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International Journal of Clinical Biochemistry and Research

Journal homepage: www.ijcbr.in

Original Research Article

Stone breaker leaf extract on the toxic effect of 2-butoxy-ethanol induction on hemolytic thrombosis

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Abstract

Background: Thrombolysis is a procedure that involve the used of medication to dissolve a blood clot, the medications used in thrombolysis are called thrombolytic agents. Stone breaker (*Phyllanthus niruri*) is a medicinal plant with long history of folkloric therapeutic use in the treatment of pathological conditions.
Objective: this study was designed to investigate the effects of leaf *Phyllanthus niruri* linn) on 2-butoxyethanol-induced hemolytic thrombosis in rat model which also provides the opportunity to clinically investigate the presence of metabolites and other constituents in the body of animals.
Materials and Methods: Twenty (20) Wistar rats were divided into four groups of five wistar rats each, 100 µl of 500 mg/kg BWT of 2- butoxyethanol solution were administered intraperitoneally as a single dose followed by oral administration of *P. niruri* leaf extract with doses of 200 mg/kg BWT and 100ml of hydroxyl urea (HU) for 28days. *In vivo* antioxidant, inflammatory, apoptosis, excitatory, and thrombolysis indices were evaluated; antioxidant assays (glutathione transferase (GST), superoxide dismutase (SOD), glutathione peroxidase (GPx), reduced glutathione (GSH)), inflammatory assays (myeloperoxidase, TNF-α, IL-1β, IL-6 and xanthine oxidase), apoptotic assay (caspase-3), excitatory assay (Na+/K+ ATPase) and thrombolysis assays (Plasma and urine zinc ion concentration, Selenium determination, RNase activity in plasma; and Red cell carbonic anhydrase) activity were determined.
Result: revealed that *P. Niuri* leaf extract significantly ameliorated the damage induced by 2-butoxyethanol in the liver and blood of the wistar rats establishing the ameliorative potential of *P. Niuri* (linn.) with the effect evident of an improved thrombolysis index. The treatments reversed the oxidative effects initiated by 2-2butoxyethanol, these were revealed by increase in the activities of antioxidant assays carried out. The treatment also suppresses the effects of 2-butoxyethanol by increasing the activity of Na+/K+ ATPase, heamatological parameters checked also confirm that the leaf extract was potent enough, which showed increase in the values observed in the parameters.
Conclusion: This study has demonstrated that the extract was able to enhance the endogenous antioxidant in the administered group, inhibiting the effect of inflammation on the tissue and increasing the hematological indices declined by the 2-butoxyethanol induction, and as such would be a therapeutic agent in the management of hemolytic thrombosis diseases.

Keywords: 2-butoxyethanol, Antioxidant, Heamatology, Hemolytic thrombosis, Stone breaker

Received: 26-11-2024; **Accepted:** 13-02-2025; **Available Online**: 22-03-2025

1. Introduction

2-Butoxyethanol (BE), an environmental toxicant is commonly used as chemicals in the manufacturing of wide range of domestic and industrial products as well as surface coatings and household cleaning agents (Lewis et al., 2005)[1]. 2-Butoxyethanol was also reported to be metabolized in Fischer 344 female rats to 2-butoxyacetic acid (BAA) causing acute hemolytic anemia (Ezov et al., 2002)[2] and disseminated thrombosis. This 2-butoxyethanol-induced acute disseminated thrombosis and infarction in female rats may be caused by vaso-occlusion, possibly initiated by acute hemolytic anemia with the possibility of release of procoagulant factors from destroyed erythrocytes, altered morphology and decreased deformability of erythrocytes, and a tendency of the red blood cells to aggregate and/or adhere to the endothelium (Ghanayem and Sullivan, 19933[3]; Nyska et al., 2003)[4]. Sickle Cell Disease also know as Depranocytosis is a genetic condition that affects hemoglobin production in red blood cells, causing them to become distorted and break down due to the substitution of the glutamate by valine at the sixth position of the β-chain of the

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DOI: https://10.18231/j.ijcbr.2025.002

hemoglobin A (HbA), which causes a structural modification that alters oxygen affinity and transport of the oxygenated sickled RBC leading to tissue infarction vis a vis hemolytic anemia and disseminated thrombosis. Unfortunately, current therapies are very limited and less efficient. Bone marrow transplant is the most promising therapy, but it is too expensive for most Africans which are most affected by this genetic disorder (Mpiana et al., 2013[5]). Anti-sickling agents such as hydroxyurea and decitibine have been developed and their modes of actions are essentially pathophysiological, by inhibiting HBS polymerization and RBCs sickling process and to protect sickle RBCs from oxidatively induced damages (Mpiana et al., 2013)[5]. Hydroxyurea has also been shown to decrease the number and severity of sickle cell crises by increasing fetal hemoglobin production significantly in patients with sickle cell anemia (Cokic et al., 2003).[6] Other clinical anti-sickling agents are tellurite, thiocyanate and folic acid had been clinically validated. Administration of folic acid, vitamins B6 and B12 were also utilized to reduce the episodes of chronic anaemia in sickle cell patients. Patients taking the vitamins-folic acid, B6 and B12 had significant improvement in their pack cell volume (PCV) but blood abnormalities and impaired growth rate associated with the disease were not improved (Ohnishi et al., 2000).[7] The desire to produce more effective but less toxic drugs fuels the continuous search for new bioactive compounds from natural samples, prompting the increasing search for more acceptable and more therapeutic options for attenuating progression of metabolic derangement and genetic disorders. To this effect there is a spontaneous evolution of clinical research to develop alternative therapies which would have both clinical and nutritional acceptability (Oyewole et al., 2008[8]; Oladipo et al., 2020).[9] Sickle cell disease causes a structural modification that alters oxygen affinity and transport of the oxygenated sickled RBC leading to tissue infarction vis a vis hemolytic anemia and disseminated thrombosis. Accelerated hallmark of sickle cell disease is oxidative damage manifested by depleted levels of selenium, glutathione, zinc, folic acid, rotenol, riboflavin (Hamdy et al., 2015)[10], pyridine and cobalamin. Studies have revealed enzymes and biomarkers which are substrates of enzyme implicated in the hemolytic and thrombotic impairments that can be inhibited to suppress the painful episodes often experienced by patients of sickle cell disease. In some parts of Nigeria, water extract of the plant leaves is taken to increases packed cell volume (PCV) in anaemic individuals (Ighodaro et al., 2020).[11]

