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To Study the Antiulcer Activity of Hydro Alcoholic Extract of Euphorbia Thymifolia on Absolute Ethanol Induced Ulcer in Rats.

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Abstract:

Pharmacological screening of Euphorbia Neriifolia Linn. (Euphorbiaceous) leaf hydro alcoholic extract were performed to explore anti-ulcer activities. All tests were conducted on rats using 100, 200 and 400mg/kg dose as LD so of extract was found to 2779.71mg/kg. Study revealed strong analgesic effect of E. Neriifolia against thermal ($P<0.001$), and in mechanical and chemical ($p < 0.01$) noxious stimuli and anti-inflammatory activity ($P<0.001$ to 0.01) at the 1000mg/kg dose. In camageen an-induced paw edema and cotton pellet induced granuloma model. Neriifolia extract showed significant ($P<0.001$ to 0.01) anti-inflammatory activity. Extract considerably increases urine volume as an effective hypermatraemic and hyperchloraemic diuretic. E. Neriifolia showed laxative property by increasing wet defecation along with castor oil. Extract showed very prominent protection against ethanol-induced ulceration as well as on pyloric ligated ulceration in dose dependent manner. Extract increases total hexodes ($P<0.001$), Hexosamine ($P<0.05$), Sialic acid and total carbohydrate content ($P<0.001$) with a decrease in total protein content ($P<0.001$) of gastric mucosa at 400mg/kg dose. Presence of Phytoconstituents like tannins, flavonoids, alkaloids and triterpenoidal saponins may be responsible for the found pharmacological activities.

Keywords: Euphorbiaceous, Antiulcer Activity of Hydro alcoholic Extract of Euphorbia Thymifolia, Hypermatraemic, Anti-inflammatory activity, Ethanol Induced Ulcer in Rats.

Introduction:

Gastro-Protective Effect:

Gastrointestinal disorders are one of the severe classes of human ailments causing maximum discomfort, morbidity and mortality. Peptic ulcer is one such gut disorder. Peptic ulcer is a benign lesion of gastric or duodenal mucosa occurring at a site where the mucosal epithelium is exposed to acid and pepsin. It is a chronic inflammatory condition involving a group of disorders characterized by ulceration in regions of upper gastrointestinal tract where parietal cells secrete pepsin and hydrochloric acid. There are several causes including, stress, alcohol consumption, cigarette smoking, H. pylori infection, ingestion of drugs and chemicals. Especially consumption of alcohol for a prolonged period, smoking of cigarettes, or chronic consumption of NSAIDs are causing peptic ulcers. The role of free radicals in the pathogenesis of peptic ulcer due to mucosal damage is established. The symptoms of peptic

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ulcer are severe pain and irritation in the upper abdomen. If it is not treated properly, it may results in perforations in the wall of the gastrointestinal tract.

Signs and Symptoms:

In peptic ulcer, patients can be asymptomatic or experience anorexia, nausea, and vomiting, bleaching and blotting and heart burn or epigastric pain.

Etymology of Chronic Ulceration:

Heredity:

Patients with peptic ulcer often have a family history of the disease. This is particularly the case with duodenal ulcers, which develop below the age of 20 years. The relatives of gastric ulcer patients have 3 times the expected number of gastric ulcer but duodenal ulcer occurs with the same frequency amongst relatives as in the general population.

Acid-Pepsin Vs Mucosal Resistance:

The immediate cause of peptic ulceration is digestion of the mucosa by acid and pepsin of the gastric juice, but the sequence of events leading to this is unknown. Digestion by acid and pepsin can't be the only factor involved, since the normal stomach is obviously capable of resisting digestion by its own secretion. The concept of ulcer aetiology may be written as "acid plus pepsin Vs mucosal resistance".

Gastric Hyper Secretion:

Ulcers occur only in the presence of acid and pepsin they are never found in achlorhydric patients such as those with pernicious anaemia. Acid secretion is more important in the etiology of duodenal than gastric ulcer. Peptic ulcer is the most common gastrointestinal disorder in clinical practice. Considering the several side effects like Arrythmias, impotence, Gynaecoemastia and hematopoietic changes, of synthetic drugs, hence their usage for a chronic period is restricted. However, comparatively indigenous drugs possessing fewer side effects. Hence, the search for better alternatives for synthetic drugs is on rise. There is evidence concerning the participation of reactive oxygen species in the etiology and pathophysiology and human disease, studies has shown alterations in the antioxidant status following ulceration indicating that free radicals, seems to be associated with the pylorus ligation and ethanol induced ulceration in rats.

Treatment of Ulcer by Using Traditional Herbs:

Ulcers are an open sore of the skin or mucus membrane characterized by sloughing of inflamed dead tissue [1].

