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CURATIVE STUDY ON THE ACTIVITY OF METHANOLIC EXTRACT OF *ACALYPHA WILKESIANA* (COPPER LEAF) IN ASPIRIN INDUCED GASTRIC ULCERATION IN SPRAGUE DAWLEY RATS.

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ABSTRACT
This study was undertaken to evaluate the activity of methanolic extract of *Acalypha Wilkesiana* in aspirin induced gastric ulceration in Sprague dawley rats. Thirty adult Sprague dawley rats weighing between 160-240g were divided into six groups consisting of five rats each. Gastric ulceration was induced with aspirin (200mg/kg). Groups I- received no treatment, while groups II-VI received various treatments consisting of aspirin only (200 mg/kg), aspirin (200mg/kg with *Acalypha wilkesiana* (100 mg/kg 200mg/kg and 300mg/kg) and *Acalypha wilkesiana* (300mg/kg). At the end of the experiment (day 7) rats were sacrificed and their stomachs were harvested and fixed in 10% formalin solution. Ulcer index, size, number, gastric acid secretion and the histological changes were observed. The reduction in ulcer index was stepwise as the dose of the extract increased. Administration of *Acalypha wilkesiana* had no significant effect on gastric acid and gastric volume. Post treatment with *A. wilkesiana* reduced the severity of aspirin induced gastric ulcer. Cimetidine (standard drug 20mg/kg) was used to treat ulcer also and compared with the extract. Methanolic extract of *A. wilkesiana* at a dose of 200mg/kg and 300mg/kg reduced ulcer incidence when compared to the control group. This work suggests that the *Acalypha wilkesiana* has antiulcer potentials due to the presence of tannins and saponins.

KEYWORDS: *Ulcer, Acalypha wilkesiana, aspirin, stomach.*

INTRODUCTION
An ulcer is basically an inflamed break in the skin or mucus membrane lining the alimentary tract.[1] Ulceration occurs when there is a disturbance of the normal equilibrium caused by either enhanced aggression or diminished mucosal resistance.[2] Peptic ulcer disease is one of the most common gastrointestinal disorders, which causes a high rate of morbidity particularly in the population of non-industrialized countries.[3] Peptic ulcers are a broad term that includes ulcers of digestive tract in the stomach or the duodenum. The formation of peptic ulcers depends on the presence of acid and peptic activity in gastric juice plus a breakdown in mucosal defenses.[4] The gastric mucosa is continuously exposed to potentially injurious agents such as acids, pepsin, bile acids, and bacterial products (*Helicobacter pylori*) and drugs.[5]

Despite the decline in the incidence of peptic ulcer disease in the recent years, the economic burden, morbidity and mortality due to the disease are massive.[6] The efficacy of the agents used to treat peptic ulcer are marred by their numerous adverse effects which include gastrointestinal dysfunction, mental state changes and an increased risk of respiratory/enteric infections.[7] With all these aforementioned effects, it is therefore necessary to find a safer and affordable alternative for the treatment of peptic ulcer.

Medicinal plants are believed to be important sources of new chemical substances with potential therapeutic effects.[8] Natural medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases, though relatively little knowledge about their mode of action is available. There have been reports that a vast majority of the population particularly those living in villages depend largely on herbal medicines.[9] Before the availability of synthetic drugs man was completely dependent on natural medicinal plants for curing diseases.[10] These plants, which abound in our environment, enjoy wide acceptability by the population and serve as cheaper alternatives to orthodox medicine.[11]; [12]

Acalypha wilkesiana (copper leaf) is a large genus of herbs and shrubs native to the tropics and subtropics.[13] A number are grown as ornamental plants either for their attractive foliage or for their showy inflorescence. It belongs to the family of Euphobiacea. *Acalypha wilkesiana* has been shown to have anti-bacterial and anti-fungal properties.[14] The pressed juice or boiled decoction is used for the treatment of gastrointestinal disorders and fungal skin infections.[15]; [16]; [17] This present study was therefore designed to investigate the activity of methanolic extract of *Acalypha Wilkesiana* in aspirin induced gastric ulceration in Sprague dawley rats.

