Available online on 15.06.2020 at http://ajprd.com

Asian Journal of Pharmaceutical Research and Development

Open Access to Pharmaceutical and Medical Research

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Research Article

Protective Effect of N-Acetyl Cysteine on *Moringa Oleifera* Aqueous Leaf Extract-Induced Hepatic Toxicity in Wistar Albino Rats

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ABSTRACT

Background: *Moringa oleifera (M. oleifera)* is a commonly used medicinal and nutritive plant. The aqueous leaf extract from this plant contains a high concentration of alkaloids and they are toxic to body organs especially the liver leading to hepatotoxicity after prolonged use. However, if N-Acetyl cysteine (NAC) is administered together with *M. oleifera* extract, it may have a hepatotoxic protective effect.

Objective: To establish the protective effect of N-Acetyl cysteine against *M. oleifera* aqueous leaf extract-induced hepatotoxicity in the Wistar albino rats.

Methods: An experimental laboratory-based study was conducted at the department of Physiology, Makerere University, College of Health Sciences. Three treatment groups of six Wistar albino rats each, were dosed with *M. oleifera* extract, Paracetamol and NAC once a day for 28 days. Group I; negative control, received 8.05g/kg bwt of *M. oleifera* extract plus 1ml of normal saline (NS), Group II; test group, received 8.05g/kg bwt of *M. oleifera* extract plus 50mg/kg of NAC. Group III; positive control, received 750mg/kg bwt of Paracetamol plus 50mg/kg of NAC. On the 14th and 29th day, three animals were selected randomly from each group and sacrificed; blood samples were collected, the liver was harvested for histopathological analysis. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), bilirubin and total protein levels were determined.

Results: There was an increase in serum ALT, AST and ALP levels in the *M. oleifera* extract plus NS group which was a sign of hepatotoxicity. The *M. oleifera* extract plus NAC group showed normal serum ALT, AST and ALP levels with no significant changes in the bilirubin (P-value = 0.9089) and total protein levels (P-value = 0.8858).

Conclusion: The results have provided evidence that NAC administration with *M. oleifera* extract effectively prevents the occurrence of *M. oleifera* leaves extract- induced hepatotoxicity.

Keywords: *Moringa oleifera*, N-Acetyl-Cysteine, hepatotoxicity, Paracetamool, Wistar rats

ARTICLE INFO: Received 10 March 2020; Review Completed 28 May 2020; Accepted 28 May 2020; Available online 15 June. 2020

Cite this article as:

Namaganda A, Kasolo J N, Bbosa G S, Lukande R, Kimuli I, Katamba G, Muwonge H, Okullo I, Nfambi J, Protective Effect of N-Acetyl Cysteine on *Moringa Oleifera* Aqueous Leaf Extract-Induced Hepatic Toxicity in Wistar Albino Rats, Asian Journal of Pharmaceutical Research and Development. 2020; 8(3):34-39 DOI: http://dx.doi.org/10.22270/ajprd.v8i3.749

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INTRODUCTION

Moringa oleifera is a plant found in many tropical and subtropical countries with significant medicinal and nutritional values[1]. Different parts of M. *oleifera* (leaves, pods, seeds, bark and flowers) have been shown to exhibit wide range of pharmacological activities, with the leaves being the most commonly used[2]. The biological activities of the plant are attributed to the

presence of many non-nutritive secondary metabolites which are bioactive in the leaves which include; polyphenols, simple sugars, tannins, vitamins, carotenoids, phytates, phenolic acids, flavonoids, alkaloids, isothiocyanates, saponins, oxalates and glucosinolates triterpenoid[3].In alternative medicine, the leaves of the *M. oleifera* are used to treat several ailments including parasitic diseases, cuts, typhoid fever, arthritis, malaria, diseases of the skin, genital urinary ailments, hypertension, diabetes mellitus and many others[4]. Previous animal studies show that *M. oleifera* leaves are safe at low doses[5], however, the aqueous extract at a lethal dose LD (16.1 g/kg) and ½ LD50 (8.05g/kg) have been reported to cause mild liver toxicity in Swiss Albino rats, when given once daily for 30 days[6]. The observed hepatotoxicity was shown by an elevation of alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and serum bilirubin and was confirmed by significant histopathological changes in the liver tissue[6].