Phyllanthus niruri L. (Euphorbiaceae) is the most tropical plant with a known medicinal properties (Ifeoma et al., 2013).[12] Globally, this plant is utilized in traditional medicine to treat various ailments, including inflammation, hepatitis, fever, malaria, and gonorrhoea, particularly in regions such as Brazil, Asia, Thailand, China, and Africa (Jantan et al., 2019a).[13] Notably, it has known to be used for malaria treatment in some areas. Such as Nigeria, the aqueous extract from the whole P. niruri plant as been used to reduce malaria symptoms. The biological and pharmacological functions reported for P. niruri include antiplasmodial, antioxidant, hepatoprotective, nephroprotective and anti-diabetic properties (Sarin et al., 2014).[14] Moreso, this plant in vitro and in vivo studies was reported of the immunomodulatory adaptation of both innate arms of the immune response. It stimulates and boosts the proliferation of lymphocytes and macrophages in experimental animals (Jantan et al., 2019b).[15] It was reported to be immunosuppressive in several conditions, as evident in the inhibition of sRBC-induced cell-mediated immune response in which interferon-gamma is involved, reduction of leukocytes in Mycoplasma gallisepticum-infected chicken (Hidanah et al., 2018)[16], inhibition of carrageen-induced oedema and neutrophil migration upon its induction by thioglycolate in Mus musculus Swiss male mice (Porto et al., 2013)[17] and regulation of serum levels of primary and secondary antibodies (Okoli, et al., 2010)[18]. P. niruri has been reported to contain various biologically active plant chemicals, including flavonoids, lignans, terpenoids, saponins, alkaloids,tannins, Catechin, quercetin, and astragalin (Bagalkotkar et al., 2006).[19] This study will investigate the erythropoietic potential of Phyllanthus niruri leaf extract on Wistar rats assaulted with 2-butoxyethanol, with emphasis on the oxidative, inflammatory and apoptotic progressions.

2. Materials and Methods

2.1. Collection of plant material

The leaves of *Phyllanthus niruri* were collected from Ilara mokey Local Government of Ondo State, identified and authenticated at Center for Research and Development (CERAD), Federal University of Technology, Akure, also was approved by The Federal University of Technology, Akure. Ethical committee.

2.2. Preparation of Phyllanthus niruri

The leaf identification and authentication was carried out in the Department of Crop, Soil and Pest Management, at the University (FUTA) Nigeria, and voucher specimen was deposited at the Herbarium. The leaves were air dried at room temperature and pulverized into powder. A thousand grams of the powdered leaves was macerated in 4000 ml of ethanol (solvent of extraction) for 48 hrs at 50^{o}C. The filtrate was collected initially through a fine linen cloth and finally through Whatman filter paper, thereafter freeze-dried. The freeze dried extract was stored in an air-tight container in a 4^{o}C refrigiretor for further analysis.

A total of twenty (20) Wistar rats were purchased from Temilade rats farm, Ogbomoso, Oyo State. All the animals were housed in well ventilated cages made of wood and wire gauze. Wood shaving were used as beddings to keep each compartment dry. Here, normal standard ambient conditions of temperature between 28-310c, relative humidity between 50%-55% and a photoperiodicity of 12h natural light and 12h

dark were maintained They were fed on standard rat chow (Vital Feeds LTD, Nigeria) and water ad libitum. The animals weighed between 150-250 g and were divided into four (4) groups of n=5 animals each.

2.3. Experimental model

Rats will be administered safe doses of the stone breaker leaf extract for 28 days post-induction of 2-butoxyethanol at 250 mg/kg bwt for 4 consecutive days.

Group 1: Negative control (without 2-butoxyethanol-induction)
Group 2: 2-butoxyethanol-induced rats without treatment (Positive control group)
Group 3: 2-butoxyethanol-induced rats with stone breaker extract (200mg/kg bwt) (administered post-induction for 28 days)
Group 4: 2-butoxyethanol-induced rats with 100 µl of 500mg/100ml of hydroxyl urea (HU) (administered post-induction for 28 days)

Rats will be sacrificed after 28 days of induction via cervical dislocation, excised and the blood and the bone marrow will be carefully collected, processed and stored prior to biochemical, hematological and histological evaluations.

3. Activity: Necropsy, Blood Collection and Bone Marrow Preparation

3.1. Activity: Morphological examination

Emmel's test and Hemolysis test will be performed as previously reported (Mpiana *et al.,* 2010). The RBCs digitize micrographs will be treated with a computer assisted image analysis system.

3.2. Activity: Haematological parameters

PCV, RBC, ESR, WBC, HB, Lymphocyte, Neutrophil, Monocyte, Eosinophil, Basophil, MCV, MCH and MCHC.

3.3. Activity: Biochemical evaluations

Measurement of Myeloperoxidase (MPO) activity; Na^+/K^+ ATPase activity; Caspase-3 activity assay (Chemicon International; CAT APT301); Xanthine oxidase activity; Glutathione peroxidase (GPx) activity (U/L); Glutathione transferase (GT) activity (U/L); Reduced glutathione (GSH) concentration; Malondialdehyde (MDA) concentration; Plasma and urine zinc ion concentration (Sigma-Aldrich kit); Selenium determination assay (AAS) ; RNase (EC 3.1.4,22) activity in plasma; and Red cell carbonic anhydrase activity. Inflammatory markers: tumor necrosis factor-α (TNF-α), interleukin-1 beta (IL-1β) and interleukin-6 (IL-6) in the bone marrow.