Ulcers are lesions on the surface of the skin or a mucous membrane characterized by a superficial loss of tissue. Ulcers are most common on the skin of the lower extremities and in the gastrointestinal tract, although they may be encountered at almost any site. There are many types of ulcer such as mouth ulcer, Oesophagus ulcer, peptic ulcer, and genital ulcer.

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Of these peptic ulcer is seen among many people. The peptic ulcers are erosion of lining of stomach or the duodenum [2].

The two most common types of peptic ulcer are called “gastric ulcer” and “duodenal ulcer.” The name refers to the site of ulceration. A person may have both gastric and duodenal ulcers at the same time. Gastric ulcers are located in the stomach, characterized by pain; ulcers are common in older age group. Eating may increase pain rather than relieve pain. Other symptoms may include nausea, vomiting, and weight loss. Although patients with gastric ulcers have normal or diminished acid production, yet ulcers may occur even in complete absence of acid [3].

Duodenal ulcers are found at the beginning of small intestine and are characterized by severe pain with burning sensation in upper abdomen that awakens patients from sleep. Generally, pain occurs when the stomach is empty and relieves after eating. A duodenal ulcer is more common in younger individuals and predominantly affects males. In the duodenum, ulcers may appear on both the anterior and posterior walls [4].

In some cases, peptic ulcer can be life threatening with symptoms like bloody stool, severe abdominal pain, and cramps along with vomiting blood [5].

The pathophysiology of peptic ulcer disease involves an imbalance between offensive (acid, pepsin, and *Helicobacter pylori*) and defensive factors (Mucin, prostaglandin, bicarbonate, nitric oxide, and growth factors) [6].

Peptic ulcers are once believed to be caused by spicy food and stress; these have been found merely to be aggravating factors and the real causes have been found by research to include bacterial infection (*Helicobacter pylori*) or reaction to various medications, particularly NSAIDS (Non-steroidal anti-inflammatory drugs) [7].

Helicobacter pylori, NSAIDS drugs, emotional stress, alcohol abuse, and smoking are the principal etiological factors associated with peptic ulcer [8].

The Gram-negative bacterium *Helicobacter pylori* remains present between the mucous layer and the gastric epithelium and is strategically designed to live within the aggressive environment of the stomach. Initially, *Helicobacter pylori* reside in the antrum but over time migrates toward the more proximal segments of the stomach [9].

Peptic ulcer is one of the world’s major gastrointestinal disorders and affecting 10% of the world population [10].

About 19 out of 20 peptic ulcers are duodenal. An estimated 15000 deaths occur each year as a consequence of peptic ulcer. Annual incidence estimates of peptic ulcer hemorrhage and perforation were 19.4–57 and 3.8–14 per 100,000 individuals, respectively. The average 7-day recurrence of haemorrhage was 13.9% and the average long-term recurrence of perforation was 12.2% [11].

In this modern era also 75–80% of the world populations still use herbal medicine mainly in developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body, and lesser side effects [12].

Histological studies revealed that these medicinal plants did not show any acute toxicity. Preliminary photochemical screening of this medicinal plant identified the presence of important secondary metabolites like flavonoids and tannins which are the active principles of antiulcer activity [13].

Present study was conducted to review medicinal plants considered as gastro protective and healing agents on ulcers in ayurvedic resources and beside that to gather evidence for their effectiveness and biological mechanisms in modern investigation. In order to achieve this aim, Indian ayurvedic book Meteria Medica and electronic databases including science direct, pubmed, scopus, and google scholar were explored for each of the medicinal plants for peptic ulcers and all retrieved articles were evaluated to achieve any in vitro, in vivo, or clinical evidence for their efficacy and possible mechanisms. The retrieved studies either demonstrate obviously effectiveness of these herbs or indirectly their efficacy on the involved mechanisms in the treatment of peptic ulcers. Meteria Medica provides lots of information about ethno medicinal herbs, which are valuable as antiulcer agents and their use experimentally was evaluated and proved by many researchers for its antiulcer activity. Following compiled data suggested that medicinal plant those are evidently reported for its antiulcer activity.

Antiulcer Effects of Methanol Extract of Euphorbia Hirta in Rats:

