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Analgesic and Anti-Inflammatory Activity of Total Crude Leaf Extract of *Phytolacca dodecandra* in Wistar Albino Rats

Loyce Nakalembe[1], Josephine N. Kasolo[2], Edward Nyatia[3], Aloysius Lubega[4], Godfrey S. Bbosa[4*]

[1]Department of Pharmacology, School of Medicine, St Augustine International University, Kampala, Uganda
[2]Department of Medical Physiology, Makerere University College of Health Sciences, Kampala, Uganda
[3]Department of Biotechnical and Diagnostic Sciences, Makerere University College of Veterinary Medicine, Animal Resources and Biosecurity, Kampala, Uganda
[4]Department of Pharmacology & Therapeutics, Makerere University College of Health Sciences, Kampala, Uganda
Email: *godfossa@gmail.com

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Abstract

Background: Pain is a common problem encountered in daily life among humans and animal and it is commonly managed conventionally using modern medicines and traditionally by use of medicinal plants. *Phytolacca dodecandra* is a common herb in Uganda, used traditionally to alleviate pain and inflammatory conditions. However, its activity in alleviating pain and inflammatory diseases has not been fully scientifically evaluated. **Aim of Study:** To evaluate the analgesic and anti-inflammatory activity of total crude leaf extracts of *P. dodecandra* in Wistar albino rats. **Materials and Methods:** It was a laboratory-based experimental study. Acetic acid-induced writhing test was used to determine the analgesic activity and the histamine-induced paw edema formation for anti-inflammatory activity of the extract. Twenty eight animals were divided into 7 groups each with 4 rats and two (2) sets of data were obtained from each animal per group as per guidance of the refinement rule of 3Rs. For analgesic activity, Group I was dosed with 1.0ml of normal saline (negative control), group II, III, IV, V, VI were dosed with 1000.0, 600.0, 150.0, 40.0 and 5.0 mg/kg bwt of extract respectively. Group VII was dosed 10.0 mg/kg bwt diclofenac sodium (Na) drug (positive control). For anti-inflammatory activity, Group I was dosed with 1.0ml of normal saline (negative control), Group II, III, IV, V, VI were dosed with 1000.0, 600.0, 300.0, 40.0 and 5.0 mg/kg bwt of extract respectively. Group VII was dosed 10.0 mg/kg bwt diclofenac Na. The percentage mean paw volume inhibition and percentage writhing protection were determined. **Results:** The mean percentage paw volume inhibitions were 29.1%, 74.2% and 32.3% at doses of

5.0, 40.0 mg/kg bwt and 10.0 mg/kg bwt diclofenac Na respectively. The mean percentage writhing protection was 8.9%, 10.4%, 78.5% and 98.7% at doses of 150.0, 600.0, 1000.0 mg/kg of extract and 10.0 mg/kg bwt diclofenac Na respectively. **Conclusions:** Total crude leaf extracts of *P. dodecandra* has analgesic and anti-inflammatory activity that may be attributed to the presence of polyphenolic, saponins and many others phytochemicals that are present in the plant.

