Received: 15th December 2024, Accepted: 16th December 2024, Published: 22nd December 2024

ANACHEM JOURNAL
ISSN: 2006-5221 (Online)

EFFECT OF CUCUMEROPSIS MANNI SEED OIL ON LIVER AND KIDNEY FUNCTION MARKERS OF CYCLOPHOSPHAMIDE INDUCED TOXICITY IN WISTAR ALBINO RATS

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DOI: https://doi.org/10.5281/zenodo.14567533

ABSTRACT

This study evaluated the hepatic and renal properties of cucumeropsis manni seed oil on cyclophosphamide-induced toxicity in Wistar albino rats. Thirty-six male Wistar albino rats were randomly assigned into 6 groups (A, B, C, D, E, and F) of 6 rats each. Group A (normal control) received 5ml/kg b.w of normal saline for 28 days; Group B (negative control) received 5ml/kg b.w of normal saline for 27 days and 100 mg/kg b.w of cyclophosphamide at day 28 only. Group C (standard control) received 300 mg/kg b.w of Omega 3 oil for 27 days and 100 mg/kg b.w of cyclophosphamide on day 28. Group D received 5 ml/kg b.w of C. mannii seed oil for 27 days, then 100mg/kg b.w of cyclophosphamide at day 28. Group E received 2.5 ml/kg b.w of C. mannii seed oil for 27 days and 100 mg/kg b.w of cyclophosphamide at day 28. Group F received 1.5 ml/kg b.w of C. mannii seed oil for 27 days and 100 mg/kg b.w of cyclophosphamide at day 28. Normal saline, Omega 3 oil and C. mannii seed oil were administered via oral intubation, while cyclophosphamide was administered intraperitoneal. The body weights of the experimental rats were measured on a weekly basis. On day 29, rats from each group (Groups A-F) were sacrificed and serum collected for biochemical analysis. Kidney function parameters such as serum creatinine, serum urea, blood urea nitrogen (BUN), serum Na^+, and K+ are determined using commercial kits following the manufacturer's instructions. Liver function parameters, serum albumin, total bilirubin and total protein were done by Doumas et al. (1971), and AST and ALT activities were assayed by the method of Reitman and Frankel (1957). In contrast, the activities of ALP were done using the method of Plummer (1978). Cyclophosphamide-induced toxicity was evidenced by significant ($p<0.05$) elevation in the activities of ALT, AST and ALP and levels of albumin, total bilirubin, conjugated bilirubin, creatinine, urea, BUN, sodium and potassium about the standard control. Administration of CMSO and Omega 3 fatty acid before administration of the cyclophosphamide in rats led to an increase in body weight. However, administration of Cucumeropsis mannii seed oil and Omega 3 oil significantly ($p<0.05$) ameliorated the toxicity caused by cyclophosphamide in a non-dose-dependent manner. This study suggests that prophylactic administration of Cucumeropsis mannii seed oil could be clinically important in managing cyclophosphamide-induced hepato-renal toxicity in albino rats.

***Keywords**:* Cucumeropsis Mannii Seed Oil, Cyclophosphamide, Hepato-Renal Toxicity, Omega 3 Oil.

INTRODUCTION

Cyclophosphamide (CPA) is an alkylating and chemotherapeutic agent employed to manage various malignancies (Khan *et al.*, 2014). CPA is active in killing tumour cells and plays a key role in immune suppression. More than 150 metabolites of CPA have been documented, but their pharmacokinetics and harmfulness are not well-demarcated (Xu *et al.*, 2016). These metabolites induce a pro-oxidant effect and increase the production of reactive oxygen species (ROS) and organ failure due to sepsis and tissue oxidative damage. The primary mechanism of CPA-induced kidney failure is almost unknown (Xu *et al.*, 2016). ROS production accompanying inflammation leads to non-programmed necrotic cell death (necrosis) (Dressler and Patel, 2015) flanked by the three types of programmed cell death, including autophagy, apoptosis, and pyroptosis (Hsu *et al.*, 2016). This is because the production of ROS can damage the glomeruli and the kidney tubules (Hsu *et al.*, 2016). Researchers have engaged in studies to overcome this toxic effect using antioxidant agents from natural plant products (El-Naggar *et al.*, 2015).

Cucumeropsis mannii, the indigenous *Egusi* of West Africa, possess ample bioactive compounds with potential pharmacological effects. The kernel of the African melon contains about 50% oil, 30% protein, 10% carbohydrate, 4% ash, and 3% fiber (Agbemafle *et al.*, 2014). It is also an excellent source of amino acids (arginine, methionine, and tryptophan), Vitamins (A and E), and minerals (phosphorus, potassium, magnesium, manganese, sulphur, calcium, iron, and zinc) (Agbemafle *et al.*, 2014). The fatty acids that it contains in abundance are linoleic (62.42 %), oleic (15.90 %), palmitic (10.27 %), and stearic (10.26 %) (Besong *et al.*, 2011). These bioactive compounds reported present in the African melon seed are potential antioxidants that possess some pharmacological characteristics such as anti-inflammatory (Mohebbati *et al.*, 2016), anti-diabetic (Beheshti *et al.*, 2017), anti-metastatic (Beheshti *et al.*, 2017), anxiolytic (Ahmad *et al.*, 2013), immune-modulatory (Ahmad *et al.*, 2013), and relaxant properties (Ahmad *et al.*, 2013).