Antioxidants such as the endogenous tripeptide glutathione, N- acetyl cysteine (NAC), vitamin C, vitamin K and lipoic acid, are protective against drug-induced toxicity. All these can directly neutralize free radicals, but they cannot replenish the cysteine required for Glutathione synthesis[7]. Therefore, it is not surprising that NAC, which supplies the cysteine are necessary for glutathione synthesis, has proven effective in treating conditions-associated with reactive free radicals that cause organ damage such as the paracetamol-induced hepatotoxicity[7]. Previous studies have reported that NAC can protect the liver from various drug-induced toxicities including anti-tuberculosis drug-induced hepatotoxicity[8], endosulfan-induced liver and kidney toxicity in rats[9] and acrylamide-induced oxidative stress[10]. However, there is limited information related to the protective effect of NAC on *M. oleifera* aqueous leaf extract-induced hepatotoxicity. The fact that people are taking *M. oleifera* leaves to treat many common ailments without a prescribed dose, it was found necessary to identify a drug that can be protective to the liver. This study established the protective effects of NAC on *M. oleifera* aqueous leaf extract-induced hepatotoxicity in Wistar Albino rats.

MATERIALS AND METHODS

Study design and setting

M. oleifera leaves were harvested during the dry season from Mukono district in central Uganda where the plant is grown by most families. The identity of the plant was confirmed by a plant taxonomist from Makerere University, Kampala, Uganda and a voucher specimen number 41302 was deposited at the Makerere University herbarium.

Extracts preparation

The collected leaves were air-dried at room temperature in the department of Physiology, Makerere University, College of Health Sciences until constant weight was attained. The dried material was pulverized to coarse powder using a motor and pestle, followed by soaking 300g of the coarse powder in 3.0L of hot water (70°C) to prevent being attacked by fungi while shaking it at 2 hours 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 Pausing a freeze dryer (Genesis 12 ES) in the department of Chemistry laboratory, Makerere University with an original temperature set at -47˚C and maintained at 0℃ to dry the extract. The dry extract obtained was stored in an air-tight bottle and wrapped in a silicon paper to prevent moisture and fungal attack. The stock solution of the extract was prepared by dissolving 100g of the dry aqueous extract in 100ml of distilled water to make a stock solution from which the daily doses were calculated according to the individual rat's body weight.

Selection of experimental laboratory animals

The experiment was performed using male disease free Wistar albino rats, aged 8 weeks, obtained from Makerere University, College of Veterinary Medicine, Animal resources and Biosecurity. The rats were housed in cages with 6 rats each. All the experimental animals were kept under standard laboratory conditions of temperature (25±1°C), relative humidity (45-55%) and light/dark cycle (12hr light: 12hr dark cycle) [11]. Standard rat food pellets and tap water were provided *ad libitum*. Rats were acclimatized in the experimental room for one week before beginning the experiment at the physiology laboratory, Makerere University, College of Health Sciences.

Drugs

N-acetyl cysteine (NAC) purchased from NOWFOODS, 244 Knollwood Dr, Bloomingdale, IL. 60108 USA was used as the antioxidant for the experiment. Paracetamol and normal saline were purchased from Friecca Pharmacy, plot 160 Wandegeya, 8472, Kampala, Uganda.