Keywords

Phytolacca dodecandra, Analgesic, Anti-Inflammatory, Total Crude, Leaf Extract

1. Introduction

Pain and inflammation are among the commonest discomforts humans and animals experience in daily life globally. They are both signs and symptoms of tissue injury or diseases [1] [2]. Globally, both these conditions are managed by use of analgesic and anti-inflammatory drugs. However, different communities worldwide use traditional medicine in form of medicinal plants to manage these conditions. And in Uganda, the country is rich in natural forest with many medicinal plants which local communities commonly use to meet their healthcare needs including in the management of pain and inflammatory-associated diseases. The various medicinal plants that have been reported to have different phytochemical compounds with analgesic and anti-inflammatory activities, and therefore because of these properties, they are commonly utilized in management of pain and inflammatory conditions [3] [4]. These plants contain various phytochemical compounds that include phenolic acids, alkaloids, saponins, flavonoids, glycosides, terpenes, lignans, steroids, coumarins, anthraquinones, edotides, xanthones and sesquiterpene lactone [5] [6]. Previous studies have reported that flavonoids, tannins, saponins, phenolic acids and alkaloids present in different medicinal plants have both analgesic and anti-inflammatory activities [7] [8]. Among the medicinal plant used include *P. dodecandra*. This herb is an ever green plant that belongs to the family Phytolaccaceae [9] [10] [11]. It is commonly known as African soap berry. It is a scrambling dioecious, semi-succulent shrub that commonly grows in forest edges, wetter bush lands, termites mounds and riparian forest [11]. The herb is used in the treatment of various skin disorders such as sore scalp, psoriasis, eczema, boils and many other conditions using the leaf extracts; preparation of good luck charm, detergent and soap in some countries like Uganda, Ethiopia and Somalia [9] [11] [12]. The plant has also been reported to have various pharmacological activities including; antifungal activity [13], molluscidal activity, anti-epileptic effects, anti-malarial activity and analgesic activity [9] [10] [11] [12]. The leaf sap is used in the treatment of otitis [11]. It is also used traditionally in the induction of abortion

by the traditional herbalists and local communities [9]. Phytochemical screening of *P. dodecandra*, show the presence of various chemical compounds including the saponins, alkaloid, phenolics, steroids, terpenoids and many others [13] [14] and these could be responsible for the pharmacological activities of the plant and its toxic properties [10] [11] [15]. However, whereas *P. dodecandra* is commonly used by the local communities to relieve pain and inflammatory conditions attributed to various pathological conditions and diseases, its pharmacological activity in management and controlling of these conditions has not been fully scientifically evaluated and documented. The study therefore, evaluated the anti-inflammatory and analgesic activities of the total crude leaf extracts of *P. dodecandra* in Wistar albino rats.

2. Materials and Methods

2.1. Study Design

A laboratory based experimental study design was used to determine the anti-inflammatory and analgesic activity of total crude leaf extracts of *P. dodecandra* in Wistar albino rats. The analgesic and inflammatory activities of *P. dodecandra* were determined using already established methods [8] [16] [17] [18] [19].

2.2. Study Setting

The field work was carried out in central Uganda where *P. dodecandra* was collected. The processing of the herb and the laboratory experiments were done at the department of Pharmacology and Therapeutics, Makerere University, College of Health sciences.

2.3. Medicinal Plant Collection

Fresh leaves, roots, flowers and fruits of *P. dodecandra* herb were collected around the fence of the Makerere University College of Veterinary Medicine, Animal Resources and Biosecurity. The herb was identified by a taxonomist by its local name, common name, scientific name and morphological descriptions. The voucher specimen, number NL808 was given for future reference and the specimen was kept at Makerere University herbarium.

2.4. Chemicals

Various chemicals and drugs such as methanol, normal saline, acetic acid, petroleum ether, disinfectants, histamine and diclofenac among others, used in the study were purchased from BDH Laboratory Suppliers (U) Ltd and Sigma-Aldrich, St. Louis, Missouri, USA.

2.5. Medicinal Plant Processing and Extraction

The collected leaves were dried in a solar drier until constant weight was obtained. They were pounded into powder using a mortar and pestle. Serial extraction was done using petroleum ether and methanol. About 400 g of the plant powder was

soaked again in one liter of petroleum ether at room temperature and the mixture was shaken regularly for 2 - 3 days to maximize the extraction of the active compounds. It was then decanted and filtered using whatman No 1 filter paper. The filtrate obtained was then stored until recovery of the solvents was done. The residue was dried for approximately one hour at room temperature and then soaked in one liter of absolute methanol for about 2-3 days followed by filtration as above. The solvents used were recovered using a rotary evaporator (Buchi, RotorVap R model R-205, BÜCHI Labortechnik AG Flawil, Switzerland) to produce semi-solid ether and methanol extracts respectively. The two semi-solid extracts of ether and methanol were then mixed uniformly to produce a total crude extract which was left in open space at room temperature to dry completely.

