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Anti-Inflammatory and Analgesic Activities of Soft Drink Leaf Extract of *Phyllanthus amarus* in Some Laboratory Animals

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Authors' contributions

This work was carried out in collaboration between the two authors. Author AAA designed the study, performed the statistical analysis, wrote the protocol, and wrote the first and final draft of the manuscript while author SOO carried out the study under the supervison of author AAA. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aim: *Phyllanthus amarus* Schum (Euphorbiaceae) is an annual herbal shrub which has been used in traditional medicine in Nigeria to treat some disease conditions. The aim of this study is to evaluate the anti-inflammatory and analgesic activities of the aqueous extract of *Phyllanthus amarus* in experimental animal models hence confirming its folkloric use.

Study Design: Forty healthy white Wister strain albino rats (100–200g) and forty mice (15–30g) of either sex bred in the experimental animal house of the Faculty of Veterinary Medicine, University of Ibadan, Nigeria were used for the study. Forty rats were used for anti-inflammatory study while forty mice used for the analgesic study. In anti-inflammatory study, carrageenan and histamine-induced paw oedema were used while acetic acid-induced writhing test and formalin-induced paw lick test were deployed for analgesic test.

Place and Duration of Study: Faculty of Veterinary Medicine, University of Ibadan, Nigeria; 2 months.

Methodology: Soft drink extract (SDE) was prepared by dissolving ground plant materials (200g) in 1 L seven up (7 UP®) for 48 h, filtered, lyophilized and then used for the pharmacological investigations. Standard phytochemical methods were used to test for the presence of phytoactive compounds in the plant. Acute toxicity was carried out in mice

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to determine safe doses for use. The anti-inflammatory activities were conducted using carrageenan and histamine to induce oedema in rats while analgesic activities were embarked upon using acetic acid- induced writhing test and formalin-induced paw lick test.
Results: The extract in doses of 100 and 200 mg/kg at 3 hr showed 15.1 and 16.4% inhibition of histamine induced-paw oedema respectively while ibuprofen caused 9.6% inhibition at the same period. In the case of carrageenan induced paw oedema, the extract in doses of 100 and 200 mg/kg at 4 hr showed 10.5 and 12.0% inhibition respectively while ibuprofen only caused 3% inhibition. In the acetic acid- induced writhing test, the extract showed a good analgesic activity characterized by a significant reduction in the number of writhes with 100 and 200 mg/kg doses used when compared to the control group. The result was also similar to the formalin-induced paw lick test.
Conclusion: The soft drink leaf extract of *Phyllanthus amarus* has both analgesic and anti-inflammatory potential. The activities of this extract were comparable to that of ibuprofen, the reference drug used in this study.

Keywords: Phyllanthus amarus; anti-inflammatory; analgesic oedema; rats; mice.

1. INTRODUCTION

Inflammation is the response of living tissues to injury and it begins when a stimulus such as infection, physical or chemical insult produces cellular damage [1]. This damage initiates the activation of transcription factors that control the expression of many inflammatory mediators including the eicosanoids, biological oxidants, cytokines, adhesion factors, digestive enzymes (proteases, hyaluronidase, collagenase and elastase). The first three mediators are therapeutic targets for the anti-inflammatory drugs.

The inflammatory response changes with time and can be divided into different phases. The acute phase is characterized by the induction of inflammatory genes by NFKB and other transcription factors [2]. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes (especially granulocytes) from the blood to the injured tissues. Chronic inflammation on the other hand leads to a progressive shift in the type of cells present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process. The chronic inflammation occurs over months to years and is marked by drastic increased production of inflammatory mediators. The secondary chronic phase of inflammation occurs after years of oxidative damage of degraded blood vessels and tissues. Such chronic inflammation appears to play a role in many diseased states such as arteriosclerosis and cancer [3]. Causes of inflammation include burns, frostbite, toxins, infection by pathogens, physical injury (blunt or penetrating), immune reactions due to hypersensitivity, ionizing radiation, foreign bodies (including splinters, dirt and debris), stress, trauma etc [3].

