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Effects of Aqueous Extract of *Moringa olifera* Leaves on Arsenic Trioxide Induced Hepato-Toxicity in Adult Wistar Rats

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Abstract

Arsenic trioxide is an amphoteric oxide with highly toxic potential when inhaled or ingested. The potential effect of *Moringa olifera* (*M. olifera)* as a chelating agent of plant origin has not been determined hence the present study. Arsenic intoxication was induced by oral administration of arsenic trioxide to the rats. Thirty (30) rats weighing within the range of 250g-300g were randomly divided into groups (A, B, C, D, E, F) of five per group. Group A was the control being orally administered 1ml of distilled water, Group B was orally administered 10mg/kg body weight of arsenic trioxide only, Groups C, D and E were orally administered graded doses of aqueous *Moringa olifera* leaves extract of 200mg/kg, 400mg/kg and 800mg/kg respectively and 10mg/kg body weight of arsenic trioxide, Group F was orally administered 800mg/kg body weight of aqueous *Moringa olifera* leaves extract only, for a period of twenty-eight (28) days with an orogastric tube. Thereafter, we studied the effects of arsenic trioxide on liver function enzymes, haematological parameters and liver histomorphology. In the group administered with arsenic trioxide only, there was a significant increase ($p<0.05$) in serum alanine transferase (ALT). Histological slides of caffeine intoxicated rats showed hepatic portal congestion, zonal necrosis, and periportal infiltrates of inflammatory cells). *Moringa olifera* extract at a high dose of 800mg/kg body weight was able to restore the levels of these parameters to normal. These findings therefore prove that high dose of *M. olifera* leaf extract was effective in reducing the resultant effects produced in the arsenic trioxide induced liver damage.

Key words: Arsenic, moringa, hepatoxicity.

Introduction

Arsenic trioxide is a metalloid with the formula As_2O_3 (Sun, 2010). It is used mainly as a medication for acute promyelocytic leukemia (Mayo Clinic, 2024). As an industrial chemical, its major uses include the manufacture of wood preservatives, pesticides, and glass (Lander, 2012). Inorganic arsenic compounds are in soils, sediments and ground water. These compounds occur naturally, or as a result of mining, and ore smelting (Center for Disease Control, 2009). Arsenic poisoning can occur due to long-term ingestion of arsenic trioxide either as a medical treatment or in drinking water from contaminated water sources. Arsenic trioxide is readily absorbed by the digestive system. Ingestion of as little as 0.1 grams can be fatal (Grund *et al.,* 2008). Its adverse effects range from increased peripheral blood WBC (Wu *et al.,* 2006), burning sensation to the nose, mouth, and eyes and cause coughing, shortness of breath, headache, sore throat, and dizziness (ATSDR, 2005; HSDB, 2007), QT prolongation and encephalopathy (Medlineplus, 2003). The liver is a peritoneal organ positioned in the right upper quadrant of the abdomen, responsible for metabolic many metabolic processes in the body such as nutrient and energy metabolism and removes toxins in blood. Arsenic trioxide induced hepatoxicity is common in patients exposed to arsenic trioxide medications (Zhang *et al.,* 2023). Arsenic induces liver toxicity via the formation of excess reactive oxygen species (ROS) and disruption of the prooxidant/ antioxidant balance in the body (Flora, 2011;

Kumar *et al.,* 2016). ROS has been shown to induce autophagy and the regulation of autophagy involves multiple pathways, and the deregulation of autophagy has been linked to many liver diseases (Doherty and Baehrecke, 2018; Allaire *et al.,* 2019). Treatment of arsenic trioxide-induced hepatoxicity entails using chelation therapy such as 2-3-dimercapto-1-propanesulfonate (DMPS) or meso 2, 3-dimercaptosuccinic acid (DMSA) (Agency for Toxic Substances and Diseases Registry, 2023). The use of these drugs has several limitations as the drugs produces severe adverse effects on the body (Erden *et al.,* 2019), and compromised with a number of shortcomings (Flora *et al.,* 2022). Most plants used for treatment of liver diseases are effective antioxidants (Rice-Evans *et al.,* 1996; López *et al.,* 2003) and these oxidants play an important role in Arsenic chelation (Gupta *et al.,* 2005; Flora *et al.,* 2007; Bhattacharya, 2017). This has made the search for drugs from plant origin with antioxidant properties has become an important focus on hepatoprotection studies. *Moringa olifera* commonly called (Moringa , n. d), is a fast-growing deciduous *tree (Encyclopædia Britannica, n. d),* it is drought-resistant tree of the family Moringaceae, native to the Indian subcontinent and used extensively in South and Southeast Asia (Encyclopedia Britannica, n. d.). In local Nigerian languages, Moringa is known as 'Barambo' in Hausa, 'Odudu Oyibo' in Igbo and 'Ewele' in Yoruba (Ekong, 2021). The anti-epileptic (Amrutia *et al.,* 2011), anti-inflammatory Paikra and Gidwani, 2017), and anti-diabetic (Ndong *et al,* 2007) properties of the plant has been evaluated. The present study seeks to investigate the effects of aqueous extract of *Magnifera oliefera* on arsenic trioxide induced hepatoxicity in Wistar rats.

