

Effects of MOF6 Fraction from Ethanolic Extract of the Leaves of *Moringa oleifera* against Sodium Arsenite-Induced Hepatotoxicity in Rats

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Moringa oleifera (MO) is a plant of significant medicinal importance. The dried leaves of MO were pulverized, extracted with ethanol and fractionated using column chromatography to provide seven fractions (MOF1-7) with MOF6 having the best preliminary antioxidant potential. Therefore, this study evaluated the hepatoprotective potentials of MOF6 in sodium arsenite (SA)-induced hepatotoxicity in rats. Thirty-five adult male Wistar rats were randomly divided into seven groups of five rats each. Control Group I received normal saline. Groups II and III received 20 mg/kg body weight (bw) of SA for 3 and 6 weeks, respectively. Groups IV and V received 20 mg/kg bw of SA for 3 weeks followed by treatment with 5.0 and 7.5 mg/kg bw of fraction MOF6, respectively, for 6 weeks. Groups VI and VII received only 5.0 and 7.5 mg/kg bw of fraction MOF6, respectively, for 6 weeks. Antioxidant (lipid peroxidation) and biochemical analyses of liver enzymes of all rats were carried out after the completion of experimental procedures. Results showed statistically significant lower mean values ($p \leq 0.05$) of malondialdehyde (MDA), acid phosphatase (ACP) and γ -glutamyl transferase (GGT) in rats of Groups IV and V compared with Group III. However, there were statistically significant higher mean values ($p \leq 0.05$) of alkaline phosphatase (ALP) in Groups IV and V compared with Groups I and III. In conclusion, these results implied that fraction MOF6 has antioxidant and hepatoprotective potentials. However, results of ALP analyses implied that MOF6 possibly augmented SA-induced hepatotoxicity in rats.

Keywords: *Moringa oleifera*, column fractions, sodium arsenite, hepatotoxicity, lipid peroxidation

INTRODUCTION

Plants are employed traditionally in many countries for therapeutic purposes, and plants have been sources for the development of drugs such as artemisinin derived from *Artemisia annua* for the treatment of malaria (Akinlolu *et al.*, 2012). This is due to the fact that plants contain chemical components which elicit biochemical and physiological actions in living systems. These phytochemicals have been of tremendous values to studies in pharmacology, biochemistry and medical sciences (Ujowundu *et al.*, 2010). Plants have limitless ability to synthesize these important chemicals of which about 10% have been isolated and characterized (Mallikharjuna *et al.*, 2007).

Moringa oleifera Lam. (MO) is widely grown in Nigeria and used traditionally for medicinal purposes (Akinlolu *et al.*, 2014; Omotoso *et al.*, 2018a). MO contains biologically active compounds of interests such as glucosinolates and isothiocyanates (Bennett *et al.*, 2003), as well as vitamins, minerals and carotenoids (including β -carotene or provitamin A) (Fahey, 2005). In addition, 4-(4'-O-acetyl- α -L-rhamnopyranoyl-oxy) benzyl isothiocyanate, 4-(α -L-rhamnopyranosyloxy) benzyl isothiocyanate, niazimicin, pterygospermin, benzyl isothiocyanate, and 4-(α -L-rhamnopyranosyloxy) benzyl glucosinolate are MO-derived compounds with reported hypotensive, antibacterial and anticancer activities (Bharali *et al.* 2003; Fahey, 2005).

Arsenic toxicity globally affects humans and animals via the generation of free

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radicals or reactive oxygen species (Walvekar *et al.*, 2007; Hughes *et al.*, 2011; Jomova *et al.*, 2011), resulting in the confinement of oxidative stress on the affected organs, multiple organ failure (Ratnaik, 2003), hepatotoxicity, neurotoxicity and cardiac dysfunction (Jana *et al.*, 2006; Singh *et al.*, 2011).