MATERIALS AND METHODS

Oral Administration

Group A (Control rats) were administered 5 ml/Kg b.w of normal saline for 28 days.

Group B Rats were administered 5 ml/kg b.w. of normal saline for 27 days and then injected intraperitoneally with 100 mg/Kg b.w. of cyclophosphamide on day 28.

Group C Rats received via oral intubation 300 mg/Kg b.w of Omega 3 oil for 27 days, and then injected intraperitoneally 100 mg/Kg b.w of cyclophosphamide at day 28.

Group D Rats were administered 5 ml/Kg b.w. of C. mannii seed oil for 27 days and 100 mg/Kg b.w. of cyclophosphamide at day 28.

Group E Rats received 2.5 ml/Kg b.w of C. mannii seed oil via oral intubation for 27 days and then injected intraperitoneally 100 mg/Kg b.w of cyclophosphamide at day 28.

Group F Rats received 1.5 ml/Kg b.w of C. mannii seed oil via oral intubation for 27 days and injected intraperitoneally 100 mg/Kg b.w of cyclophosphamide at day 28.

Kidney Function Parameters

Serum creatinine, urea, blood urea nitrogen (BUN), and some electrolytes (Na^+ and K^+) were determined using commercial kits following the manufacturer's instructions.

Determination of the Liver Function Parameters

Serum albumin, total bilirubin, and total protein were measured using the methods of Doumas *et al.,* (1971). AST and ALT activities were assayed using the methods of Reitman and Frankel (1957), whereas the activity of ALP was measured using the method of Plummer (1978).

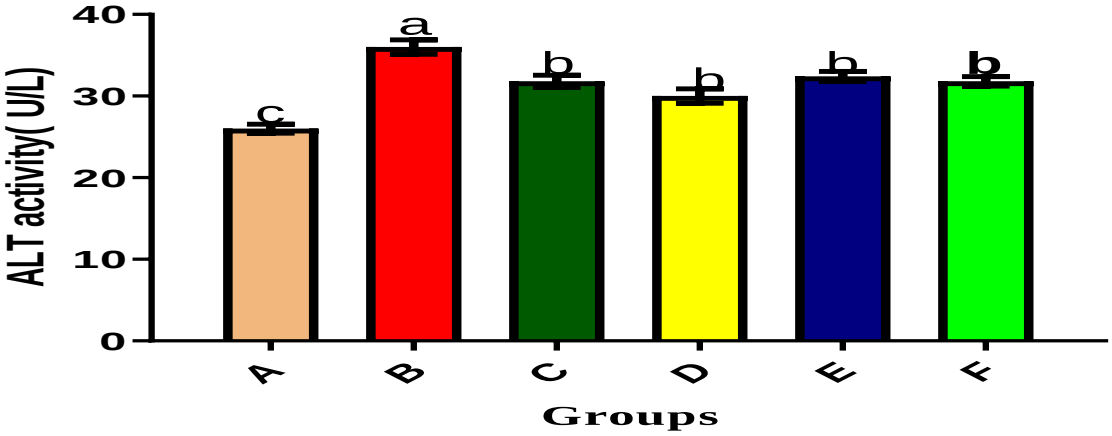
Statistical Analysis

All results were analyzed using Graph-pad Prism 5. Data were expressed as mean ± standard deviation. The means of the parameters were compared using one-way ANOVA in the Post-hoc format at $p<0.05$.

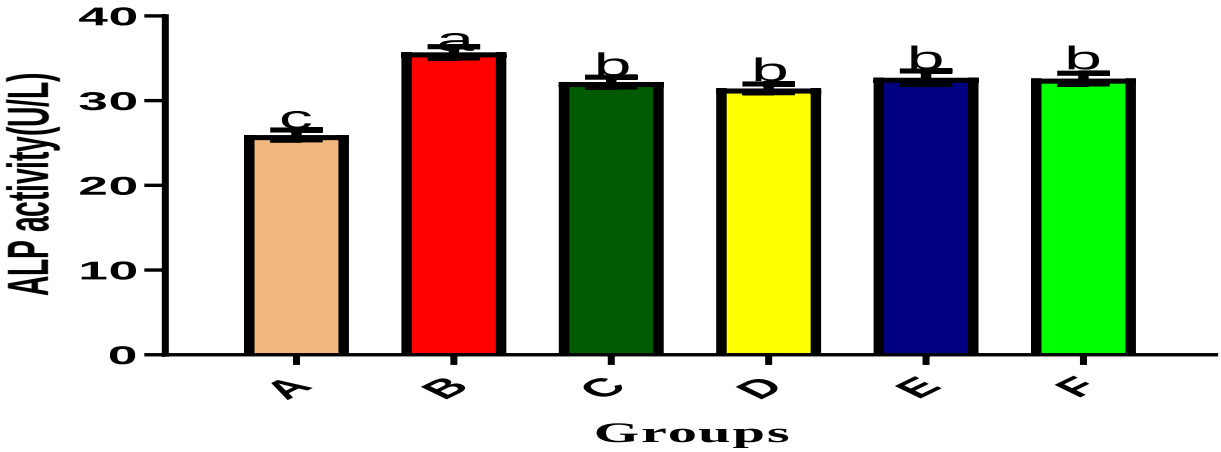
RESULTS

Effect of CMSO on Liver Function Parameters in Cyclophosphamide-induced Hepato renal toxicity in Rats