4. Result

4.1 Antioxidant potentials

The effects of 100 µl of 500mg/100ml of hydroxyl urea (HU) and 200 mg/kg BWT of *Phyllanthus niruri* extract on the activity of glutathione transferase were investigated in the management of hemolytic thrombosis in wistar rats as revealed in **Figure 1**. The results revealed that hemolytic thrombosis (Group 2: 0.943) caused reduced activity of glutathione transferase significantly compared to the treatment groups ($p \leq 0.05$). There was significant increase in GST activity by both doses of *Phyllanthus niruri* and hydroxyl urea which was the standard drug (Group 3: 1.367; Group 4: 1.940) ($p \leq 0.05$).

The effects of 100 µl of 500mg/100ml of hydroxyl urea (HU) and 200 mg/kg BWT of *Phyllanthus niruri* extract on the activity of superoxide dismutase (SOD) were investigated in the management hemolytic thrombosis in wistar rats as revealed in **Figure 2**. The results revealed that hyperlipidemia (Group 2: 1.729) caused reduced activity of superoxide dismutase (SOD) significantly compared to the treatment groups ($p \leq 0.05$). There was significant increase in SOD activity by both doses of *Phyllanthus niruri* and hydroxyl urea which was the standard drug (Group 3: 3.422; Group 4: 3.647) ($p \leq 0.05$).

Figure 3. The effects of 100 µl of 500mg/100ml of hydroxyl urea (HU) and 200 mg/kg BWT of *Phyllanthus niruri* extract on the activity of reduced glutathione were investigated in the management of hemolytic thrombosis in wistar rats. The results revealed that hemolytic thrombosis (Group 2: 1.941) caused reduced concentration of GSH significantly compared to the treatment groups ($p \leq 0.05$). There was significant increase in GSH concentration by both doses of *Phyllanthus niruri* and hydroxyl urea which was the standard drug (Group 3: 3.349; Group 4: 3.8793) ($p \leq 0.05$).

The effects of 100 µl of 500mg/100ml of hydroxyl urea (HU) and 200 mg/kg BWT of *Phyllanthus niruri* extract on the concentration of MDA were investigated in the management of hyperlipidemia in wistar rats as revealed in **Figure 4**. The results revealed that hemolytic thrombosis (Group 2: 0.924) caused elevated concentration of MDA significantly compared to the treatment groups ($p \leq 0.05$). There was significant decrease in MDA concentration by both doses of *Phyllanthus niruri* and hydroxyl urea which was the standard drug (Group 3: 0.557; Group 4: 0.488) ($p \leq 0.05$).

The effects of 100 µl of 500mg/100ml of hydroxyl urea (HU) and 200 mg/kg BWT of *Phyllanthus niruri* extract on the activity of glutathione peroxidase (GPx) were investigated in the management of hemolytic thrombosis in wistar rats as revealed in **Figure 5**. The results revealed that hemolytic thrombosis (Group 2: 4.133) caused reduced activity of GPx significantly compared to the treatment groups ($p \leq 0.05$). There was significant increase in GPx activity by both doses of *Phyllanthus niruri* and hydroxyl urea which was the standard drug (Group 3: 6.984; Group 4: 7.185) ($p \leq 0.05$).

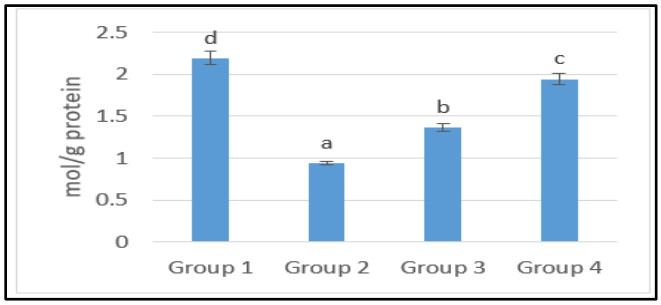

Figure 1: Effects of *Phyllanthus niruri* extract on the activity of Glutathione transferase (GST) in hemolytic thrombosis wistar rats. Results were presented as mean±standard deviation where n=5. Values with different superscripts are significantly different ($p \leq 0.05$).

5. Treatments

1. Group 1: Negative Control;
2. Group 2: Hemolytic thrombosis assault without treatment (Positive Control);
3. Group 3: Hemolytic thrombosis assault and 200mg/kg BWT of PE extract; and
4. Group 4: Hemolytic thrombosis assault with 100 µl of 500mg/100ml of hydroxyl urea (HU)

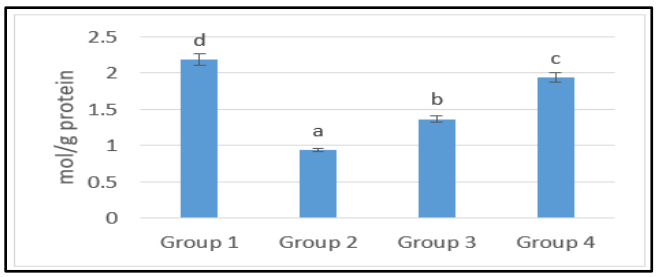

Figure 2: Effects of *Phyllanthus niruri* extract on the activity of Superoxide dismutase (SOD) in hemolytic thrombosis wistar rats.

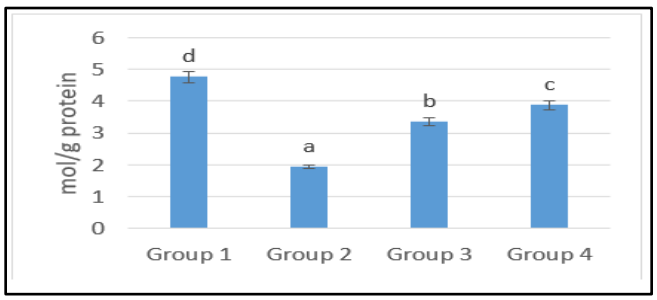

Figure 3: Effects of *Phyllanthus niruri* extract on the concentration of Reduced glutathion (GSH) in hemolytic thrombosis wistar rats.

