Study of the Effect of *Hibiscus Esculentus* Linn (Okra) Extract on Indomethacin-Induced Gastric Mucosal Damage and Gastric Secretion in rats

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Abstract: Gastric ulcers are occurring due to the imbalances between offensive and defensive factors of the gastric mucosa. The anti-ulcerogenic activity of the many plant products is reported due to an increase in mucosal defensive factors rather than decrease in the offensive factors. *Hibiscus esculentus* linn is an evergreen shrub which is indigenous to the Nile, Mediterranean, Balkans and India. A total of sixty (60) adult male albino rats were used in this study. The rats were divided into control, indomethacin- (20 mg/kg), extract- (100 and 200 mg/kg) and cimetidine (50 and 100 mg/kg)-treated groups for the study of gastric mucosal integrity and gastric secretion using indomethacin as the necrotizing agent for ulcers induction. Cimetidine was used as a reference drug. Preliminary phytochemical analysis of the extract revealed the presence of flavonoids, glycosides, tannins, alkaloids and phenolics. LD_{50} study of the extract in rats was found to be above 5000 mg/kg. The extract of *Hibiscus esculentus linn* administered at the two different doses of 100 and 200mg/kg subcutaneously produced appreciable effects on the different parameters of gastric secretion and gastric mucosal damage. Ulcer index showed a dose-dependent significant reduction and consequently a dose-dependent marked increase in the preventive index. The effect on gastric secretion was more prominent with the smaller dose of the extract (100mg/kg). The proposed mechanism of action of the extract of *Hibiscus esculentus linn* on gastric ulceration and gastric secretion may be explained to be resulting from the different phytochemical constituents found such as flavonoids, alkaloids and tannins.

Key words: *Hibiscus esculentus linn*, gastric mucosal integrity, phytochemicals, anti-ulcerogenic.

1. Introduction

The gastrointestinal or alimentary tract provides the body with continual supply of water, electrolytes, and nutrients. To achieve this requires: movement of food through the alimentary tract, secretion of digestive juices and digestion of food, absorption of the digested products together with water and the various electrolytes, circulation of blood through the gastrointestinal organs to carry away the absorbed substances. All these functions are controlled by the nervous and hormonal systems (Guyton and Hall, 2011).

Okra (*Hibiscus esculentus linn*) is a multibranched evergreen herb, erect, coarse, robust and growing up to 1 to 1.5m tall. It has pod fruits that are beaked, ridged, more or less oblong hairy capsule that dehisces longitudinally. The fruits are light green and sometimes purple in color (Gill, 1992). It is indigenous to the Nile, Mediterranean, Balkans and India where it is known in many English-speaking countries as lady's fingers. It is sliced, boiled or fried and eaten as food. It has high mucilage content and is used as such in soup and gravy. Some constituents of the okra tree include the following: the Pod is the fleshy part of the fruit with the seeds inside. This edible portion of the tree contains approximately 86.1% water, 2.2% protein, 0.2% fat, 9.7% carbohydrate, 1.0% fiber and 0.8% ash. It plays a role in the prevention of constipation by absorbing water and ensuring bulk in stools. This action is seen on the okra slippery mucilage which many people abhor. It also helps in preventing hypercholesterolemia by binding excess cholesterol. Besides, it is reported to have antipyretic, hypoglycemic, neuroprotective, antioxidant and antigonorrheal property. Thus, the plant has a wide range of medicinal values in treating many diseases (Sabitha *et al.,* 2012).

Peptic ulcers are breakages in the continuity of the mucosal epithelial lining of the stomach, duodenum and at times lower esophagus and Merkel's diverticulum. It can also occur in areas of ectopic gastric tissue, and in small and large bowels in Zollinger-Ellison syndrome (Falase and Akinkugbe, 2007). Peptic ulcer is considered the most common gastrointestinal disorder in clinical practice. It results from an imbalance between the damaging (i.e. acid, pepsin, free radicals and Helicobacter pylori) and the mucosal protective (i.e. mucus, bicarbonate and prostaglandins) factors in stomach (Pallavi and Balaraman, 2011). Although, prolonged anxiety, emotional stress, haemorrhagic shock, burns and trauma in addition to physical,

chemical and psychological factors are known to cause severe gastric irritation / ulceration in human and experimental animals, the mechanism is still very poorly understood. Oxygen derived free radicals have been implicated in the pathogenesis of gastric damage. The treatment of peptic ulcers is based on inhibition of gastric acid secretion using proton-pump inhibitors, H_2-receptor antagonists, and antimuscarinics, as well as acid independent therapy provided by antimicrobials against Helicobacter pylori. However, these drugs are expensive and cause numerous adverse effects as hypersensitivity, arrhythmia and hematopoietic changes, thereby limiting their usefulness. Thus, it is important that studies be carried out to investigate potential antiulcerogenic herbs which may lead to the discovery of new potential antiulcer drugs (Kalia *et al.,* 2013).

