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Effect of cimetidine on cyclophosphamide-induced liver toxicity in albino rats

Elias Adikwu[1], Bonsome Bokolo[2]

Lecturer [1]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Niger Delta University, Nigeria, Lecturer [2]Department of Pharmacology, Faculty of Basic Medical Sciences, Niger Delta University, Nigeria

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ABSTRACT

Background: The clinical use of cyclophosphamide (CP) has been characterised by liver toxicity. **Aims and Objectives:** This research assessed the effect of cimetidine against CP-induced liver toxicity in a rat model. **Material and Methods:** Forty eight albino rats divided into 8 groups (A-H) of 6 rats per group were used for this study. Group A (control) was administered with water while groups B-D were administered with 5, 10, and 20 mg/kg/day of cimetidine intraperitoneally (ip) for 5 days respectively. Group E was administered with 150 mg/kg of CP ip on the 5th day whereas groups F-H were administered with 5 10, and 20 mg/kg/day of cimetidine for 5 days and CP ip on the 5th day. Rats were subjected to an overnight fast and sacrificed on the sixth day. Serum was extracted from blood and liver function parameters were evaluated. Liver was excised and evaluated for biochemical parameters and histology. **Results:** CP treatment had no significant ($P>0.05$) effects on body and liver weights, but significant ($P<0.05$) increases in alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, lactate dehydrogenase, gamma, glatamyl transferase, total bilirubin, conjugated bilirubin and malondialdehyde levels were observed when compared to control. Furthermore, significant ($P<0.05$) decreases in liver superoxide dismutase, catalase, glutathione and glutathione peroxidase were obtained in CP-administered rats when compared to control. The Liver of CP-treated rat shows hepatocyte necrosis around the central veins. However, CP-induced liver damage was significantly ($P<0.05$; 0.01) ameliorated in a dose-dependent manner in rats administered with cimetidine prior to the administration of CP. **Conclusion:** Cimetidine ameliorates cyclophosphamide-induced liver toxicity in albino rats.

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Key words: Cyclophosphamide; Cimetidine; Liver; Toxicity; Rats

INTRODUCTION

Cancer is a disease that is associated with the abnormal proliferation and growth of living cells.[1] A variety of approaches and methods are employed clinically for the treatment of cancer, however each of these approaches or methods have some significant limitations especially adverse effects.[2] The methods used for the treatment of cancer include chemotherapy, surgery, hormone therapy and radiation. Chemotherapy, is one of the commonest and most frequent approaches used for the treatment of cancer, it delivers anticancer drugs systemically to patients for quenching the uncontrolled proliferation of cancerous cells.[3] However, the success of chemotherapy has been limited by lack of selectivity and differentiation between tumor cells and normal cells resulting in insufficient drug concentrations in tumors, appearance of drug-resistant tumor cells and systemic toxicity.[4] Cyclophosphamide (CP) is one of the commonly and widely used drugs for cancer chemotherapy. It is a cytotoxic alkylating agent with antitumor and immunosuppressant properties use for the treatment of chronic and acute leukemia, multiple myeloma, lymphomas, and solid tumours.[5] CP undergoes bio-activation by hepatic microsomal cytochrome P450 mixed function oxidase system to its active metabolites; phosphoramide mustard and acrolein.[6] The antineoplastic effect of CP has been associated with phosphoramide mustard, whereas acrolein is linked to its toxic effects like

Address for Correspondence:
Dr. Elias Adikwu, Lecturer, Department of Pharmacology and Toxicology, Faculty of Pharmacy, Niger Delta University, Nigeria.
Mobile: +23407068568868. **E-mail:** adikwuelias@gmail.com

cell death, apoptosis, oncosis and necrosis.[7] In spite of its therapeutic importance, a wide range of adverse effects including hepatotoxicity have been reported with the use of CP. CP-induced hepatotoxicity has been characterised by altered liver function markers and the distortion of liver architecture.[8] The precise mechanism by which CP causes hepatic injury is poorly known. However, studies suggest the action of acrolein through the generation of oxidative radicals like superoxide anion, hydroxyl radical, and hydrogen peroxide[9] which are mutagenic to mammalian cells[10] and can attack DNA, proteins, and lipids causing pathogenesis.[11]