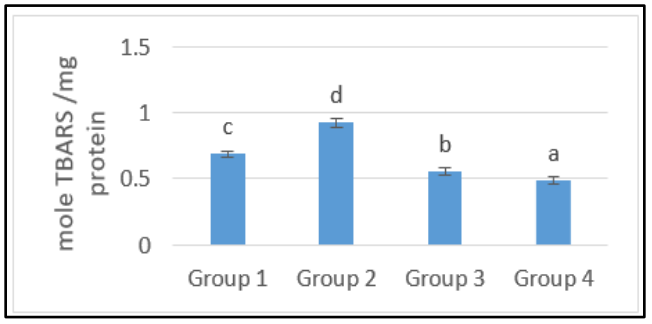

Figure 4: Effects of *Phyllanthus niruri* extract on the concentration of myelodyaldehyde (MDA) in hemolytic thrombosis wistar rats.

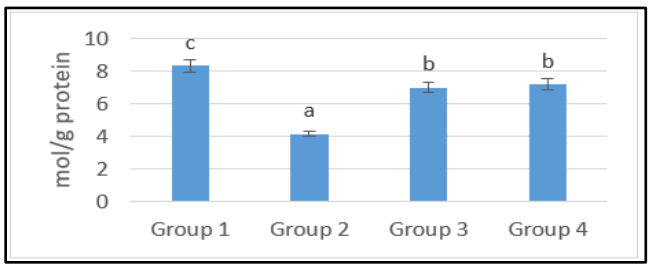

Figure 5: Effects of *Phyllanthus niruri* extract on the activity of Glutathione peroxidase (GPx) in hemolytic thrombosis wistar rats.

5.1. Cyto-excitatory potential

The effects of 100 µl of 500mg/100ml of hydroxyl urea (HU) and 200 mg/kg BWT of *Phyllanthus niruri* extract on the activity of Na^+/K^+ ATPase were investigated in the management of hemolytic thrombosis in wistar rats as revealed in **Figure 6**. The results revealed that hemolytic thrombosis (Group 2: 35.414) caused reduced activity of Na^+/K^+ ATPase significantly compared to the treatment groups ($p \leq 0.05$). There was significant increase in Na^+/K^+ ATPase activity by both doses of *Phyllanthus niruri* and the standard drug group (hydroxyl urea) (Group 3: 45.895; Group 4: 70.443) ($p \leq 0.05$).

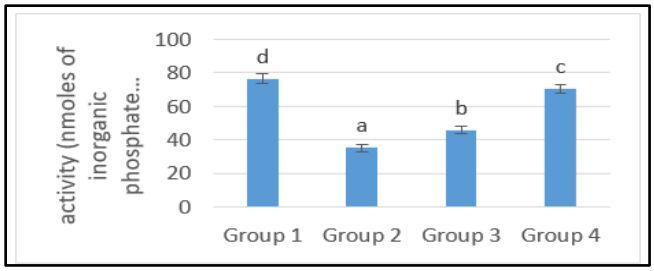

Figure 6: Effects of *Phyllanthus niruri* extract on the activity of Sodium potassium ATPase (Na^+/K^+ATPase) in hemolytic thrombosis wistar rats.

5.2. Anti-inflammatory potentials

The effects of 100 µl of 500mg/100ml of hydroxyl urea (HU) and 200 mg/kg BWT of *Phyllanthus niruri* extract on the activity of xanthine oxidase were investigated in the management of hemolytic thrombosis in wistar rats as revealed in **Figure 7** The results revealed that hemolytic thrombosis (Group 2: 0.765) caused increase in activity of xanthine oxidase significantly compared to the treatment groups ($p \leq 0.05$). There was significant decreased in xanthine oxidase activity by both doses of *Phyllanthus niruri* (Group 3: 0.483; Group 4: 0.513) which was the standard drug ($p \leq 0.05$).

The effects of 100 µl of 500mg/100ml of hydroxyl urea (HU) and 200 mg/kg BWT of *Phyllanthus niruri* extract on the activity of myeloperoxidase were investigated in the management of hemolytic thrombosis in wistar rats as revealed in **Figure 8** The results revealed that hemolytic thrombosis (Group 2: 2.129) caused increase in activity of myeloperoxidase significantly compared to the treatment groups ($p \leq 0.05$). There was significant decreased in myeloperoxidase activity by both doses of *Phyllanthus niruri* and the standard drug group (Group 3: 1.552; Group 4: 1.709) ($p \leq 0.05$). This same trend was observed in **Figure 9** - 4.12. (Interleukin 1-6, 1β, and tumor necrosive factor-α (TNF-α,)).

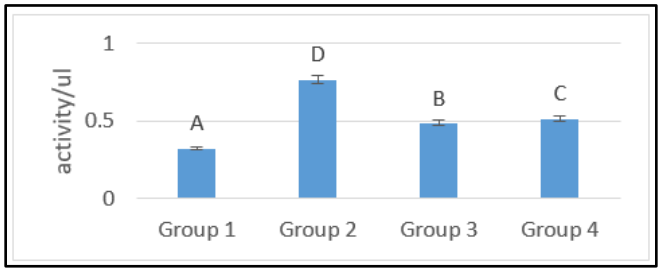

Figure 7: Effects of *Phyllanthus niruri* extract on the activity of xanthine oxidase in hemolytic thrombosis wistar rats.

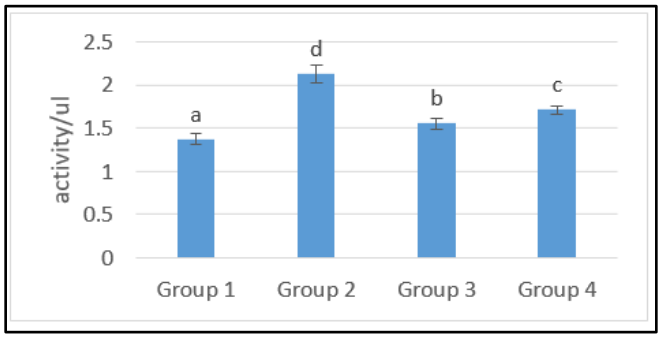

Figure 8: Effects of *Phyllanthus niruri* extract on the activity of myeloperoxidase (MPO) in hemolytic thrombosis wistar rats.

