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Toxicity, Anti-inflammatory and Anti-ulcer Studies of Nigerian *kalanchoe pinnata* (lam.) Plant

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Abstract *Kalanchoe pinnata (Bryophyllum pinnatum)* is one of the medicinal plants used for the treatment of many ailments in the North-central and Southern parts of Nigeria. In continuation of our studies of the pharmacological activities of the plant, the leaf, stem and root extracts were evaluated for toxicity as well as anti-inflammatory and anti-ulcer activities. Wistar albino rats were used to determine the acute toxicity, anti-inflammatory and antiulcer activities. The results of acute toxicity assay showed its LD_{50} to be greater than 5,000 mg/kg body weight, hence the doses of 250, 500, and 1000 mg/ body weight were chosen for the anti-inflammatory and anti-ulcer activity studies. The anti-inflammatory activity was evaluated using Diclofenac sodium (20 mg/kg) as the standard drug and the anti-inflammatory activity results showed that the experimental groups with the lowest test dose (250 mg/kg) of the leaf (% inhibition of 88.07 %) and root (% inhibition of 90.51 %) crude extracts exhibited significant anti-inflammatory effect at the third hour compared with the standard drug (% inhibition of 83.86 % and 79.93 % respectively) while the stem potentiated the inflammation of the egg white with % inhibition of 19.53 % compared with the standard drug Diclofenac sodium (% inhibition 52.53 %). The anti-ulcer activity examined against ethanol induced gastric ulcer in the rats used Omeprazole (20 mg/kg) as reference drug. It was observed that ulcer was inhibited by the treatment with the extracts of *K. pinnata* as compared to Omeprazole with a % protection of 81.54 – 82.11 % at 250 mg, 76.22 – 80.00 % at 1000 mg. When compared with the findings of previous studies, the results of the leaf and root extracts exhibited comparable anti-inflammatory and anti-ulcer activities while the stem extract showed lower anti-inflammatory activity.

Keywords *Kalanchoe pinnata*, Toxicity, Anti-inflammatory, Anti-ulcer, Crude plant extract

Introduction

Ulcers (gastric or peptic) occur when stomach acid damages the lining of the digestive tract as a result of any of the following: infection with *Helicobacter pylori*, use of non-steroidal anti-inflammatory drugs (NSAIDs), alcohol consumption or cigarette smoking. The damage could be through disturbance of the protection of the mucosal layer resulting in sub-mucosal erosion and inhibition of cyclooxygenase [1-2]. Ethanol has been administered to experimental rats to cause ulcers and gastric lesions which occur through impairing defense factors such as mucus

secretion, mucosal circulation and endogenous antioxidant system, and this leads to damage in gastric tissues and infiltration of inflammatory cells [3].

Inflammation can also occur as a response of an organism to protect and defend itself against foreign bodies like bacteria, other parasites and viruses; leading to local increase of plasmatic fluid and blood cells with symptoms like tissue injury, edema formation, and leukocyte infiltration [4]. Inflammation precedes the onset of major diseases, like cancer, rheumatoid arthritis, sprains, bronchitis, muscle pains, chronic inflammatory bowel disease, persistent asthma and liver fibrosis [5].

There are different types of drugs such as antacids, proton pump inhibitors and anti-histamines used for the conventional treatment management of ulcers, and in some cases of *H. pylori* infections, antimicrobials are used [6-9]. This treatment is targeted at therapeutically controlling acid secretion. The management of inflammation also uses similar treatment regimen. However, these drugs are expensive and have adverse side effects. Therefore, alternatives are sourced for in medicinal plants which exhibit biological and pharmacological activities, such as antimicrobial, anti-ulcer, anti-inflammatory and anti-cancer properties [10].

K. pinnata is used by the local residents because of its medicinal properties and has been reported to exhibit antimicrobial and antioxidant activities [11-13].

The present study is in continuation of our previous studies, and was performed to establish the protective effect of *K. pinnata* crude extracts against ethanol induced gastric ulcer and its anti-inflammatory effect in rats.