Pain on the other hand is part of a defensive reaction against dysfunction of an organ or imbalance in its functions against potentially dangerous stimulus and pain-relieving chemicals produced at the site of injury caused pronounced side-effects on the physiology of the body [4]. Analgesia is the reduction of pain and analgesic is any agent that selectively blunts pain by acting either in the CNS or on peripheral pain mechanisms without significantly altering consciousness [5].

Phyllanthus amarus Schum belongs to the family Euphorbiaceae (the spurge family) of which the largest genus is the genus Euphorbia. Leaves are very small, 6-8 mm, subsessile, glabrous, elliptic-obovate, oblong or linear, tip rounded, obtuse or acute. Flowers are minute, very numerous, shortly pedicelled. Capsules are also minute, depressed globose [6]. *P. amarus* is usually a small herb, usually under 30 cm tall, with numerous small oblong-elliptic or squarish leaves about 6-12 mm long; the cymules are hidden under the leaves and are bisexual, consisting of 1 male and 1 female flower. The calyx-lobes are about 5 and are acute; the pedicels are 2 mm long. *P. amarus* has a small capsule, which is depressed and globose [7]. The seeds are 5-7-ribbed" [8].

The plant has a history of use in Ayurvedic medicine for over 2000 years as well as a wide variety of traditional applications [9]. *Phyllanthus amarus* is an important folk remedy used in the treatment of a variety of ailments [10]. In India, it is predominantly used as a cure for liver disorders [11]. The aqueous extract from *Phyllanthus amarus* has been reported to inhibit DNA polymerase of Hepatitis-B and woodchuck hepatitis virus [12]. Various activities have also been reported in animals and these include lipid lowering [13, 14], contraceptive [15], antiplasmodial [16] and antitumor effects [17]. The plant is often used by human for beneficial effect on kidney stones in the region of South East Asia [18]. It was also reported that the plant is used in treating arthritis and asthma [19].

The widespread usage of this herb has prompted several investigations [20,21,22]. The plant contains several phytochemical elements including: glycosides, flavonoids, alkaloids, ellagitannins, phenylpropanoids, sterols, lipids, astragalin, brevifolin, carboxylic acids, corilagin, cymene, ellagic acid, niranthin, nirurin, rutin, saponins, triacontanal, tricontanol, quercetol, niruriside, lignans, fatty acids, vitamin C, tannins, geranin, limonine, Ascorbic acid, hypophyllatin, linoleic acid, phyttetralin, phyllanthin, estradiol, gallic acid, linnanthin, nururine, phyllanthenol, lupeol, astragalin [23]. The major bioactive lignan constituents are phyllanthin and hypophyllathin, amarinic acid, nynphyllin, phyllarurin, neolynan etc. Phyllanthin and hypophyllanthin plant chemicals help in carrying out liver protecting activities. *Phyllanthus amarus* which is otherwise called “Eyin-olobe” in South Western Nigeria has healing effects on hypertensive patients. The hypertensive effects were attributed to a specific phytochemical in the plant called geranin. The plant chemical can inhibit several neurotransmitter processes that relay and receive pain signals in the brain. Geranin is said to contain anti-ulcerous properties [19].

The use of non-steroidal anti-inflammatory drugs (NSAIDs) in the treatment of diseases associated with inflammatory reactions is fraught with many adverse effects which then pose a major problem in their clinical use. There is therefore a continuous search for more potent anti-inflammatory and analgesic remedies from medicinal plants. It is in the light of this that we evaluated the anti-inflammatory and analgesic potentials of the soft drink leaf extract of *Phyllanthus amarus* in some laboratory animals because in some parts of Nigeria, this plant is dissolved in this soft drink and then use for medicinal purpose.

2. MATERIALS AND METHODS

2.1 Plant Collection and Extract Preparation

Fresh leaves of *Phyllanthus amarus* Schum were collected from the campus of the University of Ibadan, Nigeria (7º 23' 16" North, 3º 53' 47" East) in March 2012. The leaves were identified by Dr. Abiodun Ayodele (a botanist) and a voucher specimen (UIH

ADE/003/2012) deposited at the herbarium of the Department of Botany, University of Ibadan. The leaves were dried under shade and ground into powder. The powdered plant material (200 g) was dissolved in the 7up brand of soft drink and filtered, the filterate was then concentrated in a rotary evaporator at 40ºC under reduced pressure to obtain 25.5 g of the soft drink extract (12.75% yield). Graded solutions of the extract were prepared (labelled SDE) and used for the experiments.