Experimental procedure

The Wistar albino rats (n=18) were divided into three groups. Group I, Group II and Group III. Each group comprised of six rats (n=6). After an overnight fast, Group I rats were administered orally using an intra-gastric tube with single dose of 8.05 g/kg body weight (bwt) *M. oleifera* leaf extract plus 1ml Normal saline. Group II rats received *M. oleifera* leaf extract at a dose of 8.05 g/kg b.wt plus 50 mg/kg bwt NAC. Group III rats received 750mg/kg bwt Paracetamol plus 50 mg/kg bwt NAC. The drugs were given once day for 28 days. On day 14, three rats from each group were sacrificed by carbon dioxide asphyxiation. At the end of intervention period (28 days), the three remaining rats from each group were sacrificed by carbon dioxide asphyxiation. Blood was collected by heart puncture, serum harvested, liver tissue was removed for histopathology analysis.

Blood analysis

The blood samples were collected in non-heparinized vacutainers, centrifuged at 500 gram force for 10 minutes, serum was harvested and analyzed using an automated clinical chemistry analyzer Roche diagnostics, Germany, using COBAS-e-411 Clinical chemistry analyzer at the Clinical chemistry laboratory, Mulago National Referral Hospital, Kampala, Uganda. The Alanine amino transferase (ALT), Aspartate amino transferase (AST), Alkaline Phosphatase (ALP), Bilirubin and Total protein levels were measured.

Histopathology of the liver

The harvested liver tissue was exposed to histopathology examination done at the pathology laboratory, Makerere University, College of Health Sciences. Tissues were fixed in 10% buffered formalin and then processed. tissue sections (4-5mm thickness) were prepared, stained with hematoxylin and eosin (H & E) and examined by light microscopy (Olympus CX21, Japan).

Statistical analysis

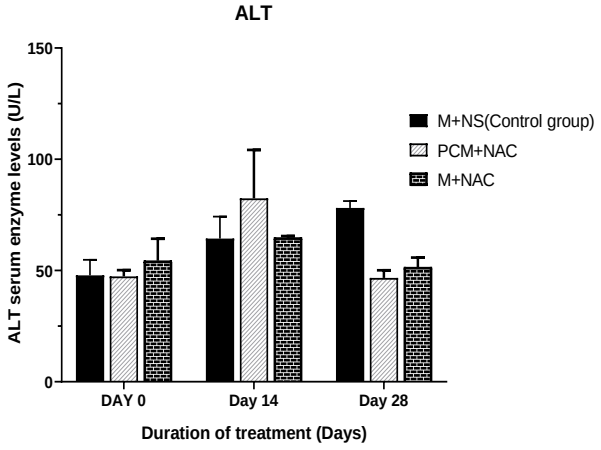
Statistical analysis was performed using Graph Pad prism version 8.0a Software (Graph Pad Software Inc., California, USA). Comparisons between groups were done by two-way analysis of variance (ANOVA) using Dunnett's multiple comparison's test. The level of significance was fixed at P-value=0.05.

Results

The study findings were; an increase in serum liver enzymes for the rats that were treated with a toxic dose of 8.05g/kg bwt *M. oleifera* extract plus 1ml of normal saline However, the rats that received 8.05g/kg of *M. oleifera* extract plus 50mg/kg bwt of NAC and those that received 750mg/kg bwt of Paracetamol plus 50mg/kg of NAC showed normal serum liver enzymes, bilirubin and total protein levels at the end of the intervention period.

Serum ALT levels

The rats that were treated with *M. oleifera* plus NS had mean serum ALT 47.80 ± 7.00 U/L on day 0, 64.35 ± 9.85 U/L on day 14 and increased to 82.40 ± 21.80 U/L on day 28. However, the rats that were treated with *M.oleifera* extract plus NAC, had mean serum ALT 54.40 ± 9.90 U/L on day 0, 64.65 ± 0.65U/L on day 14 and reduced to 51.50 ± 4.30U/L (P-value =0.1239) on day 28. The rats that were treated with Paracetamol plus NAC, had mean serum ALT 47.35 ± 2.85 U/L on day 0, increased to 82.40 ± 21.80U/L on day 14 and decreased to 46.70 ± 3.40U/L (P-value =0.0694) on day 28 (Figure 1).