Aqueous extracts of MO leaves had been demonstrated to ameliorate chemical-induced oxidative stress and hepatotoxicity in animal models of previous studies (Ajilore *et al.*, 2012; Abd Eldaim *et al.*, 2017; Farid and Hegazy, 2020). In addition, despite the great therapeutic ethnopharmacological potentials of MO, previous studies have noted abortifacient potentials of MO leaf extracts usually in the first trimester (Ekhatior and Osifo, 2015). The reported abortifacient potential of MO leaf extracts raises concerns on the safety of consumption of MO leaves preparations by women, who may sometimes not even be aware that they are pregnant. It, therefore, becomes relevant to search for MO-derived compounds which have strong pharmacological potentials and are not abortifacient.

Hence, we evaluated preliminary abortifacient potentials of MO fractions (MOF1 - MOF7), in order to identify which fraction(s) will have strong antioxidant and pharmacological potentials without being abortifacient. MOF6 was observed not abortifacient, and had the best preliminary antioxidant potential. MOF6 also had a positive antioxidant effect on superoxide dismutase levels in cuprizone-induced neurotoxicity in adult female rats (Omotoso *et al.*, 2018a). In addition, MOF6 equally had neuroprotective potentials against cuprizone-induced neurotoxicity of the pre-frontal and cerebellar cortices in adult female rats (Omotoso *et al.*, 2018a-c). Furthermore, MOF6 had neuroprotective potentials against sodium arsenite (SA)-induced decreased acetylcholinesterase levels in adult male rats (Akinlolu *et al.*, 2020). Although, previous studies examined the hepatoprotective potentials of different parts of MO, there is paucity of studies which examined the hepatoprotective potentials of

MO leaves on levels of malondialdehyde (MDA), acid phosphatase (ACP), alkaline phosphatase (ALP) and γ -glutamyl transferase (GGT) in SA-induced hepatotoxicity. Therefore, in this study, MOF6 was purified and isolated from ethanol extract of MO leaves in order to further determine its hepatoprotective potentials on sera levels of MDA, ACP, ALP and GGT in SA-induced hepatotoxicity in adult male Wistar rats.

In addition, is SA-induced toxicity reversible without treatment? It is quite possible for the body's defense mechanisms to reverse drug-induced toxicity depending on dosage and duration of exposure. Hence, we developed appropriate study design in order to determine if SA-induced hepatotoxicity is reversible without treatment and to eliminate confounding factors in the evaluation of hepatoprotective potentials of MOF6 in SA-induced toxicity in rats.

MATERIALS AND METHODS

Materials:

Chemicals and reagents:

Sodium arsenite (SA) was a product of Sigma-Aldrich Japan Co. (Tokyo, Japan). Normal saline was obtained from MOMROTA Pharmaceutical Company in Ilorin, Kwara State, Nigeria.

Plant collection

Fresh leaves of *Moringa oleifera* were collected in Ilorin, Kwara state and identified at the Herbarium Unit of the Department of Plant Biology, University of Ilorin, where a voucher specimen with voucher number UILH/001/1008 was deposited.

Experimental animals:

Thirty-five Wistar albino rats (8 weeks old and average weight of 200 g) obtained from the Experimental Animal Unit of the Department of Anatomy, College of Health Sciences, University of Ilorin, Nigeria were used in this study. The animals were

randomly assigned into 7 Groups, each comprising of 5 rats. The number of rats per group was determined in accordance with the guidelines for laboratory animal use of the University of Ilorin Ethical Review Committee. All rats were housed in a well-ventilated animal house and were fed with standard rat pellets and allowed free access to drinking water. The rats were allowed to acclimatize for 10 days before extract administration commenced.

Methods:

Preparation of plant extracts:

Fresh MO leaves were dried at room temperature, powdered and sieved. The powdered leaves (1.289 kg) were then extracted with ethanol. The extract was collected, concentrated under vacuum at 50 °C, weighed and kept in a labeled sample bottle. Absolute ethanol was used for the extraction and fractionation procedures in order to eliminate aqueous soluble fraction as much as possible.