MATERIALS AND METHOD

Experimental animals

Thirty Sprague dawley rats (160 to 240g) of both sexes were procured from the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria. They were fed with commercial pellet diet and water *ad libitum*. All animals were acclimatized to the laboratory environment for two weeks before the commencement of experiment. All procedures described were reviewed and approved by the institutional committee for ethical use of animals.

Collection of Plant Samples and Preparation of Plant Extract

Samples of fresh leaves of *Acalypha wilkesiana* were collected from within the Choba Campus of University of Port Harcourt, Port Harcourt, Nigeria. After due identification at the University of Port Harcourt Herbarium, Port Harcourt, Nigeria, they were cleaned and their leaves removed. The leaves were air dried and ground into powder and 140g of the powdered sample was exhaustively extracted with 420ml of methanol (analytical grade) for 12 h, after which the resultant mixture was filtered and solvent was removed at 55^{o}C using a water bath to give a dark green solid extract weighing 49g.

Phytochemical Screening

The methanolic extract of *Acalypha wilkesiana* were tested for the presence of flavonoids, tannins, carbohydrates/ glycosides, saponins, resins, terpenoids and alkaloids using standard procedures as described by Trease and Evans.[18]; [19]

drugs and chemicals

Aspirin was purchased from Juhel Pharmaceutical Industries and Emzor Pharmaceutical Industries respectively. All other reagents used for the experiment were of analytical grade.

assessment of `antiulcer activity

The antiulcer activity of *A. wilkesiana* was evaluated using aspirin induced ulceration model according to the method of Wang *et al*., 2011.[20] The rats were fasted for 48 hours but allowed free access to water *ad libitum*. They were randomly selected and divided into six groups of 5 rats each. Ulceration was induced in the stomach of rats fasted for of 24 hrs. All the animals were sacrificed 2hr after the last administration of extract (day 7). Ulcer were scored according to the arbitrary scale used by Singh *et al* where 0 = no lesion, 0.5 = hyperaemia, 1 = one or two slight lesions, 3 = very severe lesions, 4 = mucosa full of lesions.[21] Ulcer index was calculated as mean ulcer scores.[22]

ulcer indexing (ui)

The dissected stomachs were cut open along the greater curvature and the inner surface was examined for ulceration. The open stomachs were studied by individuals who were blind to the test drugs and control animals.[23]

ulcer number

Total numbers of ulcers in each stomach were noted and petechial hemorrhage congestion, etc. was also noted.

ulcer size

With the help of a magnifying lens the size of each ulcer was measured. Mean ulcer score for each animal was expressed as ulcer index. The percentage of ulcer protection was determined as follows according to the method of Dashputre and Naikwade; 2011.[24]

$$\% \text{ inhibition of ulcer index} = \frac{\text{Control mean ulcer index} - \text{Test mean ulcer index}}{\text{Control mean ulcer index}} \times 100$$

gastric acid secretion

The gastric acid secretion of the rats administered aspirin, the extracts or saline were collected after the pylorus was ligated in accordance with the method used by Nafeeza *et al*. 2002.[25] The content of the stomach was washed using saline, and centrifuged at 3000 rpm for 5 min. The clear supernatant was titrated against 0.01mol/L solution of sodium hydroxide (NaOH) at pH 7.0. The acid content of the stomach was calculated according to the method of Shay *et al.* (1954)[26] and expressed as MEq/L.

gastric volume

10mls of distilled water was used to wash the stomach content out and into a 10ml measuring cylinder. A rise in the initial volume was recorded and served as the gastric volume.

histological studies

A portion of the ulcer region in the stomach was dissected out and fixed in 5% buffered neutral formalin solution for histological observations. After fixations, tissues were embedded in paraffin; solid sections were cut at 5 µm and stained with hematoxylin and eosin. The sections were examined with the help of a light microscope and photomicrographs were taken.

statistical analysis

Mean values ± S. E. M. were calculated for each parameter. For the determination of significant

intergroup differences, each parameter was analyzed separately and one-way analysis of variance (ANOVA) was carried out. $p<0.05$ was considered significant.