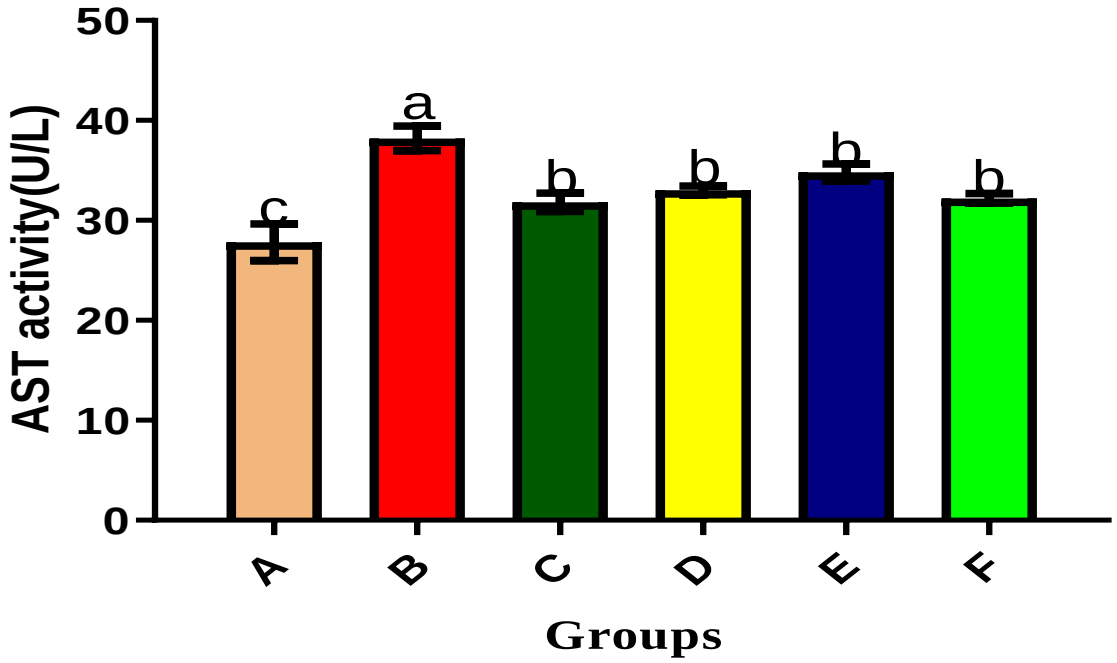
Administration of cyclophosphamide in rats significantly ($p<0.05$) elevated the activities of ALT, AST, ALP and the levels of albumin, total bilirubin and conjugated bilirubin. Interestingly, the administration of CMSO and Omega 3 fatty acid for 27 days before administration of the cyclophosphamide in rats significantly ($p<0.05$) reduced the levels of albumin, total bilirubin, conjugated bilirubin and activities of AST, ALT and ALP as shown in Figures below;



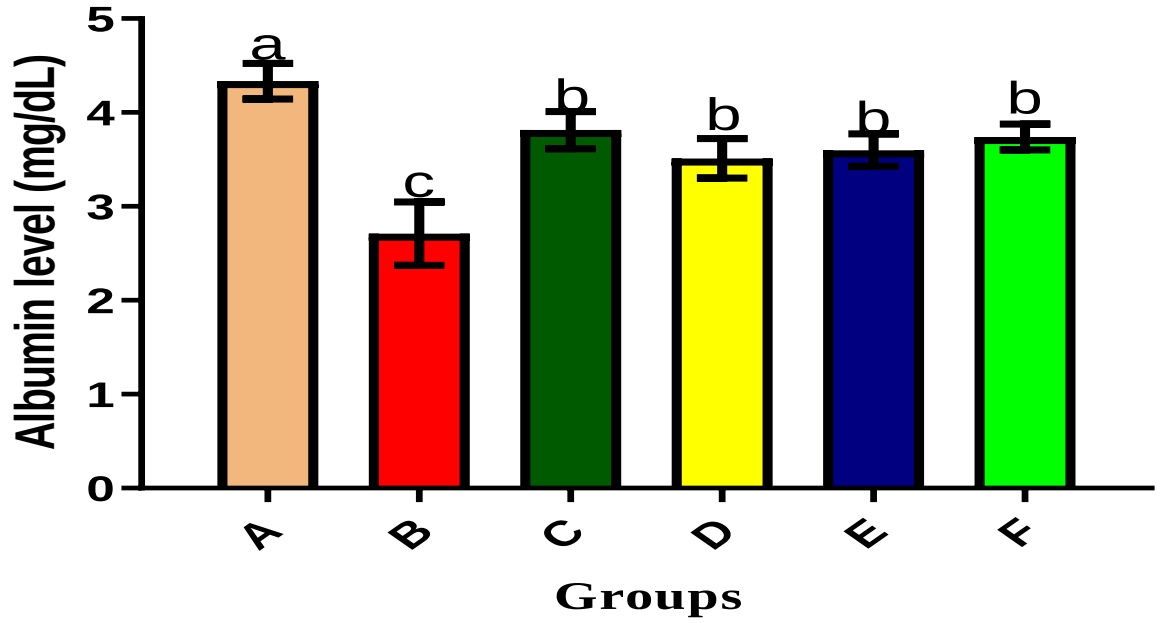
Effect of CMSO on plasma ALT Activity in Cyclophosphamide-induced hepato-toxicity in albino rats. Data are shown as mean ± S.D (n = 6). Mean values with different alphabets are significantly different at p<0.05



