The histological effects of Moringa extract on mercury induced hepatotoxicity in adult wistar rats

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Abstract
This work focuses primarily on the histological effects of aqueous extract of moringa on mercury induced wistar rats. Twenty four adult wistar rats weighing between 190-270g were allocated into four groups of six animals each. Group A served as the control and received 0.5ml of distilled water, group B received 0.5ml of moringa extract, group C received 0.35ml of mercury while group D received 0.35ml of mercury and 0.5ml of moringa extract. The oral administration lasted for twenty eight days between the hours of 12-3pm. Twenty four hours after the last administration; the animals were sacrificed under chloroform vapour and dissected. Liver tissues were removed, weighed and trimmed down for histological studies. The results showed that following administration of extract of moringa, there are no degenerated cells; the hepatocytes were not distorted even when administered with mercury shows reduced generation compared to those induced with mercury only. Mercury exposed animals showed distortion of the liver cells. The liver weight of animals in group C was significantly higher (0.001) then group A (control) and group B and D.

Keywords: Moringa, liver enzymes, mercury, hepatotoxicity, wistar rats

1. Introduction
The liver is the largest of the abdominal viscera, occupying a substantial portion of the upper abdominal cavity. It performs a wide range of metabolic activities necessary for homeostasis, nutrition and immune defence. It is composed largely of epithelial cells (hepatocytes), which are bathed in blood derived from the hepatic portal veins and hepatic arteries. There is continuous chemical exchange between the cells and the blood. Hepatocytes are also associated with an extensive system of minute canals, which form the biliary system into which products are secreted. The liver is important in the removal and breakdown of toxic, or potentially toxic, materials from the blood14,15. In adults the liver weighs 2% of body mass7,15.

Moringa oleifera is the most widely cultivated species of a monogeneric family, the Moringaceae that is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan. Moringa oleifera or the horseradish tree is a pantropical species that is known. It is called Zogole in Hausa language. It is believed to have variety usages which include combating malnutrition, anticancer and is being promoted as a panacea1,2,3,4,8. Mercury is a toxic heavy metal which is widely dispersed in nature. Most human exposure results from fish consumption or dental amalgam10. Thus this study was undertaken to investigate the hepatoprotective nature of Moringa oleifera on induction of mercury known to cause liver damage in wistar rats since it has been used non-conventionally in the treatment of certain diseases associated with liver, kidney, cough, diarrhea etc. Cirrhosis can be induced in animals by administration of mercury or a toxin or several chemical carcinogens5,7.
Cells of the liver include hepatocytes, hepatic stellate cells - also known as perisinusoidal lipocytes, or Ito cells - sinusoidal endothelial cells, macrophages (Kupffer cells), the cells of the biliary tree - cuboidal to columnar epithelium - and connective tissue cells of the capsule and portal tracts.

The liver is a highly sensitive organ which plays a major role in maintenance and performance of the homeostasis in our body. It is the chief organ where important processes like metabolism and detoxification take place. Thus the liver is prone to injury due to the chronic exposure to drugs, environmental toxicants and other xenobiotics.

The liver disorders are one of the serious health problems, throughout the world. More than 350 million people were affected with chronic hepatic infections and in India above 20,000 deaths were reported every year due to liver disorders. Hepatocellular carcinoma is one of the most common tumors in the world with over 250,000 new cases each year.

2. Materials and Methods

2.1 Procurement of Plant

The leaves of *Moringa oleifera* was procured from Nibo in Awka south (Anambra state, Nigeria) and authenticated at the department of Botany Nnamdi Azikiwe University, Awka, Nigeria.

2.2 Preparation of extract

Fresh leaves of *Moringa oleifera* were collected, shade-dried and pounded into powder of net weight of 450g before extraction. The powder was macerated into absolute alcohol at room temperature. The filtrate was concentrated under reduced pressure and later evaporated in a water bath using evaporating dish at 45°C. A greenish paste of moringa extract measuring 0.5litre was obtained.

2.3 Experimental animals

Twenty (20) adult Wistar rats weighing 150 to 220g were obtained for the study. The animals were fed with standard diet and water and were adapted to the laboratory environment in the Department of Human Anatomy for two weeks in order to acclimatize. The administration lasted for twenty eight days between the hours of 12pm – 3pm using intubation method.

Wistar rats weighing between 190g and 270g were grouped into four (4) groups of A, B, C and D of five animals each. Group A served as control and received 0.5ml of distilled water. Group B, C and D received different doses of mercury and moringa extract as follows:

- Group B received 0.5ml of extract.
- Group C received 0.35ml of mercury.
- Group D received 0.35ml of mercury and 0.5ml of extract.

Oral route of administration was used and the administration lasted for twenty eight days.

2.4 Tissue processing and staining

Twenty four hours after the last administration, liver tissues were removed and weighed. Blood for serum preparation were collected through cardiac puncture. Serum samples were separated from clot by centrifugation using bench top centrifuge. They were then dissected and the liver tissues were removed, and fixed in zenker’s fluid for histological studies. The tissues were transferred into an automatic processor where they went through a process of dehydration in ascending grades of alcohol (ethanol) 70, 80, 95% and absolute alcohol for 2 changes each. The tissues were then cleared in Xylene and embedded in paraffin wax. Serial sections of 5 micron thick were obtained using a rotatory microtome. The tissue sections were de-paraffinised, hydrated and stained using the routine haematoxylin and eosin staining method (H&E). The stained sections were examined under the light microscope.

