

Reversal Effects of N-Acetyl Cysteine on *Moringa oleifera* Leaves-Induced Sub-Acute Hepatotoxicity in Wistar Albino Rats

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Abstract

Background: *M. oleifera* is a highly valued medicinal plant used widely from time immemorial to treat various ailments. However, with continued un-standardized use of the plant leaves, studies have reported organ toxicity to the liver, kidney and the heart. As communities continue to use *M. oleifera* leaves for its medicinal and nutritional values, there is need to find an antidote for its hepatotoxicity. **Aim:** The study established the reversal effect of N-Acetyl Cysteine (NAC) on *M. oleifera* aqueous leaf extract-induced hepatotoxicity in Wistar albino rats. **Methods:** Twenty-four (24) rats received a toxic dose (8.05 g/kg bwt) of *M. oleifera* leaf extract for 28 days to cause sub-acute hepatotoxicity. They were divided into 4 groups of 6 rats each. Group I received 1 ml normal (control group), Group II received 1000 ng/kg NAC, Group III received 1200 mg/kg NAC and Group IV received 1500 mg/kg NAC. Another group of 6 rats (Group V) received 0.75 mg/kg Paracetamol to cause hepatotoxicity. Group V (a positive control) received the prescribed clinical dose of 1200 mg/kg NAC which reverses the hepatotoxicity. All the NAC doses were given once a day intragastric for 7 days. On days: 1, 3 and 7 of receiving NAC, liver serum enzymes and bilirubin were measured. On day 7 the animals were sacrificed and liver tissue harvested for histopathology analysis. **Results:** A dose of 8.05 g/kg of *M. oleifera* leaf extract and 0.75 mg/kg Paracetamol were able to induce hepatotoxicity in Wistar albino rats in 28 days. The *M. oleifera* extract induced hepatotoxic rats treated with NAC at doses of 1000 mg/kg, 1200 mg/kg and 1500 mg/kg, had a reduction in mean serum liver enzymes, plus reduced mean serum bilirubin levels. The liver histopathological analysis

showed reduced inflammation after treatment with NAC for 3 and 7 days in the *M. oleifera* and paracetamol induced hepatotoxic rats. **Conclusion:** NAC can reverse *M. oleifera* leaf aqueous extract-induced sub-acute hepatotoxicity in Wistar Albino rats.

Keywords

M. oleifera, Sub-Acute Hepatotoxicity, N-Acetyl Cysteine, Wistar Albino Rat

1. Introduction

Traditional medicines especially using medicinal plants still remain a key component in human life. However, there are growing concerns related to the safety and toxicities of these herbal medicines derived from the medicinal plants. These herbal medicines cause various organ toxicities such as liver damage with a high incidence of mortality and morbidity. Among the commonly used medicinal plants is *Moringa oleifera* (*M. oleifera*). It was initially grown in tropical & subtropical countries and more recently, worldwide [1]. It is part of a consortium of medicinal plants that 80% of the world's population depends on as first line therapy for disease alleviation [2]. In many regions of Africa, it is widely consumed as self-medication by patients with diabetes, hypertension, or HIV/AIDS [3]. All the plant parts are traditionally used for different purposes, but the leaves are generally the most used. The rural communities in Uganda, use its leaves to treat 24 common medical conditions, including diabetes mellitus, asthma, bronchitis, mastitis, skin conditions, worm infestations, and HIV/AIDS symptoms among others [4]. *M. oleifera*'s leaves ability to treat most of these conditions has been attributed to the various phytochemical compounds present in the plant parts including its leaves [4]. They are reported to be a rich source of alkaloids and oxalates, and these have been reported to be toxic to the body including the liver [5], thus requiring other medications to reverse these toxic effects. Studies done earlier found out that the LD50 of *M. oleifera* leaves' aqueous extract was 16.10 g/kg [6]. A sub-acute toxicity profile done on rats whereby 1/2 lethal dose (8.05 g/kg), 1/4 LD50 (4.0 g/kg) and 1/16 LD50 (2.0 g/kg) of *M. oleifera* leaves aqueous extract was given intragastrically to different groups of rats for 28 days. They found hepatotoxicity, renal toxicity and heart toxicity in rats that received 1/2 lethal dose while the doses below that were not affected. It was concluded that a dose of 8.05 g/kg caused sub-acute organ toxicity [7]. The reversal of these hepatic toxic effects induced by phytochemical free radical can be achieved by use of antioxidants. Several antioxidants have been used with varying degrees of success. However, not surprisingly, N-acetyl cysteine (NAC), which supplies the cysteine necessary for glutathione synthesis, has proven more effective in treating disease-associated to reactive free radicals organ damage [8]. N-Acetyl cysteine belongs to a family of antioxidant compounds that are commonly used to manage a number of drug or disease-related toxicities, including glutathione defi-