Materials and Methods

Plant material: Fresh Moringa leaves were obtained from New Benin market in Edo State, Nigeria. It was identified and authenticated in of the Department of Plant and Biology and Biotechnology, University of Benin, Benin City. The voucher specimen was deposited in the herbarium with voucher number UBH-M218**.**

Preparation of plant extract*:* The leaves were air-dried at room temperature and pulverized into fine powder with a British milling machine Viking Exclusive Joncod (Type YL112M-2), the powder was soaked in absolute methanol for 24 hours and then filtered using a filter paper, the filtrate was evaporated at 40ºC using a water bath and the eventual yield was weighed. An aliquot portion of the yield was dissolved in measured quantity of distilled water for use.

Animals: Thirty (30) adult Wistar rats weighing between 250-300g were bred in the animal house of the Department of Anatomy, University of Benin, Benin City. They were acclimatized under 12 hours light/ dark cycle and room temperature was 22° C to 25 ° C and they were allowed free access to food and water.

Experimental design*:* Induction of arsenic trioxide intoxication was by single dose administration of 2mls arsenic trioxide orally to the rats at a dose of (0.01mg/kg body weight) The rats were divided into six groups (A, B, C, D, E and F) of five rats each. Group A served as the normal control rats Group B were negative control (arsenic trioxide only) Group C were arsenic intoxicated rats treated with low dose of moringa extract (200mg/kg body weight). Group D were arsenic intoxicated rats treated with medium dose of moringa extract (400mg/kg body weight) Group E were arsenic intoxicated rats treated with high dose of the moringa extract (800mg/kg body weight). Group F were treated with (800mg/kg body weight of moringa extract only.

Extracts were administered daily for thirty (30) days. At the end of the experimental period, the animals were anaesthetized under chloroform, blood samples were collected through cardiac puncture into plain sample tubes for biochemical investigations (liver function tests) and into EDTA sample bottles for haematological assay. The blood samples for serum biochemical assays were centrifuged at 3000 g for 10 minutes to obtain serum, which was later used for the estimation of biochemical parameters. The liver tissue was also collected for histopathological examinations.

Alanine amino transferase (ALT) and aspartate amino transferase (AST) activities were measured by Reitman and Frankel (1957) method while serum ALP was assayed by the

method of Englehardt *et al*. (1970). Serum protein levels were assayed using Tietz *et al.,* (1991) method, serum albumin levels were measured using Doumas and Biggs (1972) method. Serum total and direct bilirubin levels were assayed Jendrassik and Grof (1938) method. All parameters were assayed using commercially available kits.

Histopathological examinations: Excised liver samples were cleaned with normal saline and fixed for two days in 10 % buffered neutral formalin. Sections (5 µm thick) were paraffin-embedded and stained with hematoxylin and eosin. The sections of the liver were obtained and examined under Leica DM750 research microscrope with a digital camera (LeicaICC50) attached. Digital photomicrographs of the tissue sections were taken at x400 magnifications.

Statistical Analysis: Data were subjected to statistical analysis using GraphPad prism version 8.1 statistical package and relevant statistical values were obtained were converted into graphical representation in form of bar charts.

Results and Discussion

Arsenic is known to be a naturally toxic substance capable of eliciting a variety of dangerous adverse effects such as induction of arsenic trioxide overload in the body (World Health Organization, 2000). The liver is the first target organ in arsenic metabolism in which the metal is subjected to methylation (Elkin, 2022).

