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Evaluation of the Gastric Antiulcer Efficacy of *Helianthus annuus* L, Leaves Extract and Fractions

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Abstract

Peptic ulcer disease is one of the most common gastrointestinal diseases worldwide. Its treatment is becoming increasingly difficult due to the implication of *Helicobacter pylori* and its increasing resistance to antimicrobial agents leading to a cyclical course of exacerbation and remission. This study evaluated the antiulcer effects of methanol leaf extract and fractions of *Helianthus annuus*. The crude extract was prepared by cold maceration with methanol. A portion of the extract (300 g) was partitioned with n-hexane, ethyl acetate and methanol to yield n-hexane (HF), ethyl acetate (EF) and methanol (MF) fractions respectively. The extract was subjected to oral acute toxicity (LD_{50}) and phytochemical studies. The antiulcer activity of the extract and fractions was evaluated in rat experimental ulcer models induced with aspirin, ethanol and histamine. Omeprazole and Tween 80 served as the standard drug and negative control respectively. Activities related to antiulcer properties such as effect on gastrointestinal transit and antimicrobial activities were investigated. Outcome measures were ulcer index, percentage ulcer protection, percentage gastrointestinal inhibition and antimicrobial potency. Ulcers induced by the three ulcerogens were significantly ($p < 0.05$) ameliorated by the extract and the fractions. The extract and fractions significantly ($p < 0.05$) inhibited gastrointestinal motility in mice. They exhibited potent antibacterial activity but were devoid of anti-fungicidal property. At a dose of 5000 mg/kg (p.o), the extract caused no obvious signs of toxicity or death in mice. Phytochemical analysis revealed the presence of saponins, tannins, flavonoids, steroids, glycosides, terpenoids, as well as reducing sugars. The results show that the leaves of *Helianthus annuus* possess antiulcer properties with n-hexane fraction offering best ulcer protection. Cytoprotection, antispasmodic, as well as antibacterial actions may account for the mechanisms of the antiulcer effects.

Keywords: *Helianthus annuus*; Ulcer Protection; Cytoprotection; Antibacterial; Rats

Introduction

Peptic ulcer disease is a heterogeneous disorder of the gastrointestinal tract caused by a disruption of the mucosal lining manifesting with burning sensation, gnawing pain, belching, abdominal discomfort and epigastric tenderness [1]. The etiology of peptic ulcer is influenced by imbalance in the equilibrium of various aggressive factors such as acid-pepsin secretion, blood flow, cellular regeneration, and defensive factors like the endogenous protective agents (prostaglandins and endothelial growth factor) [2]. Factors such as poor dietary habits, excessive intake of non-steroidal anti-inflammatory drugs(NSAIDS), alcohol, stress, burns, hereditary predisposition and *Helicobacter pylori* infection

are responsible for development of peptic ulcer [3]. A peptic ulcer variant called marginal ulcer results frequently from surgical intervention where portions of the gastrointestinal tract are formed as in gastrojejunostomy where an anastomosis is made between the stomach and some portion of the jejunum [4]. Social factors such as alcohol [5,6], smoking [7] and stress [8] increase gastric acid secretion and compromise mucus synthesis which enhances the risk and potentials of epithelial damage and attendant ulcerations.

Several orthodox pharmaceutical drugs such as antacids, histamine H_2 receptor blockers, anticholinergic agents, prostaglandin analogues and more recently proton pump inhibitors have been employed either as a single agent or in combination for the management of peptic ulcer but with limited success, because none has been shown to have absolute healing property. They are also associated with high incidence of adverse effects such as diarrhoea, abdominal cramps, preterm contractions, gynaecomastia, dizziness, alopecia, decreased libido, mental confusion and muscle pains [9-13]. Poor accessibility and high cost are other limiting factors.

These negative effects are the rationale for the development of new antiulcer drugs and the search for novel molecules from natural products. Plants have been a valuable source of new molecules, and considered as alternative strategy in search for new drugs. There is rich abundance of plants used in traditional medicine known to possess antiulcer properties [14]. A variety of botanical products like *Aloe vera* [15]. *Allium sativum* [16], *Mangifera indica* [17], *Moringa oleifera* [18], *Ocimum sanctum* [19] and *Persea americana* [20] have been reported to possess antiulcer activity.

