Niger. J. Physiol. Sci. 35 (December 2020): 173 - 179
www.njps.physiologicalsociety.com

Niger. J. Physiol. Sci.

Research Article

Hepato-Genoprotective Activities of Methanol Extract of The Stem Bark of *Adansonia Digitata* LINN. In Wistar Rats Challenged with Sodium Arsenite

Adegoke A.M., Gbadegesin M.A., *Odunola O.A

Cancer Research and Molecular Biology Laboratories, Department of Biochemistry, College of Medicine, University of Ibadan, Ibadan, Nigeria.

Summary: Arsenic exposure is an issue of concern in developing countries, consequently leading to arsenicosis which has been implicated in the development of cancers. There are various traditional medicinal applications of the stem bark of Adansonia digitata (SBAD). The focus of this study was to explore the hepatoprotective and antigenotoxic properties of methanol extract of SBAD (MESBAD) against sodium arsenite - induced toxicities in Wistar rats. Phytochemical investigation of the extract was done according to established procedures. Hepato-genoprotective properties were assessed using the liver function tests with histology and micronucleus induction assay respectively. Thirty (30) rats distributed into six groups (five rats each) were used for the experiment. Negative control (distilled water and rat pellets only), positive control [2.5 mg/kg body weight of sodium arsenite (SA)]. Test animals were challenged with SA and treated with 300 or 400 mg/kg body weight of MESBAD. Phytochemical analysis showed that MESBAD possess high concentration of alkaloids, saponins, flavonoids and total polyphenols. The SA increased the activities of ALP, GGT and the frequency of micronucleated polychromatic erythrocytes (nMPCEs) induced in rat bone marrow when juxtaposed with the negative control. Treatment with MESBAD significantly ($p<0.05$) reduced these parameters, histological examination of the liver showed that MESBAD reduced the severe portal and central venous congestion induced by SA, methanol extract of the stem bark of Adansonia digitata mitigates SA-induced toxicities probably through radical scavenging activities.

Keywords*: Adansonia digitata, hepatotoxicity, genotoxicity, methanol extract, phytochemical analysis, sodium arsenite*

**Address for correspondence: ronodunola@yahoo.com; Tel:* +234 8023387152

Manuscript Received – October, 2019; Accepted - July, 2020

INTRODUCTION

Arsenic is abundant in the earth's crust but occur in small quantities in rock, soil, water and air. It is a common environmental pollutant, as it is the case in Bangladesh with arsenic contaminated groundwater. Exposure to arsenic occur via inhalation, absorption through the skin and majorly by ingestion of arsenic contaminated food and water (Fulladosa *et al* ., 2007). Long-term arsenic exposure can lead to several sicknesses, including, arterial hypertension and cardiovascular disease, skin lesions, pulmonary disease etc (Smith *et al* ., 2000; Flanagan *et al* ., 2012), and different cancers, such as cancer of the lung, bladder, kidney and skin (Chen *et al* ., 1992; Smith *et al* ., 1998).

Natural products, on the other hand, play crucial roles in the therapy and care of various ailments. The high cost and toxicities associated with synthetic drugs make the search for potent antitoxic agents from plant origin a necessity. *Adansonia digitata* (Linn) is a member of the *Bombacaceae* family, commonly known as Baobab, Monkey Bread Tree, Cream of Tartar Tree, etc. The tree is mostly found in the savanna regions of the world (Keay, 1989). There are reports about the folkloric uses of *Adansonia digitata* both for dietary and several medicinal purposes (Chadare *et al* ., 2009). Some of the reported medicinal uses are as antimalaria, treatment of intestinal problems and skin disorders, anti- inflammatory, antipyretic and analgesic (Sidibe and Williams, 2002; De Caluwe *et al* ., 2010; Ramadan *et al* ., 1994; Ajose, 2007; Karumi *et al* ., 2008). The stem bark has been reported to possess antibacterial properties (Yushua'u *et al* ., 2010; Atawodi *et al* ., 2003), and hypoglycemic activity (Tanko *et al* ., 2008). The stem bark posses the alkaloid "Adansonin" which is suggested to be the potent active component for the treatment of malaria and other pyrexia (Sidibe and Williams, 2002). The antiviral and anti-trypanosomal activities of baobab extracts have also been reported (Vimalanathan and Hudson, 2009; Sulaiman *et al* ., 2011).

There is inadequate information in literature on the hepatoprotective and antigenotoxic activities associated with the methanol extract of the stem bark of *Adansonia digitata* (MESBAD). This study was designed to explore the hepato-genoprotective potentials of MESBAD against sodium arsenite (SA) - induced toxicities in Wistar rats.