2.2 Experimental Animals

Forty healthy white Wister strain albino rats (100–200g) and forty mice (15–30g) of either sex bred in the experimental animal house of the Faculty of Veterinary Medicine, University of Ibadan, Nigeria were used for the study. The animals were kept in cages within the animal house and allowed free access to water and standard livestock pellets. The animals were examined and found to be free of wounds, swellings and infections before the commencement of the experiment. All experimental protocols were conducted in compliance with the National Institute of Health Guide for Care and Use of Laboratory Animals.

2.3 Chemical and Drugs

Chemicals used include carrageenan, acetic acid, and formalin, all from Sigma–Aldrich Chemie Gmbh (Steinheim, Germany). The standard drugs used were ibuprofen and histamine which were also purchased from Sigma–Aldrich Chemie Gmbh (Steinheim, Germany). All the chemicals and drugs used were of analytical grade.

2.4 Phytochemical Screening

The phytochemical analysis was performed on the ground (powered) leaf of *P. amarus* for identification of the constituents. The constituents tested for were alkaloids, tannins, saponins, anthraquinones, cardiac glycosides and flavonoids as described by Shale et al. [24], Moody et al. [25] and Sawadogo et al. [26].

2.4.1 Test for alkaloids

About 0.5g of each extract was stirred with 5ml of 1% aqueous hydrochloric acid on a steam bath; 1ml of the filterate was treated similarly with Dragendorff's reagent (Mayer's reagent, Wagner's reagent, Picric acid solution or Tannic acid solution can also be used). Turbidity or precipitation with either of these reagents was taken as preliminary evidence for the presence of alkaloids in the extract [24]. A positive reaction is indicated by an orange–red precipitate.

2.4.2 Tests for tannins

About 5g each portion of plant extract was stirred with 10ml of distilled water and filtered and ferric chloride was added to the filtrate. A blue–black, green, or blue green precipitate was taken as evidence for the presence of tannins [25].

2.4.3 Test for saponins

The ability to produce frothing in aqueous solution and to haemolyse red blood cells was used as screening test for these compounds. For the frothing test, the method described by

Moody et al. [25] was used. About 0.5g of the plant extract was shaken with distilled water in a test tube, frothing which persist on warming was taken as preliminary evidence for the presence of saponins.

2.4.4 Test for anthraquinones

Borntrager's test was used for the detection of anthraquinones; 5g of the plant extract was shaken with 10ml benzene, filtered and 5ml of 10% ammonia solution added to the filterate. The mixture was shaken and the presence of a pink, red or violet colour in the ammoniacal (lower) phase indicated the presence of free hydroxyl-anthraquinones. For bound anthraquinones, 5g of the plant extract was boiled with 10ml aqueous sulphuric acid and filtered while hot. The filterate was shaken with 5ml of benzene, the benzene layer separated and half its own volume of 10% ammonia solution added. A pink or violet colouration in the ammonia phase (lower layer) indicates the presence of anthraquinones derivatives [25].

2.4.5 Test for free cardiac glycosides

2.4.5.1 Limbermanns test

0.5g of the extract was dissolved in 2ml of acetic anhydride and cooled well in ice. Sulphuric acid was then carefully added. A colour change from violet to blue to green indicated the presence of a steroidal nucleus (i.e. aglycone portion of the cardiac glycoside) [26].

2.4.5.2 Keller kiliani test

0.5g of the plant extract was dissolved in 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was then underlaid with 1ml of concentrated sulphuric acid. A brown ring obtained at the interface indicates the presence of a desoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout the layer [25].