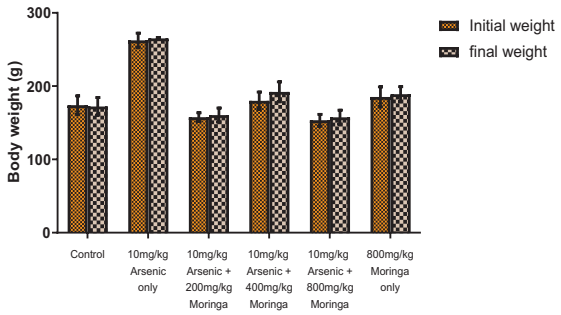

FIG 1: Effect of the Effects of Aqueous Extract of *Moringa olifera* Leaves on the body weight of the Rats

There were no significant differences between initial and final weight across the groups.

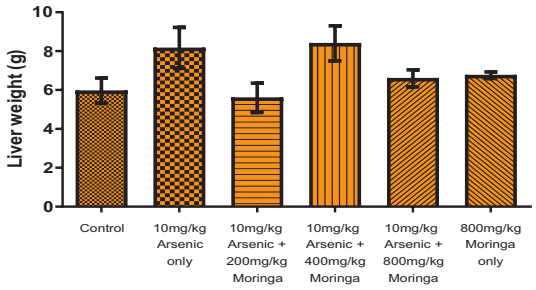

FIG 2: Effect of the Effects of Aqueous Extract of *Moringa olifera* Leaves on the liver weight of the Rats

There were no significant differences across the groups.

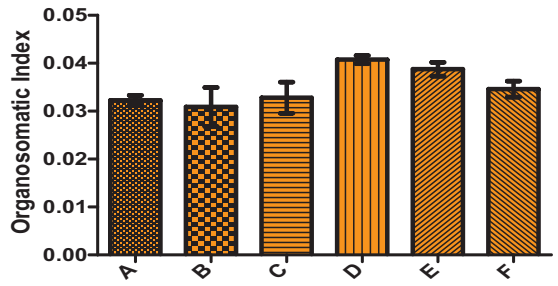

FIG 3: Effect of the Effects of Aqueous Extract of *Moringa olifera* Leaves on the organ somatic indices of the Rats.

There were significa nt increases in organo - somatic indices in 400mg/kg and 800 mg/kg *Moringa oliefera* groups when compared with control. Though, there were no significant differences in groups on arsenic trioxide only and Moringa oliefera only compared with control.

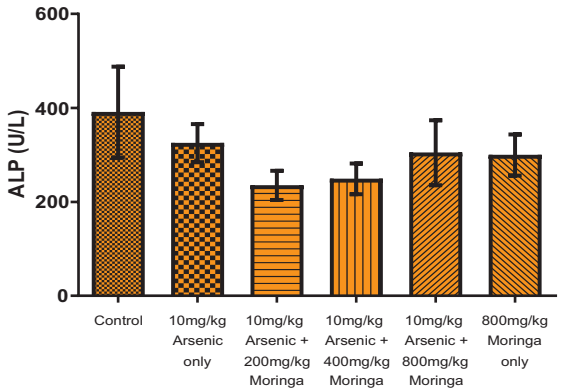

FIG 4: Effect of the Effects of Aqueous Extract of *Moringa olifera* Leaves on the ALP of the Rats.

There were no significant differences across the groups.

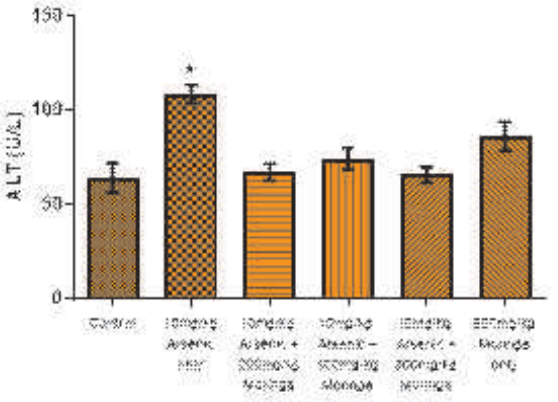

FIG 5: Effect of the Effects of Aqueous Extract of Moringa olifera Leaves on the ALT of the Rats.

There was a significant increase in group that were induced with Arsenic trioxide only compared with control. However, there were no significant differences in other groups compared with control respectively.