2. Materials and Methods
2.1. Drugs and Chemicals
Indomethacin (Cheisi[®]) ampoule used in a dose of 20mg/kg S.C. as reported by Okabe *et al.* (1977) and El-Sokkary *et al.*(1991a). **Cimetidine** (Lek Pharma. Slovenia) in a dose of 50mg/kg S.C. (Moron *et al.,* 1982) and 100mg/kg S.C. (Satoh *et al.*, 1983). **Sodium hydroxide** (NaOH) (BDH Poole, England) was used for the preparation of 0.01N NaOH solution. **Phenol red** (BDH Poole, England) powder was used for the preparation of phenol red indicator. **Chloroform** (BDH Poole, England) for preservation of the stomachs after being removed from the rats.

2.2. Plant material and preparation of extract
The fresh pods of 'Okra' *Hibiscus esculentus* were purchased from the local vegetable market in Cairo and its identity was confirmed by expert taxonomist of the Department of Pharmacognosy, Al-Azhar University, Cairo where a voucher specimen number 607 of the plant has been kept in the Herbarium. Shade dried pods (500 g) were coarsely powdered and macerated in 3 L of 96% ethanol for 72 hrs using percolation method. The solvent was then removed at 40 °C under reduced pressure in a rotavapor. The extract was then suspended in distilled water before its administration (Alqasoumi, 2012).

2.3. Phytochemical screening
The preliminary qualitative phytochemical screening of okra was conducted for the presence and/or absence of alkaloids, cardiac glycosides, flavonoids, tannins, anthraquinones, saponins, volatile oils, cyanogenic glycosides, coumarins, sterols and/or triterpenes (Fransworth, 1966).

2.4. Animals
A total of sixty (60) adult male albino rats were used in this study ranging in weight from 165-180 grams at the time of the research. The animals were housed under similar standard environmental conditions in large cages with wide meshed raised floors to prevent coprophagia. They were kept on *ad libitum* food and tap water at room temperature and 12: 12 light/dark cycle. They were divided into 4 groups as follows:

Group I: Control Group (10 rats)
(i) Five (5) rats received subcutaneous distilled water (2 ml/rat) to study gastric ulceration.
(ii) Five (5) rats received subcutaneous distilled water (2 ml/rat) to study basal gastric secretion.

Group II: Indomethacin-treated Group (10 rats)
(i) Five (5) rats received indomethacin 20 mg/kg body weight (b.w.) subcutaneously for the study of gastric ulcer formation
(ii) Five (5) rats received indomethacin 20 mg/kg b.w. subcutaneously followed by pyloric ligation for the study of the effect of indomethacin on gastric secretion.

Group III: Extract plus indomethacin-treated Group (20 rats)
(i) Ten (10) rats received two different doses of the extract (100 and 200 mg/kg b.w. subcutaneously) when given prior to indomethacin to study their effects on gastric secretion (5 rats for each dose).
(ii) Ten (10) rats received the same two different doses of the extract to study the effect on indomethacin-induced gastric ulceration (5 rats for each dose).

Group IV: Cimetidine plus indomethacin-treated (reference**) group (20 rats)**
(i) Ten (10) rats received two different doses of cimetidine (50 and 100 mg/kg b.w. subcutaneously) when given prior to indomethacin, to study the effect on gastric secretion (5 rats for each dose).
(ii) Ten (10) rats received the same two different doses of cimetidine to study the effect on indomethacin-induced gastric ulceration (5 rats for each dose).

2.5. Acute Toxicity Study
The median lethal dose (LD_{50}) determination was conducted using the method of Lorke (1983). In the initial phase, male rats were divided into three groups of 3 rats each. They were treated with the *Hibiscus esculentus linn* extract at doses of 10, 100 and 1000 mg/kg b.w. subcutaneously. Animals were observed for 24 hours and the number of death(s) was recorded. In the second phase, the animals were grouped into three groups of one rat each and treated with the extract at doses of 1600, 2900, 5000 mg/kg b.w. subcutaneously. Animals were also observed for 24 hours and the final LD_{50} was calculated.

