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RESEARCH ARTICLE

Gastroprotective Properties and Phytochemical Constituents of Leaf Methanol Extract of *Phyllanthus discoideus* on Diclofenac-Induced Gastric Ulcers in Rats

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ABSTRACT

Medicinal plant studies are important for the identification of potential therapeutic agents. Leaves of *Phyllanthus discoideus* are used in African traditional medicine as anti-inflammatory agents and also as an analgesic. This study aimed to screen the phytochemical compounds present in *Phyllanthus discoideus* leaves and subsequently evaluate the gastroprotective activity of the leaves' methanolic extract using diclofenac-induced gastric ulcer model in rats. This study employed a laboratory-based experimental study design. The leaves of *Phyllanthus discoideus* were collected, authenticated, air-dried. Solvent extraction was done using cold maceration in methanol to obtain the crude methanol extract. Liquid - liquid fractionation was used to obtain different fractions. Acute toxicity of the extract was determined using the Lorke's method. Preventive and curative methods were used for the gastro-protective activity study. For the preventive method, the animals were treated with 400 mg of the crude extract and fractions for seven days followed by induction of gastric ulcerwith diclofenac (100 mg). The animals were then sacrificed and the ulcer scores and percentage (%) preventive index determined. In the curative method, diclofenac (100 mg) was administered to the animals to induce ulcer in the rats followed by treatment with the extract and fractions of the plant. Thereafter, the animals were sacrificed and their ulcer index and % curative index determined. The results indicated that P. discoideus exhibited gastro-protective effect against Diclofenac- induced gastric ulcer. For both curative and preventive method, treatment with butanol fraction gave the highest gastro-protective effect with percentage curative index of 93.33% (ulcer index=0.2) and percentage preventive index of 88.88% (ulcer index=0.4). Preliminary phytochemical screening conducted showed the presence of alkaloids, flavonoids, tannins, steroids, and saponins. The study demonstrated that the leaves of *Phyllanthus discoideus* has potential as an alternative treatment and preventive measure for gastric ulcers.

Keywords: *Phyllanthus discoideus leaves, acute toxicity, phytochemical screening, gastro-protective activity*

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INTRODUCTION

Gastric ulcer is a break in the tissue lining of the stomach. Most ulcers can be cured without complications (Singh et al., 2023). In some cases, however, peptic ulcers can develop, such as in penetration, perforation, bleeding (hemorrhage), and obstruction (Kumadoh et al., 2021). Gastric ulcer is the most common form of peptic ulcer and the most predominant of the gastrointestinal diseases (Lakshmi et al., 2010). It is characterized by lesions in the gastric mucosa, resulting from an imbalance between aggressive factors like acid and pepsin, and protective mechanisms such as mucus and bicarbonate secretion, alongside prostaglandin synthesis (Belmamoun et al., 2023). It is a chronic and recurrent disease, with multi-etiopathological factors. Stress, smoking, Helicobacter pyloris infection and ingestion of non-steroidal anti-inflammatory drugs (NSAID) augment the gastric ulcer incidences (Vonkeman et al., 2007). Free radicals, particularly reactive oxygen species (ROS) have also been implicated in the mechanism of acute and chronic ulceration in the gastric mucosa (Alotaibi, 2023). This gastrointestinal disorder is prevalent, impacting nearly 10% of the world's populace, and also associated with considerable morbidity and economic burden (Jabbar et al., 2023). Despite the decline in the incidence of peptic ulcer disease (PUD) in the recent years, the economic burden, morbidity, and mortality due to the disease are still massive (Kiran, D. et al., 2022).

A common approach to manage gastric ulcer disease is the inhibition of gastric H^+/K^+-ATPase and the elimination of H. pylori using antibiotics (Nartey et al., 2012). Additionally, scavenging of ROS and the stimulation of the endogenous antioxidant enzymes in the stomach is also commonly employed. Despite the availability of conventional treatments like proton pump inhibitors and H2-receptor antagonists, these therapies often present undesirable side effects, including altered biochemical mechanisms with chronic use (El-dien et al., 2020). Consequently, the search for novel, safer, and more effective therapeutic agents, particularly from natural sources, has become a major scientific push in recent years (Jabbar et al., 2023). Traditional medicine, particularly phytotherapy, offers a rich supply of potential anti-ulcer agents with diverse mechanisms of action, leading to rigorous scientific evaluation of plant phytochemical extracts for gastroprotective efficacy (Sharifi-Rad et al., 2018). This necessitates studies into ethnopharmacological leads to identify compounds that can effectively counteract ulcerogenic mechanisms with reduced adverse effects (Karunakaran et al., 2017). For instance, various plant extracts have demonstrated significant anti-ulcer activity by modulating gastric acid secretion, enhancing mucosal defense, and exhibiting anti-inflammatory properties (Halim et al., 2017).