2.6. Preparation of the Extracts and Control Doses for the Experimental Studies

About 1000.0 mg of the dry total crude leaf extract of *P. dodecandra* was weighed and 3 drops of dimethyl sulfoxide (DMSO) was added to facilitate the dissolution of the extract. It was then topped up with normal saline to produce a stock concentration of 1000.0 mg/ml. Serial dilutions were then made to obtain desired concentrations of the extract that were used in the study (1000.0, 600.0, 300.0, 150.0, 40.0 and 5.0 mg/ml). Normal saline (1.0 ml) was used as negative control and 10.0 mg/ml of diclofenac Na was used as positive control drug.

2.7. Selection of Animals and Criteria of Selection for Animals to Be Used in the Study

2.7.1. Inclusion Criteria

Both males and females rats which were normal as judged by their alertness and >4 weeks of age were selected. Only healthy animals were included in this study and these were judged by the veterinary doctor and also by their physical appearance, appetite for food and mental wellbeing.

2.7.2. Exclusion Criteria

The sick, pregnant animals and nursing mothers were excluded in this study basing on the ethical guidelines on the Laboratory use of animals in Biomedical research [20] [21].

2.8. Experimental Animals Handling and Treatment

Healthy male and female Wistar albino rats (100 - 200 g) were used for evaluation of the analgesic and anti-inflammatory activities of the extracts. The animals were procured from the central animal house at Makerere University College of Veterinary Medicine, Animal Resources and Biosecurity. These were safely transported to the department of Pharmacology and Therapeutics. They were treated according to the international guidelines on use of laboratory animals in Biomedical Research [20] [21]. They were acclimatized and maintained

in clean dry cages with 12 hour light and dark cycles for a period of two weeks under standard environmental conditions of ambient temperatures (25˚C - 30˚C) and relative humidity (45% - 55%). They were also allowed to access standard feed pellets and clean water ad libitum.

2.9. Experimental Procedures

2.9.1. Determination of Analgesic Activity Using Acetic Acid—Induced Writhing Test

Animals of both sexes were divided into seven groups of four animals each. And since the standard recommended number of animals is supposed to be six (6) per group to give statistical significance, in this study, four (4) animals per group were used but two sets of data were obtained from each group animals. In this way the rule of 3Rs was applied especially the *refinement* rule [22]. Two sets of the data were obtained on the same animals a week apart. Extracts and control drugs were administered orally using intragastric tube and the dosing of the animals was as follows. Group I was dosed 1.0 ml normal saline that served as negative control, group II -VI were dosed with the extract at doses of 1000.0, 600.0, 150.0, 40.0 and 5.0 mg/kg bwt respectively. Group VII was dosed 10 mg/kg bwt of the standard drug diclofenac Na. After 20 minutes, all the animals were administered intraperitoneally [18] with 0.7% v/v acetic acid (0.1 ml) [19], an irritant which induced writhes. The writhing effect was determined by the stretching of the abdomen with simultaneous stretching of at least one hind limb and twisting of the trunk [8] [16] [17] [19]. The number of writhes that occurred between 5 to 15 minutes after administration of acetic acid were recorded for each rat and the percentage protection of the extract from pain irritation was calculated as follows:

$$\text{Percent inhibition} = \left(1 - \text{Nt}/\text{Nc}\right) \times 100$$

where, *Nc* is the average number of writhes in the control group and *Nt* is the average number of writhes in the extract group.

2.9.2. Determination of Anti-Inflammatory Activity Using Histamine-Induced Paw Edema Formation

The study was carried out using inhibition of histamine-induced paw edema formation basing on standard methods and procedures [8] [16] [17] [18] [19] [23] [24]. In this study, a similar group selection was used as for the analgesic activity above. The drugs and extract were administered sub-plantarly into the left hind limb of the animals. The initial paw volume was measured using a vernier caliper, recorded and thereafter the animals were dosed with the extract and drug. Group I received 1.0 ml of normal saline as negative control. Group VII was dosed with 10.0 mg/kg bwt of the standard drug diclofenac Na. Group II-VI were dosed the extract at doses of 1000. 600.0, 300.0 and 40.0 and 5.0 mg/kg body weight respectively.