Stomach ulcer is an endemic gastrointestinal disorder which constitutes a major public health problem all over the world. Stomach ulcer results when there is an imbalance between the protective factors (mucus and bicarbonate) and aggressive factors (acid and pepsin) in the stomach. Dried powdered leaves and stem of the phytomedicine *Euphorbia hirta* (*E. hirta*) (1000 g) was extracted with methanol using a soxhlet apparatus. The evaluation of the Phytochemical constituents of *E. hirta* and acute toxicity (to ascertain the safety of using the phytomedicine over a short period of time) was carried out. The antiulcer and gastro protective effects of crude extract of *E. hirta* combined with absolute ethanol in rats were evaluated. The study model using 0.6 M HCl model of ulceration was used to evaluate the antiulcer and Gastro protective activities of the phytomedicine. The soxhlet extraction of *E. hirta* gave a yield of 54.5 g of crude extract (5.45%). Phytochemical screening of *E. hirta* showed that the extract contains alkaloids, tannins, saponins, glycosides, flavonoids, and unsaturated steroids. Acute toxicity studies showed that LD_{50} was greater than 5000 mg/kg. The study showed that the crude extract of *E. hirta* at 200 mg/kg when administered alone had 54% inhibition of ulceration while when administered together with absolute ethanol increased to 94% inhibition of ulceration, but absolute ethanol alone had 89.47% inhibition of ulceration. This implied that *E. hirta* when combined with absolute ethanol had a synergistic effect and enhanced the inhibition of ulceration, and this could be seen by the protection of the gastric mucosa. The study of the phytomedicine *E. hirta* combined with absolute ethanol revealed that the phytomedicine has antiulcer activities against 0.6 M HCl-induced gastric ulcer in rats. This therefore validates usage and claim by

the Igbo people of the south eastern part of Nigeria that the phytomedicine of *E. hirta* combined with absolute ethanol has good antiulcer potential.

Stomach ulcer is an endemic gastrointestinal disorder which constitutes a major public health problem all over the world. Gastric ulcer occurs when there is a lack of balance between aggressive factors like the acid, pepsin, and local mucosal defense factors such as bicarbonate, mucus secretion, and synthesis of prostaglandins.

Gastric ulcer is characterized by the circumscription and complete loss of the gut epithelium in some parts of the digestive tract exposed to hyper secretion of hydrochloric acid and pepsin.

Absolute ethanol, though it has been used as a vehicle in Ayurvedic medicine, has been reported to have antibiotic and wound healing effect and also used for healing of cut and burns. Also, the antimicrobial property of absolute ethanol has been reported. Absolute ethanol has been viewed as a by-product of flower nectar and the upper aero-digestive tract of the absolute ethanol bee; it is concentrated through a dehydration process inside the bee hive. The biological activity of absolute ethanol is majorly determined by the biological sources of the nectar used by bees in the processing of absolute ethanol. Plant-based drugs and other plant-based combination therapies have been viewed to be a potential source that is relatively clean and effectively safe as drugs, though not fully tapped [Mahmood et al.].

Reported the antiulcer potential of absolute ethanol combination with *Trigonella foenum-graecum* seed extract on experimental gastric ulcer in rats. The choice of this present study was influenced by the folkloric claim by the Igbo people of South-eastern Nigeria that *E. hirta* combined with absolute ethanol cures ulcer. Moreover, no scientific research work has been done concerning the antiulcer potential and the toxicity of the *E. hirta* combined with absolute ethanol. The present study was undertaken to evaluate the acute toxicity, the preventive anti-ulcerogenic gastro therapeutic potential of *E. hirta* combined with absolute ethanol, and the Phytochemical constituents of *E. hirta.*

Plant Material (Collection and Preparation):

Fresh whole plant (leaves and the stem) of *E. hirta* were collected from the main IIMT University Meerut Uttar Pradesh, India. The plant was identified at the herbarium, Department of Botanist, Dr. Vijai Malik**,** Principle Scientist at CCS University Meerut, India, [Voucher No. (AC-11/2022). The plant materials (Leaf and Stem) were air dried at room temperature 10-14 days during the dry weather condition. The air-dried plant was ground, and 1000g of the powder was extracted using 5000ml of 70% methanol by continuous extraction in a Soxhlet apparatus. The extract was concentrated to dryness. The concentrated extract was scrapped into a sample bottle and kept in desiccators until required.

Experimental Animal Models:

Healthy adult albino Wistar (63) rats (male) weighing between 150 and 170 were obtained from the Department of Veterinary IIMT University Meerut. The animals were maintained at room temperature and humidity (25°C, 70% relative humidity) and allowed to acclimatize for

2 weeks. All the animals were fed with standard pelleted diet and water *ad-libitum*. The study followed ethical guideline of Institutional Animal Ethics Committee (Reg. No: 1678/Po/Re/2021/CPCSEA).

Other Materials:

Cytotec oral tablet manufactured by Pfizer Pharmaceuticals Ltd. was used. It contains 200mcg of misoprostol, a synthetic prostaglandin E1 analog. Misoprostol Cytotec is a water-soluble tablet. Pharmacodynamics of misoprostol has shown both anti-secretory (inhibiting gastric acid secretion) and mucosal protective properties in animals. Misoprostol can increase bicarbonate and mucus production. Misoprostol was administered at 50μg/kg. The plant extract and absolute ethanol combination was dissolved at 250 mg/ml of absolute ethanol as stock concentrations (L.T.D. Pfizer Pharmaceuticals). Jenway Spectrophotometer model 6405UV/VIS with serial number 3948, by Barloworld Scientific Limited (DUNMOW. ESSEX., CM63LB) was used.