Cimetidine is a H2 blocker that inhibits gastric acid secretion and is largely used in the treatment of peptic ulcers.[12] It works by binding to an H2-receptor located on the basolateral membrane of the gastric parietal cell, blocking histamine effects.[13] It has been demonstrated to cause dose-related inhibition of cytochrome P-450-mediated oxidation both in vivo and in vitro.[14,15] In addition to its use in the treatment of gastrointestinal ulcer, it could be reposition as a hepatoprotective agent. It has shown tremendous hepatoprotective effect in animal model of xenobiotic-induced hepatotoxicity,[16] probably by decreasing the covalent binding of xenobiotics to liver protein, gastrointestinal absorption and decreasing the rate of hepatic glutathione depletion.[17] Also, cimetidine has a strong oxidative radical-scavenging activity which reduces the generation of oxidative radicals' thereby preventing oxidative damage. In addition, cimetidine is able to reduce iron-induced lipid peroxidation.[18] The antioxidant effect of cimetidine has been attributed to its methylated imidazole with a sulfur and amino group containing side chain which is a powerful hydroxyl radical scavenger.[19] Due to the absence of literature the present study examine the effect of cimetidine against cyclophosphamide-induced liver damage in albino rat.

MATERIAL AND METHODS

Drugs and chemicals

Cimetidine used was manufactured by Shandong Shenglu Pharmaceutical Co Ltd while cyclophosphamide was manufactured by Biochem Pharmaceutical Industries Ltd India. All other chemical substances used for this study are of analytical grade.

Grouping of animals, drug administration and animal sacrifice

Forty eight (48) rats of average weight 215g which were randomised into eight groups of n=6 housed in a wire mesh cage were used for this study. The rats were allowed to acclimatize for 2 weeks in a well-ventilated room, under natural lighting condition. The directive of the 2010 European Parliament and the Council on the Protection of Animals used for scientific purposes was followed in the handling of the rats. Group A (Control) was administered with water. Groups B-D were administered with 5, 10, and 20 mg/kg/day of cimetidine ip for 5 days respectively. Group E was administered with 150 mg/kg of CP ip on the 5th day. Groups F-H were administered with 5 10, and 20 mg/kg/day of cimetidine ip for 5 days and CP ip on the 5th day respectively. The rats were subjected to an overnight fast and were sacrificed under anaesthesia on the sixth day. Blood sample was collected from the heart and serum was extracted and evaluated for biochemical parameters. Liver was harvested, weighed and rinse in ice cold potassium chloride. The liver was buffered, homogenised and centrifuged. The supernatant was collected and evaluated for biochemical parameters.

Biochemical analysis and histological evaluation of the liver

Gamma glutamyl transferase, lactate dehydrogenase, aspartate and alanine aminotransferase, alkaline phosphatase, total bilirubin and conjugated bilirubin were measured using standard laboratory test kits (Randox Laboratories Ltd., Crumlin, UK). Liver protein content was evaluated according to the method of Gonall et al., 1949.[20] while malondialdehyde was determined according to Buege and Aust 1978.[21] The method of Aebi 1984[22] was used to assess catalase activity whereas superoxide dismutase was evaluated according to Sun and Zigman, 1978.[23] Reduced glutathione was measured as reported by Sedlak and Lindsay, 1968 [24] whereas gluthathione peroxidise evaluation was performed according to Rotruck et al., 1973.[25] Liver samples were harvested and fixed in formalin for 24h and were processed and embedded in paraffin blocks. Slides were prepared (3-5 µm thick), stained with hematoxylin and eosin, and analyzed for pathology using light microscopy.

RESULTS

The administration of cimetidine did not produce significant (P>0.05) effects on body, and liver, weights and serum levels of AST, ALT, ALP, GGT LDH, TB and CB when compared to control. Also, effects were not significant (P>0.05) on liver levels of SOD, CAT, GSH, GPX, AST, ALT, ALP, GGT and LDH when compared to control (Table 1-3). The administration of CP did not produce significant (P>0.05) effects on the body and liver weights in comparison to control (Table 1). On the other hand, serum levels of AST, ALT, ALP, GGT LDH, TB and CB were significantly (P<0.05) increased in CP-administered