2.6. Induction of Gastric Ulceration
After the 48 hours starvation with only free access to water, the animals were weighed and maintained in their individual cages. This has been

proved to be non-ulcerogenic and sufficient for absolute emptying of the stomach as reported by El-Sokkary *et al.* (1991a). Then, indomethacin 20mg/kg was injected subcutaneously and the animals were deprived from both food and water for 7 hours (Urushidani *et al.,* 1979). Later on, the animals were sacrificed by decapitation (Satoh *et al.,* 1983). Their stomachs were opened along the greater curvature, rinsed slowly with water, stretched out as much as possible on a No. 1 Whatman's filter paper. The ulcerated areas on each stomach were measured with a transparent millimeter mm ruler scale, and the result for each group was expressed in mm of mean ulcer $\pm$ SE as ulcer index (U.I.) (Scepovic and Radmanovic, 1984). The preventive index percentage (P.I. %) was later calculated according to the method of Hano *et al.* (1976), which is expressed as:

$$P.I. \% = \frac{U.I. \text{ Indomethacin} - U.I. \text{ Extract (or Cimetidine) plus indomethacin}}{U.I. \text{ Indomethacin}} \times 100$$

2.7. Collection of Gastric Secretion

The gastric juice was collected according to the technique of Shay *et al.* (1954) as modified by Levine (1965) where esophageal ligation was avoided. The animals were fasted for 48 hours to ensure complete emptying of the stomach, but allowed water *ad libitum*. Each of the animals was weighed at the end of the fasting period. Then, under light anaesthesia, their abdomens were opened via a midline incision and the stomach was identified. A pyloric ligature was made using a thread with care to avoid damage to the blood vessels or traction to the stomach. The abdomens were closed by sutures, cleaned thoroughly with distilled water. The anaesthesia was discontinued and the animals were allowed to recover. After 3 hours, the animals were sacrificed, abdomen of each animal was opened, the esophagus was ligated, and the stomach was removed and washed with distilled water. An opening along the greater curvature was made, and the gastric contents were drained into a graduated centrifuge tube and then centrifuged at 3000 rpm for 15 minutes.

2.8. Analysis of Gastric Juice
2.8.1. Volume of Gastric Juice

After centrifugation, the supernatant was measured and recorded as the volume of the gastric juice.

2.8.2. Determination of the Titritable Acidity

A given volume of the gastric juice (0.2 ml) was titrated against 0.01N NaOH using an end point of pH 7.0 as determined colorimetrically through using phenol red as an indicator. The value was calculated as milliequivalents per liter (mEq/L) which is equal to the number of milliliters of 0.1 N NaOH required to neutralize 100 ml of gastric juice.

$$= \frac{\text{Volume of } 0.01 \text{ N NaOH (ml) which neutralizes 1ml of gastric juice}}{10} \times 100$$

(Grossman, 1963; Davenport, 1977).

2.8.3. Determination of the Acid Output

This was calculated as microequivalent per hour (μEq/h) by multiplying the volume of gastric juice in milliliters per hour (ml/h) of an animal by the titritable acidity in (mEq/L) of the same animal. (Okabe *et al.,* 1975).

2.9. Statistical Analysis

Results were presented as mean $\pm$ Standard Mean of Error (S.M.E.). The data was statistically analyzed using the one – way ANOVA. Results were considered to be significant when "P" values are less than 0.05 (*P*<0.05) as described by Duncan *et al.* (1977).

3. Results
3.1. Phytochemical Screening

Preliminary phytochemical analysis of the extract using the standard screening test (Treese and Evans, 1983) revealed the presence of flavonoids, glycosides, tannins and alkaloids.

3.2. Acute Toxicity Studies

The oral and subcutaneous LD_{50} study of the extract in rats was found to be above 5000 mg/kg.

3.3. Gastric Mucosal Integrity Studies

Indomethacin (20 mg/kg S.C.) produced severe gastric mucosal ulceration restricted to the corpus with an ulcer index of 16.6 ± 0.1. The ulcers appeared in the form of streaks and / or groups and went deep in the mucosa with severe necrotic and hemorrhagic bases. Administration of *Hibiscus esculentus linn* extract showed a marked dose – dependent reduction with ulcer indices of 4.10 ± 2.4 and 0.10 ± 0.1 for the small and large dose respectively and in turn, the preventive indices were

also dose – dependent with very high prevention with the large dose (99%). As regards cimetidine, both dose levels produced valuable reduction in the ulcer index nearly of the same degree with values of 2.60 ± 1.4 and 2.10 ± 1.3 for the small and large dose respectively and hence the preventive indices were 78 and 87 for both respectively. It is worthy to observe that the large dose of the extract had produced a better prevention than the large dose of cimetidine (99 and 87% respectively) as shown in table (1).

Table (1): Showing the ulcer index (mm) and the preventive index (%) in the different rat groups

O C P Parameters	Groups (20mg/kg)	Indomethacin (100mg/kg)	(200mg/kg)	Extract + Indomethacin (50mg/kg)	(100mg/kg)	Cimetidine + Indomethacin
Ulcer index (mean ± S.E.M)	16.6 ± 0.1	$4.10 \pm 2.4^{*}$	$0.10 \pm 0.1^{**}$	$2.60 \pm 1.4^{**}$	$2.10 \pm 1.3^{**}$	
Preventive Index		75	99	78	87	

Significant variation against Indomethacin – treated group at * = $P < 0.05$, **= $P < 0.01$

3.4. Gastric Secretion Studies

As regards the indomethacin – treated group, it showed marked decrease in the acid output and volume of gastric secretion with no effect on the titritable acidity as compared to the control group (Table 2). Such effects were similar to that produced by the extract plus indomethacin with the observation that the smaller dose of the *Hibiscus esculentus* linn extract was more effective in reducing the acid output and volume of gastric secretion without affecting the titritable acidity as compared with the control group (table 2). Cimetidine (the reference drug) when administered before indomethacin resulted in valuable reduction of the volume of gastric secretion without affecting neither the titritable acidity nor the acid output when compared to the control group as recorded in table (2).