Phytochemical Screening:

Phytochemical analysis was conducted using the method described to determine the presence of secondary metabolites in *E. hirta*.

Acute Toxicity Study:

Acute toxicity studies of the extract were made using the standard method of slightly modified to ascertain the acute toxicity of the methanol extract of *E. hirta*. Briefly, nine animals (mice) were randomly allocated into 3 groups of 3 rats each. Animals in groups 1, 2, and 3 were given 10, 100, and 1000 mg/kg body weights, respectively, of the extract through the oral route. Animals were therefore monitored for signs of toxicity and mortality for 2 days (48 hours). Signs of toxicity and pathological findings observed were recorded appropriately. All the animals survived, so the extract was further subjected to acute toxicity test with higher doses in the second trial. In the second trial, 4 animals were randomly allocated to 4 groups of one animal each. Animals in groups 1, 2, 3, and 4 were given 1200, 1600, 2900, and 5000 mg/kg body weight, respectively, of the extract.

0.6 M HCl induced Ulcer Model:

Gastro-protective effect of crude methanol extract of *E. hirta* combined with absolute ethanol was conducted as described by the method of Misoprostol was the standard drug used. Initially, the animals were fasted for 48 hours but were allowed free access to water *ad libitum*. They were randomly selected and divided into 9 groups of five rats each. Group 1 served as the negative control that received only water; group 2 served as the positive control that received misoprostol; groups 3 to 5 received the crude extract of *E. hirta* at doses of 200, 400, and 800mg/kg body weight, respectively; groups 6 to 8 received a combination of 1 ml absolute ethanol and crude extract of *E. hirta* at doses 200, 400, and 800mg/kg combined with absolute ethanol (1 ml), while group 9 received only 1 ml of absolute ethanol. Thirty minutes after treatment, individual rats were given 0.6 M HCl (1 ml/rat) orally as an ulcerogen. Three hours after treatment with HCl, all the rats were sacrificed in a chloroform

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chamber. On each animal, ventral midline incision was made on the abdomen to expose the stomach. The ulcerated surfaces in each stomach were measured with transparent millimeter-(mm-) scale ruler, and the result for each group was expressed in mm of mean ulcer index.

Gastric Mucus:

The concentration of gastric mucus was determined using the method. The excised glandular portion of the stomach (500 mg) was soaked in 0.1%. Alcian blue solution buffered with 0.05M sodium acetate and HCl for 2 hours. The excess dye or the un-complexes dye was removed by rinsing the stomach tissue twice in 0.25 M sucrose solution for 1hour. The dye complexes with gastric wall mucus was extracted with 0.5M-$MgCl_2$ for 2 hours. The extract was then shaken vigorously with an equal volume of diethyl ether, and the resulting blue emulsion was centrifuged at 5000 g for 10 minutes. The optical density of the solution was read against a buffer blank at 580 nm using a Jenway spectrophotometer, and the quantity of Alcian blue extract per gram wet stomach was then observed and noted.

Percentage Inhibition of Ulceration (PIU):

The percentage inhibition of ulceration was expressed as a percentage of the control by using the following formula as described by.

$$\text{Inhibition percentage (\%)} = \left[\frac{UI_{\text{ulcer control}} - UI_{\text{treated}}}{UI_{\text{ulcer control}}}\right] \times 100.$$

Statistical Analysis:

All statistical analyses were done using Graphical Prism version 4.0 for Windows from Graph Pad software, San Diego, USA. Data obtained were analysed using one-way analysis of variance. Where significant differences were observed, the Turkey post hoc test was used to identify and compare differences between groups. Values were considered significant if & 0.01. Duncan multiple range test was used to compare the means across each treatment group with untreated group.

Results and Discussion:

One thousand grams (1000g) of *E. hirta* gave an average yield of 54.5g of the extract, and this gave a percentage yield of 5.45% when extracted with the continuous extraction process of the Soxhlet apparatus.

Phytochemical Screening:

The result of the phytochemical screening is presented in Table 1. Phytochemical screenings of *E. hirta* showed that the extract contains alkaloids, flavonoids, tannins, saponins, glycosides, and unsaturated steroids while tri-terpenes and anthraquinones were absent.

Table.1: Phytoconstituents in the Crude Methanol Extract of *Euphorbia hirta*.

Phytochemical Compounds	Inference
Alkaloids	P
Tannins	P
Saponins	P
Glycosides	P
Flavonoids	P
Anthraquinones/Free Antracene	A
Unsaturated Steroids	P
Triterpenes	A

Acute Toxicity Studies:

The result of the acute toxicity studies is presented in Table 2. It showed that the LD_{50} of *E. hirta* is above 5000 mg/kg of crude extract because no death was recorded above 5000 mg/kg in the rats.

Table.2: Acute Toxicity Test of Methanol Leaf Extract of *E. hirta*.