MATERIALS AND METHODS

Plant material and preparation of extract: Leaves and fruits of baobab were used to identify and authenticate (FHI NO.109859) the forest tree and the voucher number stored at Forestry Research Institute herbarium, Jericho, Ibadan Nigeria. Fresh stem bark were harvested and dried at room temperature, it was thereafter milled and extracted

with 70% methanol for 72 hrs. The extract obtained was subjected to concentration in a rotary evaporator at 30 – 40 ^{o}C, lyophilized and kept for use (Adegoke *et al* ., 2017).

Experimental animals and treatments: Thirty (30) male Wistar rats (100-150g) were procured and kept in the animal house, Biochemistry Department, University of Ibadan, Nigeria at 29 ± 2 ^{o}C, 12 hours light/dark cycle, maintained on water and rat feeds *ad libitum* (Ladokun Livestock Feeds Limited, Ibadan, Nigeria). All the animals used for this study were handled in conformity to the guide for the care and use of experimental animals, as stipulated by the National Institute of Health (NIH publications number 85–93 revised in 1985).

The rats acclimatized for seven (7) days prior to the commencement of the study. Rats were distributed into six groups (five animals each). **Group 1** Negative control, received water and standard pelleted diet only. **Group 2** was given 400 mg/kg body weight MESBAD, **Group 3** was administered 400 mg/kg MESBAD + SA. **Group 4** received 300 mg/kg MESBAD + SA, **Group 5** was given 300 mg/kg MESBAD alone, **Group 6** was administered SA only. Sodium arsenite was administered at 2.5 mg/kg body weight (10 % oral LD_{50}) (Preston *et al* ., 1987). All treatments were done by gavage for 14 days.

Reagents and kits: Kits for alkaline phosphatase (ALP) and gamma glutamyl transferase (γGT) were procured from Randox Laboratories, UK. Sodium arsenite ($NaAsO_2$) was product of BDH chemicals Ltd poole England, other chemicals and reagents used for this study were of analytical grade, and were products of Sigma Chemical Co. St. Louis, MO., USA.

Phytochemical investigations: The MESBAD was subjected to the phytochemical screening carried out according to standard procedures, to test for polyphenols, flavonoids, alkaloids, tannins, saponins, carotenoids, Terpenoids, Oxalate, Anthocyanins, steroids, protease inhibitors and cyanogenic glycosides.

Determination of Total Phenol: The method of Singleton *et al* (1999), was employed in the determination of total phenol content.

Determination of Total Flavonoid: Total flavonoid content was evaluated using a colorimeter assay described by Bao (2005).

Determination of Alkaloid: Alkaloid content was determined by the method described by Harborne (1973).

Determination of Tanins: Tanins content was determined by employing the method described by Van-Burden and Robinson (1981).

Determination of Saponins: Saponins content was determined according to the method described by Obadoni and Ochuko (2001).

Determination of Carotenoids: Carotenoids content was evaluated according to the method described by Harbone (1998).

Determination of Terpenoids: Terpenoids content was evaluated according to the method earlier described by Ejikeme *et al* (2014).

Determination of Oxalate: Oxalate was evaluated by using the method previously reported by Ejikeme *et al* (2014); Munro and Bassir (1969).

Determination of Anthocyanin content: Anthocyanin content was determined using a method earlier described by Connor *et al* (2002).

Determination of Steroid: Analytical method used is according to the method of Ejikeme *et al* (2014).

Determination of Protease inhibitor: Activity of protease inhibitor against protease was assayed according to the procedure described by Kunitz (1947).

Determination of Cyanogenic Glycoside: Cyanogenic glycoside was determined according to the method earlier described by Amadi *et al* (2004).

Liver function enzymes assays

γ-glutamyl transferase (γ-GT) activity. The γ-GT was evaluated in the serum using the reconstituted γGT diagnostic reagent following the previously described method of Szasz (1974).

Alkaline phosphatase (ALP) activity: The ALP was determined according to the optimized recommended method of the Deutsche Gesellschaft fur Klinische Chemie (DGKC, 1972).

p-nitrophenylphosphate + H_2O ⟶ ALP phosphate + p-nitrophenol (Tietz *et al* ., 1983).

Liver histological examination: The liver sections were fixed in 4 % p-formaldehyde and cleansed in phosphate buffer for 12 hours pH 7.4 at 4 ^{o}C. After drying out, the tissue was immersed in paraffin, and then cut into segments; the sections were stained with haematoxylin–eosin dye and viewed under a microscope.

Micronucleus (MN) assay : Rat femurs were excised and each bone marrow aspirated with a needle and syringe. Microscopic slides were prepared from the bone marrows according to the procedure previously described by Matter and Schmid, 1971. Slides prepared were fixed in methanol, air-dried and then pre-treated with May-Grunwald solution, thereafter air-dried again. These slides were further stained in 5% Giemsa solution and then induced in phosphate buffer for about 30 seconds. It was thereafter rinsed in distilled water and air-dried again. The slides were mounted and scored for micronucleated polychromatic erythrocytes (MPCEs) under a microscope, according to the standard procedure at a specified X40 magnification.