ciency and acetaminophen toxicity [9]. Although toxic levels of *M. oleifera* are known to cause organ toxicity, limited studies have examined the possibility of reversal of these effects. Moreover, no study has been done to document the effect of NAC in *M. oleifera* aqueous leaf extract induced hepatotoxicity. Therefore, this study established the reversal of N-acetyl cysteine on *M. oleifera* aqueous leaf extract induced-sub-acute hepatotoxicity in Wistar Albino rats.

2. Materials and Methods

Study design and setting

It was a laboratory-based experimental study whereby *M. oleifera* leaves were collected from Mukono a central Uganda district, collected at 11:00 am during the dry season. The processing of the plant leaves and the laboratory experiments were done at the department of Medical Physiology, Makerere University College of Health Sciences. Experiments were done according to internationally approved methods [10] [11].

Plant collection and identification

M. oleifera leaves were harvested plus having the family and species of the leaves confirmed by a Makerere University plant taxonomist and a voucher specimen number (41,302) was deposited at the Makerere University herbarium. The leaves were air dried in a shade until constant weight was attained. They were pound using a motor and pestle into coarse powder for extraction.

Extracts preparation

The extraction process followed the already established extraction procedure on the plant materials [11]. About 300 g of *M. oleifera* leaf powder were weighed using an electronic weighing scale (Mettler PJ3000, Mettler-Toledo GmbH, Ock-erweg, Germany), and then soaked in 1.3 litres of hot water (96°C) to prevent fungal attack and allowed to cool while being shaken at 3 hourly intervals for 12 hours. The resulting suspension was filtered using a Whatman No. 1 filter paper in a Buchner funnel. The filtrate was freeze dried at 32 Pa with an original temperature set at -47°C and then maintained at 0°C to dry the extract. A freeze dryer (Genesis 12 ES) in the Chemistry department laboratory at Makerere University was used to dry the extract. The extracts obtained were stored in an air tight bottle, wrapped in a silicon paper to prevent moisture and was dissolved in distilled water to make stock solution.

Drugs: N-acetyl cysteine (NAC) used as the antioxidant for reversing the *M. oleifera* and paracetamol induced-hepatotoxicity. N-acetyl cysteine (NAC) was purchased from NOWFOODS, 244 Knollwood Dr, Bloomingdale, IL. 60108 USA. Normal saline was used as the negative control and paracetamol caused hepatotoxicity and was used as a positive control.

Animal handling

Thirty six disease-free Wistar albino rats aged between 6 and 8 weeks of which 24 were randomized into experimental groups. They were bred at the Makerere University College of Veterinary Medicine Animal Resources and Biosecurity

(CoVAB), animal house, transferred to Department of Medical Physiology animal house at Makerere University College of Health Sciences for the study. The rats were kept at standard laboratory conditions of temperature ($25^{\circ}\text{C} \pm 1^{\circ}\text{C}$), relative humidity (45% - 55%) having 12 hr light and 12 hr darkness. Standard commercial rat pellets and clean water were provided *Ad libitum* [10].

Dosing of animals

Twenty-four (24) rats received a toxic dose (8.05 g/kg bwt) of *M. oleifera* leaf extract for 28 days to cause sub-acute hepatotoxicity. They were divided into 4 groups of 6 rats each. Group I received 1 ml normal saline which acted as a control group, Group II received 1000 ng/kg NAC, Group III received 1200 mg/kg NAC and Group IV received 1500 mg/kg NAC. Another group of 6 rats (Group V) received 0.75 mg/kg Paracetamol to cause hepatotoxicity. For Group V which was a positive control received the prescribed clinical dose of 1200 mg/kg NAC which reverses the hepatotoxicity. All the NAC doses were given once a day intragastric for 7 days.