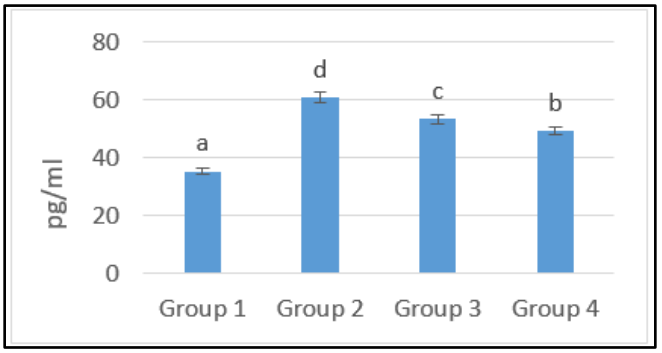

Figure 9: Effects of Phyllanthus niruri extract on the concentration of Interleukin 1-6 (IL-6) in hemolytic thrombosis wistar rats.

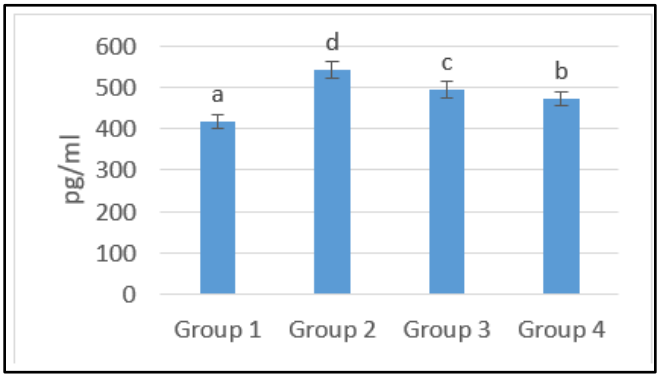

Figure 10: Effects of Phyllanthus niruri extract on the concentration of Interleukin 1β (IL-1 β) in hemolytic thrombosis wistar rats.

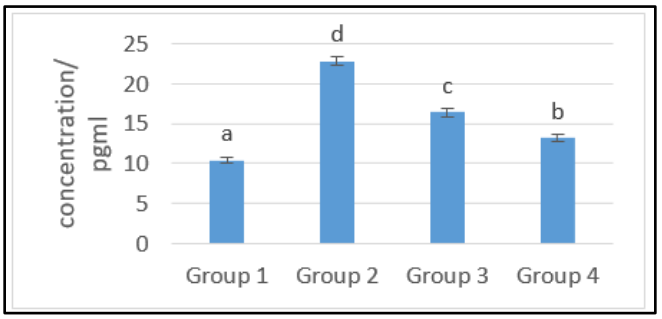

Figure 11: Effects of Phyllanthus niruri extract on the level of Tumor necrotive factor (TNF-α) in hemolytic thrombosis wistar rats.

5.3. Apoptotic marker

The effects of 100 µl of 500mg/100ml of hydroxyl urea (HU) and 200 mg/kg BWT of Phyllanthus niruri extract on the activity of caspase-3 were investigated in the management of hemolytic thrombosis in wistar rats as

revealed in

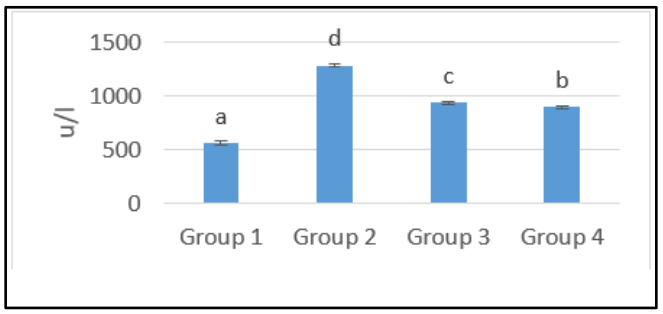

Figure 12 The results revealed that hemolytic thrombosis (Group 2: 1282.524) caused increase in activity of caspase-3 significantly compared to the treatment groups (p≤0.05). There was significant decreased in myeloperoxidase activity by both doses of Phyllanthus niruri and the standard. drug group (Group 3: 933.648; Group 4: 894.922) (p≤0.05).

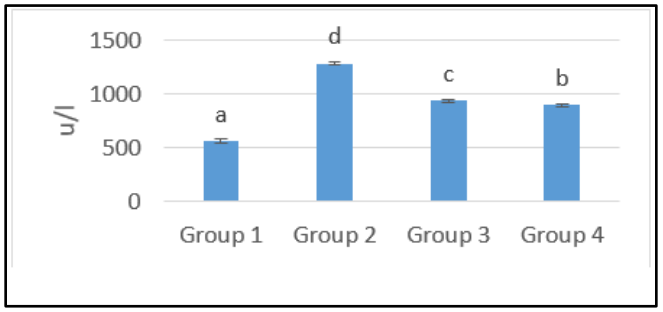

Figure 12: Effects of *Phyllanthus niruri* extract on the activity of Caspase-3 in hemolytic thrombosis wistar rats.

6. Hemolytic Thrombosis Assays

The effects of 100 µl of 500mg/100ml of hydroxyl urea (HU) and 200 mg/kg BWT of *Phyllanthus niruri* extract on the activity of plasma zinc ion concentration were investigated in the management of hemolytic thrombosis in wistar rats as revealed in **Figure 13**. The results revealed that hemolytic thrombosis (Group 2: 131.729) caused reduced activity of plasma zinc ion concentration significantly compared to the treatment groups (p≤0.05). There was significant increase in plasma zinc ion concentration level by both doses of *Phyllanthus niruri* and hydroxyl urea which was the standard drug (Group 3: 183.422; Group 4: 191.647) (p≤0.05).