Data analyses: Results are expressed as mean ± Standard deviation. The differences between the groups were analyzed using one-way analysis of variance (ANOVA) with Statistical Package for Social Sciences (SPSS) software, SPSS Inc., Chicago, Standard version 10.0.1. $p < 0.05$ was considered statistically significant for differences in means.

RESULTS

Phytochemical analysis of methanol extract of the stem bark of *Adansonia digitata* (MESBAD): There is abundant of alkaloids present in MESBAD, while saponins, flavonoids and total polyphenols are present in significant amounts (Figure 1). Alkaloids are abundant with the highest value of 330.00±0.00 mg/100g followed by saponins (153.33±2.89 mg/100g). Flavonoids 121.67±2.89 mg/100g and total polyphenols 121.67±2.89 mg/100g.

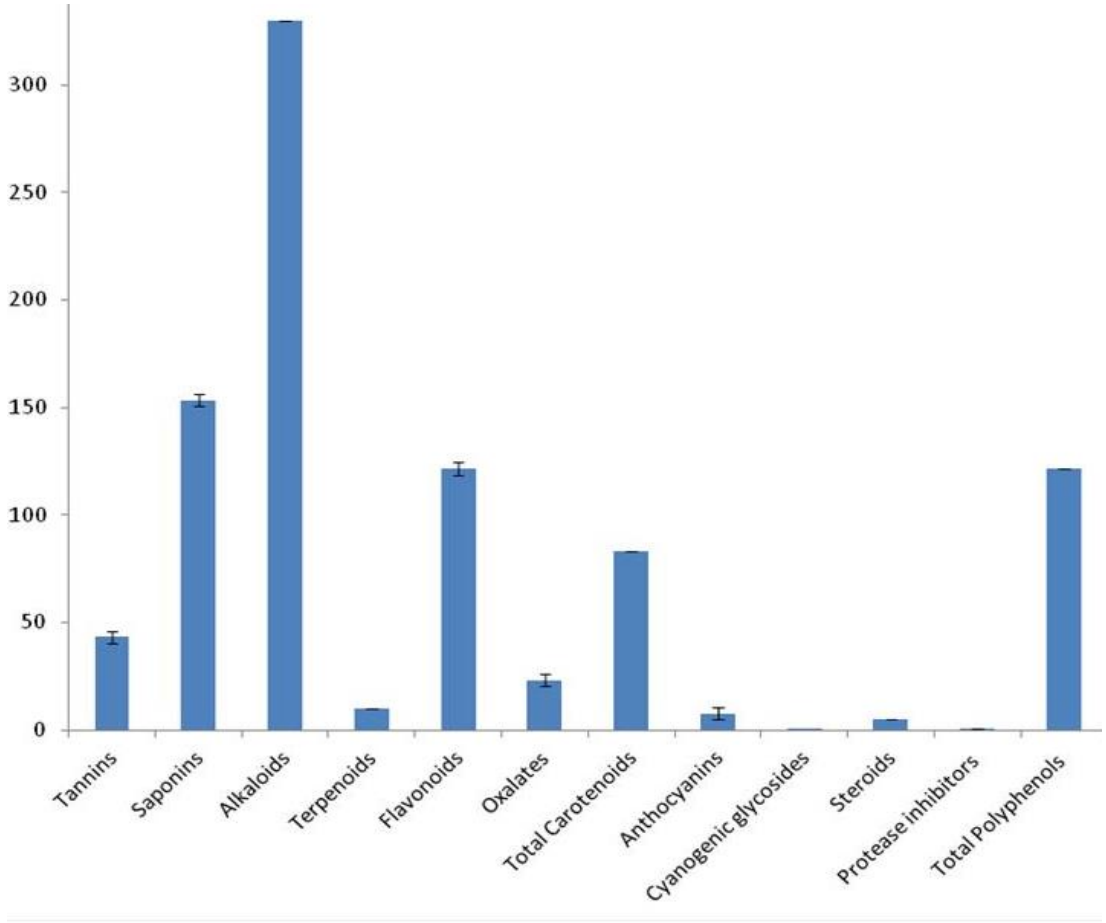

Figure 1:
The concentration of some phytochemicals in methanol extract of the stem bark of *Adansonia digitata.* Each bar represents mean ± S.D of 3 determinations * indicate a significantly high amount ** indicate abundance

Table 1:
The effect of MESBAD *and/or* SA on weight of liver and relative weights of liver of the treated rats. (values are mean ±SD)

Group	WoL (gs)	RWoL
Distilled water only	6.28±0.85	4.64±0.54
400mg/kg extract	7.00±1.60	4.26±0.78
400mg/kg extract+SA	6.38±1.13	4.06±0.48
300mg/kg extract+SA	6.00±0.21	4.48±0.79
300mg/kg extract	5.06±0.61	4.09±0.20
Sodium arsenite alone	5.54±0.33	3.03±0.11

Values are expressed as mean +or – stdev. a = the mean difference is significant (p<0.5) when compared with group a. gs = grammes

Hepatoprotective activities of MESBAD in Wistar rats challenged with SA. : Hepatoprotective effect of the stem bark of *Adansonia digitata* was investigated by assessing the activities of serum enzymes; γ-glutamyltransferase (γGT) and alkaline phosphatase. Administration of SA resulted in an increase in the mean serum ALP and γGT activities when compared with negative control, which received distilled water only (Table 2). The MESBAD alone at the doses of 300 and 400 mg/kg body weight did not induce significant ($p<0.05$) higher level of serum ALP and γGT activities when compared with the negative control.