Reversal effects of NAC on *M. oleifera* sub-acute hepatotoxicity

Groups II to IV were used to test reversal effect of ANC on *M. oleifera* leaves extract induced-hepatotoxicity and Group V rats were used to confirm the reversal effect of ANC on paracetamol induced hepatotoxicity. Group I acted as the control group. After 28 days of receiving the *M. oleifera* extract and Paracetamol, hepatotoxicity was confirmed by elevated liver enzymes: ALT(U/L) 95.60 ± 14.40 , over 2 times upper limit of normal; AST(U/L) 216.30 ± 2.30 ; over 5 times upper limit of normal; ALP(U/L) 181.05 ± 18.95 ; Total BIL($\mu\text{mol/L}$) 0.35 ± 0.04 ; Total protein (g/L) 64.65 ± 5.75 .

NAC was given intragastric once a day for 7 days. Blood was withdrawn from a tail vein and serum harvested on days 1, 3 and 7 for liver enzymes and bilirubin measurements.

On day 7 they were then sacrificed, blood taken from the heart puncture, centrifuged and serum harvested. Serum proteins, bilirubin, AST, ALP, and ALT were measured by the COBA-e-411 Clinical chemistry analyzer instrument (Roche diagnostics, Germany) using methods described by the manufacturer.

Histopathology of the liver

The liver tissues were harvested by soaking cotton wool in concentrated ethyl ether and putting it close to their nostrils which caused them to sleep for good. The abdominal walls were opened and a piece of liver tissue was removed. They were placed into formalin buffer 10%, processed and sections of 5 mm thickness were prepared by a histopathologist, stained with Hematoxylin and Eosin and examined under light microscope at $\times 10$ magnification.

Data management & Analysis

Data was entered into a Microsoft Excel spread sheet and exported to GraphPad prism 8.0a Software (GraphPad Software Inc., California, USA) for analysis. Comparisons of means and standard deviations of serum enzymes and serum bilirubin were made between day one against those of day 3 and 7 by the two-way

analysis of variance (ANOVA) using Dunnett's multiple comparison's test. Mean values of $P \leq 0.05$ were considered to be statistically significant.

Ethical considerations

Permission to conduct the study was sought from the Department of Physiology and the School of Biomedical Sciences Institutional Review Board (IRB). The protocol was approved and numbered SBC-HDREC-575. Ethical practices that govern handling of laboratory animals were adhered to as per international biosafety guidelines and the guidelines for the care and use of laboratory animals (Gordon, 2001).

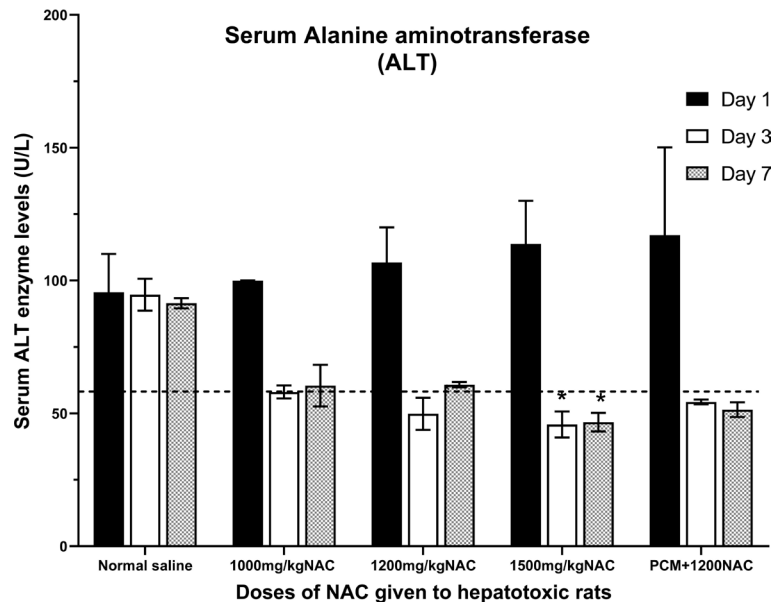
3. Results

In this study, the rats that were pre-treated with a toxic dose of 8.05 g/kg of *M. oleifera* leaf extract and those that received 0.75 mg/kg Paracetamol, showed a significant increase in mean serum liver enzymes and bilirubin levels on day 1. However by day 7 there was reduction in the mean serum liver enzymes and bilirubin

