



Full Length Research Paper

## Anti-ulcer effect of *ocimum gratissimum* on indomethacin induced ulcer and percentage of superoxide dismutase on wistar rats

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### Abstract

This study was aimed to elucidate the effect of methanolic extract of *Ocimum Gratissimum* on indomethacin induced ulcer and its antioxidant properties. Thirty (30) albino rats weighing between 140-180g were used for this study. They were divided into five groups with six in each group and group 1 and 2 (n = 6) were pretreated with 100mg/kg and 300 mg/kg methanolic extract of *Ocimum Gratissimum* for two weeks, then indomethacin on the 14<sup>th</sup> day. Group 3 was treated with 40 mg/kg indomethacin alone, group 4 served as reference group (cimetidine treated, 100mg/kg), the group 5 was the control and was given 1ml of saline. On the day 14, the leaves extract was administered thirty minutes before ulcer was induced. Ulcer was induced using indomethacin (40mg/kg). The animals were sacrificed by cervical dislocation after four hours. The percentage of superoxide was measured by Martin's method of hematoxylin. The stomach was homogenized, centrifuged and decanted. The result shows that *ocimum gratissimum* reduces ulcer score and ulcer index, also increases the percentage of superoxide dismutase in the treated animals. It is therefore concluded that methanolic extract of *ocimum gratissimum* has gastro-protective properties and antiulcer properties.

**Keywords:** *Ocimum gratissimum*, ulcer score, ulcer index, superoxide dismutase.

### INTRODUCTION

Peptic ulcer disease is one of the most common gastrointestinal disorders, which causes a high rate of morbidity particularly in the population of non-industrialized countries (Falk, 2001). Peptic ulcer occurs due to an imbalance between the aggressive (acid, pepsin and *Helicobacter pylori*) and the defensive (gastric mucus and bicarbonate secretion, prostaglandins, innate resistance of the mucosal cells) factors (Tripathi, 1999). Number of drugs including proton pump inhibitors, prostaglandins analogs, histamine receptor antagonists and cytoprotective agents are available for the treatment of peptic ulcer. But most of these drugs produce several adverse reactions including toxicities and even may alter biochemical mechanisms of the body upon chronic

usage. There is evidence concerning the participation of reactive oxygen species in the etiology and pathophysiology of human diseases, such as neurodegenerative disorders, inflammation, viral infections, autoimmune pathologies and digestive system disorders such as gastrointestinal inflammation and gastric ulcer (Vinothapooshan and Sundar, 2010). Reactive oxygen species are responsible for oxidation of tissues leading to lipid peroxidation and tissue damage, mutagenic cells that leads to cancers (Ito et al., 1996). Hence, a search for new anti-ulcer agents that retain therapeutic efficacy and are devoid of adverse drug reaction. Several natural drugs have been reported to possess anti-ulcerogenic activity by virtue of their predominant effect on mucosal defensive factors (Sairam et al., 2001a, 2001b, Nwafor et al., 2005). A study of the efficacy of methanolic extract of *ocimum gratissimum* in gastric ulcer with indomethacin induced ulcer and its antioxidant properties was undertaken in rat's model.

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*Ocimum gratissimum* is a shrub of versatile use in folk medicine and is commonly called tea bush, fever plant or scent leaf. It belongs to the family of plant known as Lamiaceae. They are erect herbs and have characteristic pleasant aroma due to their volatile oil (Dalziel, 1993). The plant is indigenous to tropical areas especially India and also in West Africa. In Nigeria, it is found in the Savannah and coastal areas (Illoh et al., 2000). *Ocimum gratissimum* leaf or the whole herbs are popular treatments, for diarrhoea. *Ocimum gratissimum* is particularly effective in the management of upper respiratory tract infection, diarrhoea, headache, skin disease, pneumonia, fever, and conjunctivitis (Dekkers et al., 1996). However, from previous studies plants that are rich in tannins have been traditionally used for their medicinal effects and several studies have demonstrated their anti-ulcer effects. *O. gratissimum* has been found to possess a higher percentage of tannins (Simides et al., 2003). Tannins are used in medicine primarily because of their astringent properties; they react with the proteins of the tissue layers and precipitate as micro proteins at the site of the peptic ulcer, forming a protective pellicle that prevents absorption of toxic substances, and promote resistance to the action of proteolytic enzymes, an associated activity against *Helicobacter pylori* (Vasconcelos et al., 2008).