2.4.5.3 Kedde test

1ml of 8% solution of the extract in methanol was mixed with 1ml of a 2% solution 3, 5-dinitrobenzoic acid in methanol and 1ml of a 5.7% aqueous sodium hydroxide. An immediate violet colour indicated the presence of cardenolides in the extract, the colour fading gradually through reddish-brown to brownish-yellow with the precipitation of a whitish crystalline solid. This test indicated the presence of a lactone ring in the cardenolide [26].

2.4.6 Test for flavonoids

1 gram of powdered plant sample was detanned by washing several times with acetone. The acetone was evaporated from the sample on hot water bath. Distilled water was then added and filtered. To 5ml of the filterate, 5ml of 20% sodium hydroxide was added and to another 5ml of the filterate, 5ml of lead acetate solution was added. Yellow colouration with either reagent was positive for flavonoids [26].

2.4.6.1 Shinoda test

This test is to identify the presence of flavonoids in the extract; few magnesium turnings and concentrated hydrochloric acid were added dropwise to the extract, presence of pink scarlet

or green to blue colour which appeared after some minutes indicated the presence of flavonoids [26].

2.5 Acute Toxicity Test

The acute toxicity of soft drink leaf extract of *P. amarus* was determined in mice according to the method of Hilaly et al. [27] with slight modifications. Mice fasted for 16 h were randomly divided into 6 groups of five mice per group. Graded doses of the plant's extract (100, 200, 400, 800 and 1600 mg/kg p.o.) and control (3ml/kg distilled water) were separately administered to the mice in each of the groups by means of bulbed steel needle. All the mice in the groups were then allowed free access to food and water and observed over a period of 48 h for signs of acute toxicity. The number of deaths within this period of time was recorded.

2.6 Anti-Inflammatory Activities

2.6.1 Histamine-induced rat paw oedema test

Using the method of Perianayagam et al. [28], the paw oedema was produced by sub–planter administration of 0.1% freshly prepared solution of histamine into the right hind paw of the rats. The paw oedema was measured at 0, 1, 2, 3 and 4 hr after administration of the histamine using thread and ruler. Increase in the linear diameter of the hind paw was taken as indication of paw oedema. Four groups of animals A, B, C and D (n=5) were pre-treated with 2 ml/kg vehicle control (normal saline), ibuprofen (10 mg/kg) and different doses of plant extract (100 and 200 mg/kg) respectively. The drug and extract were administered orally 1 hr before induction of paw oedema. The percentage inhibition of inflammation was calculated using the formula:

$$\%\, inhibition = \frac{Do-Dt}{Do} X\ 100$$

where Do is the average inflammation (hind paw oedema) of the control group of rats at a given time and Dt is the average inflammation of the drug treated (i.e. extract or reference ibuprofen) rats at the same time [25,26,29].

2.6.2 Carrageenan-induced rat paw oedema test

Four groups of rats A, B, C and D (n=5) received vehicle control (normal saline, 2 ml/kg body weight), ibuprofen (10 mg/kg body weight) and plant extract (100 and 200 mg/kg body weight) respectively. These were administered orally. Acute inflammation was then induced after 60 mins by the sub-planter administration of 0.1ml of 1% carrageenan in normal saline that contains Tween-80 in the right hind paw of the rats. The paw volume was measured at 0, 1, 2, 3 and 4 hr after carrageenan injection using thread and ruler. Increase in the linear diameter of the right hind paws was taken as an indication of oedema which was assessed in terms of the difference in the zero-time linear diameter of the injected hind paw and its linear diameter at time *t* (i.e. 1, 2, 3, 4 hr) following carrageenan administration [25]. The percentage inhibition of the inflammation (hind paw oedema) was calculated as for histamine-induced oedema.

2.7 Analgesic Activity

2.7.1 Acetic acid-induced writhing response in mice

To evaluate the analgesic effect of the plant extract, the method described by Sawadogo et al [26] was used though with slight modification. Four groups of mice A, B, C and D (n=5) each received orally administered vehicle control (normal saline 2 ml/kg) (i.e. control), ibuprofen (10 mg/kg) and plant extract (100 and 200 mg/kg) respectively. Sixty mins later, 0.6% acetic acid (10 ml/kg) solution was injected intraperitoneally to all animals in the different groups. The number of writhes occurring between 5 and 20 mins after acetic acid injection was counted. The percentage inhibition of the writhing response was calculated from the formula:

$$\%\, inhibition = \frac{Do-Dt}{Do} X\ 100$$

where Do was the average writhing response of the control group, while Dt was the average writhing response of the treated group. A significant reduction of the writhes in the tested animals compared to those in the control group was considered as an antinociceptic response.