Fractionation of the crude extract:

Column chromatography (CC) was carried out on silica gel (70 - 230 and 240 - 300 mesh size, Merck, Germany) and Merck alumina (70 - 230 mesh ASTM). Thin layer chromatography (TLC) was carried out on pre-coated silica gel 60 F₂₅₄ aluminum foil (Merck, Germany) for the establishment of the purity of isolates. Spots on TLC were examined with a UV lamp operating at a wavelength of 366 nm for fluorescence and at 254 nm for fluorescence quenching spots.

The ethanol extract of MO leaves was fractionated in a silica gel open column, using *n*-hexane, dichloromethane, ethyl acetate and ethanol in an increasing order of polarity (*n*-hexane; *n*-hexane: dichloromethane [3:1, 3:2, 1:1, 1:2, 1:3]; dichloromethane; dichloromethane: ethyl acetate [3:1, 3:2, 1:1, 1:2, 1:3]; ethyl acetate; ethyl acetate: methanol [3:1, 3:2, 1:1, 1:2, 1:3] and methanol, to afford fifty-five eluents of 200 ml each. The eluents were pooled based on

the colour of the eluents and the solvents that elute them to give a total of seven combined fractions (MOF1 - MOF7) as previously established (Omotoso *et al.*, 2018a-c; Akinlolu *et al.*, 2020).

The fraction MOF6 had the best antioxidant potential during our preliminary screening of the fractions. In addition, the fraction MOF6 had positive antioxidant effect on superoxide dismutase levels in our previous study on cuprizone-induced neurotoxicity in adult female rats (Omotoso *et al.*, 2018a). Furthermore, preliminary screenings showed that MOF6 was not abortifacient. Hence, fraction MOF6 was used in this study to evaluate the hepato-protective potentials of MO on SA-induced hepatotoxicity in rats.

Experimental procedures and drug administration:

Normal saline was used as a vehicle to dissolve drugs before administration to rats. MOF6 and SA were dissolved in normal saline. All doses of drugs and plant extracts were orally administered to rats as detailed in Table 1. Control Group I received normal saline. Group II received 20 mg/kg bodyweight (bw) of SA only for 3 weeks. Group III received 20 mg/kg bw of SA only for 6 weeks, and left untreated for another 3 weeks. Groups IV and V received 20 mg/kg bw of SA for 3 weeks followed by treatments with 5.0 and 7.5 mg/kg bw of fraction MOF6, respectively, for another 6 weeks. Groups VI and VII received only 5.0 and 7.5 mg/kg bw of fraction MOF6, respectively, for 6 weeks. The doses of MOF6 used in this study were determined from previous studies on the neuroprotective potentials of MOF6 in cuprizone- and SA-induced neurotoxicity in rats (Omotoso *et al.*, 2018a-c; Akinlolu *et al.*, 2020).

Animal sacrifice:

Rats of different groups were sacrificed by cervical dislocation at the end of the three phases of the experimental procedure, for example, 3rd week (Group 2), 6th week

Table 1: Concentrations (mol/ml) of acid phosphatase (ACP), alkaline phosphatase (ALP), γ -glutamyl transferase (GGT) and malondialdehyde (MDA) levels in rats serum of control and experimental groups