Phase	Dose mg/kg	No. of Animals	Death Ratio
Phase-1	10	3	0/3
	100	3	0/3
	1000	3	0/3
Phase-2	1200	1	0/1
	1600	1	0/1
	2900	1	0/1
	5000	1	0/1

Ulcer Index:

The result of the ulcer index of rats is presented in Table 3 and Figure 1. The result showed that the rats pre-treated with misoprostol, absolute ethanol alone, *E. hirta* alone, and in

combination with absolute ethanol at doses of 200, 400, and 800 mg/kg had a significantly reduced area of gastric ulcer formation compared to ulcer in the negative control group that received only normal saline. The result from examination of gross ulceration of the stomach showed that the negative control had the highest level of ulceration as indicated by the ulcer index followed by rats that received 400 mg/kg, 800 mg/kg, and 200 mg/kg of the crude extract of *E. hirta* respectively.

Table.3: Antiulcer Effect of Crude Methanol Extract of *E. hirta* Combined with Absolute Ethanol in Rats.

Dosage	Ulcer Index	Stomach wt.	Gastric Mucus	PIU
200mg/kg	12.14^{c}	1.29^{a}	0.034	54.09^{d}
400mg/kg	14.19^{c}	1.28^{a}	0.038	45.34^{b}
800mg/kg	13.25^{d}	1.28^{a}	0.035	48.94^{c}
200mg/kg+Honey	1.4^{a}	1.29^{a}	0.052	94.74^{f}
400mg/kg+Honey	3.2^{b}	1.28^{a}	0.048	87.97^{e}
800mg/kg+Honey	3.2^{b}	1.28^{a}	0.035	87.97^{e}
Mis 50µg/kg	1.6^{b}	1.31^{b}	0.035	93.99^{f}
Honey (1ml)	2.8^{b}	1.29^{a}	0.034	89.47^{e}
NS ml/kg	26.6^{f}	1.31^{b}	0.038	-

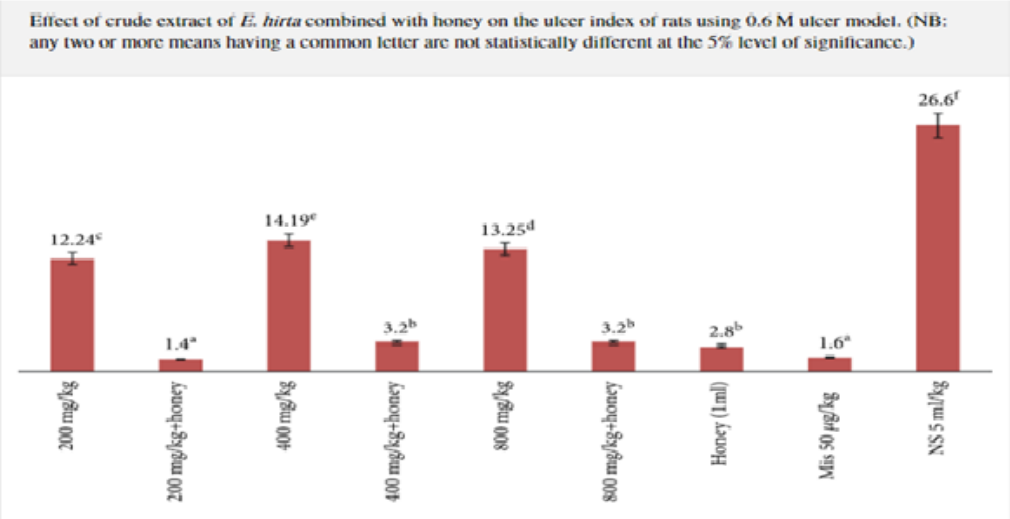

Figure.1: Effect of Crude Extract of *E. hirta* Combined with Absolute Ethanol on the Ulcer Index of Rats using 0.6 M Ulcer Model. (NB: Any Two or More Means Having a Common Letter are not Statistically Different at the 5% Level of Significance).

The result showed that rats that were pre-treated with 200mg/kg plus absolute ethanol (1.4) combination had the least ulcer index followed by the rats that received misoprostol (standard drugs) (1.6), 400 (3.2), and 800mg (3.2) of extract combined with absolute ethanol when compared to the rats that were pre-treated with 200, 400, and 800mg/kg of extract alone and the negative control group.