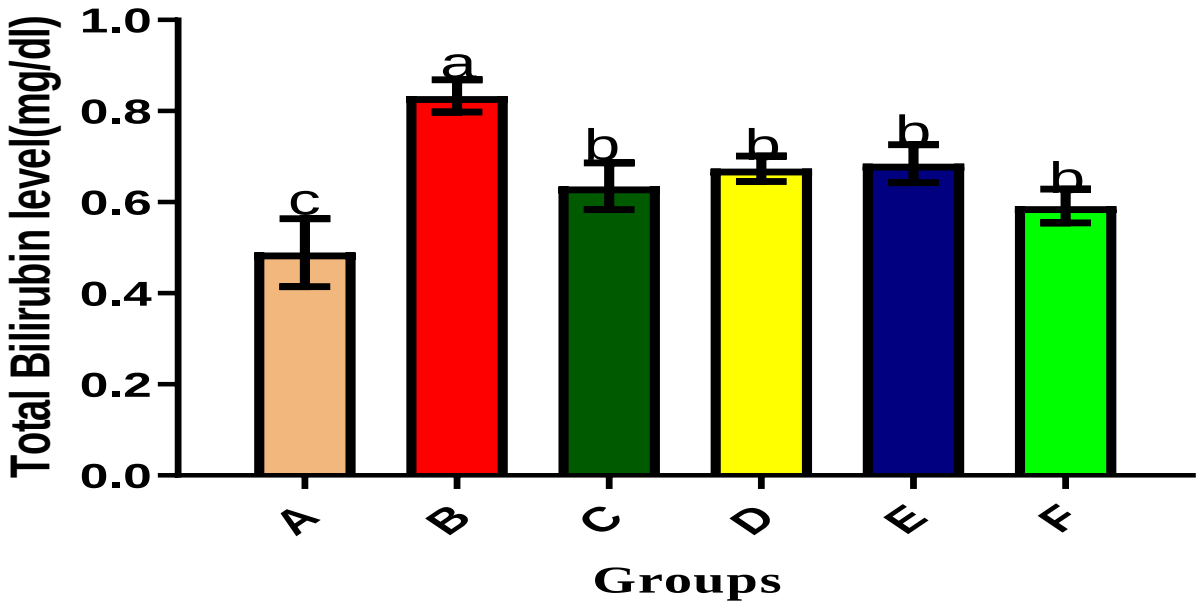
Effect of CMSO on plasma ALP Activity in Cyclophosphamide-induced hepato-toxicity in albino rats. Data are shown as mean ± S.D (n = 6). Mean values with different alphabets are significantly different at p<0.05.



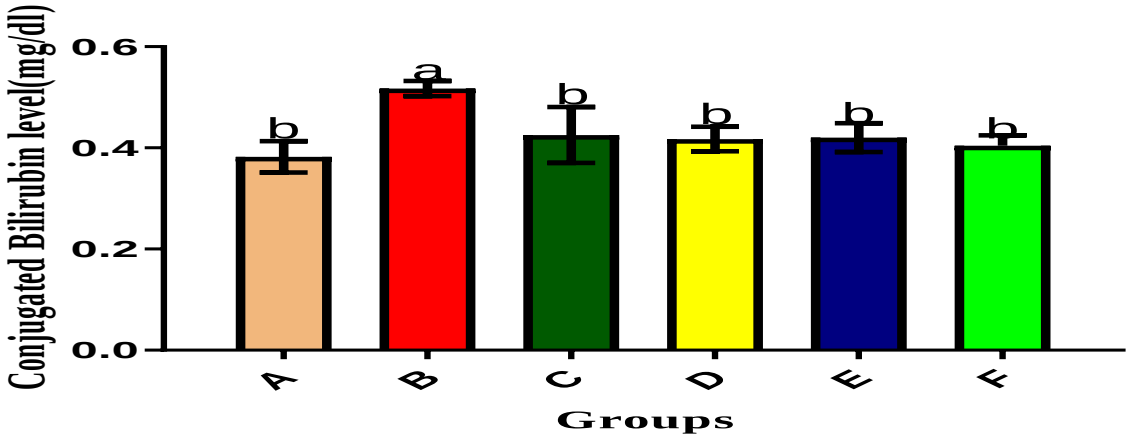
Effect of CMSO on plasma AST Activity in Cyclophosphamide-induced hepato-toxicity in albino rats. Data are shown as mean ± S.D (n = 6). Mean values with different alphabets are significantly different at P < 0.05.