The use of the leaves of *Helianthus annuus* for peptic ulcer management is a common practice in Unubi Community in Nnewi South L.G.A of Anambra state, Nigeria. *H. annuus* (Sunflower) is a tall, fast-growing annual plant with broad, oval to heart-shape, roughly hairy leaves. Traditionally, sunflower is used as a remedy for pulmonary affections, and a preparation of the seeds has been widely used for cold and coughs. In the Caucasus the seeds have served as a substitute for quinine in the treatment of malaria, and as a diuretic and expectorant [21]. Sunflower pith has been used by the Portuguese in making moxa, which was used in the cauterization of wounds and infections. A tea obtained from the leaves is used as astringent, diuretic and expectorant. The crushed leaves are used as poultice on sores, swellings; snake and spider bites.

Many bioactive secondary metabolites including phenols, tocopherols, ascorbic acid, tannins, saponins, glycosides, flavonoids etc. [22-24] have been reported to occur in *H. annuus.* These phytoconstituents have been associated with biological effects of the plant like, antitumor [25], antidiabetic [26], antimicrobial [27,28], anti-inflammatory [29,30], antihypertensive [31], antiashmatic [32], antioxidant [33] and wound healing [34]. In Nigeria, *Helianthus annuus* leaves are boiled in clean water for 4-6 hours, allowed to cool for and its extract drank three times a day for two weeks for treatment of peptic ulcers. However, no scientific study has been carried out to establish the veracity or otherwise of this claim and this study was designed to fill this knowledge gap.

Materials and Methods

Collection, Identification and Preparation of Plant Material

Fresh leaves of *Helianthus annuus* were collected from the Botanical Garden of the Department of Agricultural Economics, Federal Polytechnic Nekede, Owerri, Nigeria and authenticated by Dr. Clinton Emekoma a botanist in the Department and a herbarium specimen, FPN/AE. 086 are kept in the herbarium. The leaves were washed thoroughly, air-dried for two weeks and pulverized to coarse powder.

Extraction and Fractionation

The powder (1 kg) was thoroughly extracted with 2.5 litres of methanol using cold maceration. It was allowed to stand for 48 hours with intermittent agitations, and then filtered first by passing through a cotton plug and further filtered with filter paper (Whatman filter paper No. 1). The filtrate was concentrated to dryness over water bath at 60°C. A portion (100 g) of crude methanol extract (ME) was introduced into a separating funnel and successively extracted with n-hexane, ethyl acetate and methanol in order of increasing polarity. After extraction, the different filtrates were dried to a constant weight under reduced pressure using rotary evaporator to afford the n-hexane (NF), ethyl acetate (EF) and methanol (MF) fractions.

Animals

The study was carried out using adult albino Wsitar rats (180-210 g) of both sexes bred in the Animal House of the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka. The animals were allowed to acclimatize for 7 days at Nnamdi Azikiwe University, Awka, Animal House. The rats were fed with feed pellets, (Top Feed, Premier Feed Mills, Sapele, Delta State, Nigeria). The animals were given food and water *ad libitum* throughout the experiments. All animal experiments were conducted in compliance with NIH guide for care and use of laboratory animald (Pub. No. 85-23 Revised) as approved by the Nnamdi Azikiwe University, Awka Ethical Committee for the use of laboratory animals.

Phytochemical Analysis and Acute Toxicity (LD_{50}) Study

Qualitative phytochemical screening of the crude methanol extract (ME) and fractions were performed to ascertain the presence of secondary metabolites using standard procedures [35,36]. The median Lethal Dose (LD_{50}) of the extract (ME) was determined as described by Lorke [37].