Table 2: The effect of MESBAD *and/or* SA on liver function enzymes [Alkaline phosphatase (ALP) and gamma glutamyl transferase (γGT)] of treated rats.

Group	ALP	γGT
Distilled water only	22.52±1.59	28.65±16.24
400 mg/kg extract	20.68±15.45	31.51±9.71
400 mg/kg extract + SA	31.72±0.00	30.16±10.64
300 mg/kg extract + SA	35.86±1.95	38.26±0.82
300 mg/kg extract alone	34.48±0.00	28.42±21.29
Sodium arsenite alone	42.07±39.32	40.87±3.19

Data are expressed as mean ± sd. (n=5), DW=Distilled water, SA=Sodium arsenite.

Mean serum activities of ALP and GGT of rats after treatment with MESBAD or SA

The histological assessment of the liver cells gave robust information that buttressed the serum enzyme activities (Figure 2). There was severe portal and central venous congestion in the liver of rats given SA only (positive control). No visible lesion in the negative control, the groups that received 400 mg/kg extract+SA and the group administered 300 mg/kg extract alone (groups 1, 3 and 5 respectively).

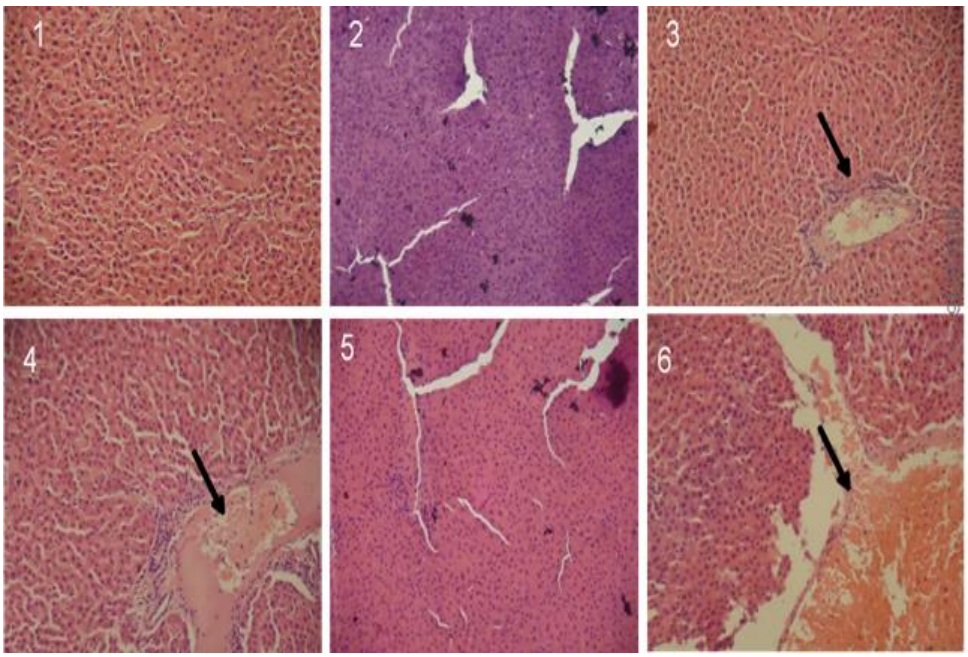

Plate 1:
Representative photomicrograph of the of the liver of rats treated with sodium arsenite and/methanol extract of the stem bark of *Adansonia digitata.* (Magnification= x400). 1. Distilled water only, normal hepatocytes, no visible lesion. 2. 400mg/kg MESBAD, mild portal congestion 3. 400mg/kg MESBAD+SA, normal hepatocytes. 4. 300mg/kg MESBAD+SA, very mild portal congestion, diffuse periportal cellular infiltration by mononuclear cells. 5. 300mg/kg MESBAD alone, no visible lesion 6. SA alone, severe portal and central venous congestion.