Effect of CMSO on plasma Albumin Level in Cyclophosphamide-induced hepato-toxicity in albino rats. Data are shown as mean ± S.D (n = 6). Mean values with different alphabets are significantly different at p<0.05.



Effect of CMSO on Plasma Total bilirubin Level in Cyclophosphamide-induced hepato-toxicity in albino rats. Data are shown as mean ± S.D (n = 6). Mean values with different alphabets are significantly different at p<0.05

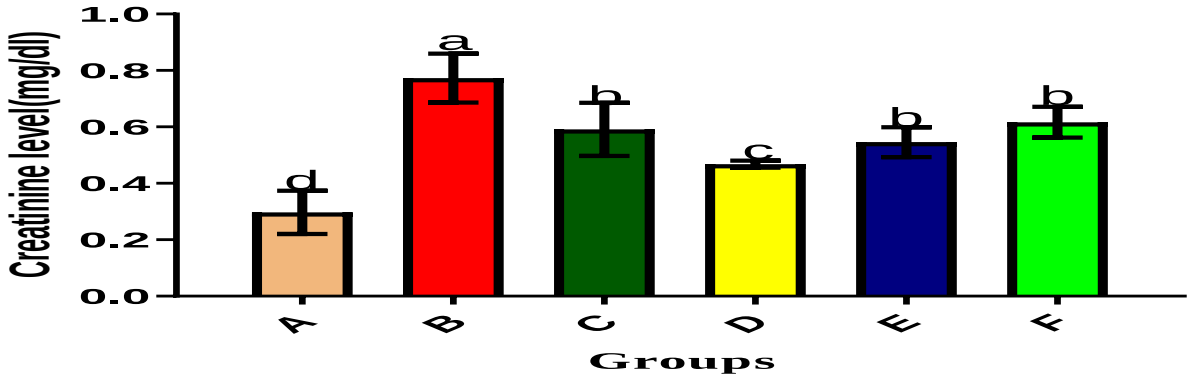


Effect of CMSO on plasma Conjugated bilirubin Level in Cyclophosphamide-induced hepato-toxicity in albino rats. Data are shown as mean ± S.D (n = 6). Mean values with different alphabets are significantly different at p<0.05.

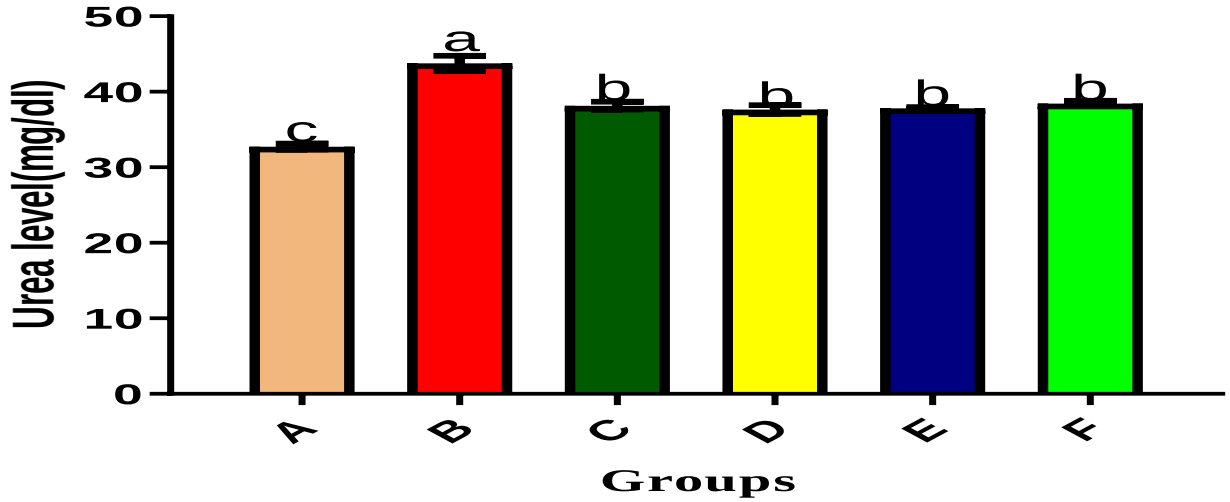
Effect of CMSO on Kidney Function Indices and some Serum Electrolytes in Cyclophosphamide-induced Nephrotoxicity in rats

Administration of cyclophosphamide in rats significantly (p<0.05) elevated creatinine, urea, BUN, Sodium and