Antiulcer Studies

Three models of ulcer; aspirin-, ethanol- and histamine-induced ulcer models were used to evaluate the ulcer protective effect of the extract and fractions in rats. Prior to the experiment a pilot study was conducted to establish the possible antiulcer property and determine the doses with significant antiulcer property using the crude methanol extract (ME). The equivalent doses of the fractions were similarly employed. For each ulcer model, the animals were randomly grouped into 10 (n=6) and treated orally as follows;

- Group 1 Tween 80 (10 ml/kg, negative control)
- Group 2 Omepraole (20 mg/kg, positive control)
- Group 3 ME (200 mg/kg)
- Group 4 ME (400 mg/kg)
- Group 5 NF (200 mg/kg)
- Group 6 NF (400 mg/kg)
- Group 7 EF (200 mg/kg)
- Group 8 EF (400 mg/kg)
- Group 9 MF (200 mg/kg)
- Group 10 MF (400 mg/kg)

The animals were treated once daily for two weeks after which ulcers were induced using the respective ulcerogen.

Effect of the extract and fractions on aspirin-induced ulcer: Ulcers were induced with aspirin (150 mg/kg p.o) and the animals sacrificed 4 h later and the ulcer grading carried out as described below.

Effect of the extract and fractions on ethanol-induced ulcer: The animals were starved for 24 hr and absolute ethanol (96 % v/v) was administered (0.5 ml/100 g p.o) to each rat as a single dose. The animals were sacrificed 1hr later for ulcer determination.

Effect of the extract and fractions on histamine-induced ulcer: Ulcerations were induced by subcutaneous administration of histamine (100 mg/kg) [38]. After 2 h, the animals were sacrificed for ulcer determination. For each model, the animals were humanely sacrificed with excess anesthetic ether. Macroscopic evaluation of the glandular portions of the stomach was made by opening the stomach along the greater curvature, rinsed under a stream of water, pinned flat on a corkboard and viewed macroscopically with a hand lens (magnification x10). Each stomach was given a severity rating from which the ulcer index was calculated [39].

- Normal stomach: 0
- Red colouration: 0.5
- Sport ulcers: 1.0
- Hemorrhagic streak: 1.5
- Ulcers: 2.0
- Perforation: 3.0

$$\text{Ulcer index (UI)} = \frac{US}{UN} \times 10^{-1}$$

Where, UI= Ulcer Index; UN = total number of ulcers per animal; US = total number of severity score for each animal.

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

Effect of the extract and fractions on gastro-intestinal transit time: Thirty six (36) rats were randomized into 6 groups (n=6) and fasted for twenty-four hours prior to the experiment. They were allowed unrestricted access to clean drinking water. The effect of the extract (ME) and fractions (400 mg/kg) on gastro-intestinal motility was also evaluated. The animals were treated orally as follows;

- Group 1- Tween 80 (10 ml/kg)
- Group 2 –Atropine (10 mg/kg)
- Group 3 – ME (400 mg/kg)
- Group 4 – NF (400 mg/kg)
- Group 5 – EF (400 mg/kg)
- Group 6 – MF (400 mg/kg)

Thirty minutes after pretreatment, each animal received 0.5 ml of charcoal meal (5% charcoal in 10% tragacanth mucilage) orally. The animals were sacrificed after 30 minutes and the intestines removed and displayed. For each animal the intestinal distance traveled by the charcoal meal was measured from the pylorus to the ileocecal junction. Percentage inhibition is calculated from the expression [39];

$$\textbf{Percentage inhibition} = \frac{Dc - Dt}{Dc} \times 100$$

Where Dc is the intestinal distance traveled in the control, Dt the distance traveled in treatment group

Anti microbiological Studies

Antimicrobiological studies were undertaken to determine the sensitivities of some gram positive and gram negative bacteria, as well as fungi and mould (yeast) to the methanol extract and fractions of *Helianthus annuus*. The inhibition zone diameters (IZD) were determined. Two

different culture media were used for this study viz; nutrient broth agar (bacteria) and Saboraud´s Dextrose agar (fungi and mould). The two media were reconstituted according to Oxoid manual specifications.