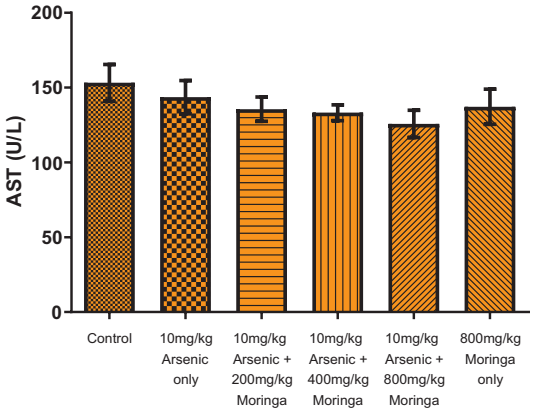

FIG 6: Effect of the Effects of Aqueous Extract of *Moringa olifera* Leaves on AST of the Rats.

There were no significant differences across the groups.

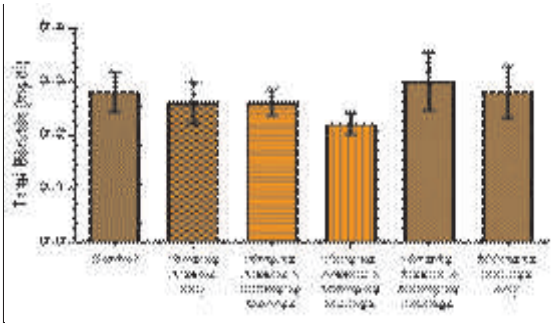

FIG 7: Effect of the Effects of Aqueous Extract of *Moringa olifera* Leaves on the total bilirubin of the Rats.

There were no significant differences across the groups

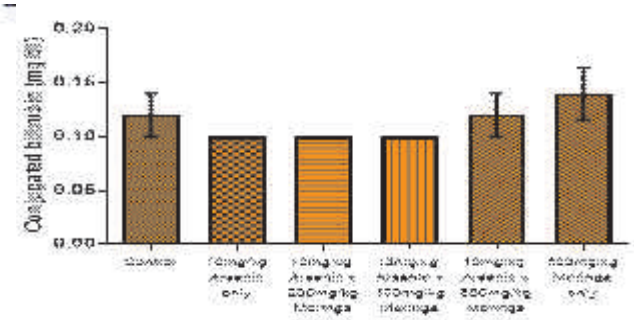

FIG 8: Effect of the Effects of Aqueous Extract of *Moringa olifera* Leaves on the conjugated bilirubin of the Rats.

There were no statistically significant differences across the groups.

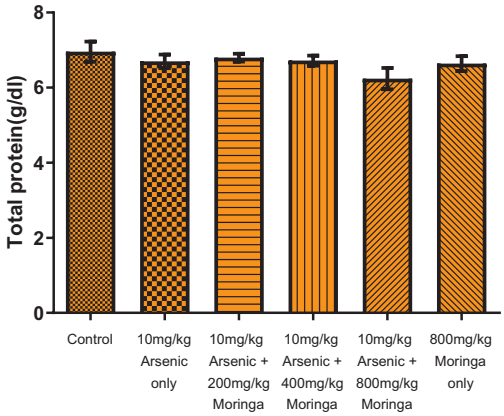

FIG 9: Effect of the Effects of Aqueous Extract of *Moringa olifera* Leaves on the total protein of the Rats.

There were no significant differences across the groups.

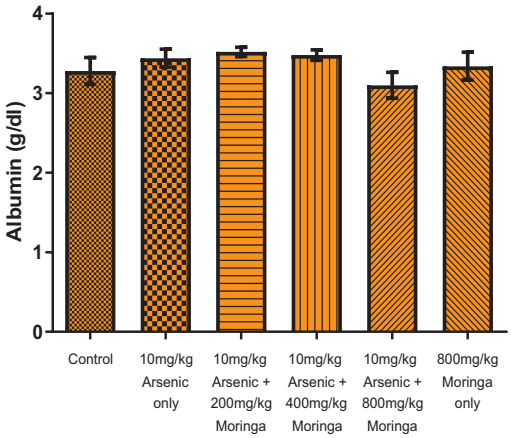

FIG 10: Effect of the Effects of Aqueous Extract of *Moringa olifera* Leaves on the albumin of the Rats.

There were no significant differences across the groups.