On day 1 before giving NAC, the mean serum ALT was 99.95 ± 0.05 U/L and on receiving 1000 mg/kg NAC the mean serum levels had reduced to 91.45 ± 1.95 (P-value 0.0288) on day 7. On day 1 before giving NAC, the mean serum ALT was 106.80 ± 13.20 U/L and on receiving 1200 mg/kg NAC the mean serum levels had reduced to 60.80 ± 1.00 (P-value 0.0162) on day 7; on day 1 before giving NAC, the mean serum ALT was 113.80 ± 16.20 U/L and on receiving 1500 mg/kg NAC the mean serum levels had reduced to 46.70 ± 3.50 U/L (P-value 0.0288) on day 7. Regarding the group that received paracetamol received 1200 mg/kg NAC, the mean serum ALT was 117.05 ± 33.05 U/L on day one and reduced to 51.40 ± 2.80 U/L (P-value 0.0009) by day 7 (**Figure 1**).

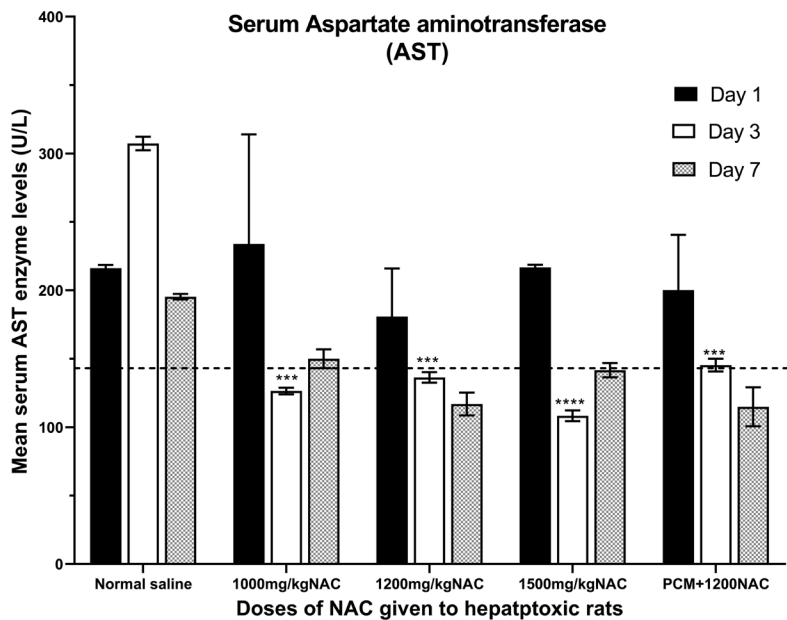
The rats that received 1000 mg/kg NAC had mean serum AST level of 233.95 ± 80.05 U/L on day 1 and 91.50 ± 2.40 U/L (P-value 0.0002) on day 3, and rose again to 150.04 ± 6.87 U/L on day 7. However the rats that received 1200 mg/kg NAC had mean serum AST level of 180.90 ± 35.10 U/L on day 1, 136.35 ± 3.85 U/L (P-value 0.0003) on day 3 and reduced again to 117.00 ± 8.40 U/L on day 7. The rats that received 1500 mg/kg NAC had mean serum AST level of 216.85 ± 1.85 U/L on day 1, 108.35 ± 3.95 (P-value 0.0001) on day 3, and rose again to 141.73 ± 5.22 U/L on day 7. The Paracetamol group received 1200 mg/kg NAC, had a mean serum AST of 200.20 ± 40.30 U/L on day 1 and reduced to 114.85 ± 14.25 U/L by day 7 (**Figure 2**).