3. Result

In this study relative liver weight of group B and C increased significantly relative to the control group. The histological results reveal distortion of liver cells in group C and normal cell architecture in group B and C. The extract has hepato-protective effects on carbon tetrachloride induced hepatotoxicity.
4. Discussion

Liver is an organ involved in many metabolic functions and is prone to xenobiotic injury because of its central role in xenobiotic metabolism. Hepatotoxic drugs cause damage to the liver.

The results of the present study showed that the ethanolic extract of Moringa oleifera leaves have some degree of hepatoprotective ability as seen in Group C, where there was fewer necrotic cells and wider sinusoidal spaces when compared with the negative control group C that showed marked distorted hepatic cords, necrotic cells and obliterated sinusoids. Mercury was used in this study to induce the liver damage.
Based on the results obtained, we therefore inferred that *Moringa oleifera* leave extract has some protective effect on the liver as shown by the reduced damage in group C. The reduced necrosis of cells in the group C study might be due in part to the presence of chemical constituents which have hepatoprotective properties. Liver protective herbal drugs contain a variety of chemical constituents like phenols, coumarins, lignans, essential oil, monoterpenes, caro tinoids, glycosides, flavonoids, organic acids, lipids, alkaloids and xanthenes, this may be present in the *Moringa oleifera* and so responsible for this effect.

From this study, we therefore inferred that ethanolic leave extract of *Moringa oleifera* has an appreciable ability to prevent damage to the liver.

**Reference**

1. Fahey, J.W., A.T. Dinkova-Kostova and P. Talalay. The ‘Prochaska’ Micro titer Plate Bioassay for Inducers of NQO1. In: Sies, H. and L. Packer (Eds.), Methods in Enzymology. Chap. 14, Elsevier Science, San Diego, CA. CAN, 2004; 382(B): 243-258.

2. Fuglie, L.J. The Miracle Tree: *Moringa oleifera*: Natural Nutrition for the Tropics. Church World Service, Dakar, 1999 pp: 68. Revised in 2001 and published as the Miracle Tree: The Multiple Attributes of Moringa, pp: 172. Retrieved from: http://www.echotech.org/bookstore/advanced_search_result.php?keywords=Moringa+Tree.

3. Fuglie, L.J. New Uses of Moringa Studied in Nicaragua. ECHO Development Notes#68, June, 2000. Retrieved from: http://www.echotech.org/network/modules.php?name=News&file=article &sid=194.GEN NUT.

4. Galan, M.V., A.A. Kishan and A.L. Silverman. Oral broccoli sprouts for the treatment of *Helicobacter pylori* infection: A preliminary report. *Digest. Dis. Sci.*, 2004; 49(7-8): 1088-1090.

5. Guo, Q.E. Mechanism of some liver injuries induced by toxicants. A written Report for PH687: Environmental Toxicology. 1995.

6. Gupta, A.K. and N. Misra, Hepatoprotective Activity of Aqueous Ethanolic Extract of Chamomile capitula in paracetamol intoxicated albino rats. *Am. J. Pharm. Toxicol.*, 2006; 1: 17-20.

7. Kumar, G., B.G. Sharmila, P.P. Vanitha, M. Sundarajan, and P.M. Raja sekara. Hepato protective activity of *Trianthera portulacastrum* against paracetamol and thioacetamide intoxication in albino rats. 2004; *J. Ethnopharmacol.*, 92: 37-40.

8. Rappaport, A.M. Physioanatomic considerations Inc: Schiff, L. Schiff, E.R. eds. Diseases of the Liver, 5th ed Philadelphia: Lippincott 1982 pp 1-57.

9. Ruckmani, K., S. Davimani, B. Jayakar and R. Anandan,. Anti-ulcer activity of the alkali preparation of the root and fresh leaf juice of *Moringa oleifera* Lam. Ancient Sci. Life, 1998; 17 (3): 2 20-223.

10. Robin A, Mercury Toxicity and Treatment: A Review of the Literature. *J Environ Public Health* 2012: 460508.

11. Amacher, DE. A Toxicologist’s Guide to Biomarkers of Hepatic Response. *Hum Exp Toxicol*, 21(5), 2002; 253-262.

12. Salhab, M and Canelo, R. An Overview of Evidence-Based Management of Hepatocellular Carcinoma: A Meta-analysis. *J Cancer Res Ther*, 7(4), 2011; 463-475.

13. Sturgill, M., and G. H. L. a mbert, 19 97. X enobiotics induced hepatotoxicity; Mechanism of liver injury and method of monitoring hepatic function. *Clin. Chem.*, 43: 1512-1526.

14. Ujah O. F, Ujah I. R, Johnson J.T, EKAM V.S and Udenze E.C.C. *J. Nat prod. Plant Resour*-2013,3(2):15-22.