Medicinal plants have also been used in traditional medicinal practices since prehistoric times (Shady et al., 2022). Many people in developing countries still rely on traditional healing practices and herbal medicine for their healthcare, in spite of the advancement in conventional medicine (Nabil et al., 2021). With the variety of climate and vegetation, African herbal medicine forms an important part of the communal culture (Erinoso and Aworinde, 2018). Medicinal plants are used at an early stage of the disease at low cost and can conveniently replace the indiscriminate consumption of drugs without prescription (Baker, D. A. 2020). Emerging research suggests that conventional pharmacological interventions for peptic ulcers may face challenges such as increasing resistance to antibiotics used against Helicobacter pylori, necessitating the development of novel, safer, and more effective therapies from medicinal plants (Kumadoh et al., 2021).

Phyllanthus discoidea (Baill.) G. L. Webster (Euphorbiaceae) is a discoideus tree which can grow up to 30 m tall depending on its location. It is widely distributed in African region where it is used for treatment of various ailments (Ahmed et al., 2021). In Nigeria and Ghana, the decoction of the bark is used traditionally for the treatment of wounds and ulcers. Previous pharmacological studies on P. discoideus have shown that the plant displays acaricidal ((Ahmed et al., 2021)), anti-inflammatory and analgesic (Adedapo et al., 2009), filaricidal (Cho-Ngwa et al., 2010) and cytotoxic activities (Johnson-Ajinwo et al., 2015). This present study focuses on evaluating the phytochemical composition and gastroprotective efficacy of *Phyllanthus discoideus* leaf methanol extract in mitigating diclofenac-induced gastric ulcers in a rat model, thereby providing scientific data to support its traditional medicinal use. To achieve this, the methodology of this study was designed to carefully assess the gastroprotective potential of *Phyllanthus discoideus* phytochemical extracts as a viable alternative (Ahmed et al., 2021). This approach involves in-vivo study using a diclofenac-induced gastric ulcer model in rats. This allowed for detailed examination of ulcer inhibition, gastric secretion modulation, and histopathological changes, providing comprehensive insights into the extract's efficacy (Jabbar et al., 2023).

METHODS

Materials

Solvents, Chemicals and Reagents

Methanol (analytical grade), Ethyl acetate (BDH Chemicals LTD, Poole England), n-Hexane (JHD) by GHTECH Guandong Guanghua Sci-Tech., Co., LTD., Normal Saline (JUHEL) by JUHEL Nigeria Ltd Anambara Nigeria, Distilled water (Pharmaceutical chemistry laboratory, Enugu state University of Science and Technology, Nigeria), Safe drinking water [(Aqua Rapha drinking water) by Aqua Rapha Investment Nigeria Ltd., Enugu Nigeria], Mercuric iodide, potassium iodide, Hydrochloric acid, Sulphuric acid, Ferric chloride, Diclofenac sodium [(Clofenac 100mg) by Hovid Bhd. Ipoh Malaysia], Omeprazole 40mg (Mecure Industries Ltd., Lagos Nigeria), Acetic acid, Sodium hydroxide, Tween 80, Picric acid, Gentian violet (MOKO) by New-Health Co. Ltd Lagos Nigeria.

Equipment

Porcelain mortar and pestle, water bath (SWB 1002 series) by Stuart, Rotary evaporator (model 320 HNXZIB), Conical flasks (ADARSH), Muslin cloth, Dissecting board and kit, Test tubes, spatulas, Metal (aluminum) animal cages (locally made), Refrigerator (Nexus NX-235), Glass bottle container (200 ml), Filter paper (Whatman No. 1 by GE Healthcare UK Ltd), Hand gloves (AGARY) by Sari Trang Agro-Industry Public Co. Ltd Thailand), Measuring cylinder, Separately funnel (500ml), Beakers (100ml, 250ml, 500ml) (ADARSH, BURTECH) , Glass stopper conical flask (ADARSH), Foil, Retort stand and clamps, Glass funnel, Syringes (1ml, 2ml, 5ml)[Frantastik Ject Anhui Kangning Industrial (Group) Co., Ltd], Electronic weighing balance (S.METTLER 221).