After 30 minutes of administration of the extracts and standard drug, a sub-plantar injection of 0.1 ml of 1% w/v freshly prepared histamine in normal

saline was administered in the left hind paw of the rats to induce inflammation [17] [18] [19] [23] [24]. The final paw volume was measured and recorded after 30 minutes. The increase in paw volume in the extract groups was compared with group I and then the percentage inhibition of paw edema volume was calculated using standard methods [8] [16] [17] [18] [19] as below.

$$\%\ \text{inhibition} = \left(1 - Vt/Vc\right) \times 100$$

where, Vc is the increase in paw volume of negative control group and Vt is the increase in paw volume in treated groups.

2.10. Statistical Data Analysis

Statistical analysis of the data was done using GraphPad Prism version 6 (GraphPad Software Inc., San Diego, CA, USA), and the means difference between the extracts and diclofenac Na control drug were compared using the t-test. The $P \leq 0.05$ was considered statistically significant. The results were presented in form of tables and bar graphs. The error bars on bar graphs were used to show the mean group differences for all the treatment groups at 95% confidence interval.

3. Results

3.1. Findings of the Analgesic Activity

The analgesic activity of the total crude leaf extracts of *P. dodecandra* was determined by acetic acid induced writhing method. The findings show that there was a dose-dependent reduction in the recorded mean number of writhes with increase in dose from 20.5 ± 9.8, 22.0 ± 3.0, 18.0 ± 1.5, 17.8 ± 2.3, 4.3 ± 2.3, 0.25 ± 0.38 and 19.8 ± 4.3 for doses of 5.0, 40.0, 150.0, and 600.0, 1000.0 mg/kg bwt of extract, 10.0 mg/kg of diclofenac Na and 1.0 ml of normal saline respectively. However, the mean number of writhes for 40.00 mg/kg bwt concentration was higher than that recorded for 5.0 mg/kg bwt (**Table 1**, **Figure 1**). Also a dose-dependent increment in percentage protection of 8.9%, 10.1%, 78.5% and 98.7% for extract doses of 150.0, 600.0, 1000.0 and 10.0 mg/kg bwt of diclofenac Na were observed respectively. The extract dose of 5.0 and 40.0 mg/kg bwt, however exacerbated the writhes and the findings show a negative percentage protection of −3.8% and −11.4% respectively at those doses (**Table 1** and **Figure 1**).

3.2. Findings of the Anti-Inflammatory Activity

For the anti-inflammatory activity, the findings show also a dose-dependent increment in the mean paw volume (mm) after 30 minutes of dosing from 0.22 ± 0.05, 0.08 ± 0.07, 0.34 ± 0.08, 0.51 ± 0.06 and 0.42 ± 0.10 mm for the doses of 5.0, 40.0, 300.0, 600.0 and 1000.0 mg/kg bwt respectively. However, the mean paw volume recorded for 40.0 mg/kg bwt extract was lower than that recorded for 5.0 mg/kg as well as that recorded for doses of 300.0, 600.0 and 1000.0 mg/kg bwt (**Table 2** and **Figure 2**). The standard diclofenac Na drug and normal saline produced a mean paw volume of 0.21 ± 0.05 and 0.31 ± 0.03 mm respectively.

Table 1. Analgesic activity of the total crude extract of *P. dodecandra* by usingacetic-acid induced writhing method.