2.7.2 Formalin paw lick test in mice

In this experiment, pain was induced by formalin. Following an overnight fast, four groups of mice A, B, C and D (n=5) each received orally administered vehicle control (normal saline 2 ml/kg) (i.e. control), ibuprofen (10 mg/kg) and plant extract (100 and 200 mg/kg) respectively. Thirty mins after treatment, 0.05 ml of 2.5% formalin was injected subcutaneously into the sub–plantar surface of the mice left hind paw, then the number of paw licks by the mice were recorded both at the early and late phases, the time interval between the paw licks was also noticed [30, 31]. The percentage inhibition of the paw licks for both phases was calculated from the formula:

$$\%\, inhibition = \frac{Do-Dt}{Do} X\ 100$$

where *Do* was the average number of paw licks of the control group, while *Dt* was the number of paw licks of the treated group.

2.8 Statistical Analysis

The data generated were presented as mean ± SD; statistical analysis was carried out by using graph pad prism 5. The results were further subjected to one way analysis of variance (ANOVA), the variance means were separated using student t-test and differences between means were considered significant at $P \leq 0.05$.

3. RESULTS AND DISCUSSION

In the acute toxicity test, no death was recorded in all the groups. All the mice appeared to be normal and none of them showed any visible signs of toxicity. It means that at the doses administered in this study, the plant is safe for use medicinally.

The results of the anti-inflammatory and analgesic activities of the soft drink leaf extract of Phyllanthus amarus are presented in Tables 1 – 2 and Figs. 1 – 2 respectively.

Table 1. Anti-inflammatory activity of the soft drink leaf extract of *Phyllanthus amarus* on histamine-induced oedema in the right hind-limb of rats. Data is presented as mean ± S.D., n = 5

Time(hr)	Control	Ibuprofen 10mg/kg	Extract (aqueous) 100mg/kg	Extract (aqueous) 200mg/kg
1	1.52±0.04	1.42±0.07 (6.6)	1.34±0.08 (11.8)	1.28±0.12 (15.8)
2	1.48±0.07	1.36±0.12 (8.1)	1.28±0.04 (13.5)	1.24±0.14 (16.2)
3	1.46±0.08	1.32±0.07 (9.6)	1.24±0.08 (15.1)	1.22±0.13 (16.4)
4	1.42±0.04	1.32±0.07 (7.0)	1.24±0.05 (12.7)	1.22±0.13 (14.1)

Percentage inhibitions of the carrageenan-induced inflammation (oedema) are in parenthesis.

Table 2. Anti-inflammatory activities of soft drink leaf extract of *Phyllanthus amarus* and ibuprofen on carrageenan-induced oedema in the right hind paw of rats. (n=5), mean ± SD

Time(hr)	Control	Ibuprofen 10mg/kg	Extract (aqueous) 100mg/kg	Extract (aqueous) 200mg/kg
1	1.50±0.06	1.56±0.05 (4.0)	1.44±0.10 (4.0)	1.42±0.04 (5.3)
2	1.44±0.05	1.44±0.08 (0)	1.36±0.10 (5.6)	1.32±0.04 (8.3)
3	1.36±0.09	1.32±0.07 (2.9)	1.24±0.10 (8.8)	1.22±0.07 (10.3)
4	1.34±0.05	1.30±0.09 (3.0)	1.20±0.09 (10.5)	1.18±0.07 (12.0)

Percentage inhibitions of the carrageenan-induced inflammation (oedema) are in parenthesis.