The result showed significant reduction of ulceration (ulcer index) among rats treated with 200 mg/kg of the leaf extract of *M. oppositifolius* combined with absolute ethanol when compared with all other treatment regimen. No significant difference was observed between the rat group treated with 800 mg plus absolute ethanol and 400 mg plus absolute ethanol compared with the group that received only absolute ethanol (1 ml). Also, there was a statistical difference between the groups treated with absolute ethanol (1 ml) and the group treated with the leaf extract at 200, 400, and 800mg/kg alone. There was enhanced significant reduction in ulceration among the group treated with 200mg+absolute ethanol when compared with absolute ethanol alone (1 ml) and when compared with 400 and 800mg/kg plus absolute ethanol. This implied that the crude leaf extract of *M. oppositifolius* at 200mg+absolute ethanol combined had enhanced significant therapeutic effect against ulceration in rats. This implied that the combination of absolute ethanol and *E. hirta* crude leaf extract at 200mg+absolute ethanol showed significant synergistic effect in gross reduction of ulceration in 0.6 M hydrochloric acid-induced ulcers in rats.

Percentage Inhibition of Ulceration:

The result of the percentage inhibition of ulceration is presented in Table 3 and Figure.2. The result of percentage inhibition of ulceration showed that rats pre-treated with a combination of absolute ethanol and *E. hirta* at 200mg (94.74%) had the highest (significant) percentage inhibition of ulceration when compared with the rats pre-treated with 200 (54.09%), 400 (45.34%), and 800 mg/kg (49.94%) of *E. hirta* alone and in 400 (88%) and 800mg (88%) combinations with absolute ethanol, respectively, while absolute ethanol alone had 89.47% inhibition of ulceration, and this is significantly different. Also, the result clearly showed the synergy in percentage inhibition and antiulcer activity of the rats pre-treated with *E. hirta* combination with absolute ethanol when compared with those treated with only 200, 400, and 800mg//kg of *E. hirta* extract alone. This implied that crude extract of *E. hirta* when combined with absolute ethanol had better inhibition of ulceration when compared with the crude extracts that were administered alone to the rats. Although no significant difference was seen between the group that received absolute ethanol alone (1 ml) when compared with the group that received *E. hirta* leaf extract at 400 and 800 mg/kg, respectively, combined with absolute ethanol. There is significant difference between the group that received 200mg *E. hirta* leaf extract plus absolute ethanol when compared with the group that received absolute ethanol alone (1 ml) and groups that received extract at 400 and 800mg/kg plus absolute ethanol, respectively. This implied that 200mg/kg of the leaf extract of *E. hirta* plus absolute ethanol worked synergistically to inhibit ulceration. There is no statistical difference between the group treated with 200mg/kg of the crude extract plus absolute ethanol when compared with the standard drugs; hence, they have similar therapeutic effect.

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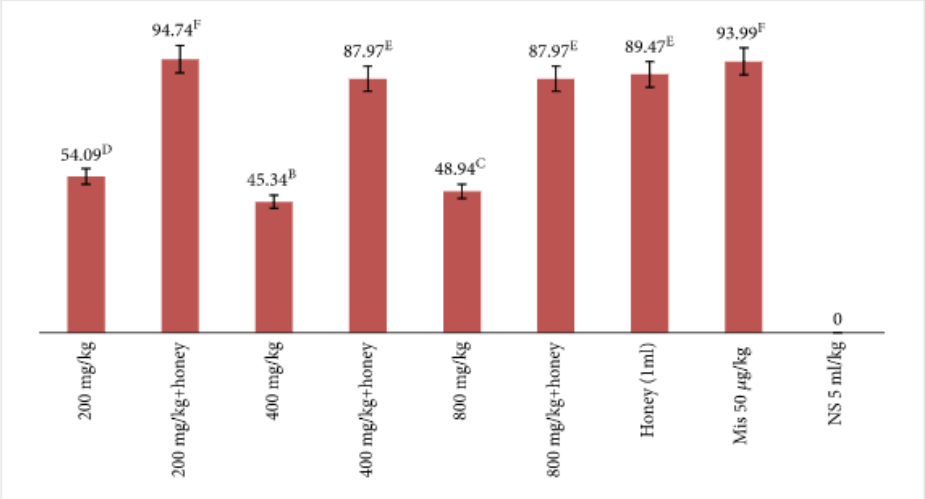

Figure.2: Percentage Inhibition of Ulceration of *E. hirta* Combined with Absolute Ethanol in 0.6 M HCl Model Ulceration in Rats.

Stomach Weight:

The result of the effect of *E. hirta* when combined with absolute ethanol on the stomach weight of rats is presented in Table.3 and Figure.3. The result showed that there was no significant difference in the stomach weight of rats among the entire treatment group. This implies that the weight of the stomach of the rats does not depend on the treatments. The result showed that the rats pre-treated with 200mg/kg and 200mg/kg combined with absolute ethanol had 1.2g of the stomach weight while those that received 400mg and 400mg/kg combined with absolute ethanol had 1.28g of the stomach weight. The weight of the stomach of the rats pre-treated with 800mg/kg and 800mg/kg combined with absolute ethanol also was 1.28g while absolute ethanol alone had 1.29g of stomach weight. The result further showed that misoprostol and distilled water had 1.31g each of the stomach weight.

