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Peptic Ulcer Diseases (Pud) Treatment Using Ocimum Gratissimum

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ABSTRACT:*Methanol leaf extract of Ocimum gratissimum was investigated for gastroprotective properties. The anti-ulcer effect was evaluated in three experimental ulcer models induced by ethanol, indomethacin and hypothermic restraint stress in rats. Anti-ulcer related properties of the extract of the plant such as gastrointestinal transit and the activity of the isolated gut tissue preparation of guinea pig and rabbit were as well determined using 200, 400 and 800 mg/kg^{-1}of the extract which showed significant level of ($p<0.05$). This reduced the ulcer to some extent. The extract showed higher level of gastroprotective properties of ethanol-induced rats and indomethacin treated rats. The motility rate of the rats was significant ($p<0.05$) reduced by the mice. On the rabbit jejunum, ME produced a concentration-dependent relaxation and inhibited contractile responses. The extract, ME on guinea pig ileum but inhibited the contractions produced by histamine. The extract showed no sign of acute toxicity of the rats. The result showed that methanol leaf extract of Ocimum gratissimum is of high level of protective property to ulcerations and could be used for the treatment of peptic ulcer diseases.*

KEYWORDS: *Extract, gastro-protective and histamine.*

I. INTRODUCTION

Herbal medicines:

The World Health Organization (WHO) defines herbal medicines as finished labeled medicinal products that contains as active ingredients,aerial or underground parts of plants or other plant materials or combination(s) of such whether in the crude form or as plant preparations (WHO, 1991). Man has used plants as medicine and food and for many other purposes since ancient times. In industrialized countries, there has been an important loss of the traditional knowledge of plants uses transmitted from parents to children. There is also a consensus that we are now at a critical moment in which the transmission chain is at risk. It is necessary therefore to make efforts to avoid the definitive erosion of this knowledge not only to preserve a part of cultural heritage, but also to conserve the information on useful plants because it could be relevant for developing new sources of medicine or be of other benefits to the population (Raja et al, 1997). Because of this, the process of learning the beneficial and harmful aspects of plant use which has taken many centuries to develop and accumulate are being continuously documented and update (Rabiu, 2002).The untapped wealth of the plant kingdom has become a target for institutions in an attempt to seek new drugs or lead compounds especially with the wave of new epidemiological conditions the world is faced with today. Diseases such as AIDS, herpes, malaria, tuberculosis and other emerging multi-drug resistant disease continue to encourage research efforts into herbal medicine (Rabiu, 2002). Based on these scientists all over the world are focusing attentions medicinal plants because of the potential these plants possess in combating various ailments (Amos et al, 2001).

Peptic Ulcer Disease

Gastrointestinal disorders are among the most important causes of morbidity and mortality among the rural poor (Germano et al, 1998; Zavala et al, 1998) and frequent complications of the disorder have stimulated considerable research.

Peptic ulcers are benign focal lesions of gastric or duodenal mocosa occurring at a site where the mucosal epithelium is exposed to acid and pepsin (Dhuley and Naik, 1998). Peptic Ulcer Disease (PUD) is characterized by a gnawing or burning sensation in the abdomen, between the breastbone and the navel; because of the position of the pain, it is commonly referred to as heartburn. Other symptoms include nausea, vomiting, weight loss, anorexia. Bleeding from the ulcer could occur which often goes unnoticed because blood loss is slow and may not be obvious in the stool. These patients may experience fatigue and weakness. However, in cases where bleeding could be heavy, blood appears in vomit or stool (NIH, 1994).

Disease Burden

PUD is a common health problem worldwide with the first episode of duodenal ulcers occurring at about the age of 20 while gastric ulcers are more likely to develop in people over the age of 40; morbidity rate of duodenal ulcer is more in men that in women while the reverse is the case with stomach ulcer (Havard, 1987; NIH, 1994).

Pathogenesis

Although in most cases the aetiology of PUD is unknown, investigations into the pathogenesis have developed the concept that ulceration results from imbalance between factors that produce epithelial and deep mucosal injury and those that protect the mucosa and facilitate its repair (Venkatarnganna et al, 1998). There is a constant confrontation in the stomach and upper small bowel between acid-pepsin aggression and mucosal defense. Usually, the gastrointestinal mucosa is able to resist acid proteolytic digestion and remain healthy. However, when there is a shift in the balance between the aggressive action of acid-peptic secretion and the maintenance of the mucosal integrity through the endogenous defense mechanism ulcer develops. The endogenous defense mechanism include the mucous layer of mucin-bicarbonate secretion, phospholipids layer, tight junctions, cell proliferation, prostaglandins and the urogastrone/epidermal healing factors. Emotional and physical stress, hormonal imbalance, hypermortility, vascular spasm, thrombosis, allergy, reduced altered mucous production, over indulgence in spicy foods and beverages containing caffeine and long term fasting are some of the possible factors contributing to increased susceptibility of mucosa to ulceration (Ibu et al, 1994). Coffee and foods that contain caffeine seem to stimulate acid secretion in the stomach aggravating the pain of existing ulcer. Bicarbonates neutralize and break down digestive fluids into substances that are less harmful to stomach lining; it also promotes cell renewal.