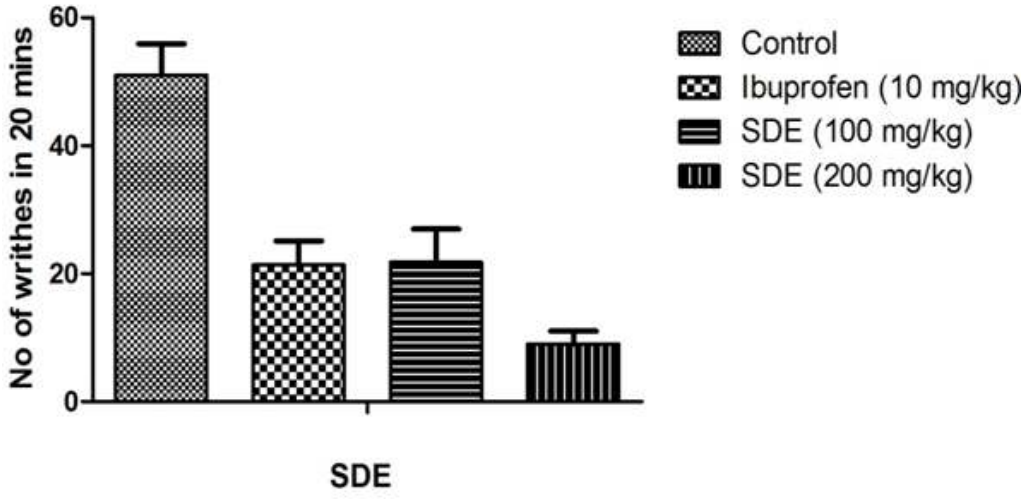

Fig. 1. Analgesic effect of soft drink leaf extract of *Phyllanthus amarus* and ibuprofen on mouse writhing reflex induced by acetic acid (n=5), mean ± SD

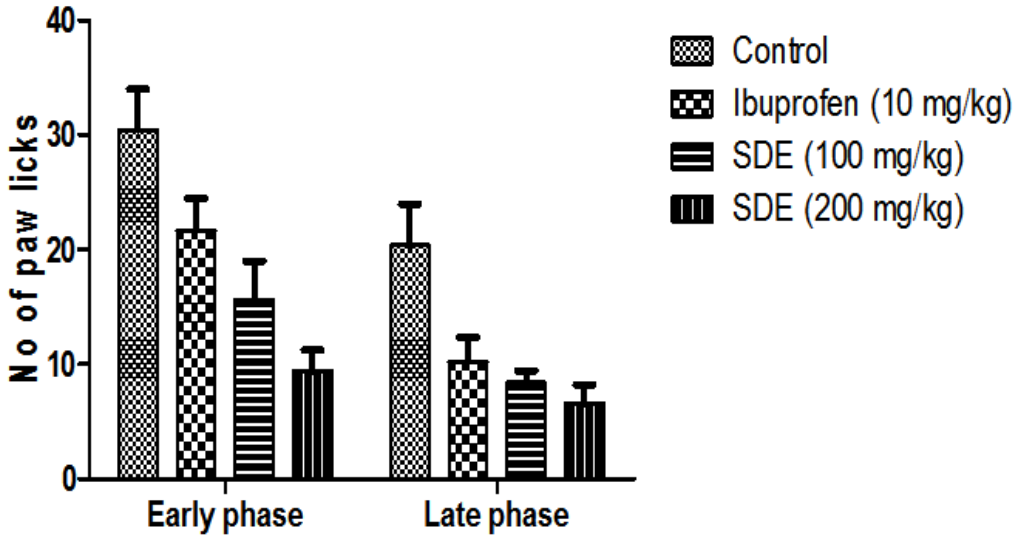

Fig. 2. Analgesic effect of soft drink extract of *Phyllanthus amarus* and ibuprofen on paw lick test on mouse induced by formalin (n=5), mean ± SD