Group	Dose	ACP	ALP	GGT	MDA
I	1 ml/200 mg bw of normal saline	2.74 (± 0.81)	86.2 (± 22.85)	1.75 $\pm$ (0.94)	4.12 (± 0.91)
II	20 mg/kg bw of SA for 3 weeks	23.21 (± 39.11) ^{**a+b+}	163.62 (± 16.35) ^{**a+b+}	2.22 $\pm$ (2.05) ^{*a-b+}	4.68 (± 0.34) ^{*a-b+}
III	20 mg/kg bw of SA for 6 weeks	15.51 (± 9.98) ^{*a+}	238.35 (± 53.56) ^{*a+}	9.61 $\pm$ (14.59) ^{*a+}	7.91 (± 3.14) ^{*a+}
IV	20 mg/kg bw of SA + 5 mg/kg bw of MOF6	1.58 (± 1.50) ^{*a-b+}	320.42 (± 21.23) ^{**a+b+}	1.60 $\pm$ (0.98) ^{*a-b+}	4.08 (± 0.32) ^{*a-b+}
V	20 mg/kg bw of SA + 7.5 mg/kg bw of MOF6	2.77 (± 1.60) ^{*a-b+}	431.53 (± 11.19) ^{**a+b+}	1.12 (± 1.58) ^{*a-b+}	4.17 (± 0.51) ^{*a-b+}
VI	5 mg/kg bw of MOF6	1.89 (± 0.39) ^{*a-b+}	118.77 (± 18.32) ^{**a+b+}	2.90 (± 0.58) ^{*a-b+}	3.71 (± 0.12) ^{*a-b+}
VII	7.5 mg/kg bw of MOF6	0.67 (± 0.48) ^{*a+b+}	352.72 (± 26.93) ^{*a+b+}	2.22 (± 0.93) ^{*a-b+}	3.82 (± 0.24) ^{*a-b+}

bw: Body weight, SA: Sodium arsenite, MOF6: *Moringa oleifera* leaf ethanol extract (fraction 6); ^a: Statistical comparison of mean values of Groups II - VII with Group I, ^b: Statistical comparison of mean values of Groups I, II and IV - VII with III, ⁺: Statistically significant difference at $p \leq 0.05$, ⁻: Statistically non-significant difference at $p \leq 0.05$, *: Mean value of statistically significant difference (^a or ^b), **: Mean value of statistically significant difference (^a and ^b).

(Groups 6 and 7) and 9th week (Groups 1 and 3 - 5). No form of anaesthesia was used, to avoid further introduction of foreign materials in the rat system and consequent enzymatic actions on the liver.

Antioxidant analysis:

Antioxidant property of MOF6 was determined in lipid peroxidation of MDA levels in rats. The end products of lipid peroxidation are reactive aldehydes, such as MDA. The levels of MDA were determined in sera samples, using thiobarbituric acid method and the results were obtained in nmol/g based on the protocol described by Akinlolu *et al.* (2013).

Biochemical evaluations:

The thoracic cavity of each rat was opened surgically, blood samples were drawn by cardiac puncture and 5 ml of the blood was allowed to clot at room temperature. The clotted blood samples were centrifuged at 3000 rpm for 15 min to obtain the serum, which was used for biochemical analyses. Sera obtained from the clotted blood samples of rats were analyzed for the

concentrations of ACP, ALP and GGT. ACP, ALP and GGT concentrations were measured based on the protocols described in the assay kits of Sigma-Aldrich (USA).

ACP activity was determined by the conversion of the non-fluorescent 4-methylumbelliferyl phosphate disodium salt (MUP) to the fluorescent product 4-methylumbelliferone, as proportional to the ACP activity present. ALP activity was determined by non-destructive and repeated measurements of the mutated version for the human placental alkaline phosphatase (SEAP) secreted out of the cells using an aliquot of the culture medium. GGT activity was determined by a coupled enzyme assay, in which the GGT transfers the γ -glutamyl group from quenched substrate L- γ -glutamyl-7-amido-4-methylcoumarin (L- γ -glutamyl AMC), liberating the fluorescent group, 7-amino-4-methyl coumarin (AMC) as proportional to the GGT activity present.

Statistical analyses:

The mean $\pm$ S.E.M value of each of the measured parameters in rats of control Group I were compared with rats of

experimental Groups II - VII for any significant difference using one-way analysis of variance (ANOVA), and Tukey post hoc test was used for group comparison as appropriate. The level of significance was set at $p \leq 0.05$.