The rats that received 1000 mg/kg NAC had mean serum ALP level of 175.10 ± 5.10 U/L on day 1 and 119.30 ± 5.40 U/L (P-value 0.0022) on day 3, and 109.60 ± 9.60 (P-value 0.001) on day 7. However the rats that received 1200 mg/kg NAC had mean serum ALP level of 175.10 ± 5.10 U/L on day 1, 117.20 ± 4.90 (P-value 0.0016) on day 3 and 110.90 ± 5.60 (P-value 0.0006) on day 7. The rats that received 1500 mg/kg NAC had mean serum ALP level of 181.70 ± 21.70 U/L on day 1, 103.80 ± 5.30 (P-value 0.0003) on day 3, and 110.20 ± 8.00 (P-value 0.0005) on day 7. The Paracetamol group received 1200 mg/kg NAC, had a mean serum



*P < 0.05 was marked significant, NS = Normal saline, NAC = N-acetyl cysteine, and PCM = paracetamol; the dotted line indicates the normal ALT level.

Figure 1. Serum ALT levels in hepatotoxic rats treated with different doses of NAC.



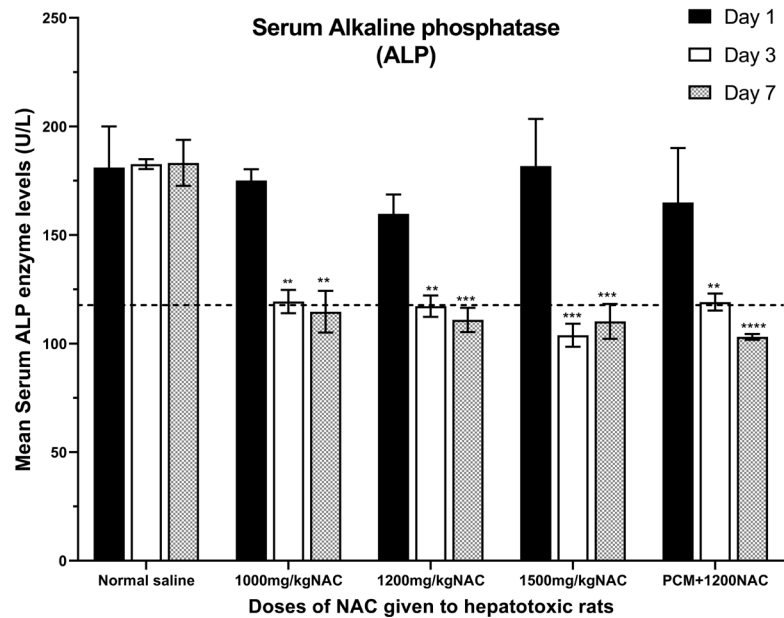
*P < 0.05 was marked significant, ***P < 0.05 was marked highly significant, NS = Normal saline, NA = N-acetyl cysteine, PCM = Paracetamol; the dotted line indicates the normal AST levels.

Figure 2. Serum AST levels in hepatotoxic rats treated with different doses of NAC.

ALP of 164.95 ± 25.05 U/L on day 1 and reduced to 103.05 ± 1.35 (P-value 0.0005) by day 7 (Figure 3).

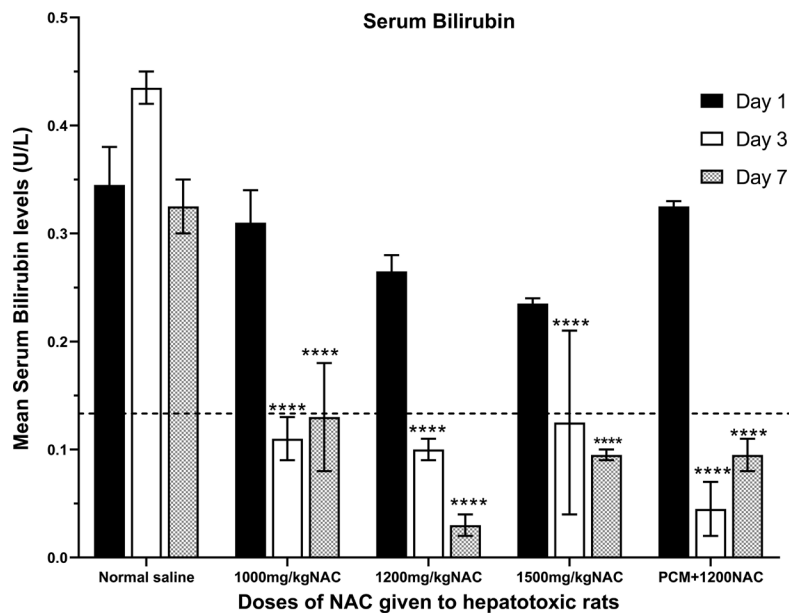
The rats that received 1000 mg/kg NAC had mean serum bilirubin level of 0.31 ± 0.03 $\mu\text{Mol/L}$ on day 1, 0.11 ± 0.02 $\mu\text{Mol/L}$ (P-value 0.0001) on day 3, and 0.13 ± 0.05 $\mu\text{Mol/L}$ (P-value 0.0009) on day 7. However the rats that received 1200 mg/kg NAC had serum bilirubin level of 0.27 ± 0.02 $\mu\text{Mol/L}$ on day 1, 0.10