Gastric and duodenal ulcers have been causally associated with infection of gastrointestinal mucosal tissue by *Helicobacter pylori* (Suerbaum and Mitchetti, 2002).

Drug management of PUD

Many drugs are available for the treatment of peptic ulcer diseases. The antacids are a group of drugs that are able to neutralize the gastric acids because of their alkaline nature thus attenuate the corrosive effect and reduce ulcer pains and promote healing. Although their principal mechanism is reduction of intragastric acidity, they may also promote mucosal defense mechanisms through stimulation of mucosal prostaglandin production (Mc Quaid, 2004).

Treatment with cytoprotective agents (Sucralfate, Colloidal bismuth), the prostaglandin analogues (misoprostol and carbenoxolone) is directed at strengthening this mucosal defense at the stomach and duodenum. Although the mode of action of sucralfate is unclear, it has been proposed that the base of ulcers or erosions forming a physical barrier that restricts further caustic damage and stimulates mucosal prostaglandin and bicarbonate secretion. It may also bind epithelial growth factor and fibroblast growth factor thereby enhance mucosal repair (Mc Quaid, 2004).

Since a high proportion of PUDs occurs with infection by *H. pylori* eradication of the bacterium with antibiotics such as amoxicillin, clarithromycin is recommended in patients with active PUDs (new, recurrent duodenum or gastric). Eradication of *H. pylori* reduces the need for maintenance therapy in patients with duodenal or gastric ulcers. Patients with complicated ulcer disease should continue maintenance therapy until the eradication of *H. pylori* (Souney and Zimmermann, 2004).

Surgery:

In cases where the ulcer does not respond to adequate medical treatment, or if the ulcer leads to complications such as severe pyloric obstruction, perforations of the ulcer or narrowing of GIT passages, surgery may be performed. In vagotomy where a branch of the vagus nerve is severed, the pains of peptic ulcer are relieved by decreasing the volume and acidity of gastric secretion and the motility of the stomach is also reduced. An antrectomy removes the antrum thereby removing some acid-secreting mucosa as well as the major source of gastric (Souney and Zimmermann, 2004).

Objective of the study

Extensive studies have been carried out on *Ocimum gratissimum;* there is however, no reference in literature on the possible anti-ulcerogenic potential of this plant either in man or animals. This present work was therefore designed to evaluate the effects of *Ocinum gratissimum* leaf extract and its fractions on gastric lesions and to determine its possible application as an anti-ulcer agent.

II. MATERIALS AND METHODS

Plant Materials

The leaves of *Ocinum gratissimum* were collected around Awo-Omamma, Imo State, Nigeria between the months of June and September, 2012. The plant was identified and Ibrahim Muaxiam of the Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NJPRD), Abuja.

Preparation of Extract

The fresh leaves were cleaned, air-dried and crushed into coarse powder using a mortar and pestle. The powder (350g) was cold macerated with 2.5 litre of methane). The mixture was allowed to stand with occasional shaking for 24 hr, after which, it was filtered. The filtrate was concentrated to dryness in vacuum at 40 ^{0}C using a rotaryevaporator to give a residue of 6.5 ± 0.25 % w/w referred to as the crude extract (OGE.).

Phytochemical Screening

Phytochemical analysis of the extract to detect the presence of carbohydrates, tannins, glycosides, flavonoids terpenes, steroids, resins, balsams, volatile oil, saponins, anthraquinones and alkaloids were performed according to the methods of Evans (2004).

Animals

20 adult Wistar rats (180 - 200g), adult Swiss albino mice (28 - 32g) of both sex adult guinea pigs (350 - 400g) and New Zealand rabbits (1.5 - 3.0kg) bred at uniform condition at the Animal Facility Centre of the National Institute for Pharmaceutical Research and Development (NIPRD), were used in this study. They were housed under standard condition of temperatures (25 ± 2°C), 12 hr light/dark cycle and fed on standard feeds (Ladokun Feeds, Ibadan, Nigeria) and water *ad libitum.*

Hypothermic Restraint-stress

The 20 rats were deprived of food for 24 hr, but allowed free access to water. The animals were randomly grouped into five groups of 4 animals per group. One (1) hr after oral drug administration, the rats were immobilized individually in restraining cages at a temperature of 4 ± 1 °C for 2 hr (Al-Mashhadani et al,1991). The animals were then sacrificed with chloroform. The stomach were opened along the greater curvature washed and stretched on cork plates and the inner surface was examined for the presence of lesions with a hand lens. The gastric ulcers were scored on the following arbitrary scale.

0 - No ulcer;
1 - Punctiform ulcer;
2 - Ulcers < 2 mm;
3 - Ulcers > 2 $\leq$ 4 mm.

Mean score for each group was calculated and expressed as ulcer index (UI).