RESULT AND DISCUSSION

qualitative phytochemical analysis of acalypha wilkesiana

The preliminary qualitative phytochemical screening of the leaf of A. *wilkesiana* showed the presence of important phytochemical constituents as summarized in Table 1.

Table 1: Result of the Preliminary Qualitative phytochemical analysis of *Acalypha wilkesiana.*

ACTIVE INGREDIENTS	METHANOLIC EXTRACT
Tannins	+++
Saponins	++
Resins	-
Alkaloids	+
Glycosides	+
Flavonoids	+
Steroids and Terpenoids	+
Protein	-
Carbohydrates	+

+ = slightly Present, ++ = moderately Present, +++ =highly present, - = Not detected.

The result of the phytochemical analysis obtained from the methanolic leave extract of *A. wilkesiania* indicated a high level of tannins and saponins.

quantitative phytochemical analysis of *acalypha wilkesiana*

The result of the preliminary quantitative analysis for Flavonoids, flavonol, phenol and tannins is shown in Table 2. The results showed that the methanolic extract of *A. wilkesiana* had high level of tannins and phenol content (0.066±0.024 and 0.331±0.024 mg gallic acid equivalents (GAE)/ mg dry weight plant extract) respectively.

Table 2: Result of the Preliminary Quantitative Phytochemical Screening of *Acalypha wilkesiana*

Active ingredients/metabolite	Methanol extract
Flavonoids $^{++}$	0.0506±0.035
Flavonol $^{++}$	1.575±0.044
Phenol *	0.331±0.024
Tannins *	0.066±0.024

Data represented as Mean+- SEM of triplicate analyses
**Expressed as mg gallic acid equivalents (GAE)/ mg dry weight plant extract*
$^{++}$Expressed as mg quacitin equivalents (QE)/ g dry weight plant extract

antiulcer studies

The effects of *Acalypha wilkesiana* on Aspirin induced mucosal ulceration are in Table 4. The result shows that *Acalypha wilkesiana* significantly reduced the ulcer index from 2.32±0.26 to 0.60±0.15. The reduction in ulcer index was stepwise as the dosage of the extract increased. Administration of *Acalypha wilkesiana* had no significant reduction in the amount of gastric acid and the gastric volume.

Table 3. Effect of methanolic extracts of *A. wilkesiana* against Aspirin induced gastric ulcer in rats.

TREATMENT	DOSE (mg/kg)	ULCER INDEX	% INHIBITION (%)	pH	GASTRIC VOLUME GV (ml)
Control	200	2.32±0.26	–	4.53±0.36	5.54±0.07
Low dose	100	1.30±0.15*	5	4.57±0.16	5.42±0.27
Medium dose	200	0.90±0.44*	27	4.63±0.09	5.46±0.07
High dose	300	0.60±0.15*	59.57	4.62±0.12	5.44±0.17
Standard Cimetidine	20	1.04±0.68*	25	4.63±0.21	5.54±0.13

Results are mean ± S.E.M. (n = 5). Statistical comparison was performed by using ANOVA coupled with student't' test.
** P<0.05 were consider statistically significant when compared to control group.*