Materials and Methods

Plant material and extraction

K. pinnata (Lam) plants were collected from Yandev, Gboko LGA, Benue State and identified at the Federal college of Forestry, Jos, Nigeria. A specimen was deposited at the herbarium with voucher number FHJ 263. The fresh roots were cut, cleaned, air dried and the dried plant materials were stored in sterile containers for further use.

The crushed plant materials (leaves, stems and roots) were extracted. The leaf (300g) was macerated using 70% ethanol with frequent agitation to improve the extraction efficiency. The process was repeated for the stems and the roots to obtain yellowish green crude extract from the leaves and brownish crude extracts from the stems and roots. The percentage yields of the extracts were calculated and noted.

Animals

Wistar albino rats (70 – 120 g) of either sex were obtained from the animal house of the Department of Pharmacology, University of Jos, Jos, Nigeria. They were housed in cages under standard conditions, and fed standard rodent diet and water *ad libitum*. All the experimental protocols were duly approved by Institutional Animal Ethics Committee (Reference number: UJ/FPL/F17-00379; dated 22/02/2021).

Acute Toxicity Testing

The acute toxicity of the crude extracts of *K. pinnata* was determined to establish the dose range producing any toxic effect [14]. It involves the use of thirteen animals in 2 phases; in the first phase, nine animals were divided into three groups of three animals each and were administered 10, 100 and 1,000 mg/kg body weight of the test substances in order to establish the dose range producing any toxic effect. The number of deaths in each group was recorded after 24 hours. In the second phase, three animals which were distributed into three groups of one animal each were administered higher doses of (1600, 2900 and 5000 mg/kg body weight) the test substances and then observed for 24 hours for behaviour and mortality.

The LD_{50} was calculated as: $LD_{50} = \sqrt{D_0 \times D_{100}}$

Where D_0 is the highest non-lethal dose and D_{100} is the least toxic dose.

Anti-Inflammatory Activity

The anti-inflammatory activity of the crude extracts was studied using fresh egg white-induced paw oedema in rats [15-16]. Inflammation was produced by a subcutaneous injection of 0.1 mL of freshly prepared 10% (v/v) egg white

in normal saline into the right hind paws of the rats. The swelling of the paws were measured by digital vernier caliper in one hour intervals. The observations were tabulated, and the percentage of inhibition of paw oedema was calculated at the end of the third hour. The increase in paw thickness and percentage of inhibition in control/treatment were calculated using the following formula:

Increase in paw thickness in control or treatment group = PC or PT = Pt – Po

Percentage of inhibition in paw thickness in the treatment group = PC – PT / PC×100

Where, Pt = paw thickness at time t, Po = initial paw thickness, PC = increase in thickness of paw of the control group and PT = increase in thickness of paw of the treatment group.

Antiulcer Activity

Ethanol induced ulcer: The rats were fasted for 24 hours before the experiment. Gastric ulcer was induced by 50% ethanol given orally. Omeprazole (20 mg/kg body weight) used as standard drug. The animals were sacrificed after 1 hour and their stomachs were excised and the gastric contents were aspirated. The intensity of gastric lesions was assessed and the ulcer index was calculated [17].

Ulcer index: Scoring of ulcer was done as follows: No ulcer = 0; Superficial ulcers = 1; Deep ulcers = 2; Perforation = 3. Mean ulcer score for each animal will be expressed as ulcer index.

The percentage of ulcer protection was determined by formula:

% Protection = [(Control mean ulcer index - Test mean ulcer index)/Control mean ulcer index] 100.

Statistical analysis

The results were expressed as means ± standard deviation (SD), n = 5. The differences between groups were considered significant at $p<0.05$, using the Graph Pad Prisms Software (version 6.0, Graph Pad Software, Inc., USA).