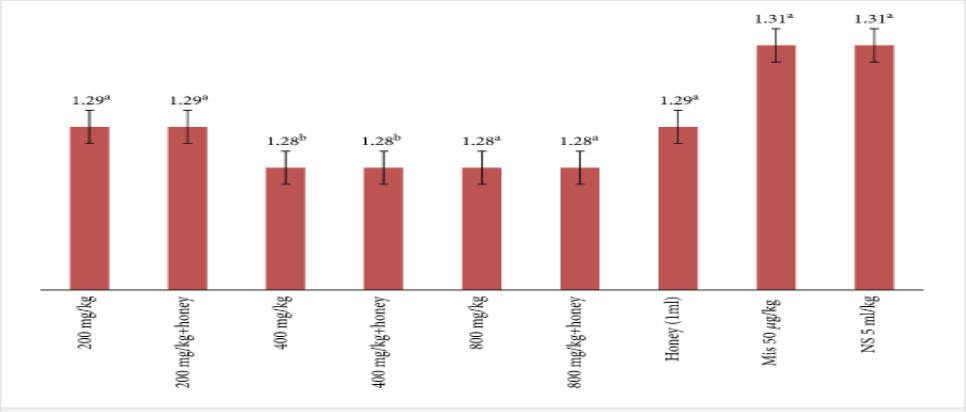

Figure.3: Effect of Crude Extract of *E. hirta* Combined with Absolute Ethanol on the Stomach Weight of Rats Using 0.6 M HCl (NB: Any Two or More Means Having a Common Letter are not Statistically Different at the 5% Level of Significance.)

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Gastric Mucus:

The result of the effect of *E. hirta* on gastric mucus secretion on the stomach is presented in Table.3 and Figure.4. The study showed that the absolute ethanol combined with *E. hirta* at 200mg/kg enhanced the gastric mucus secretion, though there was no significant difference with the other treatment groups. Rats administered with 200mg/kg combined with absolute ethanol had the highest concentration of gastric mucus (0.052μg) while the rats administered with 200mg/kg of the crude extract alone had the least 0.034μg of gastric mucus concentration. The result showed that rats administered with 200mg alone had 0.034μg concentration of gastric mucus while the rat administered with 200mg/kg combined with absolute ethanol had 0.052034μg concentration of gastric mucus. This implied that absolute ethanol combined with the crude extract of *E.hirta* had effect on the gastric mucus concentration which could be attributed to the cytoprotective nature of absolute ethanol and the wound healing effect of absolute ethanol which worked in synergy with crude extract of *E. hirta* to inhibit ulcers in rats.

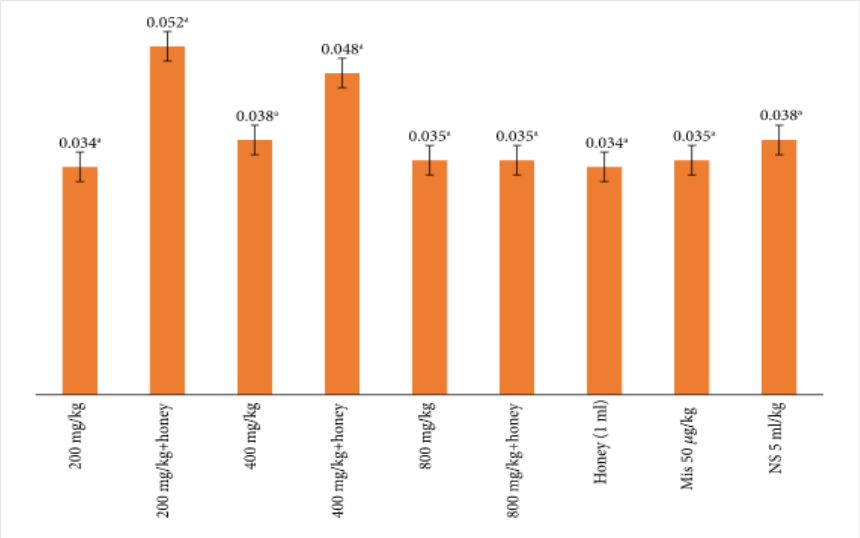

Figure.4: Effect of Crude Extract of *E. hirta* Combined with Absolute Ethanol on the Stomach Gastric Mucus of Rats Using 0.6 M HCl.

The present work evaluated the Phytoconstituents, acute toxicity, and antiulcer effect of methanol extract of *E. hirta* combined with absolute ethanol in rats. The result of phyto-chemical screening of *E. hirta* revealed the presence of alkaloids, tannins, saponins, glycosides, flavonoids, and unsaturated steroids. This is in agreement with the findings of who reported similar Phytoconstituents in *E. hirta*.