Experimental animals

Adult albino Wister rats of either sex weighing about 75-225g were obtained from Enugu State University of Science and Technology, Agbani, Enugu, Nigeria. They were allowed to acclimatize to animal house standard condition prior to the study.

Methods

Plant Collection, Authentication and Preparation

The fresh young leaves were collected from Ezza in Ebonyi State, Nigeria. The plant was authenticated by Mr. A. Ozioko, a taxonomist with the Bioresources Development and Conservation Programme (BDCP) centre in Nsukka. A herbarium: (PCG/125/A/024) was deposited at the Department of Pharmacognosy Enugu State University of Science and Technology. The clean leaves were shade dried for three weeks under room temperature.

Extraction and fractionation

Extraction and fractionation were carried out according to the method of Ali et al. (2025). Cold maceration was used to extract 2 kg of P.discoideus leaves using 95% methanol. The coarsely powdered leaves of P. discoideus were macerated in 1 litre of methanol for 48 hours with intermittent agitations. After 48 hours, the filtrate was separated from the marc by filtration using Whatman (no.1) filter paper. The crude extract was concentrated in a rotary evaporator at 40 ºC. Liquid-liquid fractionation of the crude methanol extract was carried out using n-hexane, ethyl acetate, n-butanol and water to get fractions of the extract soluble in those solvents.

Preliminary phytochemical analysis of Phyllanthus discoideus leaves

The crude methanol extract was subjected to qualitative analytical tests to evaluate the constituents present in the extract. The standard analytical phytochemical screening procedure below as described by Ahmed et al., (2021) and Ibrahim et al., (2012)was adopted for identification of the phytoconstituents. The filtrate used for the analysis was prepared by dissolving 20 g of methanol extract in 100 ml of distilled water, it was heated for five minutes in a water bath, then, allowed to cool after which it was filtered. The filtrate was used for the following tests:

Test for Alkaloids: Meyer's test

To 2 ml of the filtrate solution in a test tube, 2 ml dilute Hydrochloric acid was added then, Mayers reagent (potassium mercuric iodide solution) was added. Precipitate formation was observed.

Test for saponins: Frothing test

To 1 ml of the extract solution in the test tube, 2 ml of distilled water was added and vigorously agitated for few seconds. Formation of froth which persists for 30 minutes was observed.

Test for phenolic compounds: Ferric chloride test

To 2 ml of extract solution in the test tube, 3 drops of 5% ferric chloride was added. Bluish brown color was observed.

Test for flavonoids

To 2 ml of extract solution a test tube, concentrated Sulphuric acid was added in drops through the wall of the test tube in slanted position. Orange coloration was observed.

Test for tannins: Ferric chloride test

To 2 ml of extract solution in a test tube, few drops of 5% ferric chloride was added. Dark green coloration was observed.

Preparation of extract and fractions for administration to animals

The extract and fractions were found insoluble in water but soluble in Tween-80 which was used as a wetting agent. Stock solution of ethyl acetate , butanol, n-haxane, aqueuous, and methanol fractions were prepared by dissolving 2 g of each in 15 ml of 3 % Tween 80. The stock solution of diclofenac, 10 mg/ml was prepared in distilled water. The stock solution of omeprazole, 20 mg/ml was also prepared in distilled water.

Gastro-protective activity study

The animal sample size for the in vivo study was selected according to Halim et al., (2017) and Jabbar et al., (2023). Total of eight seven Albino Wistar rats were carefully selected for the study. Seventy of them were selected randomly into two groups, which represented the preventive and the curative groups, and seventeen were used for toxicity study. The groups were further divided into seven sub groups which contained five animals per sub group that were randomly selected. For each of the groups (preventive and curative respectively), groups 1 and 2 represented the negative and positive control respectively, the remaining groups (groups 4-7) served as the treatment groups for the different fractions and the crude extract.

For the preventive group, all the sub groups were pre-treated with their designated fractions and extract for seven days, except group 2 which was pre-treated with a standard drug (Omeprazole) and group 1 which was allowed access to water and food only. On the seventh day all the animals were administered diclofenac 100 mg/kg body weight orally to induce gastric ulcer an hour after the last dose of the test samples and standard drug. Twelve hours prior to the day of ulcer induction, the animals' feed was withdrawn and they were fasted but received water ad libitum. The administration of the fractions and extract were performed once a day in all the experiment groups. After twelve hours of ulcers induction,the animals were sacrificed by cervical dislocation, their abdomen were opened through midline incision using a dissecting kit on a dissecting board while the stomach was incised along the greater curvatures.