rats when compared to control. However, the serum levels of these parameters were significantly (P<0.05) restored in a dose-dependent manner in rats administered with cimetidine +CP. The restored levels of these parameters differ significant at P<0.01 in rats administered with 20mg/kg of cimetidine + CP in comparison to CP-treated rats (Table 3). Furthermore, significant (P<0.05) increases in the liver levels of AST, ALT, ALP, GGT and LDH were obtained in CP-administered rats when compared to control. It is worthy of note that the levels of these parameters were significantly (P<0.05) restored in a dose-dependent manner in rats administered with cimetidine + CP. Significant difference was observed at P<0.01 in 20mg/kg of cimetidine +CP administered rats when compared to CP-administered rats (Table 4). Furthermore, the liver levels of SOD, CAT, GSH and GPX were significantly (P<0.05) decreased in CP-treated rats when compared to control. Interestingly, SOD, CAT, GSH and GPX levels were significantly (P<0.05) increased in a dose-dependent manner in cimetidine +CP administered rats with most pronounced effects which were significant at P<0.01 observed in rats administered with 20mg/kg of cimetidine +CP (Table 4). The liver of the control rat and cimetidine administered rats showed normal histology (Fig 1 a-d) while the liver of CP-administered rat shows hepatocytes necrosis around the central veins (Fig 1 e). On the other hand, the liver of rat administered with 5mg/kg of cimetidine +CP, 10 mg/kg of cimetidine + CP and 20 mg/kg of cimetidine

Table 1: Effects of cimetidine and on body and liver weights of cyclophosphamide- administered albino rats

Dose (mg/kg)	Initial body weight (g)	Final body weight (g)	Absolute Liver weight (g)	Relative Liver weight (%)
Control	217.2±10.6	230.4±12.6	8.94±0.19	3.88±0.19
CD 5	219.0±12.5	221.8±10.0	8.73±0.31	3.93±0.19
CD 10	213.8±10.0	220.8±11.0	8.79±0.12	3.98±0.14
CD 20	219.2±14.2	217.2±13.7	9.03±0.29	4.15±0.18
CYP 150	209.4±12.0	210.6±12.2	8.49±0.26	4.03±0.13
CD 5+CP	213.0±14.4	210.4±10.7	8.79±0.20	4.17±0.18
CD 10+CP	210.6±15.2	220.0±14.1	9.00±0.13	4.09±0.11
CD 20+CP	218.8±12.2	215.4±12.9	9.01±0.09	4.18±0.07

CD=Cimetidine, CP=Cyclophosphamide, values are expressed as Mean ± SEM, n=6

Table 2: Effects of cimetidine on serum liver function markers of cyclophosphamide-administered albino rats

Dose (mg/kg)	AST (U/L)	ALT (U/L)	ALP (U/L)	GGT (U/L)	CB (g/dL)	TB (g/dL)	LDH (U/L)
Control	59.0±4.89	66.0±3.14	58.1±5.78	0.78±0.01	3.99±0.63	7.95±0.40	65.5±3.90
CD 5	58.0±3.89	64.2±4.25	58.5±4.22	0.76±0.03	3.91±0.61	7.85±0.46	66.8±3.89
CD 10	57.4±4.99	62.9±4.82	55.8±5.87	0.83±0.02	3.93±0.54	7.87±0.34	65.4±4.03
CD 20	56.1±3.25	61.2±4.26	56.6±3.42	0.81±0.07	3.91±0.52	7.890.32	65.5±4.57
CP 150	345.0±13.3[a]	245.8±12.4[a]	228.9±10.8[a]	6.04±0.48[a]	15.4±1.20[a]	40.3±4.89[a]	290.2±12.2[a]
CD5+CP	248.3±11.5[b]	159.3±10.4[b]	158.8±8.64[b]	4.71±9.49[b]	10.4±0.75[b]	30.9±2.21[b]	183.7±13.7[b]
CD10+CP	160.7±8.65[b]	96.4±5.71[b]	98.7±5.51[b]	2.49±0.19[b]	7.57±1.01[b]	21.6±1.54[b]	110.1±5.61[b]
CD20+CP	90.5±4.77[C]	69.0±5.61[c]	60.6±20.6 [c]	1.00±0.05[c]	4.03±0.68[c]	10.1±0.56[c]	70.8±4.94[c]