The effects of 100 µl of 500mg/100ml of hydroxyl urea (HU) and 200 mg/kg BWT of *Phyllanthus niruri* extract on the activity of selenium were investigated in the management hemolytic thrombosis in wistar rats as revealed in **Figure 14**. The results revealed that hyperlipidemia (Group 2: 110.543) caused reduced activity of selenium significantly compared to the treatment groups (p≤0.05). There was significant increase in selenium level by both doses of *Phyllanthus niruri* and hydroxyl urea which was the standard drug (Group 3: 131.367; Group 4: 139.940) (p≤0.05).

Figure 15. The results revealed that hemolytic thrombosis (Group 2: 145.235) caused reduced activity of RNase significantly compared to the treatment groups (p≤0.05). There was significant increase in RNase activity by both doses of *Phyllanthus niruri* and hydroxyl urea which was the standard drug (Group 3: 164.526; Group 4: 157.288) (p≤0.05).

The effects of 100 µl of 500mg/100ml of hydroxyl urea (HU) and 200 mg/kg BWT of *Phyllanthus niruri* extract on the activity of red cell carbonic anhydrase were investigated in the management of hyperlipidemia in wistar rats as revealed in **Figure 16**. The results revealed that hemolytic thrombosis (Group 2: 131.832) caused reduced activity of red cell carbonic anhydrase significantly compared to the treatment groups (p≤0.05). There was significant increase in red cell carbonic anhydrase activity by both doses of *Phyllanthus niruri* and hydroxyl urea which was the standard drug (Group 3: 176.433; Group 4: 182.734) (p≤0.05).

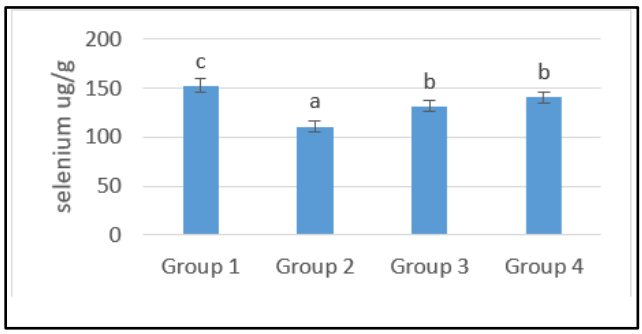

Figure 13: Effects of *Phyllanthus niruri* extract on the level of Selenium in hemolytic thrombosis wistar rats. Results were presented as mean±standard deviation where n=5. Values with different superscripts are significantly different (p≤0.05).

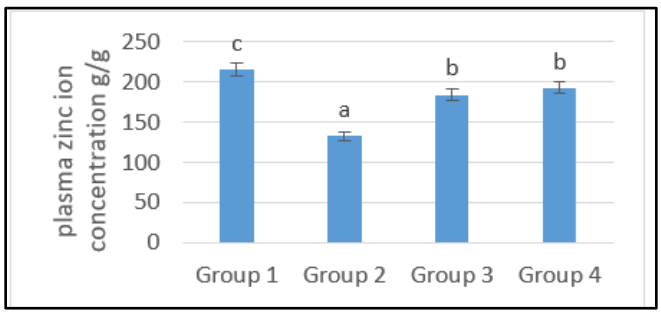

Figure 14: Effects of *Phyllanthus niruri* extract on the level of Plasma zinc ion concentration in hemolytic thrombosis wistar rats.

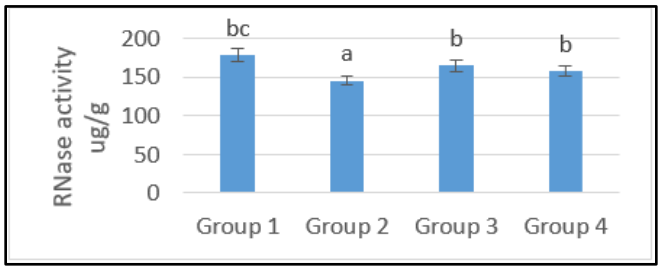

Figure 15: Effects of *Phyllanthus niruri* extract on the activity of RNase in hemolytic thrombosis wistar rats.

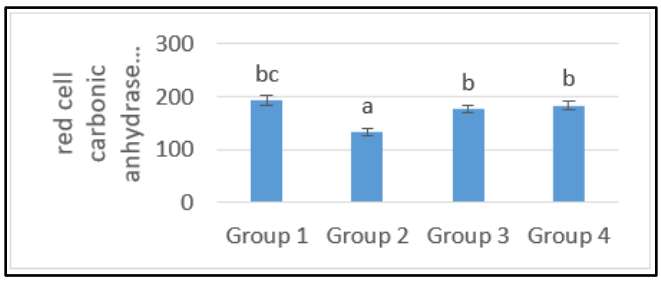

Figure 16: Effects of *Phyllanthus niruri* extract on the activity of Red cell carbonic anhydrase in hemolytic thrombosis wistar rats

4.17 Haematology result

	ESR	PCV	RBC	WBC	Hb	Lym	Neu	Mon	Eds	Bas	MCH	MCV	MCHC
Group 1	0.5±0.035	42.480±3.0331	1840.252±131.394	565.839±40.400	13.593±0.970	27.178±1.940	42.098±3.005	8.635±0.6166	4.073±0.2909	0	17.926±1.279	39.166±2.796	56.074±4.003
Group 2	0.5±0.035	21.032±1.5017	911.146±65.05583	280.158±20.003	6.730±0.4805	13.456±0.960	20.843±1.488	10.404±0.742	4.908±0.3504	1±0.0714	27.763±1.982	8.875±0.6337	19.392±1.384
Group 3	0.5±0.035	32.392±2.3128	1403.237±100.1911	431.466±30.806	10.365±0.740	20.724±1.479	32.101±2.292	9.217±0.6582	4.348±0.3104	1±0.0714	42.757±3.052	13.669±0.976	29.865±2.132
Group 4	0.5±0.035	34.668±2.3325	1515.21±100.1911	475.147±31.069	11.454±0.746	20.901±1.492	34.3742.3116	9.285±0.6631	4.380±0.3128	1±0.0714	43.122±3.078	14.786±0.984	30.120±2.150

From the heamtological parameters obtained, this shows that the PLE and the standard groups displays a level of repair on Lymphocyte, Neutrophils, Monocyte, Eosinophils, Basophils, ESR, PCV,RBC, HB, MCV, MCH, MCHC over the 2-butoxyethanol induced group.

