row differ significantly (P<0.05)

CK: Creatininekinase; LDH: Lactate dehydrogenase; Pros Phos : Prostatic Phosphatase; Acid Phos : Acid Phosphatase

Table 4: Effects of *Mangifera indica* extract on Sex hormones of male Wistar rats exposed to sodium arsenite.

PARAMETERS	GROUP A	GROUP B	GROUP C	GROUP D	GROUP E	GROUP F
Testosterone (ng/mL)	3.13±0.06 ^a	1.02±0.02 ^b	3.02±0.06 ^a	3.10±0.10 ^a	2.43±0.06 ^a	2.84±0.05 ^a
LH (iu/L)	15.33±0.88 ^a	12.25±0.47 ^b	14.00±0.70 ^a	15.00±1.00 ^a	13.67±0.66 ^a	14.80±0.37 ^a

Means bearing different superscripts ab along the same row differ significantly (P<0.05)

Table 5: Phytochemical screening of *Mangifera indica* leaves.

	Constituents	Test	Observation
1.	Alkaloids	Dragenduff: Meyer's Wagner's	+ve +ve +ve
2.	Cardenolides	Keller-killiani Kedde	-ve -ve
3.	Anthraquinones	Chloroform/Ammonia	+ve
4.	Saponins	Frothing	+ve
5.	Tannins	Ferric chloride	+ve
6.	Flavonoids	Ammonia/H ₂ SO ₄ Aluminum solution	+ve +ve
7.	Terpenoids	Ethyl acetate/ ammonia Chloroform/ H ₂ SO ₄	+ve -ve

+ve A colour change indicating presence; -ve No colour change indicating absence.

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