CTD=Cimetidine, CP=Cyclophosphamide, values are expressed as Mean±SEM, n=6
[a]Significant (P<0.05) difference when compared to control
[b]Significant (P<0.5) difference when compared to CP
[c]Significant (P<0.01) difference when compared to CP

Table 3: Effects of cimetidine on liver biochemical parameters of cyclophosphamide-administered albino rats

Dose (mg/kg)	AST (U/L)	ALT (U/L)	ALP (U/L)	GGT (U/L)	LDH (U/L)
Control	99.9±8.56	122.3±9.93	110.1±9.11	2.23±0.34	110.3±8.81
CD 5	97.5±6.14	119.8±8.75	108.7±8.03	2.41±0.76	104.9±8.27
CD 10	94.5±8.54	119.2±9.91	104.3±9.56	2.29±0.52	104.7±9.94
CD 20	94.4±7.33	123.8±10.4	102.4±7.69	2.24±0.46	104.6±9.64
CP 150	519.2±11.3[a]	560.8±15.2[a]	440.9±10.6[a]	10.6±1.43[a]	369.0±12.9[a]
CD 5+CP	320.5±11.1[b]	350.9±14.3[b]	310.3±11.7[b]	6.58±0.37[b]	290.4±10.0[b]
CD 10+CP	215.7±8.69[b]	230.8±10.8[b]	230.7±21.1[b]	4.82±0.33[b]	193.5±9.24[b]
CD 20+CP	130.8±6.96[c]	165.5±9.08[c]	145.2±4.91[c]	2.56±0.27[c]	122.0±7.43[c]

CD=Cimetidine, CP=Cyclophosphamide, Values are expressed as Mean±SEM, n=6
[a]Significant (P<0.05) difference when compared to control
[b]Significant (P<0.05) difference when compared to CP
[c]Significant (P<0.01) difference when compared to CP

+ CP showed fatty change and disorganisation of liver architecture (Fig 1 f-h).

DISCUSSION

Chemotherapy has been used for many years and is still one of the most common treatments for cancer. Cyclophosphamide (CP) is a cytotoxic alkylating agent that is widely used as a chemotherapeutic agent in the treatment of cancer and autoimmune disorders. However, hepatotoxicity is one of the drawbacks that could limit the use of CP and deprived patients of therapeutic benefits.[26]

Studies using animal model of xenobiotic-induced hepatotoxicity have reported the potential of cimetidine as a hepatoprotective agent[27]. This study was designed to examine the possible benefit that could be derived from cimetidine as a remedy for cyclophosphamide-induced liver toxicity in albino rats. The use of CP can cause alterations in body and organ weight as a sign of toxicity.[28] In this study, body weight, relative and absolute liver weights were not altered in CP-administered rats. The aminotransferases (ALT and AST) are the most frequently utilized and specific indicators of hepatocellular necrosis. These enzymes catalyze the transfer of alpha amino acids of aspartate and alanine respectively to the alpha

Table 4: Effects of cimetidine on liver oxidative stress parameters of cyclophosphamide- administered albino rats

Dose (mg/kg)	SOD (U/mg protein)	CAT (U/mg protein)	GSH (µg/mg protein)	GPX (U/mg protein)	MDA (nmol/mg protein)
Control	34.8±2.89	54.9±3.20	18.5±2.70	18.2±3.19	0.17±0.04
CD 5	35.8±2.99	55.8±3.84	20.5±1.15	18.6±1.38	0.14±0.02
CD 10	36.5±3.33	56.6±3.14	19.9±2.36	17.7±2.22	0.13±0.01
CD 20	37.7±2.03	57.3±5.44	19.6±1.42	17.7±1.71	0.12±0.02
CP 150	14.7±1.24[a]	18.1±1.92[a]	4.05±0.65[a]	4.79±0.78[a]	1.53±0.13[a]
CD 5+CP	15.1±1.84[b]	26.3±1.80[b]	7.34±0.33[b]	6.98±0.39[b]	0.90±0.46[b]
CD 10+CP	23.3±1.57[b]	33.7±2.33[b]	11.3±1.32[b]	9.09±0.53[b]	0.52±0.05[b]
CD 20+CP	31.2±2.26[c]	49.9±1.12[c]	16.6±1.12[c]	13.3±1.68[c]	0.22±0.08[c]