Indomethacin-induced Ulcer

20 rats were fasted of food for 24 hr but allowed free access to water. The least effective dose (p.o.) of indomethacin that would produce 100 % gastric ulceration was obtained by administering various doses of indomethacin (40, 60, 100 mg/kg p.o.). 100 mg/kg produced gastric-ulceration in all rats in 4 hr. This dose was repeated to verify if the degree of ulceration will be reproducible (Nwafor et al,2000). The rats were randomly placed into five groups of 4 rats each. Indomethacin 100 mg/kg was administered orally 1 hr after drug treatment. The animals were sacrificed with chloroform 4 hr after indomethacin administration, their stomachs were dissected out, opened along the greater curvature, rinsed and stretched on cork boards. These were examined with hand lens and gastric ulcers rated using the method as described by Evbuonwa and Bolarinwa (1990).

0 - Normal
0.5 - Punctuate or Pin-point
1.0 - Two or more small haemorrhagic ulcers;
Less than 3 mm in diameter
2.0 - Ulcers greater than 3 mm in diameter
3.0 - Several ulcers

Statistical Analysis

Results were expressed as mean ± SEM. Significance was determined using two way ANOVA and Student's t-test. Results were regarded as significant at $p < 0.05$.

III. RESULTS

Phytochemical tests

The phytochemical evaluation indicated the presence of the following chemical constituents in the crude extract and fractions of the plant; *Ocimum gratissimum* crude extract (OGE):Saponins, tannins, flavonoids, terpenes, alkaloids, carbohydrates, steroids, resins and volatile oils.

Haxane Fraction (F1) - Volatile oils

Ethylacetate Fraction (F2) - Saponins, tannins, flavonoids, terpenes and alkaloids.

Methanol Fraction (F3) - Saponins, carbohydrates, tannins, flavonoids, steroids, alkaloids.

Acute Toxicity Studies

No lethality was observed in the mice upon oral administration of doses up to 3000 mg/kg. OGE and its fractions did not produce any obvious behavioural changes and no major clinical signs of toxicity (e.g. convulsion, respiratory distress) were observed in the animal during the 48 hr observation period.

Studies on Isolated Smooth Muscle Preparations

The extract OGE showed no effect on the guinea pig ileum. Histamine (4.5×10^{-7} -7.2×10^{6}) produced a concentration dependent contraction of the guinea pig ileum. However, the extract inhibited the contractile effect of histamine in a concentration-dependent manner similar to that of mepyramine (2.5×10^{-9} M). On the rabbit jejunum, (lie extract caused a concentration-related relaxation of the spontaneous contraction, while acetylcholine (2.5×10^{-9} - 1.0×10^{-7} M) produced a concentration dependent contraction. The extract attenuated the acetylcholine-mediated contraction of the tissue in a similar manner to the effect of atropine (5.0×10^{-9} M) on acetylcholine-induced contraction.

IV. DISCUSSION

This study has provided data which suggests that the methanolic extract of *O. gratissimum* contains biologically active components that produce anti-ulcerogenic activities. Methanol, hypothermic-restraint stress and indomethacin are among the most commonly utilized experimental models for the evaluation of anti-ulcer activity in rats (Paiva et al,1998). Various mechanisms may be associated with the formation of gastric mucosal damage in these experimental models. It has been postulated that ethanol-induced gastric mucosal lesions are caused by the direct toxic action of ethanol, reduction of the secretion of bicarbonate, and depletion of gastric wall mucous (Marhuenda et al, 1993)gastric acid is virtually not involved in the formation of such lesions. Endogenous glutathione and prostaglandin (PG) levels are reduced by ethanol, while the release of histamine, influx of calcium ions and, generation of free radicals are increased. It has been shown that ethanol-induced gastric mucosal lesions are not inhibited by anti-secretary agents like cimetidine but are inhibited by agents, which enhance mucosal defensive factors (Morimoto et al, 1991*).*

The results obtained in this study showed that the extract and its fractions protected the rats against ethanol, hypothermic restraint and indomethacin-induced ulcerogenesis. It may be possible that the protective effect of *O. gratissimum* is due to an increase in production of gastric wall mucous and bicarbonate secretion that would re-enforce gastric mucosal defense and/or a possible leukotriene antagonism by prevention of the generation or the necrotic action of LTCU as reports have shown that inhibition of leucotriene synthesis was accompanied by a decrease in gastric mucosal damage in different experimental models (Osada et al, 1990). It has been proposed that mucosal protection induced by non-prostanoid compounds may be mediated through the mobilization of endogenous prostaglandin. When the cytoprotective effect of an anti-ulcer agent is significantly reduced by pre-treatment with indomethacin the cytoprotection is interpreted as being mediated by endogenous prostaglandins (Tan et al, 2000). It could be possible that *O. gratissimum* possesses the ability to mobilize endogenous prostaglandins in gastric mucosa as endogenous prostaglandins play an important role in gastrocytoprotection against the necrosis produced by irritant agents (Germano et al, 1998). It is known that flavonoids possess anti-bacterial activity and inhibit acid secretion, effects which have been related to flavonoid influence on arachidonic acid metabolism and their ability to interfere with the formation of histamine in gastric mucosa.

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