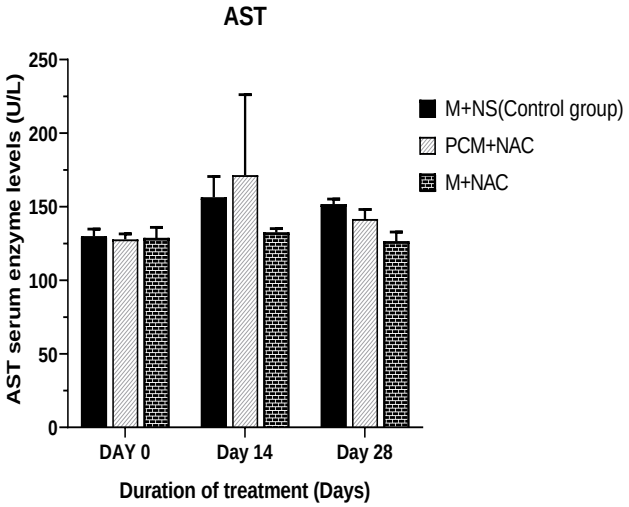


M=Moringa oleifera, NS=Normal saline, PCM=paracetamol, NAC=N-acetyl cysteine

Figure: 1. Mean serum ALT

Serum AST levels

The rats treated with *M. oleifera* plus NS had mean serum AST 129.95 ± 4.85 U/L on day 0, increased to 156.40 ± 14.10 U/L on day 14 and slightly decreased to 151.70 ± 3.50 U/L on day 28. However, the rats that were treated with *M. oleifera* plus NAC, had mean serum AST 128.70 ± 7.20 U/L on day 0, 132.45 ± 2.65U/L on day 14 and reduced to 126.45 ± 6.35U/L (P-value=0.5753) on day 28. The rats treated with Paracetamol plus NAC, had mean serum AST of 127.85 ± 3.65 U/L on day 0, increased to 171.50 ± 54.70 U/L on day 14 and decreased to 141.65 ± 6.45 U/L (P-value 0.9079) on day 28 (Figure 2).

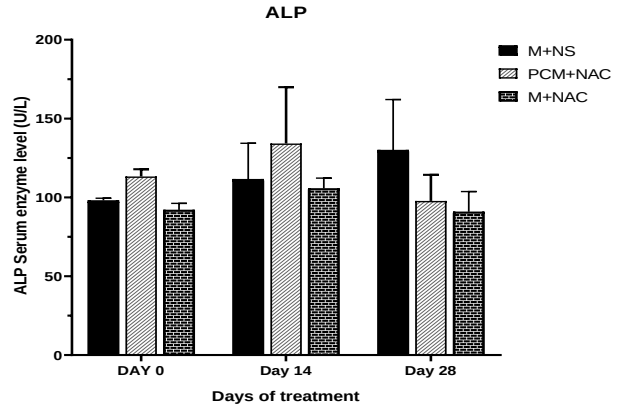


M=Moringa oleifera, NS=Normal saline, PCM=paracetamol, NAC=N-acetyl cysteine

Figure: 2. Mean serum AST

Serum ALP levels

The rats treated with *M. oleifera* plus NS had mean serum ALP 98.10 ± 1.40 U/L on day 0, 111.60 ± 22.70 U/L on day 14 and further increased to 130.05 ± 32.05 U/L on day 28. However, the rats that were treated with *M. oleifera* plus NAC, had mean serum ALP of 92.10 ± 4.10 U/L on day 0, 105.70 ± 6.50U/L on day 14 and reduced to 90.90 ± 12.80 U/L (P-value =0.3023) on day 28. The rats treated with Paracetamol plus NAC, had mean serum ALP 113.30 ± 4.60 U/L on day 0, increased to 134.20 ± 35.70 U/L on day 14 and decreased to 97.80 ± 16.50 U/L (P-value =0.4224) on day 28 (Figure 3).