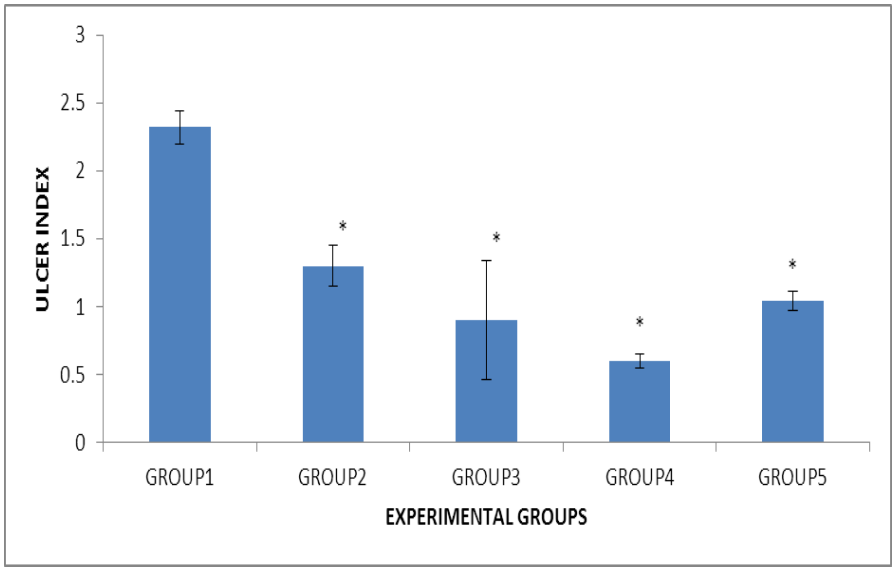

Figure 1: effect of methanolic extracts of *a. wilkesiana* against aspirin induced gastric ulcer in rats