For the curative group, all the animals were administered diclofenac 100 mg/kg body weight orally. After 24 h, the animals were treated with the fractions, crude extract and the standard drug for seven days except for group 1 which was allowed access to water and food. After treatment, the animals were sacrificed as described above in the preventive group. The grouping and administration of the drug, extract and fractions were as follows;

For Preventive Method:

Group 1: negative control; 100 mg/kg body weight of diclofenac only.
Group 2: positive control; omeprazole 40 mg/kg body weight + 100 mg/kg body weight of diclofenac.
Group 3: 400 mg/kg body weight of methanol extract + 100 mg/kg body weight of diclofenac
Group 4: 400 mg/kg body weight of aqueous fraction + 100 mg/kg body weight of diclofenac.
Group 5: 400 mg/kg body weight of n-hexane fraction + 100 mg/kg body weight of diclofenac.
Group 6: 400 mg/kg body weight of n-butanol fraction + 100 mg/kg body weight of diclofenac.
Group 7: 400 mg/kg body weight of ethyl acetate fraction + 100 mg/kg body weight of diclofenac.

For curative method:

Group 1: negative control; 100 mg/kg body weight of diclofenac only.
Group 2: positive control; 100 mg/kg body weight of diclofenac + omeprazole 40 mg/kg body weight.
Group 3: 100 mg/kg body weight of diclofenac + 400 mg/kg body weight of methanol extract.
Group 4: 100 mg/kg body weight of diclofenac + 400 mg/kg body weight of aqueous fraction.
Group 5: 100 mg/kg body weight of diclofenac + 400 mg/kg body weight of n-hexane fraction.
Group 6: 100 mg/kg body weight of diclofenac + 400 mg/kg body weight of n-butanol fraction.
Group 7: 100 mg/kg body weight of diclofenac + 400 mg/kg body weight of ethyl acetate fraction.

Measurement of Gastric Lesions

Measurement of gastric ulcerations following the induction was done by first dissecting the stomach along its greater curvature and fixing on a board or transparent glass as demonstrated bu Kiran et al. (2023). The following scores/ratings as described by Ommurugan & Rao, (2019) were used to evaluate the ulcer index as well as the severity of gastric lesions:

0 = no lesion,
1 = mucosal oedema and petechiae,
2 = one to five small lesions (1-2 mm),
3 = more than five small lesions or one intermediate lesion (3-4 mm),
4 = two to more intermediate lesions or one gross lesion (>4 mm),
5 = perforated ulcers.

The ulcer index (UI), the percentage protective ratio, and the percentage curative ratio were calculated respectively, using the following equations:

UI = total ulcer score ÷ no of animals ulcerated……Equation 1

UI of ulcerogen treated group-UI of drug treated group UI of ulcerogen treate group ×100…… Equation 2

Acute toxicity study

The acute toxicity of methanol extract of *P. discoideus* was determined by Lorke's method outlined by Mousa *et al* (2019) using seventeen rats. Rats of either sex were divided into 2 phases. In the first phase of the study, 9 rats were divided into 3 groups of 3 rats each and they were treated with methanol extract of *P. discoideus* by gavage at the doses of 10, 100 and 1000 mg/kg. In the second phase, 8 rats were divided into 4 groups of 2 rats each and they were treated with methanol extract of *P. discoideus by* gavage at the doses of 850, 1700, 3400 and 6800 mg/kg. The general behaviour of the animals was observed continuously for 1 h after treatment and then intermittently for 4 h, then hourly for the next 24 h. The LD50 was determined using the formula in Lien *et al* (2022);

$LD50 = \sqrt{a} \times b$

Where, a = least dose that killed a rat; and b = highest dose that did not kill any rat.

Data Analysis

SPSS version 20 software package was used to run analysis of variance (ANOVA) at value less than 0.005 ($p < 0.005$) level of significant. The data obtained were analysed and the significant difference between the control and treated groups was determined using the (ANOVA) followed by Dunnett's t-test to comparing all the test group against the positive control. *P*-values less than 0.05 were considered to be statistically significant.