7. Discussion

Stone breaker *Phyllanthus niruri* L. (Euphorbiaceae) is a prevalent tropical plant with recognized medicinal properties (Ifeoma *et al*., 2013).[12] It is widely used as ethnomedicine for the remedy of inflammation, hepatitis, fever, malaria, and gonorrhoea, in many parts of the world like Brazil, Asia, Thailand, China and Africa (Jantan *et al.*, 2019).[13] The biological and pharmacological functions reported for *P. niruri* include antiplasmodial, antioxidant, hepatoprotective, nephroprotective and anti-diabetic properties. (Sarin *et al.*, 2014).[14] Scientific investigation also report that *Phyllanthus niruri* has potent activity against various diseases such as hepatitis B, HIV, microbial infections, diabetes, nephrotoxicity, hepatotoxicity, and biological oxidation (Jantan *et al*., 2019a).[13] A report on the phytochemical analysis of *Phyllanthus niruri* linked the presence of the phytochemicals to the various pharmacological activities in which antioxidant activity was connected to the presence of flavonoids, antispasmodic activity to alkaloids, and antiplasmodial activity to lignans (Jantan *et al*., 2019b).[15]

Plants are a treasure trove of antioxidant-rich compounds that shield the human body from the ravages of cellular oxidation (Ozsoy et al., 2008).[20] By neutralizing free radicals, these antioxidants - including phenolic acids, polyphenols, and flavonoids - prevent oxidative stress, which is a precursor to degenerative diseases. By scavenging harmful free radicals, these plant-derived compounds halt the destructive oxidative cascade, promoting overall well-being (Subramanian et al., 2011).[21]

Hemolytic thrombosis is a complex pathological process involving the destruction of red blood cells and the formation of blood cloth, is a condition where red blood cells are destroyed and cause blood clots to form in small blood vessel, but there is limited scientific literature on the effect of 2-butoxyethanol on hemolytic thrombosis specifically. However, a study conducted on rats exposed to 2-butoxyethanol was observed to have caused a significant increase in platelet aggregation and blood clotting time (Zhao *et al.* 2013).[22] Another study on human subjects exposed to 2-butoxyethanol through inhalation reported similar findings, with increased platelet aggregation and clotting activity compared to control subjects (Kesavachandran *et al.*,2012).[23] Same trend was also seen in this study having an increase in the concentration of the hematological markers. 2-Butoxyethanol (BE) is an environmental toxicant used as chemicals in the manufacturing of wide range of domestic

and industrial products as well as surface coatings and household cleaning agents (Lewis *et al*., 2006).[1] BE was reported to be metabolized in Fischer 344 female rats to 2-butoxyacetic acid (BAA) causing acute hemolytic anemia (Ezov *et al*., 2002)[2] and disseminated thrombosis. This 2-butoxyethanol-induced acute disseminated thrombosis and infarction in female rats may be caused by vaso-occlusion, possibly initiated by acute hemolytic anemia with the possibility of release of procoagulant factors from destroyed erythrocytes, altered morphology and decreased deformability of erythrocytes, and a tendency of the red blood cells to aggregate and/or adhere to the endothelium (Nyska *et al*., 2005). Depranocytosis also known as sickle cell disease is a genetic disorder due to the substitution of the glutamate by valine at the sixth position of the β-chain of the hemoglobin A (HbA). This causes a structural modification that alters oxygen affinity and transport of the oxygenated sickled RBC leading to tissue infarction vis a vis hemolytic anemia and disseminated thrombosis. However, It is possible that exposure to 2-butoxyethanol may have indirect effects on hemolytic thrombosis through its ability to cause oxidative stress and inflammation, which are known risk factors for thrombotic events (Esmon, 2003[24]; Favaloro, 2016[25]).

Oxidative stress, defined as a disruption of the balance between oxidative and antioxidative processes, plays an important role in the pathogenesis of atherosclerosis (Yokozawa *et al*., 2006[26]). It has been reported that plant-derived antioxidant compounds can help in the prevention of diseases in which oxidative stress is implicated in their pathogenesis, and one of their mechanism of action is by interacting with regulatory factors which participate in ROS-committed signalling cascades. Intracellular antioxidant mechanisms against free radicals, reactive oxygen and nitrogen species generated in excess in the system involve the endogenous antioxidant enzymes and molecules, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione molecules etc. in tissues. They can stabilize or deactivate free radicals before they attack cellular components (Krishnamurthy and Wadhwani, 2012[27]). Enzymatic antioxidants help protect organisms from the excessive generation of oxidative stress in inflammatory processes. This action has triggered studies focusing on the role of natural products in suppressing oxidation production by increasing enzymatic antioxidants in tissues (Deng *et al*., 2011[28]).28 CAT, GPx and SOD are part of the hepatic endogenous antioxidant defense system that contributes to regulating oxidoreductive homeostasis and mitigating oxidative attacks on cells. CAT and GPx are involved in eliminating hydrogen peroxide, while SOD scavenges superoxide radicals and therefore prevents its conversion into hydrogen peroxide and molecular oxygen (Shukla & Mossman, 2006).[29] The present study demonstrated a similar pattern in expressing these endogenous antioxidant enzymes in the experimental animals' brain in a dose-dependent manner. The leaf extract of *P. niruri* was reported to display an increase in the activity of the endogenous antioxidant enzymes in the kidney (Giribabu *et al*., 2014[30]). PE significantly ($p < 0.05$) increased CAT, GPx, and SOD activities in the PE-treated rats. GSH is an intracellular antioxidant co-factor to glutathione reductase (GR) and GPx. It is an essential water-soluble antioxidant molecule which directly neutralizes ROS such as lipid peroxides. A similar trend of the result was observed with GSH. A dose-dependent increase in the amount of glutathione was observed. Na^+-K^+ ATPase is a pump that couples the active exchange of three intracellular Na+ ions for two extracellular K+ to the hydrolysis of a molecule of Adenosine triphosphate (ATP), and it is so important because almost a third of the ATP molecules generated by the mitochondria in animal cells are used to run this pump (López-López *et al*., 2011).[31] Its activity depends on the integrity of the cell membrane structure. It has been reported to decrease in mammalian tissues under oxidative stress assault, possibly because of the structure and function affected by oxidative stress. In this study, treating rats with an increasing dose of PE increased activity in a dose-dependent manner. The antioxidant-enhancing capability has been linked to the increase in the activity of this enzyme (Suru & Ugwu, 2015).[32]