Murakami et al., in 1991 showed that ellagic acid is also a component of *O. gratissimum* and the acid is a potent competitive inhibitor of gastric H<sup>+</sup>, K<sup>+</sup> - ATPase, competing with ATP at the ATP hydrolysis site, thus markedly inhibiting acid secretion, and stress-induced gastric lesions (Murakami et al., 1991). Being phenolic compounds, tannins are chemically reactive and forms inter and intra-molecular hydrogen bonds (Heil et al., 2002). In the process of healing wounds, burns and inflammations, tannins help by forming a protective layer (tannin-protein/tannin-polysaccharide complex), over injured epithelial tissues permitting the healing process below to occur naturally (Simides et al., 2003). Since reactive oxygen species (ROS) have been implicated in some of the disorders associated with the traditional uses of *Ocimum* species, such as gastrointestinal tract disorders, diabetes mellitus and inflammatory injury (Halliwell et al., 1999), and polyphenolic compounds are able to bolster biological resistance against ROS, hence in the present study, the *ocimum gratissimum* plant has been selected to investigate the anti-ulcer and antioxidant study on indomethacin-induced ulcer oxidative stress in wistar rats.

## METHOD

### Reagent

Cimetidine was purchased from Alpa laboratories

Limited, India. The solvents and other chemicals of analytical grade were used and obtained from the institute's central store. Indomethacin was purchased from Medrel pharmaceuticals (India) PVT. Limited

### Collection of leaves

The fresh leaves of *Ocimum gratissimum* were purchased from local market in Nassarawa state. It was then identified by a botanist in the department of botany, Ahmadu Bello University, Zaria.

### Preparation of Extraction

The leaves were air-dried and were ground into coarse powder. The powder was first weighed and was extracted with absolute methanol using Soxhlet extractor. The extract was concentrated using rota-evaporator. This methanolic extract was further evaporated to dryness to obtain alcoholic extract.

### Animals and Grouping

30 albino rats of male sex weighing between 120-180g were procured from the Animal House of the Bingham University for this experimental study. They were divided into five groups with six in each groups (n=6). They were acclimatized to laboratory condition seven days before the commencement of the experiment and were allowed free access to standard dry pellet diet and water ad libitum. The experimental protocol was approved by Institutional Animals Ethics Committee (IAEC), for using animals in this experiment. Animals were fasted overnight with free access to water prior to each experiment.

### Animal Grouping

Group 1- control group was given 1ml of water  
 Group 2- negative control was treated with 40mg/kg of indomethacin  
 Group 3 -standard reference was treated with indomethacin and with 100mg/kg of cimetidine  
 Group 4- was treated with indomethacin and 100mg/kg of *Ocimum gratissimum*  
 Group 5- was treated with indomethacin and 300mg/kg of *Ocimum gratissimum*

### Administration

The extract was then dissolved in 0.8% Tween 80 to make a solution of concentration 100mg/ml of *Ocimum*

*gratissimum*. The extract was then given orally to the groups of animals in the appropriate dosages of 100mg/kg, 300mg/kg. Indomethacin was dissolved in 1% sodium bicarbonate and was given orally to the appropriate groups of animals in the correct dosage (100mg/kg) and cimetidine was dissolved in distilled water.

### Acute toxicity studies

Acute toxicity was carried out on the albino rats by up and down method (425 OECD, 2001). The animals were fasted overnight and the extract at different doses was administered orally. The animals were kept under observation continuously for 3 hours for general behavioural, neurological and autonomic profiles and finally till death after 24hrs.

### Indomethacin Induced Ulcer

Following two weeks treatment of the animals with the extract, indomethacin was administered (40mg/kg) and the animals were sacrificed after 4 hours. The stomachs were opened along the greater curvature, washed in normal saline to remove any debris and pinned on a corkboard for ulcer scoring (*Elegbe, 1978*). Ulcers will be independently assessed and scored by two observers using the method of *Rao et al (1997)* as follows: 0, no ulcers (normal stomach); 1, up to 5 petechial; 2, up to 5 petechial with erosions of depth 1 mm; 3, up to 10 petechial haemorrhages with erosions of 1 mm depth; 4, up to 10 petechial haemorrhages with erosion of above 1 mm depth.