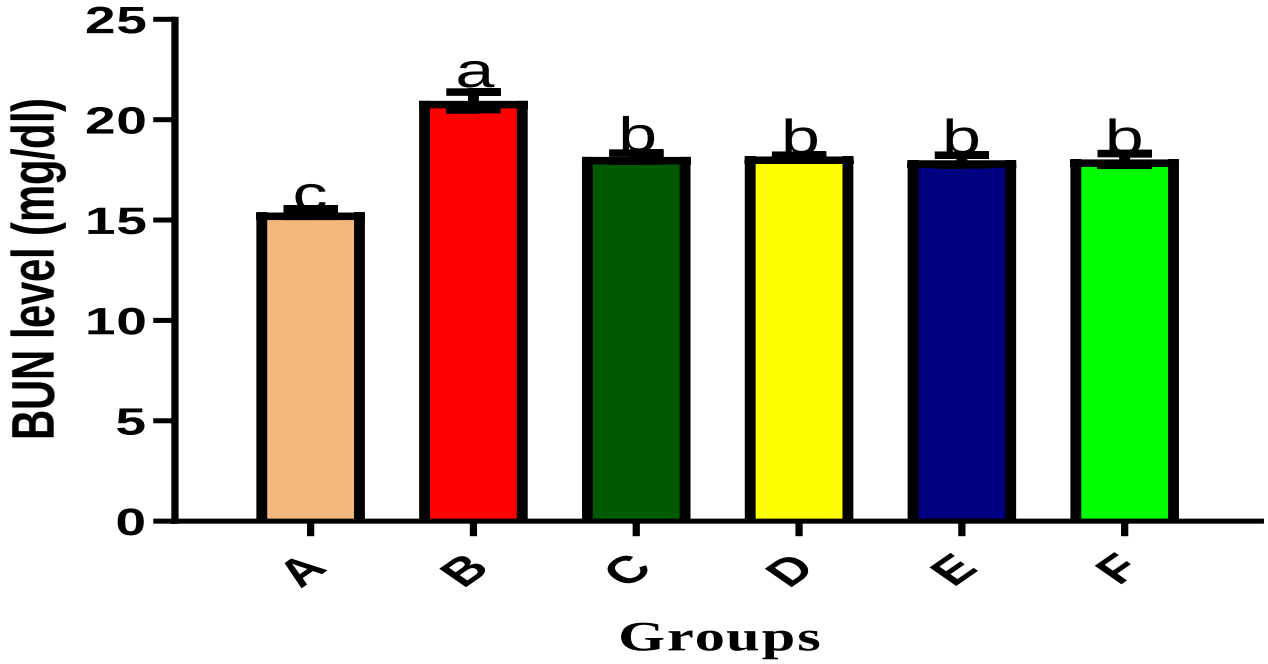
potassium levels, as shown in the Figures below. The administration of CMSO and Omega 3 fatty acid for 27 days before administration of the cyclophosphamide in rats significantly ($p<0.05$) reduced the creatinine, urea, BUN, sodium and potassium levels.



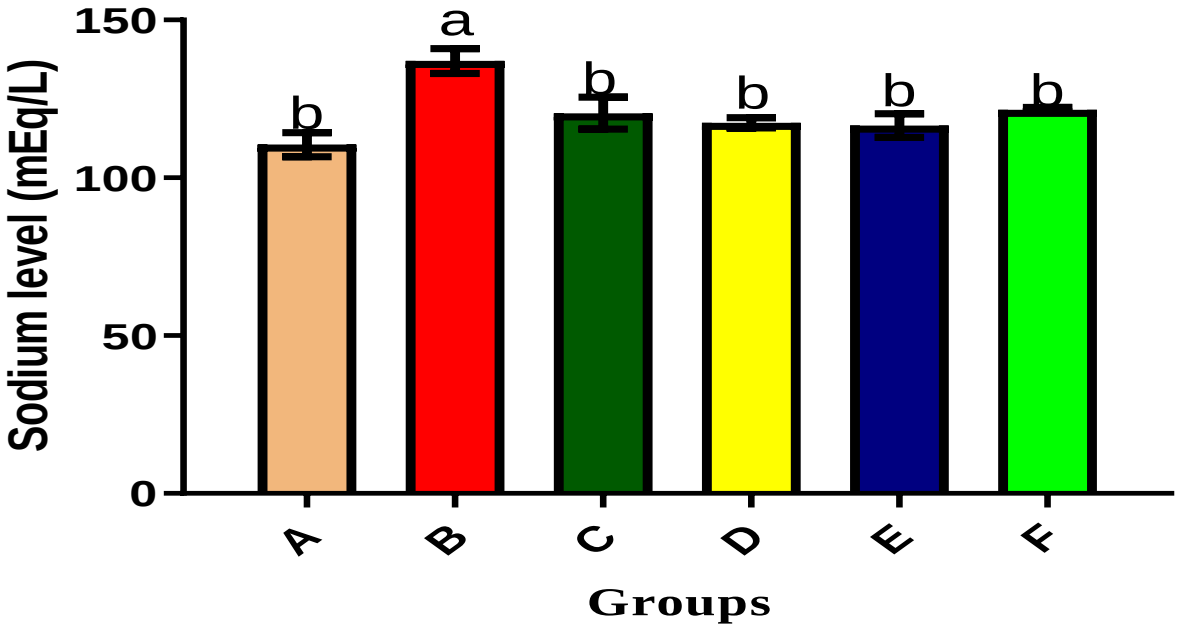
Effect of CMSO on Creatinine Level in Cyclo-induced Nephrotoxicity in albino rats. Data are shown as mean ± S.D (n = 6). Mean values with the different alphabets are significantly different at P < 0.05.