Ethical approval:

Ethical approval for this study was sought and received from the University of Ilorin Ethical Review Committee. The ethical approval number is UERC/ASN/2018/1161. This research study was conducted in accordance with the guidelines for laboratory animal use of the University of Ilorin Ethical Review Committee, the internationally accepted principles for laboratory animal use and care as provided in the European Community guidelines (EEC Directive of 1986; 86/609/EEC) and the US guidelines (NIH publication #85-23, revised in 1985).

RESULTS AND DISCUSSION

SA is an established clastogen and its toxicity has been associated with tumors of the liver, kidney, heart, bladder, prostate, lungs and uterus (Jana *et al.*, 2006; Singh *et al.*, 2011). The observed hepatoprotective potentials of MOF6 in this study (Table 1), emphasize the ethnopharmacological values of MO, and the relevance for further characterization and biological evaluations of compounds present in MOF6 in order to identify and isolate the compound responsible for the hepatoprotective activities of MO.

Evaluation of MDA levels in rats

Lipid peroxidation results from oxidative degradation of lipids and stealing of electrons from the lipid cell membrane by free radicals (Akinlolu *et al.*, 2013). It involves a free radical chain reaction mechanism terminated either by the counter-effects of antioxidants such as phospholipid hydroperoxide glutathione peroxidase or

production of reactive aldehydes such as MDA (Akinlolu *et al.*, 2013). Lipid peroxidation consequently results in increased oxidative stress, compromised cell membranes and cellular damage (Akinlolu *et al.*, 2013), hence it is increased in inflammatory conditions and carcinogenesis. In addition, arsenic toxicity is induced via generation of free radicals or reactive oxygen species (Walvekar *et al.*, 2007; Hughes *et al.*, 2011; Jomova *et al.*, 2011), and further confinement of oxidative stress on the affected organs (Ratnaike, 2003).

Is SA-induced oxidative stress reversible without treatment? It is quite possible for the body's defense mechanisms to reverse drug-induced toxicity depending on dosage and duration of exposure. Results showed statistically significant higher mean values ($p \leq 0.05$) of MDA in rats of Groups II and III compared with Group I. In addition, there were statistically significant lower mean values ($p \leq 0.05$) of MDA in rats of Group II, which received SA for 3 weeks only compared with Group III, which received SA for 6 weeks and left untreated for another 3 weeks. These findings implied that SA-induced toxicity resulted in increased oxidative stress in rats of Groups II and III. Consequently, SA-induced oxidative stress was not reversed in rats of Group III, which were left untreated for another 3 weeks.

The current results showed statistically significant lower mean values ($p \leq 0.05$) of MDA in rats of Groups IV - V compared with Group III (Table 1). These results implied that post-treatments of SA-induced oxidative stress and cellular damage with 5 and 7.5 mg/kg bw of MOF6 in rats of Groups IV and V resulted in decreased MDA levels. These observations are in agreement with those of Gupta *et al.* (2005), which noted ameliorative potentials of MO seed powder on increased thiobarbituric acid reactive substances (TBARS) level in SA-induced hepatotoxicity in rats. In addition, the present findings are in agreement with those of Farid and Hegazy (2020), which reported that *M. oleifera* leaf extract ameliorated increased MDA levels and

oxidative stress in levofloxacin-induced hepatotoxicity in rats.

Evaluation of ACP, ALP and GGT levels in rats

The liver plays significant roles in detoxification, drug metabolism and the functionality of the digestive, reproductive and other body systems. Elevation in serum levels of liver enzymes are used as biomarkers of hepatotoxicity, hepatocellular injury and in the determination of the functional status of the liver. However, some of these enzymes are expressed in the liver and other body organs. ACP, ALP and GGT are examples of such liver enzymes evaluated in the present study. ACP is a lysosomal enzyme present in many body organs including the prostate, bone, kidneys and liver. The clinical significance of serum ACP level has been noted in the diagnosis of many diseased conditions of body organs, other than the liver. In addition, increased serum level of GGT is associated with diseases that result in damage and dysfunctions of the liver or bile ducts. GGT has equally been noted as the most sensitive liver enzyme test for detecting bile duct problems, and it is a predictive or diagnostic marker for many organ diseases (Koenig and Seneff, 2015).