The result of the acute toxicity evaluation of *E. hirta* combined with absolute ethanol showed no apparent toxic (mortality) effect in rats up to a dose of 5000mg/kg when given orally. Therefore, LD_{50} of the extract combined with absolute ethanol was considered to be above 5000mg/kg of the extract. This implies that the plant is a relative safe for consumption and is used in ethno medicine, at doses not exceeding 5000mg/kg of the extract.

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Our choice of this combination therapy was informed by the traditional use of the leaves of *E. hirta* for the treatment of peptic ulcer in folk medicine and the well-established therapeutic values of absolute ethanol, particularly in the treatment of gastrointestinal disorders. A combination therapy of the leaf extract and absolute ethanol has an excellent anti-ulceration activity with a high potency.

The evaluation of anti-ulcerative effects of *E. hirta* combined with absolute ethanol at doses of 200, 400, and 800mg/kg using different ulcer models showed that the combination is effective. Gastric ulcer instillation using 0.6mol/l HCl induces gastric necrotic damage due to infiltration of inflammatory cell leading to reduction in the secretion of bicarbonate, gastric mucus, and hyper secretion of nitric oxide. Instillation by 0.6mol/l-HCl reduces the gastric blood flow and induces the oxidative stress by increasing the production of malondialdehyde thereby reducing secretion of endogenous glutathione. The significant increase in the ulcer index gastric volume as observed in the negative group that received water after the instillation of ethanol confirmed the induction of ulcer, which can be attributed to either reactive oxygen species formation or inhibition of mucus synthesis and also lipid per-oxidation. The gastric ulcer induced by 0.6mol/l-HCl could be associated with the increased purine degradation that leads to increased O_2^- radical production and ROS-mediated increased lipid per-oxidation. A low level of mucus suggests that the integrity of gastrointestinal apparatus was impaired when exposed to 0.6mol/l-HCl as seen in the negative control group. Treatment with the methanol leaf extract of *E. hirta* and the combination with absolute ethanol showed that there was significant reduction in the ulcer index, most notably in the treatment with a combination of the extract and absolute ethanol.

The antiulcer effect of *E. hirta* combined with absolute ethanol could be attributed to antioxidant and free radical scavenging effect of the Phytoconstituents of *E. hirta* and absolute ethanol, respectively, as reported by several studies. Thus, the antioxidant effect of absolute ethanol was due to its catalase content and one molecule of catalase can scavenge for 40 million free radicals. The antiulcer effect could have resulted from the cytoprotective effect of *E. hirta* combined with absolute ethanol, which could be due to increase in mucus secretion that protects the gastric mucosal membrane from corrosive effects of HCl and stomach acid overproduction. This result is in agreement to the findings who reported that ethanolic extract *Euphorbia hirta* possesses gastro-protective potential which is related partly to preservation of gastric mucus secretion and anti-secretory action. Also, the findings are similar to the findings of. Who reported that absolute ethanol in combination with *Trigonella foenum-graecum* seed has antiulcer potential [Mahmood et al].

The combination of crude extract of *E hirta* and absolute ethanol showed increased inhibition of ulceration, and this implied that there is synergy in activity. Flavonoids in *E. hirta* and Catalase in absolute ethanol by their capacity as a free radical scavenger and saponins by their capacity to produce mucus could protect the gastric mucosal membrane against the acid effects and could synergistically be responsible for the antiulcer effect of these combinations. Flavonoids enhance the protection of the gastric mucus therefore inhibiting ulceration. They stimulate prostaglandin bicarbonate and mucus secretion and prevent degrading effects of reactive oxidants in the gastrointestinal system. Flavonoids have also been reported to offer

protection in ulcer development by increasing capillary resistance and improving microcirculation. It is acknowledged that tannins protect the outermost layer of the mucosa and make it less permeable and more resistant to chemicals and mechanical injury or irritation and thus prevent ulcer development. Saponins induce mucus production, which protects the gastric mucosal membrane against the acid effects.

This finding further supports the veracity of the indigenous knowledge ethno medicinal claim and has shown that *E. hirta* combined with absolute ethanol is relatively safe for consumption since its LD_{50} exceeded 5000mg/kg and also posses gastro-protective and gastro-therapeutic effect.

Conclusion:

The result of this study suggests that the methanolic extract of *E. hirta* combined with absolute ethanol is safe for use and could protect the gastric mucosa against damage by HCl and also has ulcer healing effect. The study confirmed that crude extract of *E. hirta* and standard drugs produced a decreased ulcer index and increased percentage inhibition of ulceration in 0.6mol/l HCl-induced ulceration. Furthermore, the *E. hirta* crude leaf extract at 200mg/kg combined with absolute ethanol had more gastric mucus concentration which implied that the combination has an enhanced and synergistic effect against 0.6 mol/l HCl-induced ulceration in rats. The ulcer inhibition potential of *E. hirta* combined with absolute ethanol could justify the use of this combination by the Igbo traditional healers in South-eastern Nigeria for the treatment of ulcer.

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