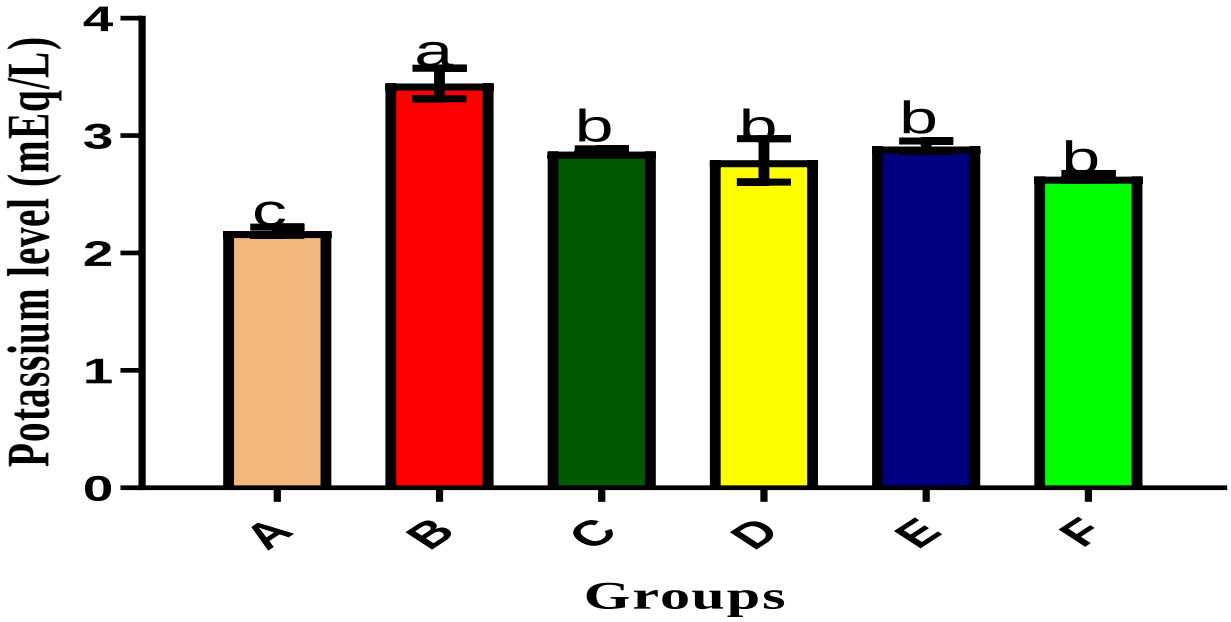
Effect of CMSO on Urea Level in Cyclophosphamide-induced Nephrotoxicity in albino rats. Data are shown as mean ± S.D (n = 6). Mean values with the different alphabets are significantly different at p<0.05.



Effect of CMSO on BUN Level in Cyclophosphamide-induced Nephrotoxicity in albino rats. Data are shown as mean ± S.D (n = 6). Mean values with the different alphabets are significantly different at P < 0.05.



Effect of CMSO on Sodium Level in Cyclophosphamide-induced Nephrotoxicity in albino rats. Data are shown as mean ± S.D (n = 6). Mean values with the different alphabets are significantly different at p<0.05.



Effect of CMSO on Potassium Level in Cyclophosphamide-induced Nephrotoxicity in albino rats. Data are shown as mean ± S.D (n = 6). Mean values with the different alphabets are significantly different at p<0.05.

DISCUSSIONS

Liver Functions Indices of Cyclophosphamide-induced Hepato-renal Toxicity in Albino Rats

The results of this study showed an increase in the activities of ALT, AST, and ALP and in the levels of albumin, total bilirubin, and conjugated bilirubin within the test group injected with cyclophosphamide compared to the control group.

The present study confirms the previous work that reported a significant increase in serum activities of liver markers following induction of hepatotoxicity induced by CPA (Nitharwal *et al.*, 2013; Germoush and Mahmoud, 2014). Akay *et al.*, (2006) and Subramanian *et al.*, (2014) have also reported that a low dose of CPA at 200 mg/kg body weight following intravenous administration can induce hepatotoxicity by increasing the activities of liver enzymes in the serum and also negatively altering other liver function markers.