Preparation and Sterilization

Each of the constituents of the media were weighed, mixed and subsequently dissolved in distilled water (1000 ml) for about 15 minutes. The mixture was distributed in Bijou bottles and sterilized by autoclaving at 121°c for 15 minutes. The sterilized media were maintained in a molten state until use. The microorganisms were maintained by weekly sub culturing and incubated at 37°c (bacteria) and 25°c (fungi and yeast). The cultures were allowed for 24 hours before use**.** The inoculums employed throughout the studies contained 1.0 ×10^6 organism/ml

Antimicrobial Screening

Five microbial agents were used in the study. These include *Staphylococcus aureus*, *Escherichia coli, Klebsiella pneumonia, Candida albicans and Aspergillus*. All the microorganisms were obtained from Microbiology Department, Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State, Nigeria, and were maintained on nutrient broth agar (bacteria) and Saboraud's Dextrose agar (fungi and mould) at 4°c respectively. Before use they were sub-cultured in nutrient broth agar and Saboraud's Dextrose agar plates at 37°C (bacteria) and 25^{0}C (fungi and mould) for 24 hours. The agar disc diffusion method was employed. Using a sterile pipette 0.1 ml 1.0×10^6organism/ml suspension of microorganism was placed at the centre of petri dish and 50 ml of molten agar poured on it. This dish was agitated gently in a centrifugal direction to ensure even distribution of the seeded organism. This was then allowed to stand so as to solidify. Thereafter, two drops of 0.02 ml of the extracts were applied to appropriately labeled wells about 6 mm made in gelled agar containing 1.0×10^6 organism/ml. The plates were incubated at 37°c for 24 hours for bacteria and at 25°c for 72 hours for fungi. The effect of the extract (ME) and fractions on the growth of the microorganism was evaluated by determining the zones of inhibitions. The experiment was carried out in triplicates and the average clear diameter of zones of inhibition was recorded in each case.

Statistical Analysis

The data were expressed as mean ± Standard error of mean (SEM).The data were analyzed using Statistical Package for Social Sciences (SPSS version 20) using one way ANOVA, followed by post-hoc Turkey's test for multiple comparisons. The difference between mean were considered significant at $p < 0.05$.

Results

Acute Toxicity Study (LD_{50})

Oral administration of the crude methanol extract (ME) up to 5000 mg/kg did not cause any lethality or any obvious sign of acute toxicity within 24 hr. The LD_{50} of the extract was therefore greater than 5000 mg/kg.

Phytochemical Constituents

The qualitative phytochemical analysis of the extract and fractions revealed the presence of some bioactive substances. These phytoconstituents - saponins, flavonoids, tannins, terpenoids, cardiac glycosides and reducing sugars were more abundant in the extract than the fractions (Table 1).

Metabolites	ME	NF	EF	MF
Alkaloids	-	+	-	-
Carbohydrates	-	-	+	+
Glycosides	++	++	-	-
Fats and oils	+	-	+	-
Flavonoids	++	-	++	+
Proteins	-	-	-	-
Reducing sugars	+	++	+	++
Resins	+	-	-	-
Saponins	+++	+++	++	++
Steroids	+	++	-	-
Tannins	+++	+++	-	-
Terpenoids	++	++	+	+

Key: +++.. in abundance. ++…in moderate quantity. +… in trace amount. -… absent.
Table 1: Phytochemical Constituents of the Extract and Fractions.

Effect of the extract and fraction on aspirin-induced ulcer: Administration of aspirin produced 100 % ulceration in the control. The extract and fractions conferred significant ($p < 0.05$) and dose-related ulcer protection against aspirin-induced ulcer (Table 2). N-Hexane fraction produced the best ulcer protection.

Effect of the extract and fractions on ethanol-induced ulcer: The extract and the fractions exhibited dose-related ulcer protection against ulcers induced by ethanol as evidenced by the significant ($p < 0.05$) reduction in ulcer index when compared with Tween 80 control ($p < 0.05$).