CD=Cimetidine, CP=Cyclophosphamide, values are expressed as Mean±SEM, n=6
[a]Significant (P<0.05) difference when compared to control
[b]Significant (P<0.05) difference when compared to CP
[c]Significant (P<0.01) difference when compared to CP

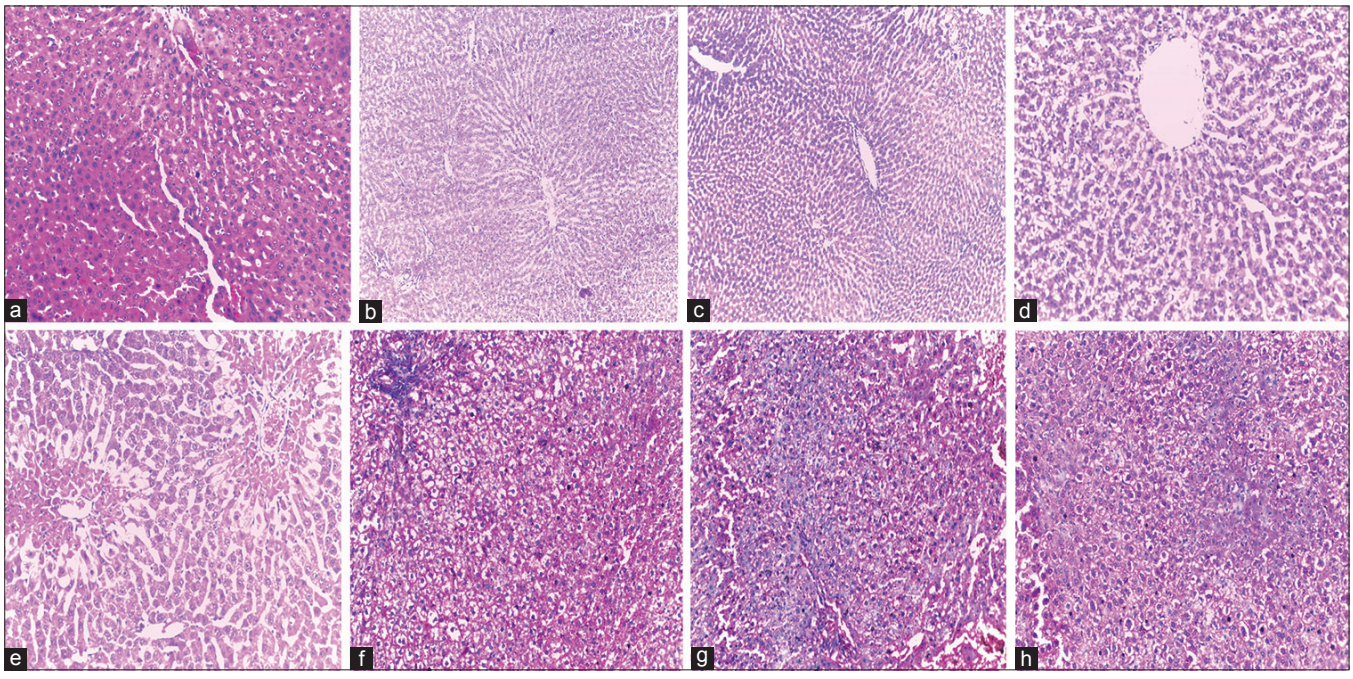

Figure 1: (a-d) Liver of control rat administered with water and the liver of rats administered with 5, 10 and 20 mg/kg of cimetidine showing normal histology. (e) Liver of rat administered with 150mg/kg of cyclophosphamide showing necrosis of hepatocytes around the central veins. (f) Liver of rat administered with 5mg/kg of cimetidine and 150mg/kg of CP showing fatty change and disorganisation of liver architecture. (g) Liver of rat administered with 10 mg/kg of cimetidine + 150mg/kg of CP showing fatty change and disorganisation of liver architecture. (h) Liver of rats administered with 20 mg/kg of cimetidine and 150mg/kg of CP showing fatty change and disorganisation of liver architecture (Hand E X200)

keto group of ketoglutaric acid.[29] Alkaline phosphatase (ALP) is a marker enzyme for plasma and endoplasmic reticulum that is often employed to assess the integrity of plasma membrane.[30] GGT and LDH are enzymes present in hepatocytes which are clinically used as yardstick for liver function.[31] Abnormal elevations in ALT, ALP, AST, GGT, and LDH levels are normally correlated with hepatic damage. One of the consequences of hepatic injury induced by CP is the leaching out of enzymes form hepatocytes resulting in increased systemic circulation and activities.[32] The current study observed marked elevations in liver and serum levels of ALT, AST, ALP, GGT, and LDH in CP-administered rats. This observation is a reflection of liver cellular damage and the alteration of membrane function.[33] However, it is noteworthy that the levels of these enzymes were restored in rats administered with cimetidine and CP.