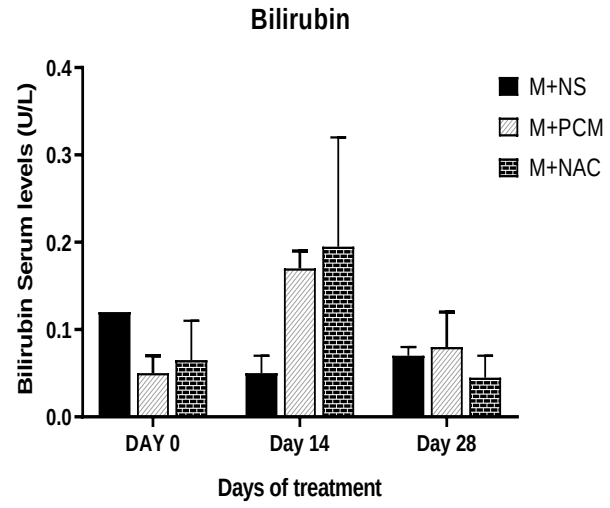
M=Moringa oleifera, NS=Normal saline, PCM=paracetamol, NAC=N-acetyl cysteine

Figure: 3 Mean serum ALP

Serum Bilirubin levels

The rats treated with *M. oleifera* plus NS had mean serum bilirubin 0.12 ± 0.00 µmol/Lon day 0, increased to 0.05 ±

0.02 µmol/L on day 14 and decreased to 0.07 ± 0.01 µmol/Lon day 28. However, the rats that were treated with *M. oleifera* plus NAC, had mean serum bilirubin 0.07 ± 0.05 µmol/L on day 0, increased to 0.20 ± 0.13 µmol/Lon day 14 and reduced to 0.05 ± 0.03 µmol/L (P-value =0.9089) on day 28. The rats treated with Paracetamol plus NAC, had mean serum bilirubin 0.05 ± 0.02 µmol/Lon day 0, increased to 0.17 ± 0.02µmol/Lon day 14 and decreased to 0.08 ± 0.04 µmol/L (P-value =0.9846) on day 28 (Figure 4).

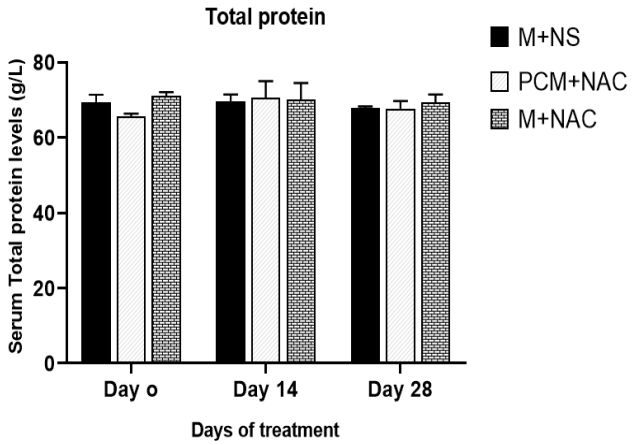


M=Moringa oleifera, NS=Normal saline, PCM=paracetamol, NAC=N-acetyl cysteine

Figure: 4. Mean serum Bilirubin

Serum Total protein levels

The mean serum total protein was within normal levels in all treatment groups on day 0, 14 and 28 that is 67.90 ± 0.40 g/L to 69.35 ± 2.15 g/L (Figure 5).