Results and Discussion

The results of the toxicity assay as shown in Table 1, shows that the LD_{50} was above 5000 mg/ kg for all the plant's extracts (leaf, stem and root) which implies that the extracts are relatively nontoxic, and this agrees with the findings of [18]. Acute toxicity study evaluates behavioural changes in organisms on exposure to doses of a test substance within 24 hours of administration and serves as a guide in dose selection for other studies involving the use of animals [18].

Table 1: Toxicity Assay

Phase 1				**Phase 2**		
Crude Extract	**Group**	**Treatment (mg/kg)**	**Mortality/No of animals**	**Treatment (mg/kg)**	**Mortality/No of animals**	**Toxicity Sign**
Leaf	1	10	0/3	1,600	0/1	-
	2	100	0/3	2,900	0/1	-
	3	1000	0/3	5,000	0/1	-
Stem	1	10	0/3	1,600	0/1	-
	2	100	0/3	2,900	0/1	-
	3	1000	0/3	5,000	0/1	-
Root	1	10	0/3	1,600	0/1	-
	2	100	0/3	2,900	0/1	-
	3	1000	0/3	5,000	0/1	-

The data in Table 2 and Fig 1, suggest significant anti-ulcer activity of *K. pinnata* plant extracts against ethanol induced gastric ulcers in rats as compared to the standard drug (Omeprazole). The % protection offered (Fig. 1) by 250 mg/kg body weight of the leaf extract (81.54 %) was slightly greater than that produced by standard drug

Omeprazole (79.46 %), indicating that the extract could probably be more potent than Omeprazole. The % protection offered by the stem (82.70 %) and root (82.11 %) extracts were slightly lower as compared to that of Omeprazole (91.35 % and 86.32 % respectively). This could be attributed to the phytoconstituents of the plant and the results are similar to the findings of previous studies [20-21]. The results of this study show that *K. pinnata* plant extracts exhibited significant anti-ulcer activity comparable with the standard drug.

Table 2: Anti-ulcer Activity

Crude Extracts	**Treatment**				
	Mean Ulcer Index (mm) / % Protection				
			Crude Extracts (mg/kg)		
	Control	**Standard drug**	**250**	**500**	**1000**
Leaf	18.50 ± 2.12	3.80 ± 3.03 (79.46 %)	3.60 ± 1.82 (81.54 %)	9.20 ± 10.10 (50.27 %)	4.40 ± 2.88 (76.22 %)
Stem	18.50 ± 0.71	1.60 ± 1.14 (91.35 %)	3.20 ± 1.48 (82.70 %)	7.60 ± 10.40 (58.92 %)	3.00 ± 2.00 (83.78 %)
Root	19.00 ± 2.83	2.60 ± 1.67 (86.32 %)	3.40 ± 2.41 (82.11 %)	7.80 ± 10.40 (58.95 %)	3.80 ± 2.17 (80.00 %)