Drug/extract	Dose (mg/kg bwt)	Mean no. of writhes recorded (±SEM)	P-value	Percentage protection
Total crude extract	5.0	20.5 ± 9.8	0.0061*	−3.8
	40.0	22.0 ± 3.0	0.0014*	−11.4
	150.0	18.0 ± 1.5	0.0008*	8.9
	600.0	17.8 ± 2.3	0.0012*	10.4
	1000.0	4.3 ± 2.3	0.085	78.5
Diclofenac Na	10.0	0.25 ± 0.38	0.0083**	98.7
Normal saline	1.0ml	19.8 ± 4.3	0.0083*	0.0

*P ≤ 0.05 was statistically significant as compared to diclofenac Na; SEM—Standard Error of Means. **P ≤ 0.05 was statistically significant as compared to the negative control.

Table 2. Anti-inflammatory activity of total crude extract of *P. dodecandra* on using histamine-induced paw edema.

Drug/extract	Dose (mg/kg bwt)	Mean paw volume increment after (±SEM)	P-value	%mean paw volume inhibition
Total crude extract	5.0	0.22 ± 0.05	0.5440	29.1
	40.0	0.08 ± 0.07	0.1830	74.2
	300.0	0.34 ± 0.08	0.0984	−9.7
	600.0	0.51 ± 0.06	0.0089*	−64.5
	1000.0	0.42 ± 0.10	0.0230*	−35.5
Diclofenac Na	10.0	0.21 ± 0.05	0.033***	32.3
Normal saline	1.0 ml	0.31 ± 0.03	0.033*	0.0

*P ≤ 0.05 was statistically significant as compared to diclofenac Na; SEM—Standard Error of Means; (−) Increment in % paw volume inhibition; (+) Reduction in % paw volume inhibition. ***P ≤ 0.05 was statistically significant as compared to normal saline.

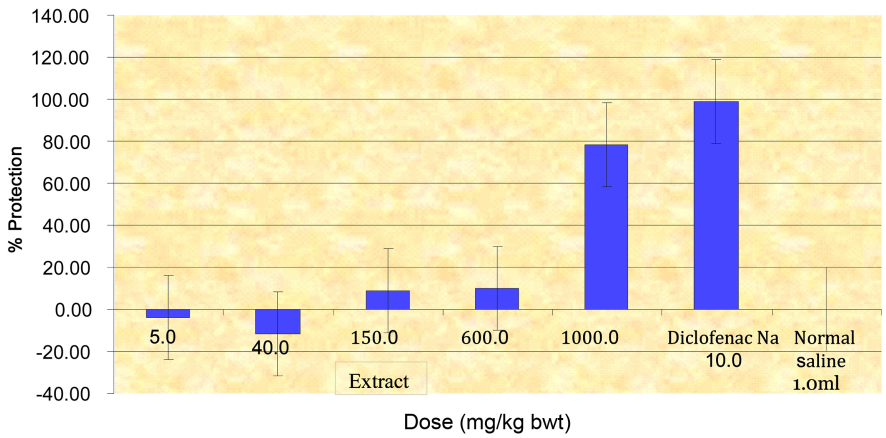

Figure 1. Percentage protection for the different concentrations of the total crude extract of *P. dodecandra* after 5 minutes of acetic acid dosing.

However, the extract dose of 5.0, 40.0 and 10.0 mg/kg bwt of diclofenac Na showed a percentage mean paw volume inhibition of 29.1%, 74.2% and 32.3% respectively while the extract dose of 300.0, 600.0 and 1000.0 mg/kg bwt exacerbated the paw volume by −9.7%, −64.5% and −35.5% respectively (Table 2 and Figure 2).