M=Moringa oleifera, NS=Normal saline, PCM=paracetamol, NAC=N-acetyl cysteine

Figure: 5 Mean serum total protein

Histopathology results

the liver tissue for all the study rats that received *M. oleifera*plus NAC and 8.05g/kg *M. oleifera* extract plus ns were analyzed. The liver tissue had vasocongestion, lymphocyte infiltration around the portal triad (Figure 6). The liver tissue for the rats that received *M. oleifera* plus NAC showed normal appearance of the hepatocytes with no lymphocyte infiltrations around the portal triad (Figure 7) and the normal liver tissue showed normal hepatocytes around the portal triad (Figure 8).The liver tissue from rats that received a toxic dose of paracetamol plus NAC showed normal liver tissue with vasocongestion (figure 9)

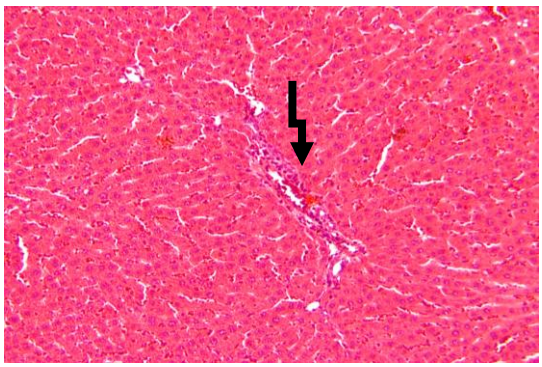


Figure 6: Histopathological appearance of the liver tissue that received *M.oleifera* plus NS; H&E-(X10): Infiltration of lymphocytes around the portal triad as shown by the arrow.

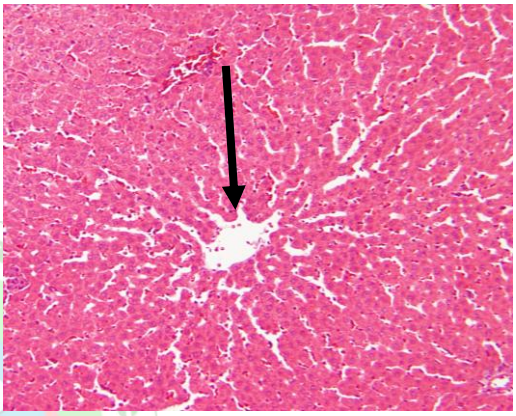


Figure 7: Histopathological appearance of the liver tissue that received *M. oleifera* plus NAC; H&E-(X10): Normal appearance with no lymphocyte infiltrations around the portal triad as shown by the arrow.

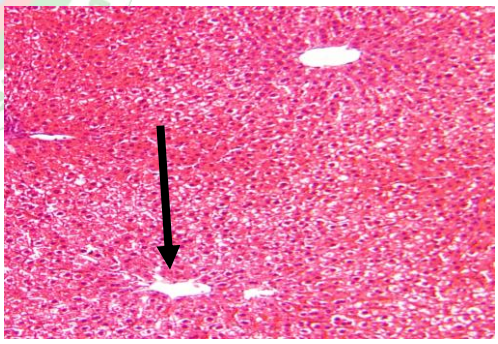


Figure 8: Histopathological appearance of a normal liver tissue H&E-(X10): Normal appearance of the hepatocytes around the portal triad as shown by the arrow.

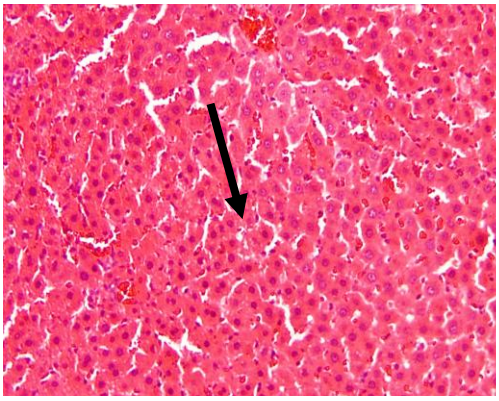


Figure 9: Histopathological appearance of the liver tissue from rats that received a toxic dose of paracetamol plus NAC: normal liver tissue with vasocongestion as shown by the arrows. H&E (X10).