Values are mean ± S.D. (n = 5). P > 0.05

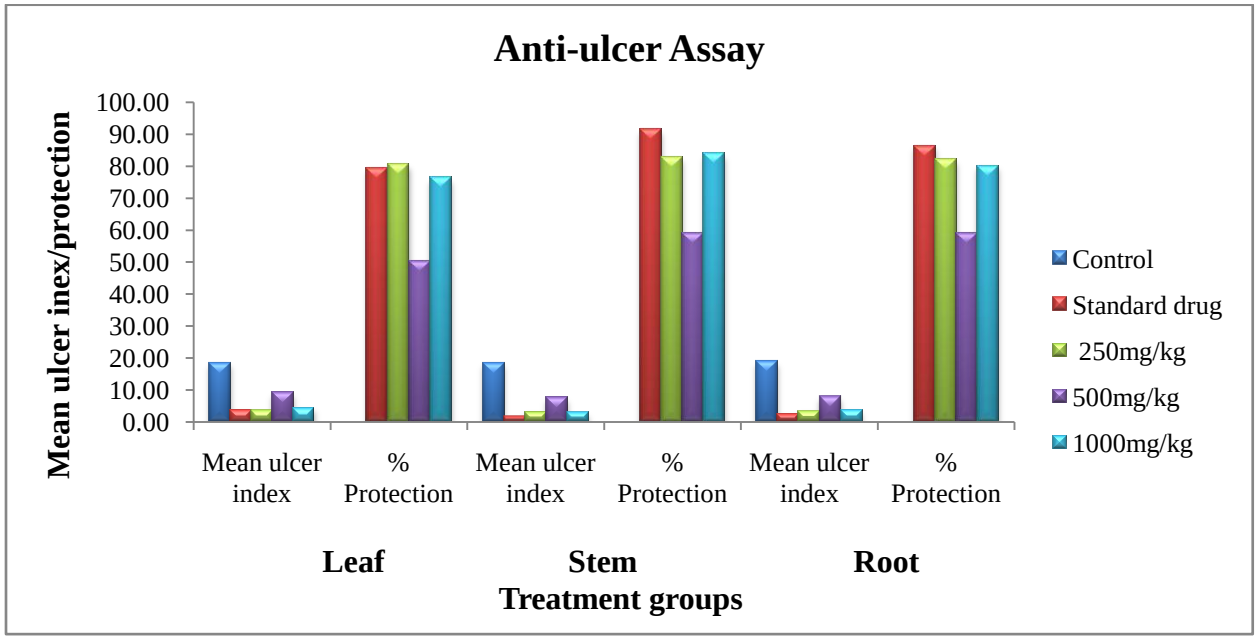

Figure 1: Effect of plant extracts (leaf, stem, root) and standard drug on ulcer surface

From the results shown in Table 3 and Figs 2 & 3, the induced paw edema in rats gradually increased up to two hours then were noticeably reduced especially with the leaf and root extracts, suggesting the release of inhibitory inflammatory mediators such as histamine, serotonin, polypeptides or prostaglandins while the stem extract potentiated the inflammation caused by the egg white, suggesting that it contains pro-inflammatory agent [22]. In Fig. 3, the experimental groups with the lowest test dose (250 mg/kg) of the leaf and root crude extracts exhibited a significant anti-inflammatory effect expressed as percentage inhibition (88.07 % and 90.51 % respectively) at the third hour ($p < 0.05$); the activity was higher than observed for the standard drug Diclofenac sodium (20 mg/kg) with % inhibition of 83.86 % and 79.93 % respectively, while the experimental groups with higher doses (500 and 1000 mg/kg) exhibited comparable effect with the standard drug. The stem extract showed the least inhibitory effect (19.53 %) lower than the value observed for the standard drug (% inhibition 52.53 %). The anti-inflammatory

activities of the leaf have been reported [23-25], however, a weak anti-inflammatory effect of the plant's leaf has been reported [18] while the present study reports the anti-inflammatory activities of the plant's leaf stem and root. Inflammatory related diseases are gradually becoming a major healthcare problem despite accessible treatments due to adverse effects from prolong usage the drugs. Alternatives are sourced for in medicinal plants which exhibit little or no adverse effects in the management of inflammatory diseases [4].