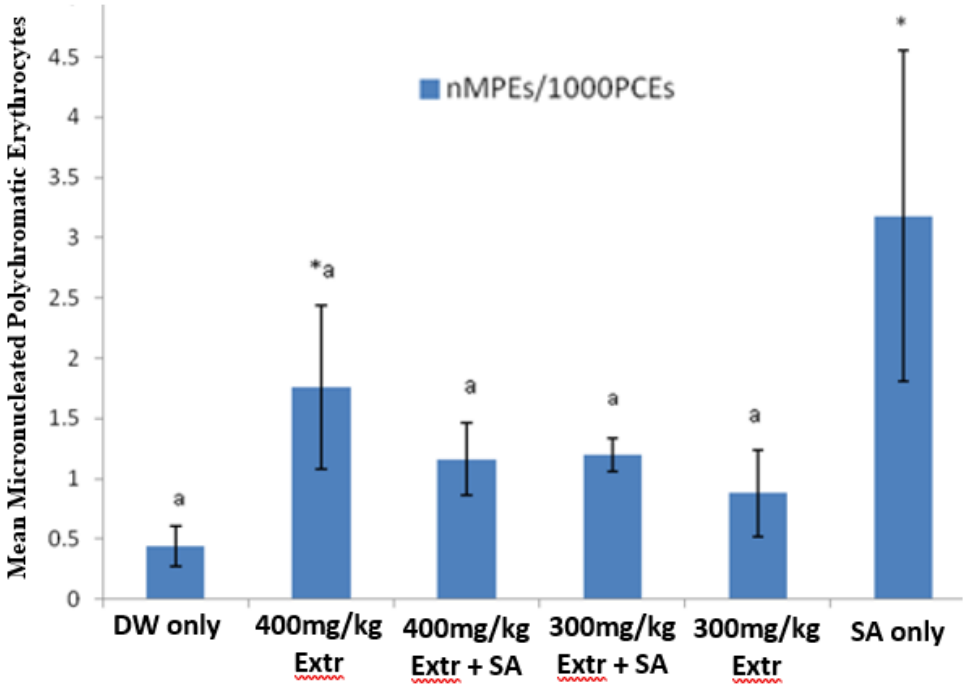

Figure 2:
Mean number of micronucleated polychromatic erythrocytes (nMPCEs) of the experimental animals after treatment with *Adansonia digitata* stem bark extract or sodium arsenite (SA). Data are expressed as mean ± sd. (n=5). DW=Distilled water, SA=Sodium arsenite. * mean difference is significant ($p< 0.05$) when compared with DW only group, [a] mean difference is significant ($p< 0.05$) when compared with SA only groups

There was mild portal congestion in group 2 administered 400 mg/kg of extracts only. However, there is very mild portal congestion and diffuse periportal cellular infiltration by mononuclear cells in group 4 administered 300mg/kg MESBAD+SA, and severe portal and central venous congestion in the group administered SA alone.

Antigenotoxic activity of MESBAD in rats challenged with SA.

The frequency of mPCEs per 1000 PCEs recorded in the bone marrow cells of rats (Figure 3) is significantly higher ($p < 0.05$) in the group that received SA alone (groups 6) when juxtaposed with the negative control (group 1). Treatments with the MESBAD led to a significant ($p<0.05$) reduction in the frequency of mPCEs scored in the groups that received both MESBAD and SA when juxtaposed with the group given SA alone. In other word, when juxtaposed with the positive control, given SA only, MESBAD significantly ($p<0.05$) reduced the number of mPCEs when co-administered with SA (groups 3 and 4).

DISCUSSION

Sodium arsenite (NaAsO2) is a known human carcinogen, its cancer-causing potential has been reported by the International Agency for Research on Cancer (IARC) (Hughes *et al* ., 2011; IARC, 1973). Some reports have also shown that sodium arsenite is genotoxic and hepatotoxic

(Mallick *et al* ., 2003; Odunola *et al* ., 2007, Gbadegesin *et al* ., 2014; Adegoke *et al* ., 2017). Adansonia digitata is a well-known forest tree with many uses in folk medicine; it is employed in the management of various diseases in certain parts of Africa. The current study investigates the hepato-genoprotective activities of MESBAD in rats challenged with sodium arsenite.

The health benefits derived from plants are numerous and they have severally been associated with many phytochemicals found in plants, phytochemicals in plants have been reported to be responsible for their medicinal properties (Hill, 1952). The MESBAD was therefore subjected to the phytochemical screening. In this study, various phytochemicals and bioactive compounds present in MESBAD were analyzed. The analysis showed that alkaloids are present in abundance, also saponins, flavonoids and polyphenols in an appreciable amount. Some of these phytochemicals have been found in many plants and had been reportedly used for medicinal purposes. The concentration of some phytochemicals in MESBAD is in this order:- Alkaloids > Saponins > Flavonoids > Total polyphenol > Tanins. Most phytoconstituents scavenge free radicals and therefore reduce oxidative stress. Examples of phenolic compounds are flavonoids and tannins, these compounds act as antioxidants or free radical scavengers. Also, terpenoids, as vitamins, play vital roles as regulators of metabolism and as antioxidants (Nair *et al* ., 1998; Agbafor *et al* ., 2014). Alkaloids possess several therapeutic activities such as antimalarial, antiasthmatic and anticancer properties as reported by Kittakoop *et al* . 2014. Flavonoids possess antioxidant, anti-inflammatory, anti allergic, anti carcinogenic, anti microbial, hepatoprotective and anti viral properties (O'Neil *et al* ., 2000; Ajuru *et al* ., 2017). Phenolic compounds prevent chronic diseases such as cardiovascular disease, some cancers, neurodegenerative disease, and also diabetes (Scalbert *et al* ., 2005). Saponins also possess anticancer properties, immunomodulatory properties, cell cycle regulation and cholesterol regulatory property (Jimoh and Oladiji, 2005). Alkaloids, flavonoids and saponins are known to possess hepatoprotective activities (Reddy *et al* ., 2015).This indicates that the extract is a good drug candidate against oxidative stress and various diseases associated with it.