The extract (100 and 200 mg/kg) and ibuprofen (10 mg/kg) significantly ($P \leq 0.05$) reduced the paw oedema at 1, 2, 3 and 4 hr after histamine injection when compared with the control (except ibuprofen at 2 hr which showed a non-significant reduction in the paw-oedema). The effect of ibuprofen and extract (100 and 200mg/kg) on histamine-induced paw oedema was most pronounced at 3hr after histamine injection. Both ibuprofen and extract (100 and 200 mg/kg) have similar antioedema effect at 3 and 4 hr after histamine injection. In addition, 100 mg/kg dose of the extract have similar effect with ibuprofen at 1 and 2 hr after histamine injection. The 200 mg/kg dose of the extract has greater anti-inflammatory effect at 1 and 2 hr after histamine injection relative to the standard drug (ibuprofen) (Table 1). The extract reduced the oedema produced by histamine. The results tend to suggest that the anti-inflammatory activity of the extract is possibly backed by its anti-histamine activity. Histamine is an important inflammation mediator, a potent vasodilator substance which increases vascular permeability [32]. Because the extract effectively suppressed the oedema produced by histamine, it shows that the extract exhibited its anti-inflammatory actions by means of either inhibiting the synthesis, release or action of inflammatory mediators, such as histamine, serotonin and prostaglandins.

The extract (200 mg/kg) significantly ($P \leq 0.05$) reduced the paw oedema at 1, 2, 3 and 4 hr after carrageenan injection when compared with the control; also 100 mg/kg dose of the extract significantly ($P \leq 0.05$) reduced the paw oedema at 3 and 4 hr after carrageenan injection but there was a non significant reduction in the paw oedema by ibuprofen (10 mg/kg) at 1, 2, 3 and 4 hr and 100 mg/kg dose of the extract at 1 and 2 hr when compared with the control. The effect of ibuprofen (10 mg/kg) and the extract (100 and 200 mg/kg) on carrageenan-induced paw oedema was highest at 4 hr after carrageenan injection. The effect of the extract at 200 mg/kg was similar to that of ibuprofen (10 mg/kg) at 2 and 4 hr, while the effect of the extract at 100 mg/kg was similar to that of ibuprofen (10 mg/kg) at 1hr after carrageenan injection (Table 2). Carrageenan-induced rat paw oedema is a suitable experimental animal model for evaluating the anti-oedematous effect of natural products and is

believed to be biphasic. The first phase (1 hr) involves the release of serotonin and histamine and the second phase (over 1 hr) is mediated by prostaglandins, the cyclooxygenase products, and the continuity between the two phases is provided by kinins [28]. It has been shown that species of *Phyllanthus* plant demonstrated inhibitory effects on the biosynthesis of prostaglandins E_2 (PGE_2) and prostaglandins D_2 (PGD_2) [33]. It is also known that *Phyllanthus* species contained tannins [34] and these compounds are known to be potent cyclooxygenase-1 inhibitors. Because the carrageenan- induced inflammation model is a significant predictive test for anti-inflammatory agents acting by the mediators of acute inflammation [25,26], these results are an indication that *Phyllanthus amarus can* be effective in acute inflammatory disorders. Carrageenan-induced oedema is a form of acute inflammation and it, involves the synthesis or release of mediators at the injured site. These mediators such as prostaglandins, especially the E series, histamine, bradykinins, leukotrienes and serotonin, all cause pain and fever [35]. Inhibitions of these mediators from reaching the injured site or from bringing out their pharmacological effects will normally ameliorate the inflammation and other symptoms. This study has shown that the soft drink leaf extract of *Phyllanthus amarus* possessed an antioedematogenic effect on paw oedema induced by histamine and carrageenan. When compared however, to other studies from our laboratory, it was discovered that the anti-oedematous effect of this plant is relatively low. For instance, the anti-inflammatory effect of *Acacia karroo* on rats at 100 and 200mg/kg doses by 1 hr caused 67.8 and 90.8% inhibition respectively to carrageenan-induced paw oedema and this is comparable to indomethacin which caused 90.4% inhibition [36]. At 3 hr, the 100 and 200mg/kg dose of *Margaritaria discoidea* caused 81.3 and 94.9% inhibition while indomethacin the reference drug produced 56.5% inhibition using carrageenan induced paw oedema [37]. These results are also similar to inflammation induced with histamine.