Table 3: Anti-inflammatory Activity

Treatment	Increase in mean paw oedema (mm)				% Inhibition
	0 hr	1 hour	2hours	3hours	
Leaf					
Control	1.10 ± 0.52	2.68 ± 0.33	2.78 ± 0.21	2.85 ± 0.19	-
Standard drug	1.30 ± 0.41	2.51 ± 0.56	1.01 ± 0.50	0.46 ± 0.54	83.86
250 mg/kg	1.64 ± 0.25	1.59 ± 0.31	0.88 ± 0.43	0.34 ± 0.40	88.07
500 mg/kg	1.16 ± 0.20	1.76 ± 0.58	1.40 ± 0.52	1.00 ± 0.50	64.91
1000 mg/kg	1.39 ± 0.14	2.35 ± 0.60	2.26 ± 0.33	1.82 ± 0.55	36.14
Stem					
Control	1.13 ± 0.55	1.98 ± 0.54	2.35 ± 0.56	2.56 ± 0.57	-
Standard drug	1.07 ± 0.48	1.50 ± 0.51	1.51 ± 0.63	1.22 ± 0.30	52.53
250 mg/kg	1.55 ± 0.26	2.19 ± 0.69	2.57 ± 0.92	2.06 ± 0.77	19.53
500 mg/kg	1.00 ± 0.21	2.81 ± 0.76	2.57 ± 0.90	2.65 ± 0.44	-3.52
1000 mg/kg	1.33 ± 0.30	2.20 ± 0.45	2.43 ± 0.41	2.29 ± 0.37	10.55
Root					
Control	0.85 ± 0.27	1.28 ± 0.35	2.32 ± 0.33	2.74 ± 0.37	-
Standard drug	0.77 ± 0.31	1.75 ± 0.42	1.23 ± 0.37	0.55 ± 0.46	79.93
250 mg/kg	0.93 ± 0.59	1.40 ± 0.78	0.81 ± 0.76	0.26 ± 0.73	90.51
500 mg/kg	1.30 ± 0.16	2.02 ± 0.73	1.50 ± 0.64	0.85 ± 0.95	68.98
1000 mg/kg	1.34 ± 0.17	2.11 ± 0.50	1.65 ± 0.40	1.05 ± 0.45	61.68

Values are mean ± S.D. (n = 5). P > 0.05

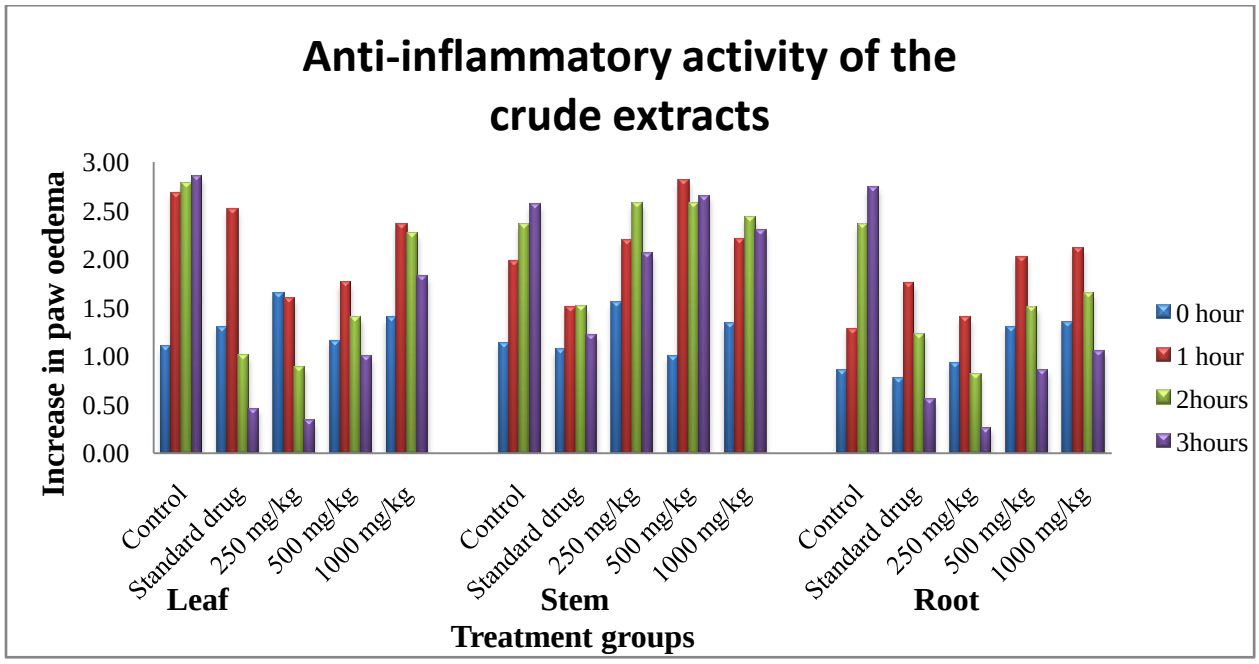

Figure 2: Increase in mean paw oedema of the leaf, stem, and root crude extracts at various time intervals