Ulcer Index				
Treatment	**Dose (mg/kg)**	**Aspirin**	**Ethanol**	**Histamine**
Tween 80	10 ml/kg	8.70 ± 3.23	10.50 ± 2.13	4.32 ± 0.16
Omeprazole	20	2.20 ± 1.21* (74.71)	2.60 ± 0.02* (75.23)	0.84 ± 0.26* (80.55)
ME	200	6.70 ± 1.80 (22.98)	7.10 ± 1.40 (32.38)	2.82 ± 0.61* (35.19)
	400	3.85 ± 0.82* (55.75)	5.00 ± 0.22* (52.36)	1.63 ± 0.42* (62.27)
NF	200	2.69 ± 0.16* (69.08)	2.51 ± 0.66* (60.10)	1.41 ± 0.44* (67.36)
	400	1.22 ± 0.61* (85.98)	1.50 ± 0.24* (85.71)	0.78 ± 0.36* (81.90)
EF	200	5.82 ± 1.03 (33.33)	4.00 ± 0.16* (61.90)	2.31 ± 0.11* (46.76)
	400	4.20 ± 1.31* (52.31)	2.12 ± 0.51* (79.81)	1.84 ± 0.32* (57.41)
MF	200	4.10 ± 0.55* (52.87)	4.33 ± 0.31* (58.76)	1.95 ± 0.41* (54.86)
	400	3.30 ± 1.20* (62.07)	2.30 ± 0.52* (78.10)	0.94 ± 0.16* (78.01)

ME=Methanol extract, NF=N-hexane fraction, EF=Ethyl acetate fraction, MF=Methanol fraction.
*$p < 0.05$, percentage inhibition in parenthesis, n=6

Table 2: Effect of the Extract and Fractions on the Ulcer Models.

Effect of the extract and fraction on histamine-induced ulcer: Histamine administration resulted in increased ulceration in the control. The ulcer protections exhibited by the extract and fractions were also dose-related and significant ($p < 0.05$).

In the three ulcer models employed the n-hexane fraction (NF 400 mg/kg) offered the best ulcer protection. The percentage ulcer protections by NF (400 mg/kg) in the three ulcer models were highest and similar to that of omeprazole (20 mg/kg) (Table 2).

Effect of the extract and fractions on gastro-intestinal propulsion

In the control group the charcoal meal moved an average distance 94.20±16.42 mm. Treatment with the extract and fractions (400 mg/kg) significantly reduced the motility of gastrointestinal tract when compared with Tween 80 control. The inhibition by NF was comparable to that of atropine (10 mg/kg) (Table 3).

Treatment	Dose (mg/kg)	Intestinal distance travelled (mm)	Percentage inhibition (%)
Tween 80	10 ml/kg	94.20±16.42	0.00
Atropine	10	40.64±0.32*	56.84
ME	400	60.14±06.26*	36.20
NF	400	42.64±04.87*	54.73
EF	400	50.40±06.22*	46.50
MF	400	46.35±11.50*	50.80

*p < 0.05 (n=6)

Table 3: Effect of Extract and Fractions on Gastrointestinal Motility in Rats.

Antimicrobial Activity of the Extract and Fractions

The bacterial growth was inhibited to various degrees by the extract and fractions of *Helianthus annuus* leaf. The order of sensitivity was *E. coli>K. pneumonia>S. aureus.* The fungi were however resistant to the extract and fractions (Table 4).

Inhibition zone diameter (mm) ±SD					
Microorganisms	**DW**	**ME**	**NF**	**EF**	**MF**
Staphylococcus aureus	NA	8.42±02.00	6.88±1.06	4.36±06.20	2.33±04.30
Escherichia coli	NA	36.04±05.30	38.06±08.37	42.01±06.22	30.62±10.70
Klebsiella pneumonia	NA	12.24±00.40	18.13±04.05	10.09±04.18	16.83±06.04
Candida albicans	NA	NA	NA	NA	NA
Aspergillus niger	NA	NA	NA	NA	NA

DW=Distilled water, NA= No activity. (n=4)

Table 4: Sensitivity of the microorganisms to 100 mg/ml of the extract and fractions.

Discussion

The aim of ulcer therapy is to restore the equilibrium between the aggressive action of acid-pepsin secretion and the maintenance of the mucosal integrity through endogenous defense mechanisms [40,41]. This can be achieved by balancing the defensive factors against aggressive factors thereby relieving patients of pain, promote healing, prevent recurrence and development of complications [42]. To achieve these therapeutic goals, certain agents are employed to either promote mucosal defense mechanism or inhibit the gastric secretion [43,44]. Unfortunately, no one antiulcer agent has all the above mentioned qualities.

