International Journal of Biomedical Research

ISSN: 0976-9633 (Online)
Journal DOI:10.7439/ijbr
CODEN:IJBRFA

Research Article

The effects of *Moringa oliferia* extract on liver enzymes of mercury induced hepatotoxicity in adult wistar rats

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Abstract

This work was carried out to determine the effect of aqueous extract of *moringa olieferia* on liver enzymes of mercury induced hepatotoxicity in adult 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 weighed and recorded. The levels of mean aspartate aminotransferase (AST), Alanin aminotransferase (ALT) and alkaline phosphotase (ALP) in group C was significantly higher ($p<0.001$) than the control and groups B and D. The result from this study shows that no adverse biochemical changes are associated with the use of *moringa extract* even when administered with mercury.

The result also shows the hepatoprotectivity of *moringa olieferia* on mercury induced hepatocytes.

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

1. Introduction

Plants are a major source of novel anti-oxidant and hepatoprotective agents since many industrial drugs are derived as a result of knowledge got from folklore medicine[1]. Some of the herbal preparations speed up the natural healing processes of the liver[12].

Moringa oleifera is the most widely cultivated species of a monogeric family, the Moringacea e that is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan. *Moringa oleifera* or the horseradish tree is a pan-tropical species that is known. It is believed to have variety usages which include combating malnutrition, anticancer and is being promoted as a panacea [3,4,5,6,11].

M. oleifera extracts or silymarin (as a reference) appears to enhance the recovery from hepatic damage induced by antitubercular drugs.

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. The liver is important in the removal and breakdown of toxic, or potentially toxic, materials from the blood. [10]

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.[17]

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[18]. One of the major functions of the liver is the manufacture and secretion of bile, which is stored in the gall bladder and released in the small intestine. It is the main organ for the synthesis and storage of carbohydrate energy in the form of glycogen. Inter-conversion of food stuff is accomplished by the liver. Normally, the liver itself stores little if any gross fat, but excess fat may accumulate in it in starvation, excessive carbohydrate feeding, and deficiency of chlorine or as a result of the action of hepatotoxins such as alcohol, carbon tetrachloride, phosphorous and mercury[19].

The liver contains thousands of enzymes some of which are also present in serum in very low concentration. These enzymes have no known function in serum other than to provide information about hepatic state and disorders. These enzymes are distributed in plasma and in interstitial fluid and have characteristic half-lives, usually measured in days. The elevation of a given enzyme activity in serum reflects its increase rate of entrance into serum from damaged liver cells like AST, ALT, ALP. Specific isoenzymes of AST are present in the liver cell mitochondria and cytoplasm whereas ALT is confined to the cytoplasm[16]. The amino transferases (transaminases) are one group of enzymes that are sensitive indicators of liver cell injury[9]. Their serum levels are especially altered in hepatocellular disease particularly in acute diseases and they are often referred to as hepatocellular enzymes[16]. Elevated serum transaminase levels are typically associated with acute hepatocellular necrosis and reflect the release of enzymes from the cytoplasm of dying cells. Other features such as fatty degeneration, infiltration by inflammatory cells are variable and reflect the severity of injury[8].

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.

2. Materials and Methods

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

2.2 Preparation of extract: Fresh leaves of *Moringa oleifera* were collected, shade-dried and pounded into powder weighing 700g 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^{o}C. A greenish paste was obtained.

2.3 Experimental animals: Twenty (20) adult Wistar rats weighing 190g to 270 g 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 between190 and 270 g 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 drugs as follows:

Group B received 0.5ml of *Moringa olieferia* extract.

Group C received 0.35ml of mercury.

Group D received 0.35ml of mercury and 0.5ml of *moringa olieferia* extract.

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

2.4 Tissue processing and staining: Twenty four hours after the 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. Activities of serum aspartate aminotransferase alanine phosphatase level and alkaline phosphatase level were determined using randox kit method. They were then dissected and the liver tissues were removed, and immediately fixed in 10% formalin. 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 rotary microtome. The tissue sections were deparaffinised hydrated and stained using the routine haematoxylin and eosin staining method (H&E). The stained sections were examined under the light microscope.