Where, Ulcer index =  $\frac{\text{Ulcerated Area}}{\text{Total surface area}} \times 100$ .

### Measurement of Superoxide Dismutase Activity

Martin method of hematoxylin (*Martin et al., 1987*) was used based on the principle that: SOD mediate decrease in the rate of auto-oxidation of hematoxylin in aqueous alkaline solution. The stomach was homogenized, centrifuged and decanted. Sample solutions (40  $\mu$ L) were added to of 920 mM phosphate buffer (pH 7.4) containing 1 mM EDTA and 40  $\mu$ L of 5 mM haematoxylin and mixed thoroughly. The mixture was incubated at 25°C for 2 min and the absorbance was measured at 560 nm. The results were expressed as the percentage (%) of inhibition of haematoxylin auto-oxidation rate with respect to the reaction mixture without the test compound.

### Statistical Analysis

Comparison between treatments were done using

ANOVA+ post Hoc, scheffe's test, the results were expressed as the mean  $\pm$  SEM, differences were considered to be statistically significant at  $p < 0.05$ .

## RESULT

The result of the study is shown in table 1 below.

## DISCUSSION

*Ocimum gratissimum*, commonly known as scent leaf, has been found to possess various medicinal properties such as anti-bacterial, anti-nociceptive, anti-hypertensive effects (*Prabhu et al., 2009*) and very effective in wound healing (*Madeira et al., 2002*).

This present experiment studied the effect of *Ocimum gratissimum* on the development of gastric ulcer and its effect on superoxide dismutase in the stomach of albino rats. It was observed that *ocimum gratissimum* reduces the ulcer index (from  $20.27 \pm 4.10$  to  $1.0300 \pm 0.16$  on administration of a 300mg/kg of the extract, with  $P < 0.05$ ) and caused an increase in percentage of superoxide dismutase in the indomethacin treated and extract treated animals (from  $28.5667 \pm 0.87$  to  $39.8333 \pm 2.55$ ,  $P < 0.05$ ).

Indomethacin induced ulcer has been reported to cause disturbances in gastric secretion, damage to gastric mucosa, alteration in permeability, gastric mucus depletion, increase in the pepsin and protein content, and generation of free radical production (*Salim, 1990*). Another mechanism through which NSAIDs may exert topical irritant effects on the mucosa is through their ability to decrease the hydrophobicity of the mucus gel layer in the stomach (*Lichtenberger, 2005*). It is observed in the study as indomethacin caused an ulcer score change of  $1.0000 \pm 0.00$  in the control group to  $14.3333 \pm 1.20$  in the indomethacin induced group ( $P < 0.05$ ). From this study, the higher dose of the extract (300mg/kg) showed a slightly higher preventive action of *ocimum gratissimum* than the 100mg/kg treated animals. On administration of a 100mg/kg of extract there was an ulcer index of  $2.0333 \pm 0.29$ , while the 300mg/kg dose elicited an ulcer index of  $1.0300 \pm 0.16$ ,  $P$  value  $< 0.01$ . Comparing the cimetidine treated with the 100mg/kg treated animals with 300mg/kg treated group, there is no significant differences, the ulcer score, ulcer index are  $1.6667 \pm 0.333$ ,  $0.5333 \pm 0.200$  vs  $2.000 \pm 0.000$ ,  $1.300 \pm 0.16$ ,  $p > 0.05$ , which shows that it is not significantly different .i.e. their actions is almost the same. This suggests that if the dose of *ocimum gratissimum* is increased than 300mg/kg used in this study, it will have a higher antiulcer effect than cimetidine i.e. it is dose dependent.

The exposure of gastric mucosa to aggressive factors such as absolute ethanol, stress, and ischemia followed by reperfusion, and the use of NSAIDs produce patholo-

**Table 1.** Effects of *ocimum gratissimum* on Ulcer score

Group	Treatment	Ulcer score(mm <sup>2</sup> )	Ulcer index (%)	Super oxide dismutase (SOD)%
1	Control ( 1ml of distilled water)	1.00±0.00	0.18±0.03	51.90±1.91
2	Indomethacin (40mg/kg) (negative control)	14.33±1.20	20.27±4.10	28.57±0.87
3	Cimetidine (100mg/kg) + Indomethacin (40mg/kg)	1.67±0.33***	0.53±0.20***	46.30±0.90***
4	<i>Ocimum gratissimum</i> (100mg/kg)	2.67±0.33*	2.03± 0.29*	29.43±1.23*
5	<i>Ocimum gratissimum</i> (300mg/kg)	2.00±0.00**	1.03±0.16**	39.83±2.55**

N=6 (animals are used in each group.)