Bilirubin is an endogenous anion derived from the degradation of hemoglobin found in red blood cells. It is transported to the liver bound to albumin. High plasma conjugated bilirubin concentration indicates impaired hepatic excretory function. Higher serum levels of bilirubin seen in hepatitis is directly proportional to the degree of histological injury of hepatocytes and the longer course of the disease.[34] This study observed that rats exposed to CP had higher levels of conjugated and total bilirubin. The higher levels of conjugated and total bilirubin observed in CP-intoxicated rats is a sign of toxicity and agrees with reported findings.[35] This observation could be attributed to CP-induced overproduction, impaired uptake, conjugation or excretion of unconjugated or conjugated bilirubin from hepatocytes to bile ducts. Comparatively, levels of conjugated and total bilirubin were restored in rats' administered with cimetidine + CP.

Histopathological alteration of the liver is a common and key factor in CP-induced hepatotoxicity.[36] In the current study, microscopic examination of the liver of CP-treated rat shows hepatocyte necrosis around the central veins. However, the liver of CP and cimetidine administered rats showed fatty change and disorganisation of architecture. SOD is involved in the dismutation of superoxide anion to H_2O_2 and O_2, this harmful H_2O_2 is further broken down to water by CAT while GSH and GPX are considered as the major antioxidants against free radicals and are vital constituents of detoxification pathways.[37] One of the primary effects of oxidative stress is the depletion of the antioxidant defence status of an organism. Therefore measurements of antioxidant levels serve as indexes for oxidative stress. In the current study, significant decreases in liver SOD, CAT, GSH and GPX levels were observed after CP administration. This observation can be correlated with similar findings.[38] In contrast, the levels of these antioxidants were restored in rats administered with cimetidine +CP.

Malondialdehyde (MDA) is one of the end products of lipid peroxidation (LPO) and its level within a tissue indicates the nature and the extent of LPO. LPO is a major marker of oxidative stress, altered membrane structure, and enzyme inactivation.[39] The present study observed elevated liver level of MDA in CP-administered rats. This finding is consistent with reports by some authors that lipid peroxidation is an integral aspect of CP-induced hepatotoxicity.[40] However, the liver levels of MDA were lower in rats administered with cimetidine +CP. The precise mechanism by which CP causes hepatic injury is poorly known; however, numerous studies have shown that CP exposure enhances the production of intracellular reactive oxygen species (ROS), suggesting that biochemical and physiological disturbances may result from oxidative stress.[41] CP requires metabolic activation by hepatic microsomal cytochrome P450 mixed function oxidase system for both its therapeutic action and adverse effects.[42] The metabolic conversion of CP leads to the formation of two cytotoxic metabolites; phosphoramide mustard and acrolein. Acrolein is believed to be responsible for CP-induced liver injury.[43] CP metabolites can stimulate oxidative stress and depress the antioxidant defence mechanisms in the liver.[44] The observed effect of cimetidine in this study could be attributed to its antioxidant activity through the scavenging of oxidative radicals generated by CP. Another possible mechanism is the inhibitory effect of cimetidine on hepatic microsomal cytochrome P450 mixed function oxidase system thereby preventing the biotransformation of CP to its toxic metabolites.

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Authors Contribution:
EA- Concept and design of the study, literature search, statistical analysis, interpretation of manuscript preparation and critical revision of manuscript;
BB- Concept, collected data and review of literature, preparation of first draft of manuscript and critical revision of manuscript.

Study Attributed to: Department of Pharmacology and Toxicology, Faculty of Pharmacy, Niger Delta University, Nigeria.

Orcid ID:
Dr. Adikwu Elias - https://orcid.org/0000-0003-4349-8227
Dr. Bonsome Bokolo - https://orcid.org/0000-0001-6426-6228

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