The liver and relative liver weights of the treated rats were taken and compared, to establish any direct effect of the treatments on the liver and body weights, we noticed a decrease in WoL of rats treated with SA and also that of 300 mg/kg extract only, when colligated with the negative control administered distilled water only. However, the group administered sodium arsenite and the other test groups did not show significant changes on the relative weight of liver (RWoL) to body weight across board, when juxtaposed with the negative control, which was administered distilled water only.

Administration of SA led to an increase in the mean serum ALP and γGT activities when juxtaposed with negative control, which received distilled water only. The extract reduced the elevated ALP and γGT close to that of the negative control. This indicates a mild induction of hepatotoxicity in the liver cells, but the simultaneous administration of the extract reduced the values close to that of the negative control. The extract at the doses of 300 and 400 mg/kg body weight did not produce any significant higher levels of serum ALP and γGT activities. The above results are corroborated and better portrayed by histological analysis of the liver obtained from animals in each group. Histological analysis revealed that, there was severe portal and central venous congestion in the rats given SA only. No visible lesion in the negative control and the groups that received 400mg/kg extract+SA and 300 mg/kg extract alone. There was mild portal congestion in the group given 400 mg/kg of extracts only. However, there is very mild portal congestion and diffuse periportal cellular infiltration by mononuclear cells in group 4 administered 300mg/kg MESBAD+SA, and severe portal and central venous congestion in the group administered SA alone. The results indicate that SA caused hepatotoxicity and degeneration of the hepatocytes. Also, MESBAD possess hepatoprotective activities against sodium arsenite-induced hepatotoxicity.

The frequency of mPCEs per 1000 PCEs scored in rat bone marrow is an index for the measurement of genotoxicity and antigenotoxic potential of substances and compounds. Our study revealed that the frequency of mPCEs was higher significantly in the rats that received sodium arsenite alone, when colligated with the negative control. Administration of the extract led to a significant reduction in the frequency of mPCEs scored in the groups that received the extract and sodium arsenite when juxtaposed with the group that received sodium arsenite only, the extract significantly reduced the frequency of mPCEs when it was given along with sodium arsenite. This study therefore, revalidates the genotoxic properties of sodium arsenite and also provided proofs about the antigenotoxic properties of the methanol extract of the stem bark of Adansonia digitata against sodium arsenite-induces genotoxicity in rats.

From the results, it could be inferred that SA possess hepatotoxic and genotoxic effect. The result also show the protective role of MESBAD on SA-induced toxicity. Methanol extract of the stem bark of Adansonia digitata showed potent hepatoprotective and angenotoxic activities in rats challenged with sodium arsenite. These observed activities may be attributed to the phytochemicals present in the extract. Future research is required to be targeted at isolation, purification and characterization of the active principle in the extract.

REFERENCES

Adegoke, A. M., Gbadegesin, M. A., Otitoju, A. P. and Odunola, O. A. (2017). Hepatotoxicity and Genotoxicity of Sodium Arsenite and Cyclophosphamide in Rats: Protective Effects of Aqueous Extract of Adansonia digitata L. Fruit Pulp. British Journal of Medicine and Medical Research Vol.8. No.11: 963 – 974.

Agbafor, K. N., Ogbanshi, M. E. and Akubugwo, E. I. (2014). Phytochemical screening, hepatoprotective and antioxidant effects of leaf extracts of Zapoteca portoricensis. Advances in Biological Chemistry 4: 35-39.

Ajose, F. O. A. (2007). Some Nigerian plants of dermatologic importance. Int. J. Derm. 46 (suppl 1): 48-55.

Ajuru, M. G., Williams, L. F. and Ajuru G. (2017). Qualitative and Quantitative Phytochemical Screening of Some Plants Used in Ethnomedicine in the Niger

Delta Region of Nigeria. Journal of Food and Nutrition Sciences. Vol. 5, No. 5. pp. 198-205.

Amadi, B. A., Agomuo, E. N. and Ibegbulem, C. O. (2004). Research Methods in Biochemistry. Supreme Publishers, Owerri. 90-115.