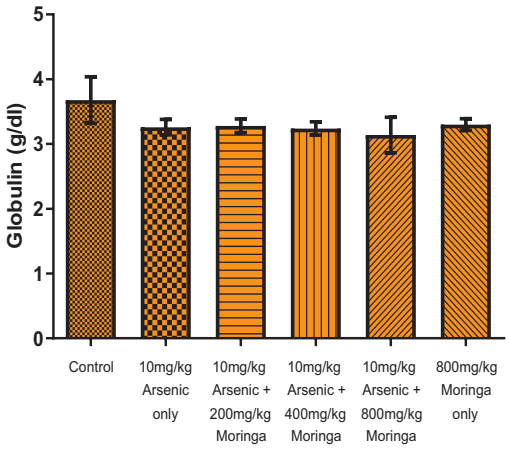

FIG 11: Effect of the Effects of Aqueous Extract of *Moringa olifera* Leaves on the globulin of the Rats.

There were no significant differences across the groups.

However, moringa is known to have various biological effects for treating various diseases such as liver diseases (Sowunmi and Gonzo, 2023). The ability of a hepatoprotective substance to reduce the injurious effects or to preserve the normal hepatic physiological mechanisms which have been upset by a hepatotoxin is the index of its protective effect (Nasir *et al,* 2013). Therefore, arsenic trioxide-induced injuries are commonly used models for the screening of hepatic plant extracts and the extent of damage is assessed by the level of released cytoplasmic transaminases (ALT and AST) and alkaline phosphatase (ALP) in circulation (Mershiba *et al.,* 2013).

Statistical results show there was significant increase in organo-somatic index i.e. organ to body weight which proves that increase in hepato-somatic index value in an ideal environment is related to normal liver growth but in cases of pollution, liver enlargement is associated with hyperplasia (Hoque *et al.,* 1998). Also, there was no significant difference in organ weight which shows the anti-inflammatory effect of moringa (Paikra and Gidwani, 2017).

Results from this study showed that in the group administered with arsenic trioxide only, there was a significant increase in serum alanine transferase (ALT) level as likely caused by the hepatotoxic properties of arsenic trioxide, therefore giving the signs of liver damage or disease as confirmed by Li *et al.,* (2015).

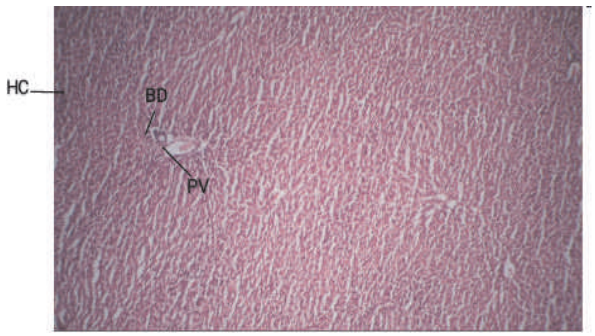

Plate 1. Rat liver. Control. Showing hepatocytes (HC), bile duct (BD), portal vein (PV), all normal: H&E x 40

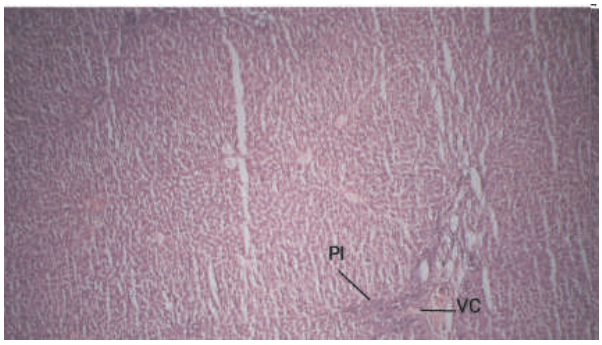

Plate 2. Rat liver g iven Arsenic only showing periportal infiltrates of inflammatory cells (PI), vascular congestion (VC): H&E x 40

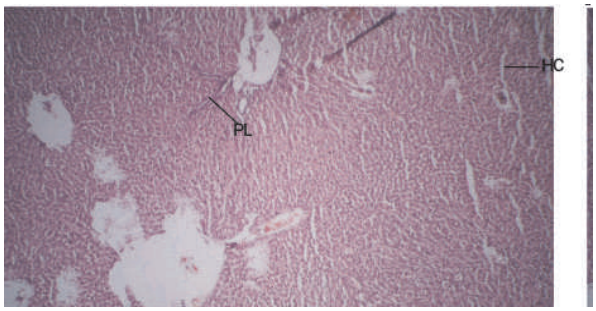

Plate 3. Rat liver given 200mg extract + Arsenic showing hepatocytes (HC), periportal mobilization of lymphocytes (PL), all normal: H&E x 40