RESULTS

Percentage yield of crude extract and fractions

The results of percentage yields of methanol crude extract and fractions of the leaves of the plant are given in Table 1. The results showed that the leaves yielded 33.46% of the crude extract. It was also noted that the aqueous gave the highest fraction yield than the others.

Table 1:

Percentage Yield of Extract and Fractions of P. discoideus.

Extract and solvent fractions	Mass of initial powder (Mx) in gram	Mass of final extract or fraction (My) in gram	%yield = My/Mx × 100
CF	200	66.92	33.46
AF	48.66	19.13	39.31
HF	48.66	4.47	9.19
BF	48.66	14.74	30.29
EF	48.66	16.43	33.76

Phytochemical Composition

The results of the qualitative phytochemical analysis of P. Discoideus is represented in Table 2. It was found out that alkaloids, saponins, phenolics, steroids, flavonoids, tannins and were present in the methanol extract of the leaves. One or more of the secondary metabolites were absent in the different fractions.

Table 2:

Results of the Phytochemical Screening of the Extract and Fractions of P. discoideus Leaves

Phytochemicals/extract or fractions	Alkaloid	Tannin	Saponin	Flavonoid	Steroid	Carbohydrate
n-hexane fraction (HF)	-	-	-	-	+	-
Ethyl acetate fraction (EF)	+	+	-	+	-	+
Butanol fraction (BF)	-	-	+	+	-	+
Methanol extract (MF)	+	+	+	+	+	+

+ = Present *- = Absent*

Acute toxicity profile

Administration of methanol extract of *P. discoideus* leaves up to 5000 mg/kg per oral did not produce any sign of toxicity in rats. There was no significant change in daily body weight or organ weight during the next 4 weeks. In addition, there was no symptoms of diarrhoea or abnormal behaviour during this period. None of the rats died. However, oral administration of the methanol extract at 6400 mg/kg caused 100 % mortality in rats. The oral LD50 was determined to be ≥ 4808.33 mg/kg body weight in adult Wistar rats.

Gastro-Protective Effect

The results for preventive study in the table below showed ulcer count for groups treated with extract, fractions and standard drug were significantly ($p < 0.05$) when compared to the group 1. $P< 0.05$ when compared to the positive control.

The analysis on the volume had it that groups 3, 5 and 6 had a significant ($p < 0.05$) decreases when compared to the group 1. However, groups 2, 4 and 7 were not significantly ($p > 0.05$) when compared to group 1 and higher compared to groups 3, 5 and 6. The analysis of the ulcer on pH showed that the group 4, 6 and 7 treated with methanolic extract, n-Hexane and ethyl acetate fractions respectively were found to be significantly ($p < 0.05$) when compared to group 1. Meanwhile, group 3 and 5 were not significantly ($p > 0.05$) when compared to group . The ulcer score analysis reveals a high significant ($p < 0.05$) when group 1 was compared to the rest of the groups. However, groups 2, 3, 4, 5, 6 and 7 were not statistically significant ($p > 0.05$) compared amongst them. Results are expressed in Means ± SD (n = 5). Mean values with different letters as superscripts across the column are considered significant at

Table 3:

Gastro-Protective Effect of Crude Extract and Solvent Fractions of Phyllanthus Discoideus Leaves Against Diclofenac Induced Ulcer in Albino Wister Rats

Groups	Treatments	No of Ulcer	Volume	pH	Ulcer Score	% Preventive Index
1	DIC	11.20 ± 5.81b	0.84 ± 0.35 b	4.06 ± 0.64 a	3.60 ± 0.55 b	0
2	Omeprazole +DIC	0.80 ± 0.84a	0.56 ± 0.28 ab	5.50 ± 0.50 bc	0.60 ± 0.55 a	83.33
3	AQF +DIC	1.60 ±1.82a	0.20 ± 0.12 a	4.44 ± 0.39 ab	1.00± 1.00 a	72.22
4	MTE +DIC	1.20 ± 1.30a	0.80 ± 0.41 ab	5.78 ± 1.83 c	1.00± 1.00 a	72.22
5	NHF + DIC	0.60 ± 0.89a	0.22 ± 0.08 a	5.08 ± 0.35 abc	0.60 ± 0.89 a	61.11
6	BTF + DIC	0.60 ± 1.34a	0.20 ± 0.00 a	6.00 ± 0.16 c	0.80 ± 1.30 a	88.88
7	EAF + DIC	2.20 ± 1.64a	0.62 ± 0.73 ab	6.02 ± 0.38 c	1.40 ± 0.89 a	77.77