The quest for a safe anti-ulcer agent is a continuous one and part of this search is the evaluation of medicinal plants for gastro-protective properties [45,46]. Traditional medicine has claimed a lot of success in the management of peptic ulcer disease and this has informed the recent screening of *H. annuus* for antiulcer properties with the aim of providing potent and safer antiulcer drugs. The phytoconstituents in the extract and fractions of *H. annuus* are known to offer protection against ulcer and promote healing of ulcers. Some reports have asserted that flavonoids have anti-secretory and cytoprotective properties [47,48]. In addition, other phytoconstituents including terpenoids, steroids and glycosides found in the extract and fractions have been reported to possess anti-ulcer properties through formation of protective mucous, and by selectively inhibiting $PGF_{2\alpha}$ [49,50].

It has also been demonstrated that flavonoids possess spasmolytic and antiulcerogenic tendencies [51], as a result of their action on arachidonic acid metabolism, vasoprotective action and their ability to inhibit formation of histamine in gastric mucosa [52]. Tannins on the other hand have soothing, astringent and emollient action on the mucosal membrane of gastrointestinal tract [53]. Tannins hasten the healing of wounds and inflamed mucous membrane due to their anti-inflammatory effects [54], and their ability to form protective layer over the exposed tissue, hence, keeping the wound from being infected [55].

The three models used in this study represent the commonest etiological agents of peptic ulcer disease in humans. Non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin cause ulcer particularly at the glandular portion of the stomach by inhibiting endogenous prostaglandin synthesis. Prostaglandins especially PGE_2 has been shown to possess high gastro-protective and

gastro-curative properties involving maintenance of gastric microcirculation, enhancement and promotion of mucous and bicarbonate secretion and inhibition of gastric acid secretion [42]. Prostaglandin inhibition by non-steroidal anti-inflammatory drugs such as aspirin is widely proclaimed to be associated with increased leukotriene production with consequent mucosal vasoconstriction with the attendant reduced local blood flow [14]. It is possible that the extract and fractions promote PGE production thereby suggesting a cytoprotective and healing mechanism.

Ethanol reduces prostaglandin level and increases generation of free reactive oxygen radicals leading to lipid peroxidation which are major pathways of ulcer progression [56]. The healing properties of the extract is buttressed by the fact that ethanol induced gastric mucosal lesions are not inhibited by antisecretory agents but by agents that enhance mucosal defense factors. The significant reduction of ethanol-induced ulcer indicates prostaglandin-mediated cytoprotective mechanism of action. This finding is also attributable to the flavonoids and tannins contents [20].

Histamine promotes gastric acid and pepsin secretion at the basolateral membrane of parietal cells leading to increased gastric ulceration and delayed healing of already formed ulcer. It also enhances the contractile response and propulsive movement of the intestine leading to worsening symptoms of ulcer [57]. In histamine-induced ulcer model, the extract and fractions offered significant ulcer protection which can be attributed to inhibition of histamine-induced contractile response of the intestine, and blockade of histamine H_2 receptors on the parietal cells leading reduced gastric acid and pepsin output. Reduction in intestinal propulsive movement and gastric motility improves ulcer pain and quickens the healing of ulcer wounds [58].

Microbial colonization of the gastro-intestinal system has been associated with a variety of peptic ulcer diseases [59]. *Helicobacter pylori* have been implicated as the microorganism involved in the pathogenesis of peptic ulcer disease and has made the use of antibiotics imperative in peptic ulcer disease management. *Helicobacter pylori* eradication provides a definitive cure than the contemporary palliative symptom alleviation [60,61]. The effects of the extract and fractions against bacteria pathogens belonging to the same class as *Helicobacter pylori* implicated in peptic ulcers is remarkable and points to their beneficial effect in ulcer.

Conclusion

The results of this study indicate that the extract and fractions of the leaves of *Helianthus annuus* have good potential for use in peptic ulcer disease. The antiulcer activity can be attributed to inhibition of acid secretion, inhibition of gut motility, antimicrobial effect and strengthening of mucosal barrier. All of these are beneficial in ulcer management.

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