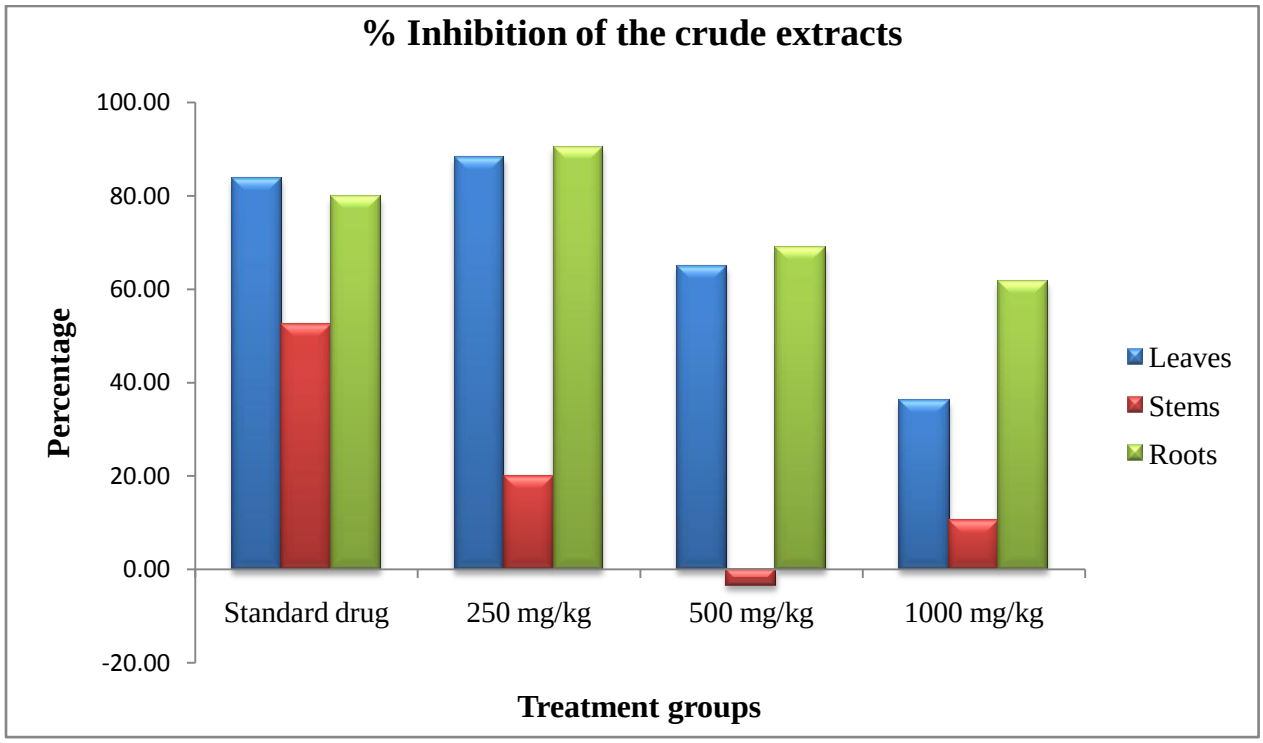

Figure 3: % Inhibition of the leaf, stem, and root crude extracts at the third hour

Conclusion

The experimental evidence obtained in this study indicates that the ethanolic crude extracts of the plant possess anti-inflammatory and anti-ulcer properties. The results show that the leaf and root extracts exhibited anti-ulcer and anti-inflammatory activities while the stem extract only showed anti-ulcer activity. This further provides scientific evidence in support of the reported folkloric uses of the plant's leaf in the management of inflammatory and ulcerogenic conditions. However further studies are ongoing toward the isolation and characterization of the active compound(s) of the plant.

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