Group 1= *Diclofenac Sodium (DIC) as Negative control;* ***Group 2=*** *Omeprazole + DIC as Standard;* ***Group 3=*** *Aqueous Fraction (AQF + DIC);* ***Group 4=*** *Methanol Extract (MTE + DIC);* ***Group 5=*** *n-Hexane Fraction (NHF + DIC);* ***Group 6=*** *Butanol Fraction (BTF + DIC);* ***Group 7=*** *Ethyl acetate Fraction (EAF + DIC)*

The curative study in the table below showed ulcer index for groups treated with extract, fractions and standard drug (groups 2, 3, 4, 5, 6 and 7) had high significant ($p < 0.05$) when compared to the group 1. The results on the volume showed that groups 3, 4 and 7 were significant ($p < 0.05$) when compared to group 1. They were found not to be significant ($p > 0.05$) compared to groups 2 and 6. However, group 5 was also not significant ($p > 0.05$) when compared to groups 2 and 6. The analysis of the ulcer on pH revealed a significant ($p < 0.05$) increases when the groups treated with extract, fractions and standard drug were compared to group 1. However, groups 2, 3 and 7 were not significantly ($p > 0.05$) compared to group 4, but lower when compared to groups 5 and 6. The analysis of the results on ulcer score showed a high significant ($p < 0.05$) increase when group 1 was compared to the rest of the groups. However, groups 2, 3, 4, 5, 6 and 7 were not statistically significant ($p > 0.05$) compared within the groups. All results were expressed in Means ± SD (n = 5). Mean values with different letters as superscripts across the column considered significant at $P< 0.05$ compared to the positive control.

Table 4:

Curative Method; Ulcer Curative Index Effect of Crude Extract and Solvent Fractions of Phyllanthus Discoideus Leaves Against Diclofenac Induced Ulcer in Albino Wister Rats.

Groups	Treatments	No of Ulcer	Volume	pH	Ulcer Score	% Curative Index
1	DIC	5.80 ± 2.28 b	1.60 ± 0.95 c	3.74 ± 0.44 a	3.00 ± 1.00 b	0
2	Omeprazole +DIC	1.20 ± 1.64 a	0.56 ± 0.27 ab	5.28 ± 0.85 bc	0.40 ± 0.55 a	86.67
3	AQF +DIC	0.60 ±0.55 a	0.36 ± 0.12 a	4.70 ± 0.38 b	0.60 ± 0.55 a	80.00
4	MTE +DIC	0.80 ± 1.30 a	0.36 ± 0.09 a	5.48 ± 0.52 bc	0.60 ± 0.89 a	80.00
5	EAF + DIC	0.20 ± 0.45 a	1.04 ± 0.25 b	5.98 ± 0.49 c	0.20 ± 0.45 a	86.67
6	BTF + DIC	0.40 ± 0.55 a	0.56 ± 0.38 ab	5.86 ± 0.73 c	0.40 ± 0.55 a	93.33
7	NHF + DIC	0.20 ± 0.45 a	0.38 ± 0.33 a	5.34 ± 0.34 bc	0.20 ± 0.45 a	60.00

Group 1= *Diclofenac Sodium (DIC) as Negative control;* ***Group 2=*** *Omeprazole + DIC as Standard;* ***Group 3=*** *Aqueous Fraction (AQF + DIC);* ***Group 4=*** *Methanol Extract (MTE + DIC);* ***Group 5=*** *n-Hexane Fraction (NHF + DIC);* ***Group 6=*** *Butanol Fraction (BTF + DIC);* ***Group 7=*** *Ethyl acetate Fraction (EAF + DIC)*

DISCUSSION

The genesis of diclofenac-induced gastric lesions is multifactorial with the depletion of gastric wall mucous content as one of the involved factors. It is also associated with significant production of free radicals, leading to an increased oxidative stress and damage to the cell and cell membrane. The results obtained showed that there was a level of gastro-protective activity following oral administration of *Phyllanthus discoideus* leaves extract and solvent fractions in rats. Two methods were adopted in this study (curative and preventive method). From the results of this study it is evident that *Phyllanthus discoideus* possesses more curative effect than preventive effect in NSAID induced gastric ulcer having butanol fraction as the best treatment with both curative and preventive effect. The dose of 400 mg/kg of the extract and fractions was used for all the treated groups.