Are the effects of SA on measured parameters exposure-dependent? Administration of SA for 6 weeks in rats of Group III resulted in increased ALP, GGT and MDA, but decreased ACP levels compared to rats of Group II which were exposed to SA for 3 weeks (Table 1). This indicates that duration of administration has a differential effect on the measured parameters.

Is SA-induced hepatotoxicity reversible without treatment? The present results showed statistically significant ($p \leq 0.05$) higher mean values of ACP in rats of Groups II and III compared with Group I (Table 1). In addition, there were statistically significant ($p \leq 0.05$) higher mean values of ACP in rats of Group II, which received SA for 3 weeks only compared with Group III, which

received SA for 6 weeks and left untreated for another 3 weeks (Table 1). Similarly, the results showed statistically significant ($p \leq 0.05$) higher mean values of GGT in rats of Groups II and III compared with Group I. There were also statistically significant ($p \leq 0.05$) lower mean values of GGT in rats of Group II compared with Group III (Table 1).

These results implied that SA-induced toxicity probably resulted in damage and dysfunctions of the liver and bile ducts in rats of Group II and III with increased ACP and GGT levels. Consequently, SA-induced hepatotoxicity was not reversed in rats of Group III, which were left untreated for another 3 weeks.

Furthermore, the present results showed statistically significant lower mean values ($p \leq 0.05$) of ACP and GGT in rats of Groups IV - V compared with Group III. These results implied that post-treatments of SA-induced hepatotoxicity and probable damage to the liver and bile ducts with 5 and 7.5 mg/kg bw of MOF6 resulted in decreased ACP and GGT levels. Hence, these results further implied that MOF6 possesses hepatoprotective potentials against SA-induced clastogenesis and hepatotoxicity in rats. The current findings are in agreement with those of previous studies, which reported decreased GGT levels and hepatoprotective effects of *M. oleifera* leaf extracts against chemical-induced hepatotoxicity in rats (Ajilore *et al.*, 2012; Farid and Hegazy, 2020). Similarly, the present findings are in agreement with those of Tanju and Madhuri (2013), who reported ameliorative effects of MO leaf powder on SA-induced increased GGT levels in broiler chickens.

ALP plays regulatory roles in many cellular processes in the body, and is of significance in liver function and bone development. Abnormal serum level of ALP is used as predictive or diagnostic marker of pathologies of the liver, gall bladder and bones as well as disease conditions such as diabetes (Chen *et al.*, 2017). Biochemical analyses of ALP levels showed statistically significant higher mean values ($p \leq 0.05$) of ALP in rats of Groups IV - V compared with

Groups I and III (Table 1). These results implied possible hepatotoxicity in rats treated with MOF6. However, further studies will be aimed at evaluating the effects of MOF6 on ALP levels since the present findings showed statistically significant decreased ACP and GGT levels following post-treatments of SA-induced toxicity with 5 and 7.5 mg/kg bw of MOF6.

CONCLUSION

The findings of this study indicate that SA-induced oxidative stress and hepatotoxicity are not reversible without treatment in rats. Therefore, the present findings imply that the hepatoprotective potentials and ameliorative effects of MOF6 on SA-induced

increased levels of MDA, ACP and GGT in rats are due to the pharmacological actions of compounds present in MOF6. This study, therefore, opined that MOF6 possesses antioxidant and hepatoprotective potentials.

Further efforts shall be made to characterize the compounds present in MOF6 in order to identify the bioactive compound responsible for the antioxidant and hepatoprotective activities of *M. oleifera*.

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