Fig. 1 showed that soft drink leaf extract of *P. amarus* caused a significant decrease in the number of writhes at doses 100 and 200mg/kg when compared to the control. The extract (100 and 200mg/kg) and ibuprofen exhibited a significant antinociceptive power of 57.3%, 82.4% and 58% respectively. The 200mg/kg dose of the soft drink extract showed a significant analgesic effect relative to the standard drug. The standard drug ibuprofen (10 mg/kg) also caused a significant reduction (P=.05) in the number of writhes when compared to the control. For the acetic acid-induced abdominal writhing, the result showed that the doses (100 and 200 mg/kg) produced a significant analgesic effect. This analgesic effect of the extract could be attributed, at least in part, to its anti-inflammatory effect as, in the visceral pain model, the processor releases arachidonic acid via cyclooxygenase and prostaglandin biosynthesis which plays a role in the nociceptive mechanism [38]. Therefore, an anti-inflammatory substance may also be involved in the peripheral analgesic activity, because inhibition of the acute inflammation by these extracts leads to their inhibitory effect on pain development. The 100 and 200 mg/kg doses of *Malva parviflora* and 10mg/kg dose of indomethacin each caused 100% inhibition of writhes produced by acetic acid indicating that the extract of *P. amarus* at the same dose caused less inhibition [39].

Fig. 2 showed that the soft drink leaf extract of *P. amarus* (100 and 200 mg/kg) caused a significant decrease in the number of paw licks induced by formalin when compared to the control both at early and late phases of the test. The analgesic effect of both 100 and 200 mg/kg doses of the soft drink extract was greater relative to the standard drug (ibuprofen) at both phases of the test. The standard drug ibuprofen (10 mg/kg) caused a significant decrease (P=.05) in the number of paw licks induced by formalin when compared to the control at both phases of the test. Formalin test is biphasic, and measures pain of both neurogenic (first/early phase) and of inflammatory origin (second/late phase). The first phase (0 – 5mins) being result of direct stimulation of nociception measures centrally mediated

effects and is insensitive to anti-inflammatory agents. The second phase (15 – 30mins) is qualitatively different from the first phase and is dependent on peripheral information and changes in central procession due to chemical mediators released from damaged cells that stimulate nociception and thus induce pain. The result showed that the number of time that the mice licked their right hind paw was significantly reduced with administration of the extract especially at the late phase when compared to that observed in the control group. This significant reduction is an indication of the analgesic effect of the plant extract. The reduction noticed in this study is however less effective when compared to that of *Malva parviflora* at the same doses [39].

Phytochemical screening of the leaves of *P. amarus* in this study showed the presence of alkaloids, tannin, flavonoids, saponin, anthraquinones and cardiac glycosides. The anti-inflammatory activities of many plants have been attributed to their high sterol/triterpene [40] or flavonoid contents [41]. Calixto et al. [20] and La Casa et al. [42] have demonstrated that various flavonoids, such as rutin, quercetin, luteolin, biflavonoids and triterpenoids, produced significant antinociceptive and or anti-inflammatory activities. Studies have shown that the plant, *P. amarus* is rich in flavonoids [22]. The mechanism of anti-inflammatory activities may be due to the presence of these flavonoids in the plant. Non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen act by the reduction of sensitization of pain receptors caused by prostaglandins at the site of inflammation [43]. The observed anti-inflammatory and analgesic activities of these extracts may be attributable to the overall effects of the plant constituents or the compounds having action similar to NSAIDs. Although the active doses of the plant extract were higher than those of the reference drug, it should be noted that the extract have different compositions of several substances.

4. CONCLUSION

The result from this study showed that the soft drink leaf extract of *Phyllanthus amarus* exhibits anti-inflammatory and analgesic potentials. The anti-inflammatory and analgesic effects are even higher than those of ibuprofen, the standard anti-inflammatory drug used in this study. In the acute toxicity test, no death was recorded in all the groups. All the mice appeared to be normal and none of them showed any visible signs of toxicity. Acute oral administration of *Phyllanthus amarus* to mice indicated that the plant is non toxic even at the dose of 1600mg/kg body weight. It thus showed that this plant is safe for medicinal use at this dose. The implication of this is that at the doses used in this study to evaluate antinociceptive and anti-inflammatory activities of the plant, no pathological changes occurred.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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