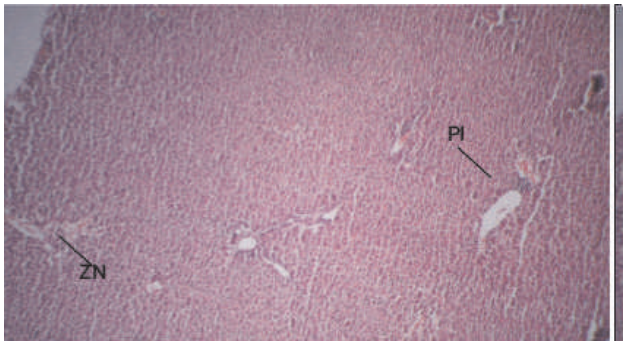

Plate 4. Rat liver given 400mg extract + Arsenic showing zonal necrosis, (ZN), periportal infiltrates of inflammatory cells (PI): H&E x 40

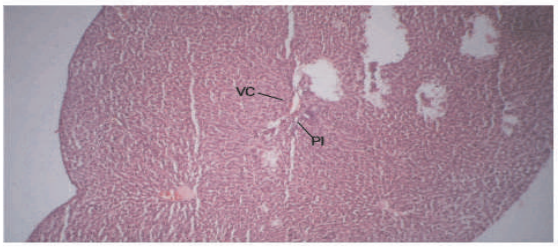

Plate 5. Rat liver given 800mg/kg extract + Arsenic showing mild periportal infiltrates of inflammatory cells (PI), Vascular Congestion (VC): H&E x40

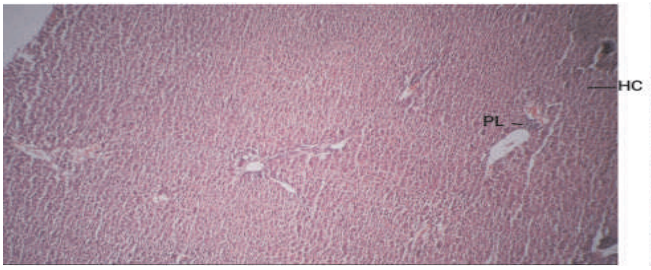

Plate 6. Rat liver given 800mg extract only showing hepatocytes (HC), periportal mobilization of lymphocytes (PL), all normal: H&E x 40

Observations from histological results of hepatic tissues further validate the result of the biochemical studies. The results of the comparative histopathology of the livers of the experimental animals are shown in the photomicrographs (plates 1-6). It was observed that arsenic trioxide treated liver sections showed vascular congestion, zonal necrosis and periportal infiltrates of inflammatory cells thus showing hepatic damage or injury when compared with control (Al-Ghanayem *et al.,* 2022). In disparity, it was observed that *Moringa oliefera* treated liver sections showed normal architecture when compared with control group. Findings indicated that low dose of *Moringa oliefera* extract have mitigating effect on the arsenic trioxide induced damages as shown in plate 3 in which a near normal architecture of liver tissues of rats was observed, hence proving the hepatoprotective nature of the extract.

However, liver sections treated with 200mg/kg of Moringa oliefera are best for protection of liver against arsenic trioxide induced hepato-toxicity. Hence this suggests that treatment with Moringa oliefera However, liver sections treated with 200mg/kg of Moringa oliefera are best for protection of liver against arsenic trioxide induced hepato-toxicity. Hence this suggests that treatment with Moringa oliefera prevented and reversed arsenic trioxide induced hepatic damage. This study corroborates previous studies that Moringa oleifera leaf extract has an appreciable ability to prevent hepatotoxicity caused by arsenic trioxide, possibly via its chemical constituents which has hepatoprotective properties (Omotoso *et al.,* 2015).

Conclusion

Results from this study showed the dose-dependent hepato-protective ability of Moringa leaves on arsenic trioxide induced hepatotoxicity in rat model.

Recommendation

We recommend firther research to elucidate the underlying mechanism of action of Moringa and explore its therapeutic effect in other areas.

Conflict of Interest: There is no conflict of interest to declare.

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