\*\*\*P<0.001, \* \*P<0.01, \*P<0.05, NS- Non Significant as compared to control One way ANOVA followed by student't't test

gical changes and the development of inflammation, hemorrhagic erosions, and ulcers with the acute involvement of free radicals, or Reactive Oxygen Species (Wada et al., 1997). These radicals are normally neutralized by the action of the antioxidant system consisting of organic substances containing thiol groups such as glutathione, vitamins C and E, NADPH, antioxidant enzymes such as peroxidase, superoxide dismutase, glutathione peroxidase, glutathione reductase and others (Chattopadhyay et al., 2006). When there is an imbalance between ROS and the antioxidant defence mechanisms, ROS lead to oxidative modifications in the cellular membrane and intracellular molecules resulting in peroxidation of membrane lipids, accumulation of lipid peroxides, and cellular damage (Cartea et al., 2012). In this experiment, the percentage (%) of superoxide dismutase is higher in the methanolic extract treated group. In the 100mg/kg treated animals, the percentage of the SOD is about 29.43±1.23,\*p<0.05 is slightly higher than those given indomethacin alone (28.5667±0.87) while the % of SOD in 300mg/kg treated animals is significantly than the indomethacin treated group and in 100mg/kg treated group. *Ocimum gratissimum* has shown antioxidant properties in this study by the % of SOD.

However, from previous studies plants that are rich in tannins have been traditionally used for their medicinal effects and several studies have demonstrated their anti-ulcer effects. *Ocimum gratissimum* has been found to possess a higher percentage of tannins (Simides et al., 2003). Tannins are poly - phenols present in plants, foods and beverages. They form complexes with water-insoluble proteins, alkaloids and gelatin.

Therefore, the high presence of these phenolic compounds explains the anti-ulcer benefits of *ocimum gratissimum*. Studies shows that many tannins act as radical scavengers, intercepting active free radicals, various degenerative diseases such cancer, multiple

sclerosis, atherosclerosis and aging process itself are associated with high concentrations of intercellular free radicals (Funatogawa et al., 2004). Cimetidine inhibit by antagonizes the activity of histamine at the H<sub>2</sub> receptors and could be possible that *ocimum gratissimum* may also use the same mechanism of action since from the results there is no significant difference in the group treated with cimetidine and 300mg/kg of *ocimum gratissimum*. Flavonoids and phenolic compounds such as cirsilinole, cirsimaritin, isothymusin, apigenin and rosmarinic acid which are present in the leaves of *ocimum gratissimum* are also known to exhibit antioxidant activities as well as scavenge superoxide radicals (Kelm et al., 2000). The increase in serum SOD produced by *ocimum gratissimum* leaf extract in animal models of peptic ulcer indicates its antioxidant property which could be a mechanism of action for it antiulcer activity.

Furthermore, from this study, it could also be suggested that *ocimum gratissimum* could also increase up regulation or increase in COX1 and COX2 which in turn would lead to increase in prostaglandin synthesis. Although the exact mechanism is not yet determined. However, it could be concluded that *ocimum gratissimum* has both anti-ulcer and anti-oxidant properties probably by increasing defensive gastric mucosa and increasing the percentage of Superoxide dismutase in the stomach.

## REFERENCES

- Albuquerque UP, Monteiro JM, Araújo EL (2005). Tannins. Int. J. Molecul. Sci.
- Cartea MA, Francisco M, Soengas P, Velasco P (2011). Phenolic compounds in Brassica vegetables. Molecules.
- Chattopadhyay I, Bandyopadhyay U, Biswas K, Maity P, Banerjee R (2006). Indomethacin inactivates gastric peroxidase to induced reactive oxygen mediated gastric mucosal injury and curcumin