The rise in serum activities of these markers has been attributed to the damaged structural integrity of the liver. This is often because they're cytoplasmic in their location and are released into circulation after cellular damage (Huang *et al.*, 2000). Enzymes are proteins found within the

body that increase the speed of chemical reactions. The liver has enzymes which perform these actions within the liver. The foremost common liver enzymes are Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline Phosphatase (ALP), which are valuable biomarkers of liver injury during a patient with a point of intact liver function (Ezejindu *et al.*, 2014).

Interestingly, the administration of CMSO and Omega 3 fatty acid for 27 days before administration of the cyclophosphamide in rats significantly ($p<0.05$) reduced the levels of albumin, total bilirubin, conjugated bilirubin and activities of AST, ALT and ALP. CMSO was chosen because it's widely used and readily available in Nigeria and many parts of Africa and the world. The observed effect of CMSO on liver enzymes could be attributed to its oil containing several groups of drugs collectively called annonaceous acetogenins and also other phytochemicals of the oil including flavonoids, alkaloids, megastigmanes (MGs), flavonol triglycosides (FTGs), phenolics (PLs), cyclopeptides (CPs) and essential oils (Kossouoh *et al.*,2007). Flavonoids are known to be antioxidants, radical scavengers and anti-lipoperoxidants, resulting in hepato-protection. The mechanism by which CMSO protects against cyclophosphamide-induced alterations within the liver could also be linked to the antioxidative and acetogenic effect of the seed oil. Similarly, Nitharwal *et al.*, (2013) and Zarei and Shivanandappa (2013), have reported some medicinal plants' hepatoprotective effects against CPA-induced toxicity.

Kidney Function Indices and Electrolytes Levels of Cyclophosphamide-induced Hepato Renal Toxicity in Albino Rats

This study showed that administration of cyclophosphamide in rats significantly ($p<0.05$) elevated the creatinine, urea, BUN, sodium and potassium levels. However, administration of CMSO and Omega 3 fatty acid for 27 days before administration of the cyclophosphamide in rats significantly ($p<0.05$) reduced the creatinine, urea, BUN, sodium and potassium levels.

Increasing serum creatinine and urea levels is an important indicator of poor glomerular filtration and has been a significant clinical marker for renal dysfunction and loss of renal integrity (Ogbeke *et al.*, 2014). Creatinine could be a matter of muscle creatine, whose quantity in blood serum is proportional to the body's muscle mass. The creatinine is sometimes constant, so elevated levels indicate diminished

nephritic operation solely since it is excreted by the kidneys (Ijeh *et al.,* 1996). Gluconeogenesis is sustained by increased proteolysis, which releases free glucogenic amino acids circulating in plasma and is deaminated in the liver due to increased urea in blood (Robinson and Johnston, 1997). Our finding is consistent with the findings of Achuba (2018), who posited that V. amygdalina extract significantly decreased creatinine and urea levels in rats, offering a reno-protective effect.

Plasma concentrations of waste substances such as urea, creatinine, and electrolytes are the most commonly used indices to evaluate renal function. The kidney eliminates waste substances and controls fluid, electrolyte and acid-base balance (Griffin *et al.,* 2008). Renal injury often results in the accumulation of waste substances in the blood and altered fluid homeostasis and acid-base balance. In the present study, the urea and creatinine concentrations were significantly elevated following the administration of cyclophosphamide.

Plasma levels of creatinine and urea are determined by the balance between their rate of synthesis and excretion although they are subject to many variables (Griffin *et al.,* 2008). Since they are eliminated through the kidney, kidney diseases would affect and alter their concentration. Significant changes were observed in the electrolyte concentration in the study compared to the control. These electrolytes are commonly monitored in clinical practice and can be used to evaluate symptoms and effectiveness of treatment of high blood pressure, heart failure, liver and kidney disease. Several conditions can lead to electrolyte imbalance; dehydration, ketoacidosis, cancer, renal diseases and injury (Griffin *et al.,* 2008). Sodium and Potassium are the principal cations in extracellular intracellular fluids, respectively, in which the kidneys regulate their physiological Concentration (Burtis *et al.,* 2012). Sodium and Potassium balance is usually maintained even in disturbances that cause major changes in kidney function (Akpanyung *et al.,* 2015). Hypokalaemia can result in muscular weakness and cardiac arrhythmia, whereas hyperkalaemia is a risk factor for cardiac arrest (Burtis *et al.,* 2012).

Phytochemicals present in the oil, such as flavonoid compounds of Vernonia amygdalina, are terpenes, coumarins, phenolic acids, lignans, and xanthones, which could be responsible for the plethora of bioactivities possessed by the plant. These bioactive principles may act singly or synergistically to produce the results for which the medicinal values of CMSO have been vigorously studied.

CONCLUSION

Administration of CMSO and Omega 3 fatty acid for 27 days before administration of the cyclophosphamide in rats significantly ($p<0.05$) reduced the levels of albumin, total bilirubin, conjugated bilirubin and activities of AST, ALT and ALP.

Administration of CMSO and Omega 3 fatty acid for 27 days before administration of the cyclophosphamide in rats significantly ($p<0.05$) reduced the creatinine, urea, BUN, sodium and potassium levels.

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