$\pm 0.01 \mu\text{Mol/L}$ (P-value 0.0001) on day 3 and $0.03 \pm 0.01 \mu\text{Mol/L}$ (P-value 0.0001) on day 7. The rats that received 1500 mg/kg NAC had serum bilirubin level of 0.33 ± 0.01 (P-value 0.0001) on day 1, 0.05 ± 0.03 (P-value 0.0001) on day 3, and 0.10 ± 0.02 (P-value 0.0002) on day 7. The Paracetamol group received 1200 mg/kg NAC, had a mean serum bilirubin of $0.24 \pm 0.00 \mu\text{Mol/L}$ on day 1, and reduced to 0.13 ± 0.09 (P-value 0.0002) by day 7 (**Figure 4**).



*P < 0.05 was marked significant. ***P < 0.05 was marked highly significant, NS = Normal saline, NAC = N-acetyl cysteine, PCM = Paracetamol; the dotted line indicates normal ALP levels.

Figure 3. Serum ALP levels in hepatotoxic rats treated with different doses of NAC.



****P < 0.0001 was marked highly significant, NS-Normal saline, NAC = N-acetyl cysteine, PCM = Paracetamol; the dotted line indicates normal bilirubin levels.

Figure 4. Bilirubin levels in hepatotoxic rats treated with different doses of NAC.

The serum proteins remained in the normal range of 73.30 ± 1.40 to 58.40 ± 4.30 g/L. This means that the liver toxicity developed did not affect the mean total serum proteins.

Histopathology of the liver

The liver histopathological changes stained with of Hematoxylin and Eosin ($\times 10$) of all the study animals that received 0.75 mg/kg paracetamol for 28 days were analyzed. The liver tissue had infiltration of lymphocytes around the portal triad (**Figure 5**). After treatment with 1200 mg/kg NAC for 7 days, the liver tissue was normal with no lymphocytes infiltration (**Figure 6**).

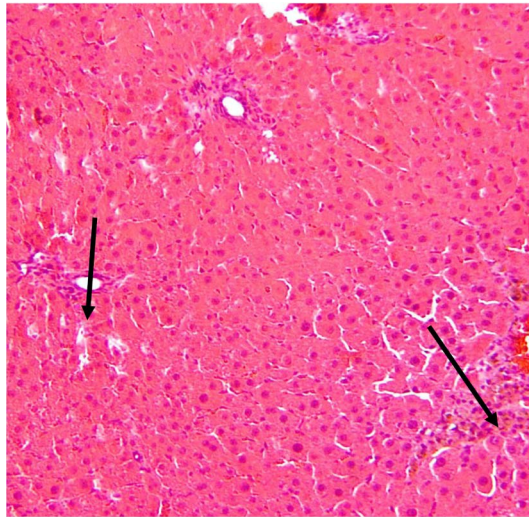


Figure 5. Histopathological appearance of the liver tissue from rats that received a toxic dose of paracetamol: infiltration of lymphocytes around the portal triad ($\times 10$).