3. Results

In our study, ALT, AST, ALP activities were used to measure the protective effect of M.oleifera on mercury induced hepatotoxicity in wistar rats was examined and interpreted and the result is presented in table 1 below; However, there was significant ($p<0.05$) increase in the level of enzymes recorded for group C (129.15±3.20), when compared with the control (90.16±2.30). Beside, significant ($p<0.05$) decreased was observed in moringa treated group B (88.05±2.0), when compared with those of mercury. Significant ($p<0.05$) decrease was observed in Group D (111.44±4.04) compared to group C. More so, no significant ($p>0.05$) changes was observed for serum ALT levels all compared with the control. There was a similar trend of observed for serum ALP levels compared with AST levels. We infer that the decrease level of the liver enzymes in the groups treated with *M. oleiferia* shows reduced cell dead, hence its hepatoprotectivity.

Table 1: Effect of crude extract of *M.oleifera* on serum enzyme activities of mercury induced hepatotoxicity induced rats.

Groups	Treatment	Enzyme activity		
		AST	ALT	ALP
A	Control 0.5ml of distilled water	90.16±2.30	23.50±2.25	1.60±0.06
B	0.5ml of *M. oleiferia* extract	88.05±2.0	23.50±2.25	1.67±0.12
C	0.35ml of Mercury	129.15±3.20	23.50±2.25	1.68±0.09
D	0.5ml of *M. oleiferia* extract and 0.35ml of mercury	111.44±4.04	23.50±2.25	1.23±0.17

Values are means ± standard deviation (n=6 for each group).

Table 2: Comparison of mean initial and final body weight and liver weight in all the groups (A, B, C &D)

	Group A	Group B	Group C	Group D	F-Ratio	Prob. of Sig.
Initial body Weight	217.33±4.79	231.60±4.64	263.86±7.63	248.50±6.94	56.32	<0.05
Final body Weight	225.50±6.60	242.51±7.21	234.25±8.53	257.65±9.01	27.52	<0.05
Liver Weight	6.17±0.120	6.05±0.161	8.75±0.225	6.71±0.172	51.84	<0.05

(Mean ± SEM given for each measurement)

The final body weight for group A (Control), groups B, and D showed a statistically significant increase ($P<0.05$). The initial body weight for group C treated with mercury was significantly higher ($P<0.05$) than the control and other experimental groups (B and D) animals. The weight change for group C showed a statistically significant increase compared with the control and other experimental groups. The liver weight of group C is significantly higher than the other groups; they could be as a result of inflammation and cellular damage.

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[13]. Hepatotoxic drugs cause damage to the liver[13,14].

Mercury was used in this study to induce the liver damage in group C. Based on the results obtained, There were significant difference ($P<0.001$) in the serum and tissues levels of AST, ALT and ALP in group C when compared with the control and groups B and D. These results indicated that the extract from *M. oleiferia did* not bring about cell death in the liver during the experimental period. Enzyme activities in the serum and tissues are often used as "maker" to ascertain toxic effects of administered foreign compounds to experimental animals[21]. ALP is a membrane bound enzyme[22] while ALT and AST are cytosolic enzymes[20].

The final body weight for group A (Control), groups B, and D showed a statistically significant increase ($P<0.05$). The initial body weight for group C treated with mercury was significantly higher ($P<0.05$) than the control and other experimental groups (B and D) animals. The weight change for group C showed a statistically significant increase compared with the control and other experimental groups. The liver weight of group C is significantly higher than the other groups treated with *M. oleiferia* and distilled water this could be as a result of inflammation and cellular damage. The liver weight of group D is significantly lower than group C showing the hepatopropectivity of *M. oleiferia* extracts.

We therefore inferred that *Moringa oleifera* leave extract has some protective effect on the liver as shown by the reduced damage in group D. The reduced necrosis of cells in the group C of this study might be due in part to the presence of chemical constituents which have hepatoprotective properties.

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