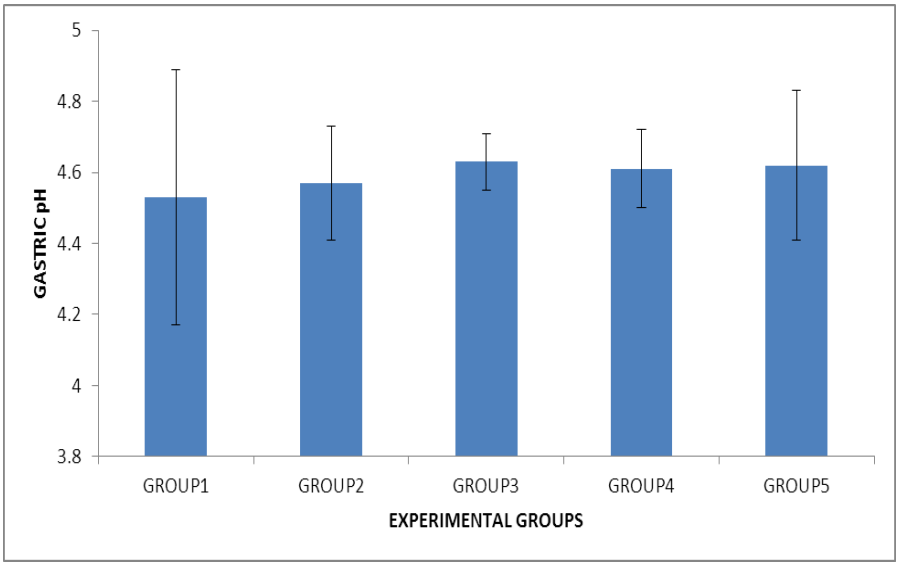

Figure 2: effect of extract, aspirin and cimetidine on ph of wistar rats

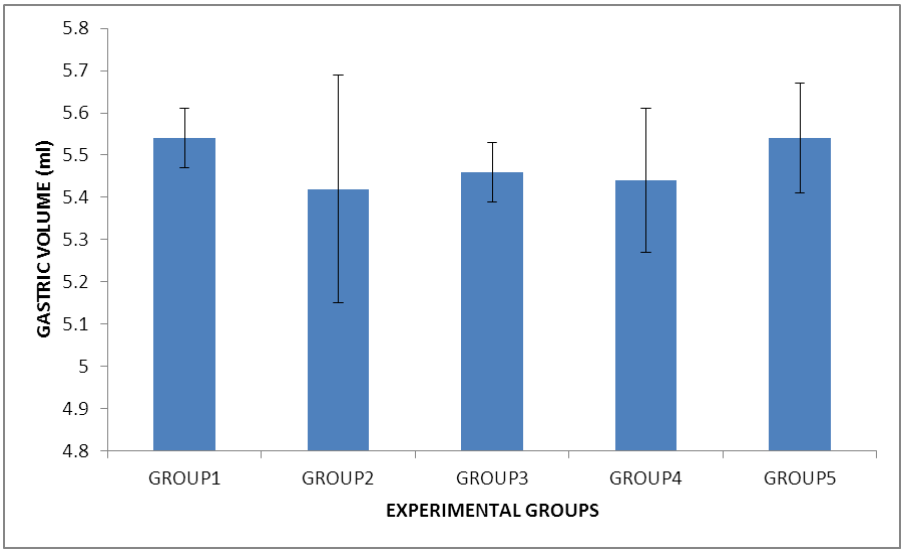

Figure 3: effect of extract, aspirin and cimetidine on gastric volume (ml) of wistar rats

macroscopical view of aspirin-induced ulcer

Figure 4 shows the representative stomachs of rats after aspirin induction of gastric ulcer. Administration of aspirin (200mg/kg) produced superficial or deep erosions, bleeding, and antral ulcers. However, post treatment with cimetidine and *A. wilkesiana* reduced the severity of aspirin induced gastric ulcer.