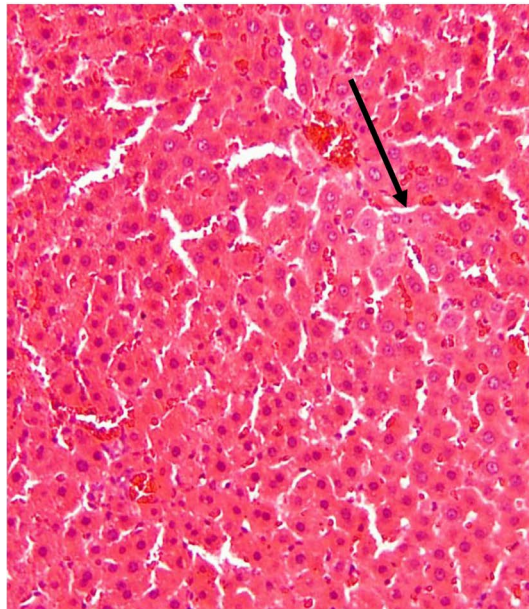


Figure 6. Histopathological appearance of the liver tissue from rats that received a toxic dose of paracetamol and NAC concurrently: normal liver tissue with no lymphocytes infiltration ($\times 10$).

Histopathology of the liver

The liver histopathological changes stained with Hematoxylin and Eosin ($\times 10$) of all the study animals that received 8.05 g/kg *M. oleifera* extract for 28 days were analysed. The liver tissue had infiltration of lymphocytes around the portal triad (**Figure 7**). After treatment with 1000 mg/kg NAC for 3 days, the liver tissue had mild infiltration of lymphocytes around the portal triad (**Figure 8**) and had normal liver tissue with no infiltration of lymphocytes around the portal triad on day 7 (**Figure 9**).

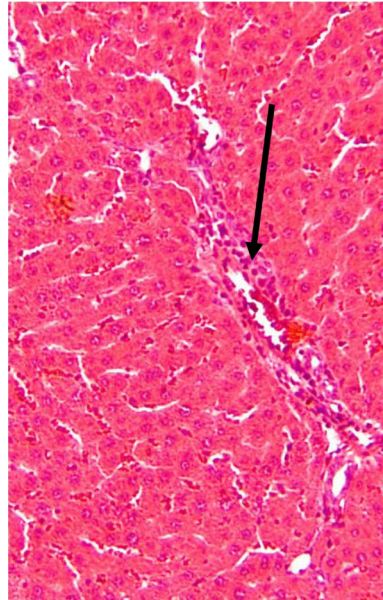


Figure 7. Histopathological appearance of the liver tissue with Moringa toxicity H & E-($\times 10$). Infiltration of lymphocytes around the portal triad.

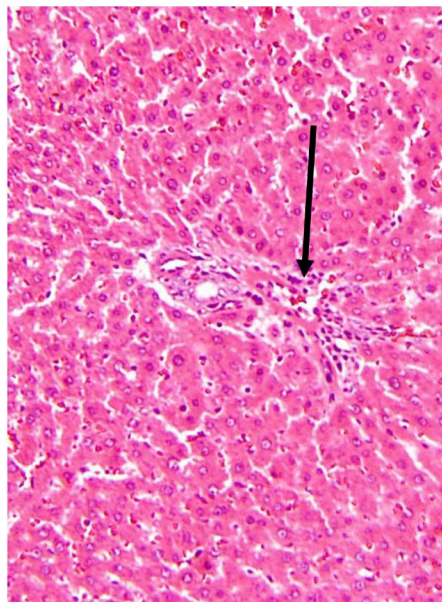


Figure 8. Histopathological appearance of the liver tissue that received 1000 NAC H & E-($\times 10$). Normal liver tissue with mild infiltration of lymphocytes around the portal triad.

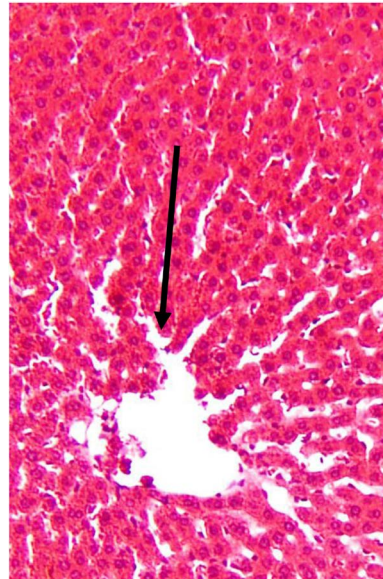


Figure 9. Histopathological appearance of the liver tissue that received 1500 NAC H & E-($\times 10$). Normal liver tissue with no infiltration of lymphocytes around the portal triad.