Atawodi, S. E., Bulus, T., Ibrahim, S., Ameh, D. A., Nok, A. J., Mamman, N., Galadima, M. (2003). In Vitro trypanocidal effect of methanolic extract of some Nigerian savannah plants. Afr. J. Biotechnol. 2: 317-321.

Bao, J. S., Cai, Y., Sun, M., Wang, G. and Corke, H. (2005). Anthocyanins, flavonols, and free radi-cal scavenging activity of Chinese bayberry (Myrica rubra) extracts and their color properties and stability. Journal of Agricultural and Food Chemistry 53: 2327-2332.

Chadare, F. J., Linnemann, A. R., Hounhouigan, J. D., Nout, M. J. R., Van Boekel, M. A. J. (2009). Baobab Food Products: A Review on their Composition and Nutritional Value. Crit. Rev. Food Sci. Nutr. 49: 254-274.

Chen, C. J., Chen, C., Wu, M. and Kuo, T. (1992). Cancer potential in liver, lung, bladder and kidney due to ingested inorganic arsenic in drinking water. Br J Cancer: 66:888-92.

Connor, A. M., Luby, J. J. and Tong, C. B. S. (2002). Variability in antioxidant activity in blueberry and correlations among different antioxidant activity assays. Journal of American Society for Horticultural Science 127: 238-244.

De Caluwé, E., Halamová, K. and Van Damme, P. (2010). Adansonia digitata L. – A review of traditional uses, phytochemistry and pharmacology. Afrika focus — Volume 23, Nr. 1. pp. 11-51

Deutsche Gesellschaft für klinische Chemie (DGKC) (1972). Empfehlungen der deutschen Gesellschaft für Klinische Chemie Standardisierung von Methoden zur Bestimmung von Enzymaktivitäten in biologischen Flüssigkeiten. (Recommendation of the German Society of Clinical Chemistry. Standardization of methods for measurement of enzymatic activities in biological fluids.) Z Klin Chem Klin Biochem: 10:182-92.

Ejikeme, C. M., Ezeonu, C. S. and Eboatu, A. N. (2014). Determination of physical and phytochemical constituents of some tropical timbers indigenous to Niger Delta Area of Nigeria. European Scientific Journal 10(18): 247–270.

Flanagan, S. V., Johnston, R. B. and Zheng, Y. (2012). Arsenic in tube well water in Bangladesh: health and economic impacts and implications for arsenic mitigation. Bulletin of the World Health Organization; Type: Policy & practice Article ID: BLT.11.101253

Fulladosa, E., Murat, J. C., Bollinger, J. C. and Villaescusa, I. (2007) Adverse effects of organic arsenical compounds towards Vibrio fischeri bacteria. Sci Total Environ: 377:207–213.

Gbadegesin, M.A., Adegoke, A.M., Ewere, E. G. and Odunola, O.A. (2014). Ethanol Extract of Irvingia gabonensis Protects Against Sodium Arsenite Induced Toxicity in Rats. Nigerian Journal of Physiological Sciences Vol. 29. No. 1: 029 – 036.

Harborne, J. B. (1973). Methods of plant analysis. In: Phytochemical Methods. Chapman and Hall, London.

Hill, A. F. (1952). Economic Botany. A textbook of useful plants and plant products. 2nd edn. McGraw-Hill Book Company Inc, New York USA.

Hughes, M. F., Beck, B. D., Chen, Y., Lewis, A. S. and Thomas, D. J. (2011). Arsenic exposure and toxicology: a historical perspective. Toxicological Sciences 123, 305–332.

IARC, (1973). Arsenic and inorganic arsenic compounds. In: Some Inorganic and Organometallic Compounds.

Jimoh, F. O. and Oladiji, A. T. (2005). Preliminary studies on Pilostigma thonningii seed: proximate analysis, medicinal composition and phytochemical screening, African Journal of Biotechnology. Vol. 4, No. 12. pp. 1439-1442.

Karumi, Y., Augustine, A. I., Umar, I. A. (2008). Gastroprotective effects of aqueous extract of Adansonia digitata leaf on ethanol-induced ulceration in rats. J. Biol. Sci. 8: 225-228.

Keay, R. W. J. (1989). Trees of Nigeria. Clarendon Press, Oxford; 137p.

Kittakoop, P., Mahidol, C. and Ruchirawat, S. (2014). "Alkaloids as important Scaffolds in therapeutic drugs for the treatments of cancer, tuberculosis and smoking cessation". Cur Top Med Chem. Vol. 14, No. 2. pp. 239-252.

Kunitz, M. (1947). Crystalline soybean trypsin inhibitor: II. General properties. The Journal of General Physiology 20: 291–310.

Mallick, P., Mallick, J. C., Guha, B. and Khuda-Bukhsh, A. R. (2003). Ameliorating effect of microdoses of a potentized homeopathic drug, Arsenicum Album, on arsenic-induced toxicity in mice. BMC Complement Altern Med 3: 7.