DISCUSSION

The findings for the rats that received 8.05g/kg *M. oleifera* leaves extract plus 50mg/kg NAC, and 750mg/kg Paracetamol plus NAC showed normal mean serum ALT, AST, ALP and bilirubin levels by day 28 (Figures 1,2,3,4), highly suggesting that NAC prevented development of *M. oleifera* leaf extract-induced hepatotoxicity in Wistar Albino rats. These results were in agreement with those from previous studies which have reported that NAC can protect the liver from various drug-induced toxicities including against anti-tuberculosis drug-induced hepatotoxicity[8], endosulfan-induced liver and kidney toxicity in rats[9], acrylamide-induced oxidative stress[10] and Cisplatin-induced toxicity[12].

In the current study, the elevation in mean of serum ALT, AST, and ALP levels in the rats that received *M. oleifera* plus NS was possibly due to the inflammation of the liver tissue caused by ingestion of a toxic dose of *M. oleifera* aqueous leaf extract (Figure 1-4). The previous studies have shown that administration of toxic doses (8.05g/kg) of *M. oleifera* leaf extract to Swiss Albino rats, induces reversible or irreversible toxicity changes in the liver, kidney and heart[6]. The aqueous leaf extract contains a high concentration of alkaloids and the plant-derived alkaloids, by function and chemical nature, are toxic to mammals[3]. This could have contributed to leakage of hepatic cell enzymes into plasma which is a sign of hepatic tissue damage[13]. However, rats that received the synthetic antioxidant NAC along with either *M. oleifera* or paracetamol were protected from hepatotoxic effects of either agent. Therefore, antioxidants, such as NAC should be administered along with *M. oleifera* leaves to prevent the toxicity effects of *M. oleifera* leaf extract.

It is also possible that NAC prevented the altered oxidative stress parameters related to exposure of the liver to a toxic dose (8.05g/kg) of *M. oleifera* aqueous leaf extract by inducing antioxidant mechanisms since NAC has strong antioxidant properties. Antioxidants appear to act against disease processes by increasing the levels of endogenous antioxidant enzymes and decreasing toxic products such as lipid peroxidation byproducts[13].The antioxidant effect of NAC is believed to arise from NAC-induced increases in local nitric oxide concentrations and promotion of microcirculatory blood flow, thereby enhancing local oxygen delivery to peripheral tissues. The microvascular effects of NAC therapy are associated with a decrease in morbidity and mortality, even when NAC is administered in the setting of established hepatotoxicity[14].

The mild non-significant elevation of ALT, AST and ALP levels noted on day 14 in rats that were co-administered with both *M. oleifera* and NAC (figure 1-4), was probably due to the low dose of NAC (50mg/kg) used in the current study due to a longer period of administration (28 days) [10], compared to a higher dose of NAC (150mg/kg) employed in earlier studies where there was shorter duration of administration (3-5 days) [9]. The mild elevation in serum liver enzymes could also be because of evidence of intestinal metabolism of NAC in the rat, which would further reduce NAC bioavailability after oral administration[12]. Therefore, these results proved that NAC probably protected the integrity of the liver cells and preserved it from leakage as ALT, AST, ALP, bilirubin and total protein levels were kept within normal ranges.

CONCLUSION

Findings have provided evidence that NAC administration with *M. oleifera* leaves extract effectively prevents the occurrence of *M. oleifera* leaves extract- induced hepatotoxicity.

Acknowledgements

This work was financially supported by the Directorate of Research and Graduate Training, Carnegie NERLP Programme, Makerere University.

Mr. Ssengendo Geofrey – Laboratory technician who assisted us with laboratory work.

Special thanks to my colleagues Rita Nakato, Leah Nabirye, Barnabas Kasule, Patrick Opio, Julius Onyango, Denis Kavuma and Bitek Brian from the Department of Pharmacology and Therapeutics, College of Health Sciences, Makerere University, Kampala, Uganda for the encouragement, opinions and suggestions rendered to the accomplishment of this experiment.

Disclosure of conflict of interest

None.

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