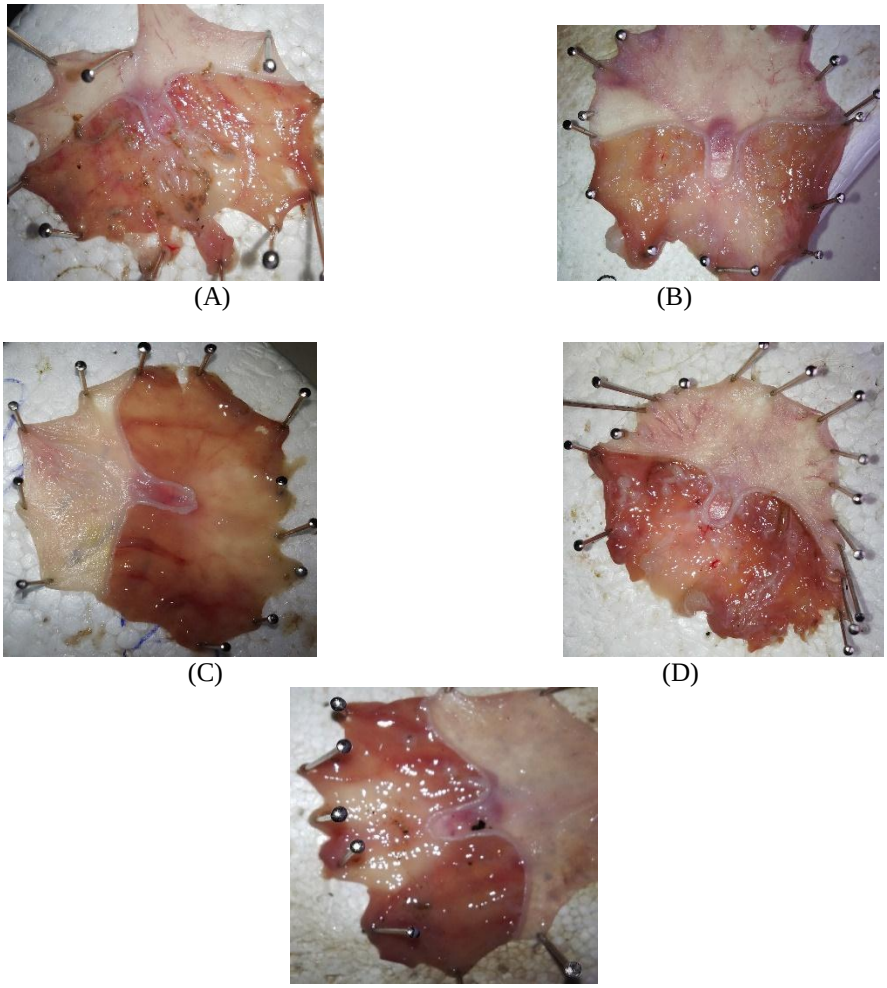

Figure 4. anti-ulcer activity of methanolic extract obtained from a. wilkesaina leaves. a: stomach of an ulcer control rat showing larger ulcer area; b: stomach of a rat treated with cimetidine; c: stomach of rats treated with extract 100mg/kg; d: stomach of rat treated with 200 mg/kg; e: stomach of rat treated with extract 300mg/kg.

The etiology of peptic ulcer is unknown in most cases, yet it is generally accepted that it results from an imbalance between aggressive factors and the maintenance of mucosal integrity through the endogenous defence mechanisms. To regain the balance, different therapeutic agents including plant extracts may be used.[27] *Acalypha wilkesiana* is one such herbal drug used in this present study to evaluate its anti-ulcer property. In most of the cases, non-steroidal anti-inflammatory drugs like indomethacin and aspirin are known to induce numerous punctiform and filiform gastric ulcers during the course of anti-inflammatory therapy and hence, aspirin induced model was used in the present study.[28]

Aspirin is a nonsteriodal anti-inflammatory drug which induces ulcers by inhibiting prostaglandin synthesis in the stomach by blocking the cyclooxygenase enzymes.[29] Nonsteroidal anti-inflammatory drugs also cause an inflammatory response increasing the reactive oxygen species in the gastric mucosa.[30]

The result of the phytochemical analysis obtained from the methanolic leave extract of *A. wilkesiania* indicated the presence of tannins, saponins, flavonoids, Alkaloids, Glycosides, Steroids, Terpenoids and Carbohydrates. In this present study the methanolic extract of *Acalypha wilkesiana* was investigated for its anti-ulcer effect on aspirin induced ulcers in wistar rats. The result shows that *Acalypha wilkesiana* significantly reduced the ulcer index from 2.32±0.26 to 0.60±0.15. The reduction in ulcer index was stepwise as the dosage of the extract increased. A reduction in the ulcer index signifies the anti-ulcer property of the methanolic extract of *Acalypha*

wilkesiana. This can be ascribed to the presence of tannins in the methanloic extract of *Acalypha wilkesiana*. Tannins react with the proteins of the tissue layers. They precipitate micro proteins at the site of the peptic ulcer, forming a protective pellicle that prevents absorption of toxic substances, and promote resistance to the action of proteolytic enzymes.[31] Saponins also have a beneficial effect on aspirin induced ulcers, due to the fact that plant extracts containing saponins have been patented for the prevention and/treatment of a variety of conditions including gastric ulcer and duodenal ulcer.[32] Flavonoids are among the cytoprotective materials for which antiulcerogenic efficacy has been extensively confirmed.[33] Therefore the anti-ulcer activity of *Acalypha wilkesiana* can also be attributed to the flavonoid content. Administration of *Acalypha wilkesiana* had no significant reduction in the amount of gastric acid and the gastric volume. The results of the present study suggest that the methanolic extract of Acalypha wilkesiana leaves may be valuable in the treatment of peptic ulcer. Further studies to identify the active moieties and elucidation of the mechanism of action are recommended.

CONCLUSION

Methanolic extract of *A. wilkesiana* at dose 200mg/kg and 300mg/kg reduced ulcer incidence when compared to the control group as evident by decrease in ulcer index in the above model. There was no decrease or significant change in gastric volume and gastric pH. The extract was found to possess antiulcer activity due to presence of tannins and saponins in it and thus justifies the local uses of the plant for the treatment of ulcers in humans.

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