Matter, B. and W. Schmid. (1971). Bone Marrow Toxicity. Mutation Res 12:417-25.

Munro, A. and Bassir, O. (1969). Oxalates in Nigerian vegetables. West African Journal of Biology and Applied Chemistry, 12: 14-18.

Nair, S., Nagar, R. and Gupta, R. (1998). Antioxidant phenolics and flavonoids in common Indian foods. Journal of the Association of Physicians of India 46: 708-71

O'Neil, L., Kim-Standage, W. S., Hughes, G. B. and Murray, K. B. (2000). In: Watson, R. R., 2000, Ed. Vegetables, Fruits and Herbs in Health Promotion, C. R. C. Press, USA.

Odunola, O. A., Akinwumi, K. A., Ogunbiyi, B. and Tugbobo, O. (2007). Interaction and Enhancement of the Toxic Effects of Sodium Arsenite and Lead Acetate in Wistar Rats. African Journal of Biomedical Research 10: 59 – 65.

Preston, R. J., Dean, B. J., Galloway, S., Hoden, H., Mcfee, A.F. and Shelby, M. (1987). Mammalian in vivo cytogenetic assays: Analysis of chromosome aberrations in bone marrow cells. Mutat Res. 189: 157-65.

Ramadan, A., Harraz, F. M. and El-Mougy, S. A. (1994). Anti-inflammatory, analgesic and antipyretic effects of the fruit pulp of Adansonia digitata. Fitoterapia, 65: 418-422.

Reddy, N., Anjum, M., Aveti, S. and Raju, G. M. (2015). Hepatoprotective Activity of Methanolic Extract of Whole Plant of Rhynchosia beddomei in Wistar Rats. J Pharm Chem Biol Sci: 3(3):378-387

Scalbert, A., Manach, C., Morand, C., Rémésy, C. and Jiménez, L. (2005). Dietary polyphenols and the prevention of diseases. Critical Reviews on Food Science and Nutrition. Vol. 45, No. 4. pp. 287-306.

Sibibe, M., Williams, J. T. (2002). Baobab – Adansonia digitata. Fruits for the future 4, International Centre for Underutilised Crops, Southampton, UK, 96p.

Singleton, V. L., Orthofer, R. and Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods in Enzymology 299: 152–178.

Smith, A. H., Goycolea, M., Haque, R. and Biggs, M. (1998). Marked increase in bladder and lung cancer mortality in a region of northern Chile due to arsenic in drinking water. Am J Epidemiol:147:660-9.

Smith, A. H., Lingas, E. and Rahman, M. (2000). Contamination of drinking-water by arsenic in Bangladesh: a public health emergency. Bull World Health Organ: 78:1093-103.

Sulaiman, L. K., Oladele, O. A., Shittu, I. A., Emikpe, B. O., Oladokun, A. T. and Meseko, C. A. (2011). In-ovo evaluation of the antiviral activity of methanolic root-bark extract of the African Baobab (Adansonia digitata Lin. African Journal of Biotechnology Vol. 10 (20), pp. 4256 – 4258.

Szasz, G. (1974). Determination of GGT activity. Methods of Enzymatic Analysis. 2nd English edition. Academic Press Inc. New York pp 715 – 720.

Tanko, Y., Yerima, M., Mahdi, M. A., Yaro, A. H., Musa, K. Y., Mohammed, A. (2008). Hypoglycemic Activity of the methanolic Stem Bark of Adansonia digitata extract on Blood Glucose Levels of Streptozocin-Induced diabetic Wistar Rats. International Journal of Applied Research in Natural Product. Vol.1(2).pp32-36.

Tietz, N. W., Burtis, C. A., Ducan, P., Ervin, K., Petitclera, C. J., Rinker, A. D., Shney, D. and Zygowicz, E. R. (1983). A reference method for measurement of alkaline phosphatase activity in human serum. Clinical Chemistry 29: 751-761.

Van Buren, J. P. and Robinson, W. B. (1981). Formation of Complexes between Protein and Tannic Acid. Journal of Agric Food Chemistry 17: 772 –777.

Vimalanathan, S., Hudson, J. B. (2009). Multiple inflammatory and antiviral activities in Adansonia digitata (Baobab) leaves, fruits and seeds. J. Med. Plan. Res. 3(8): 576-582.

Yeshua'u, M., Hamza, M. M and Abdullahi, N. (2010). Antibacterial activity of Adansonia digitata stem bark extracts on some clinical bacterial isolates. International Journal of Biomedical and Health Sciences. Vol.6.No.3 129-135.

Obadoni, B.O. and Ochuko, P.O. (2001). Phytochemical Studies and Comparative Efficacy of the Crude Extracts of Some Homeostatic Plants in Edo and Delta States of Nigeria. Global Journal of Pure and Applied Science 8: 203-208.

Harbone, J.B. (1998). Phytochemical Methods Chapman and Hall Ltd London.