4. Discussion

4.1. Analgesic Activity

The analgesic activity of the *P. dodecandra*, the findings showed that there was a dose-dependent reduction in the mean number of writhes recorded in 15 minutes with increased concentrations of the extracts. However, the 40.0 mg/kg bwt produced a higher mean number of writhes of 22.0 ± 3.0 as compared to 20.5 ± 9.8 mean writhes for 5.0 mg/kg bwt (Table 1 & Figure 1). The observed effects may be due to the dilution effects leading to the low levels of the alkaloid, phenolics, steroids, terpenoids and saponins present in the plant that have been reported to have analgesic activity [7] [8] [14] [15]. Also, the difference could have been due to the non-uniform distribution of the active compounds in the total crude extract during the process of dissolution as well as the difference in the sensitivity of the animals to the acetic acid dosed. Also observed was a dose-dependent increment in the percentage protection produced by the total crude leaf extracts against acetic acid-induced writhing from a dose of 150.0 mg/kg to 1000.0 mg/kg bwt. The observed effects could have been due to the high levels of alkaloid, phenolics, steroids, terpenoids and saponins present in the plant that have been reported to have analgesic activity [7] [8] [14] [15]. However, the 5.0 and 40.0 mg/kg bwt doses produced a negative percentage protection, where the doses just worsened the pain and hence increased the number of writhing recorded. The increased writhes at low concentrations as opposed to the protective effect at high dose concentrations could have been due to some phytochemical compounds with acetic-acid like effects present in the herb that can easily dissolve in dilute aqueous form. On the other hand, the

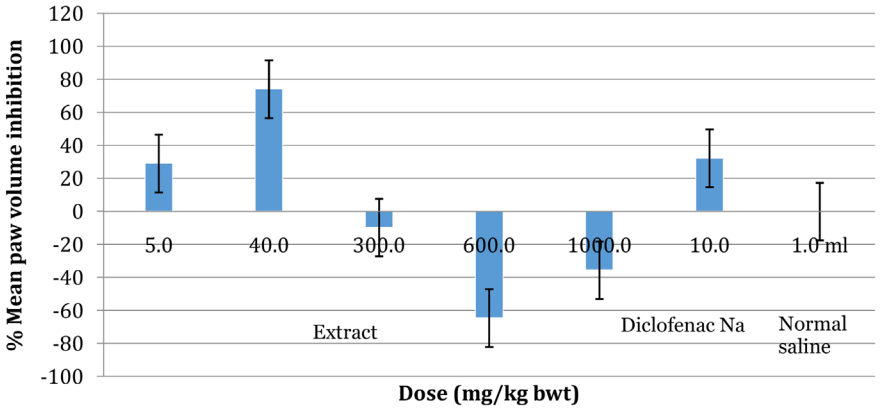

Figure 2. Percentage mean paw volume inhibition of the different concentrations of the total crude extract of *P. dodecandra* after 30 minutes of histamine dosing.

protective effect of the total crude extracts was comparable to that produced by diclofenac and this could have been due to the presence of various phytochemical compounds especially the alkaloid, phenolics, steroids, terpenoids and saponins produced by the plant in response to external aggressors especially the predators and these have been reported to have analgesic activities [11] [15] [25] [26]. And the effects observed may be due to the similarity in the mechanism of action of diclofenac Na that inhibits cyclooxygenase enzymes that are important in the prostaglandin synthesis which is one of the chemicals that mediate inflammatory conditions and pain. Various compounds in *P. dodecandra* and other members of the family like *P. americana* have been isolated and include alkaloids, flavanoids, steroids, terpenoids, tannins, phenolic compounds, glycosides and saponins [11] [15] [26]. These plants are commonly used traditionally as anti-inflammatory and analgesic agents in the treatment of arthritis and swollen throat glands [10] [11] [27] and their roots, fruits and seeds are used in the treatment of edema [10] [11] [27]. The phenolic compounds have also been reported to have analgesic activity [11] [27]. These protective effects of the extracts could be due to the reported different levels of phytochemical compounds present in *P. dodecandra* medicinal plant. The bioactive compounds may also have produced the analgesic activity by blocking the pain sensory neurons especially those mediated by A-δ and C-fibers that are responsible for nociception as well as reducing the motor neurons response from the brain [2] [25] [28] [29]. The compounds could also have caused the repolarization of the neuronal membranes or increasing the threshold of neuronal membrane depolarization and hence the reduction in the firing of the neurons leading to reduction in the nociception. Acetic acid is known to induce nociception by stimulating nociceptive fibers directly, besides promoting the release of the endogenous mediators involved in pain modulation like prostaglandins and bradykinins among others [2] [25] [28] [29]. Therefore the extract may have inhibited the activity of prostaglandins (especially PGE2) on peripheral chemosensitive nociceptive receptors. The observed effects of *Phytolacca dodecandra* could be the reason why the plant is used traditionally as anti-inflammatory and analgesic agents in the management of arthritis and swollen throat glands [11] [27].