4. Discussion

The findings from this study demonstrated that NAC has a capability to reverse the sub-cute hepatotoxicity caused by a toxic dose of *M. oleifera* extract in Wistar Albino rats. The mean serum liver enzymes (ALT, AST and ALP) and bilirubin levels in the rats treated with NAC following a *M. oleifera* leaf extract toxic dose, showed a significant ($P < 0.05$) reduction. The hepatotoxicity that was produced by paracetamol was also reversed. The findings were in agreement with previous studies that focused on the effects of NAC in a variety of biological and pathological processes especially in the management of paracetamol-induced hepatotoxicity [12] [9].

Serum liver enzymes

There was an elevation in liver enzymes (ALT, AST and ALP) in rats given a toxic dose 8.05 mg/kg of *M. oleifera* leaf extract for 28 days. These results were in agreement with several researchers who have reported perturbations in the activities of ALT, AST, and ALP in liver during *M. oleifera* leaf aqueous extract-induced hepatotoxicity [13] [14]. The liver is the most vulnerable organ in the body to toxic substances and chemicals and therefore it was affected by toxic levels of *M. oleifera* leaves aqueous extract with specific elevated transaminase enzymes; Alanine transaminase (ALT), Aspartate transaminase (AST) and Alkaline phosphatase (ALP) [14] [15] [16]. The elevated liver enzymes indicate inflammation or damage to cells in the liver. Inflamed or injured liver cells, leak higher amounts of chemicals, including enzymes such as ALP, AST and ALP, into the bloodstream, resulting into their elevated levels [17].

N-acetyl cysteine may have reversed oxidative stress that was caused by *M. oleifera* leaf extract, through increased glutathione synthesis, thus providing more substrate for the detoxification [18]. NAC may also have reduced the liver dam-

age by its ability to promote cell survival through activating extracellular signal-regulated kinase pathway plus directly modifying the activity of several proteins [11].

A rise in alkaline phosphatase indicates damage to the liver cells including the biliary system. Therefore, the increase in serum alkaline phosphate may be considered as a sensitive indicator of cholestasis which is also supported by significant increase in total bilirubin in rats that received *M. oleifera* aqueous leaf extract [14]. The reduction noted in ALP levels was because of antioxidant properties of NAC.

Serum total protein changes

Liver synthesizes plasma protein including albumin, globulin and fibrinogen. As there were no significant changes in total protein levels in control groups and treatment groups, the synthetic function of the liver was preserved.

Histopathological liver changes

Paracetamol induced-hepatotoxicity histopathological features show infiltration of lymphocytes around the portal triad (Figure 5). On treatment with NAC for 7 days, the normal liver tissue was observed (Figure 6). This indicated reversal of the paracetamol induced hepatotoxicity by NAC. In relation to the rats that received *M. oleifera* aqueous extract for 28 days, the liver tissue features of infiltration shown by of the lymphocytes around the portal triad, confirmed hepatotoxicity in the same way as what happened in the rats that received paracetamol (Figure 7). This confirms that half LD50 of *M. oleifera* aqueous extract given orally to rats for 28 days causes hepatotoxicity as was reported by other researchers [7]. The group that received 1500 mg/Kg of NAC for 3 days had normal liver tissue with mild infiltration of lymphocytes around the portal triad (Figure 8) and had normal liver tissue with no infiltration of lymphocytes around the portal triad (Figure 9). Xenobiotics and intermediates may have disturbed the redox balance and provoked excessive production of ROS in the hepatocytes, which oxidized lipids, proteins, DNA, and other macromolecules causing disruption of cell processes, thus injuring hepatocytes [18].

Due to the fact that *M. oleifera* leaves are taken by humans and animals due to its nutrition and medicinal values without standardization, there is a great chance of taking in toxic doses of the plant leaves. This study suggests an antidote for *M. oleifera* leaves over dose.

5. Conclusion

NAC reverses *M. oleifera* leaves aqueous extract-induced sub-acute hepatotoxicity in Wistar Albino rats.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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