For the preventive method, the highest activity was established in treatment with butanol fraction which gave a percentage preventive index (PI) of 88.88% (ulcer index (UI) = 0.4). Pre-treatment of the rats with ethyl acetate fraction of the leaves of *P. discoideus* offered the second highest protection of the gastric mucosa of the test animals against NSAID- induced damage. This is evident by the percentage PI of 77.77% (UI = 0.8). Pre-treatment with aqueous fraction and methanol extract of leaves of *P. discoideus* provide the third highest protection with percentage PI of 72.22% (UI=1). The n-hexane fraction provided the least protection of the gastric mucosa with percentage PI of 61.11% (UI=1.4). Although all the agents provided a percentage preventive index above average, only butanol fraction had a percentage PI higher than that produced by the standard.

For the Curative method, treatment with butanol fraction provided the highest protective activity of the gastric mucosa against NSAID-induced damage. This is evident with percentage curative index(CI) of 93.33% (UI=0.2). The second highest protective fraction was ethyl acetate fraction with percentage CI of 86.67% (UI=0.4). Treatment with aqueous fraction and methanol fraction provided the third highest protection with percentage CI of 80.0% (UI= 0.6). The n-hexane fraction provided the least mucosal protection with percentage CI of 60.00% (UI=1.2). Although the extract and fractions provided a percentage curative index above average only ethyl acetate fraction and butanol fraction had percentage curative index higher than the widely used conventional drug, Omeprazole, at 40mg/kg dose against NSAID-induced gastric ulcer.

Previous studies have shown that non-steroidal anti-inflammatory drugs (NSAIDs) decreased nutrient digestion and absorption through their direct effect on the gastric and intestinal mucosal cells (Kuna, L. et al 2019). However, the direct effect of NSAIDs on gastric and intestinal mucosal cells is as a result of inhibitory effects of NSAIDs on constitutive cyclooxygenase in the

gastrointestinal cells. An increase in gastric pH and significant decrease in gastric volume and acidity was observed in Omeprazole, methaol and fraction treated groups when compared with diclofenac group. Also, the ulcer counts and ulcer index were decreased in groups that received Omeprazole, methanol and other solvent fractions of *P discoideus* leaves when compared with diclofenac treated ulcerated rats. This suggests that P. discoideus methanol extract and solvent fractions have inhibitory effect on gastric acid secretion and its inhibitory action might mimic Omeprazole effect on gastric acid secretion. The extract may act by blocking H2 receptor and prevent histamine from binding, causing decreased in gastric acid secretion. It is established that inhibition of histamine release as a result of blockage of H2 receptors, inhibit intracellular adenylatecyclase, Na+-K+ ATPase and proton pump of parietal cells, thereby reducing the gastric acid secretion (Saski et al., 2000; Ayada et al., 2003).

Methanol extract of P. discoideus leaf has been characterized in this study to contain alkaloids, tannin, saponins, terpenoids, glycoside and flavonoids. The reduction in gastric acid secretion seen in the group treated with P. discoideus might be due to action of tannin. Tannin tends to compete with adenosine triphosphate at the ATP hydrolysis site, thereby causing the inhibition of gastric H+-K+ ATPase that is necessary for gastric acid secretion (Maity, B et al.2008). In addition, MEVA (methanol extract of vernonia amygdalina) may prevent gastric mucosal lesions through its flavonoid content (Tundis, R. et al, 2008). Flavonoids could scavenge free radicals, inhibit lipid peroxidation, and increase prostaglandins and mucosal content of the gastric mucosa; showing cytoprotective effects (Sharifi-Rad m. et al, 2018).

Conclusion

The present study indicates that n-butanol fraction of *P. discoideus* leaves administration attenuated diclofenac-induced gastric ulcer in both the curative and the preventive study methods. Its gastro-protective activity may be attributed to its antioxidant constituents. Overall, the results obtained supported the beneficial effects *P. discoideus* as a potential gastro-protective agent in experimental ulcer ailments in rats, thus opening the possibility of its usage as an alternative therapy for gastric ulcer.

Recommendations

Phyllantus discoideus can be harnessed as a potential source of lead compounds that can be used to prevent and cure NSAID induced ulcer. Therefore, further study to carry out the isolation and characterization of the lead bioactive compounds that possess gastro-protective activity in *Phyllanthus discoideus* leaves is recommended.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the works presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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