4.2. Anti-Inflammatory Activity

The anti-inflammatory activity of the total crude leaf extracts of *P. dodecandra*, the findings show also a dose-dependent increment in the mean paw volume. The increase in the concentration of the extracts had no inhibitory effect on increment in paw volume after administration. However, it was noted that the 40.0 mg/kg bwt dose produced the lowest mean paw volume as opposed to 5.0 mg/kg bwt (**Table 2** & **Figure 2**). The observed effects could have been due to the similar compounds reported to be found in the medicinal plant [7] [8]. The higher doses produced a slightly lower effect than that of the standard diclofenac Na drug which is an anti-inflammatory drug in the class of non-steroidal anti-inflammatory drugs (NSAIDS); commonly used as a pain reliever in clinical

practice that inhibits the cyclooxygenase (COX) enzymes and hence a reduction in the production of prostaglandins, thromboxanes and leukotrienes that mediate pain and inflammation [28] [29]. In terms of the mean percentage paw volume inhibition, the lower doses of 5.0 and 40.0 mg/kg bwt produced an inhibitory effect of 29.1% and 74.2% respectively and the results were similar to that produced by the diclofenac drug. The 40.0 mg/kg bwt dose produced even better results as compared to diclofenac Na. But the higher doses had no inhibitory effect and they were even worsening the paw volume. The observed effect at low doses could have been due to the ability of anti-inflammatory bioactive compounds to dissolve well in a more dilute environment and possibly also become more active in dilute form as compared to the more concentrated form. And also the observed non-protective effect at higher doses could have been due to the increased concentration of the phytochemicals that may activate the inflammatory processes in the paw as opposed to the lower doses. Therefore, the active phytochemical compounds may have reduced the production of the pain modulator cytokines like the TNF, IL-1 and High Mobility Group Box Protein 1 (HMGB1) that are responsible for mediating the inflammatory responses [2] [25] [28] [29]. They could also have facilitated the cholinergic anti-inflammatory pathways leading to a reduction in the production of cytokines [2] [25] [28] [29]. The active compounds could also have blocked the mobilization of membrane phospholipids that leads to the production of bradykinins and other kinins as well as the activation of the arachidonate pathway and their associated enzymes such cyclooxygenase (COX 1, COX 2, and COX 3) and phospholipases that leads to production of prostaglandins, thromboxanes and leukotrienes that are inflammatory mediators [2] [25] [28] [29]. The bioactive compounds in *P. dodecandra* could also have stabilized the inflammatory cells such as mast cells, basophils, eosinophils and blood platelets that produce vasoactive chemical compounds such as histamine, bradykinins and many others [2] [25] [28] [29]. However, the observed none protective effect with some doses could have been due to the exaggeration of the above effect similar to that produced by histamine reaction. Therefore the findings have provided evidence that *P. dodecandra* contains phytochemical bioactive compounds with analgesic activity at higher doses and anti-inflammatory activity at lower doses and hence its continued use by the local communities and traditional herbalists in the management of pain due to tissue injury and inflammation due to various inflammatory disease in humans and animals.

5. Conclusion

The total crude leaf extract of *P. dodecandra* possess an anti-inflammatory activity at lower doses and the opposite is true for analgesic activity at higher doses therefore supporting the claim of the ethno-medical use of the leaves of *P. dodecandra* in the management of pain due to tissue injury and inflammation due to various inflammatory disease in humans and animals. And therefore, they

have the potential to be used as alternatives in the management of various disease conditions and their associated nociceptive and anti-inflammatory reactions.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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