- protects it by preventing peroxidase inactivation and scavenging reactive oxygen. *Free Radical Biological Medicine*, 40:1397–1408
- Dalziel JM (1993). *Plants Description, Useful Plants of West Tropical Africa*, (3).
- Dekkers JC, Dorman N, Van LJ, Kemper HC (1996). The Role of Antioxidants, Vitamins and Enzymes in the Prevention of Exercise Induced Muscle Damage, *Sports Med. Sports Medicine* 21(3):213-228(1996).
- Dhuley JN, Naik SN (1998). Protection by Rhinax in various models of ulceration in rats. *J. Ethnopharmacol.*
- Elegbe RA, bamigbose SOA (1976b). The antihistaminic properties of 1-methyl-3 puyyroidy phenylclohexane glycolate methobromide *Arch. Biochem. Expo. Biol.* 12:329-332
- Falk GW (2001). *Cecil essentials of medicine*. Edn 5, Edinburgh: WB Saunders Company: 334-343.
- Funatogawa K, Hayashi S, Shimomura H, Yoshida T, Hatano T, Ito H, Hirai Y (2004). Antibacterial activity of hydrolysable tannins derived from medicinal plants against *Helicobacter pylori*. *Microbiol. Immunol.*, 48:251–261.
- Halliwell B, Gutteridge. JM (1999). *Free Radicals in Biology and Medicine*; Oxford University Press, Oxford U. K. *Int. J. Biol. Med.*
- Illoh HC, Awojide AO (2000). Systematic Foliar Anatomy of Four Species of *Bauhinia* Linn Occurring in Nigeria, *Plant Science Resources. Commun.*
- Ito N, Hirose M, Imaida K (1996). Antioxidants: Carcinogenic and chemo-preventive properties. In: Bertino JR, editor. *Encyclopedia of Cancer*, (1), California, Academic Press.51– 63.
- Kelm MA Nair MG, Strasburg GM, DeWitt DL (2000). Antioxidant and cyclooxygenase inhibitory phenolic compounds of *ocimum gratissimum*, 2000.
- Khenouf S, Benabdallah H, Gharzouli K, Amira S, Ito H, Kim T, Yoshida T, Gharzouli A (2003). Effect of tannins from *Quercus suber* and *Quercus coccifera* leaves on ethanol-induced gastric lesions in mice. *J. Agric. Food Chem.*, 51:1469–1473
- Lichtenberger LM (2005). The hydrophobic barrier properties of gastrointestinal mucus. *Ann. Rev. Physiol.* ? Provide page number
- Nwafor SV, Okoye CF (2005). Antiulcer properties of ethanol root extract of *Cissampelos mucronata*. *Pharmacol. Biol.*, 43:396-403.
- OECD (2001). Guideline on acute oral toxicity (AOT) Environmental health and safety monograph series on testing and adjustment (425).
- Rao VNFA, Santos TT, Sobrierra, MF, Souza CL Melo, silveira ER (1997). Investigation on the gastroprotectiv and antidiarrhoeal properties of Ternatin, a tetramethoxy flavone from *Eglets Viscose.Planta.Med.*63:146
- Sairam K, Rao CV, Goel RK (2001). Effect of *Centella asiatica* linn on physical and chemical factors induced gastric ulceration and secretion. *Indian J. Exp. Biol.* 39:137-142.
- Sairam K, Rao CV, Goel RK (2001). Effect of *Convolvulus pluricaulis Choisy* on gastric ulceration and secretion in rats. *Indian J Exp. Biol.* 39:350-354.
- Salim AS (1990). Removing oxygen-derived free radicals stimulates healing of ethanol-induced erosive gastritis in the rat. *Gastroenterology*.
- Simdes CMO, Henkel EP, Gosmann G, Mello JCP, Mentz LA (2003). *Farmacognosia daPlanta ao Medicamento*.
- Tripathi KD (1999). *Essentials of Medical Pharmacology*. Jaypee Brothers Medical Publishers (P) Ltd. New Delhi, pp. 628- 642.
- Vasconcelos PCP, Kushima H, Andreo M, Hiruma-Lima CA, Vilegas W, Takahira RK, Pellizon CH (2008). Studies of gastric mucosa regeneration and safety promoted by *Mouriri pusa* treatment in acetic acid ulcer model. *J. Ethnopharmacol.*
- Wada K, Kamisaki, Y Kentaro, N Kishimoto, Y Ashida, Itoh T (1997). Effect of plaunotol on gastric injury induced by ischaemia-reperfusion in rats. *J. Pharmacol.* 49:903–907.