In the context of the hemolytic assay, which encompasses RNase, selenium, plasma and urine zinc ion concentration, and red cell carbonic anhydrase, ribonucleases play a crucial role. Ribonucleases are enzymes that catalyze the degradation of RNA molecules into smaller fragments, primarily residing in the cytoplasm, nucleus, and various secretions (Singh et al., 2007).[33] Notably, exposure to 2-butoxyethanol has been shown to exert toxic effects on various enzymes and cellular processes, inducing DNA damage and oxidative stress in human lymphocytes and rat brain cells.

Selenium is known to play a role in antioxidant defense by functioning as a cofactor for various antioxidant enzymes, including glutathione peroxidases and thioredoxin reductases. It is possible that selenium may help to protect cells from the oxidative damage caused by 2-butoxyethanol, Studies have shown that exposure to 2-butoxyethanol can cause oxidative stress and damage to cellular components, including lipids, proteins, and DNA (Singh *et al.*, 2007). These effects are thought to be due to the ability of 2-butoxyethanol to generate reactive oxygen species and to cause lipid peroxidation. The result obtain shows decrease in the selenium level at the induced but not treated group but for treated group reverse was the outcome.

This study revealed that 2-butoxyethanol (BE) inhibits the activity of ribonuclease (RNase). Zinc, an essential trace element, plays a crucial role in various cellular processes, including gene expression, enzyme catalysis, and immune function. Notably, zinc homeostasis is tightly regulated through mechanisms such as dietary intake, absorption, and excretion. Our findings indicate that 2-butoxyethanol exposure led to a significant decrease in blood zinc levels,

consistent with previous observations by Rodríguez et al. (2001).[34]

Carbonic anhydrase (CA) is an enzyme that catalyzes the reversible conversion of carbon dioxide and water to bicarbonate and protons. Red blood cells contain a high concentration of CA, which plays a crucial role in regulating acid-base balance and gas exchange in the body. There is some evidence to suggest that exposure to 2-butoxyethanol can inhibit the activity of red cell carbonic anhydrase. In one study, rats were exposed to 2-butoxyethanol via inhalation for six hours per day for three consecutive days, and their red blood cell CA activity was measured. The results showed that exposure to 2-butoxyethanol led to a dose-dependent decrease in CA activity in the red blood cells and similar result was also obtain at the cause of this research.

Anti-inflammatory potential of the study plants revealed that Myeloperoxidase (MPO) which is a dervied chlorimated compounds are specific biomarkers for disease progression (Malle *et al.,* 2007[35]), it help other inflammatory marker to oxidizes chloride through MPO production of hypochlorous acid (HOCL) then the excessive production of these radicals can cause oxidative stress leading to oxidative tissue injury. In this study, it was observed that *p. niruri.* reduced the level of MPO produced in the treated groups, thereby control the production of HOCL which may result in oxidative stress and oxidative tissue injury.

The effect of *P. niruri.* Leaf extract on BE induced hemolytic thrombosis on the pro-inflammatory assay, showed an obvious inflammation response of TNF-α, IL-6, and IL-1β during induction. NF-κB is a critical factor for the expression of various pro-inflammatory cytokines, such as TNF-α, IL-6 and IL-1β (li and Verma, 2002.[36] TNF-α, IL-6, and IL-1β were significantly decreased in *p. niruri.* treated groups and as well the standard drug group, these results suggest that *p. niruri.* will be useful in the management of hemolytic thrombosis, but there was an increase in the level of MPO, TNF-α, IL-6, and IL-1β in the induced but not treated group. From the result obtained, *P. niruri.* Leaf extract has the potentials of ameliorating the effect caused by BE induction, thereby making this plant (*P. niruri.)* potent for the management of hemolytic thrombosis.

8. Conclusion

This study has proven the therapeutic effects of Stone Breaker (*Phyllanthus niruri)* extracts through its contributions against oxidative stress, inflammation, apoptosis and hemolytic thrombosis dysfunction. *P. niruri* leaf extract possess various bioactive components and these have conferred its therapeutic ability by reduction of oxidative stress, thus, elevating the antioxidant enzyme activities, thereby, reducing the level of ROS and protecting the liver from oxidative damage. It was also observed that the plant extact inhibited lipid peroxidation and improved the activity of Na^+/K^+ ATPase. *P. niruri* leaf extract demonstrated the activation Zinc which is an essential trace element that plays important roles in various cellular processes, including gene expression, enzyme catalysis, and immune function.

9. Source of Funding

There are no funding sources.

10. Conflicts of Interest

The authors have no actual or potential conflict of interest to declare.

11. Acknowledgement

The authors thank Victoria and Uduak Umoh on his contribution during the laboratory work.

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Cite this article:. Umoh EU, GOyubu G, Ojo OO, Dimas JJ, Ayodele A. Olowogbade, Stone breaker leaf extract on the toxic effect of 2-butoxy-ethanol induction on hemolytic thrombosis. *Int J Clin Biochem Res*. 2025;12(1):11-19.