Table 2: Showing the different parameters tested on 3 hourly collected gastric secretions for different rat groups.

Groups (20mg/kg) Parameters	Control (100mg/kg)	Indomethacin (200mglkg)	Extract + indomethacin (50mg/kg)		Cimetidine + Indomethacin (100mg/kg)	
Volume (ml/3h)	3.70 ± 0.1	$1.04 \pm 0.4^{***}$	$1.22 \pm 0.2^{***}$	$1.44 \pm 0.2^{*}$	$2.12 \pm 0.2^{*}$	$2.14 \pm 0.3^{*}$
Titritable acidity (mEq/L)	30.00 ± 5.20	23.40 ± 4.6	15.20 ± 3.2	20.00 ± 1.5	41.40 ± 4.3	33.40 ± 1.9
Acid Output (µEq/h)	35.20 ± 5.0	$8.60 \pm 2.0^{***}$	$5.90 \pm 1.2^{***}$	$9.52 \pm 1.5^{**}$	30.14 ± 5.4	24.50 ± 4.7

-Values indicate mean ± S.E.M.
-Significant variation against control group at * = $P < 0.05$, **= $P < 0.01$ and *** = $P < 0.001$

4.0. Discussions

The models of gastric ulcer induction according to the methods of Shay *et al.* (1954) and Rebecca and Raymond (1995) were used in this study, hence the use of indomethacin as the ulcer- inducing agent. The results indicated that the extract of *Hibiscus esculentus linn* is an important source of antiulcer agents.

Non steroidal anti-inflammatory drugs (NSAIDs) and aspirin use, is the second most common etiologic factor for peptic ulcer disease and a major factor for peptic ulcer complications (Voutilainen *et al.*, 2001). Indomethacin which is one of the NSAIDs has the ability to cause gastroduodenal ulceration, and this effect is related to the ability of these agents to suppress prostaglandin synthesis through inhibition of prostaglandin

synthetase enzyme and also reduces bicarbonate secretion and gastric mucosal blood flow in animals, thereby increasing susceptibility of the stomach to mucosal injury and ulceration John-Africa *et al.,* 2012).. Experimental evidence has shown that the incidence of ulcers in patients that use this drug is resulting from failure of the mucosa to develop resistance to the damaging effect of these drugs (Wallace *et al.*, 2000).

Several studies have reported that indomethacin induced gastric mucosal damage and hemorrhagic gastric secretion as we reported in our work. Soll *et al.* (1989), El-Sokkary *et al.* (1991a), Magaji *et al.* (2007) and Okasha *et al.* (2008) also reported that haemorrhage is the most common complication of NSAIDs.

The results of the present study showed that *Hibiscus esculentus linn* extract produced a dose-dependent valuable reduction of the ulcer index, which is similar to that produced by the reference drug cimetidine. However, the large dose of the extract (200 mg/kg) reported even a greater reduction in the ulcer index and consequently it revealed a greater prevention than that produced by the large dose of cimetidine (99 and 87% respectively) with a subsequent greater gastroprotection. The results of our study as regards cimetidine is consistent with the study of Okabe *et al.* (1977) where they reported that cimetidine treatment produced marked inhibition of gastric damage induced by the adverse effects of aspirin and indomethacin. Similarly, El-Sokkary *et al.* (1991b) and Mequanente *et al.* (2006) reported the gastroprotective effect of cimetidine at 100 mg/kg in rats.

Acid output as one of the principal aggressive factors, in addition to the volume of gastric secretion has been markedly decreased by indomethacin in this study. Our results in this aspect are consistent with that of Nicoloff (1967), Kobayashin *et al.* (1985) and El-Sokkary *et al.* (1991a). Therefore, the role of acid in ulcerogenesis caused by indomethacin is negligible or needs further evaluation. On the contrary, some other studies reported that the ulcerogenic dose of indomethacin had no effect on basal or stimulated acid output (Ueki *et al.*, 1988; Filaretova *et al.*, 2002). Thus, from our studies and previous studies, the effect of indomethacin on gastric secretion is not yet settled. As regards the effect of cimetidine pretreatment, it showed significant reduction of the volume of gastric secretion without affecting the titritable acidity or the acid output. The *Hibiscus esculentus linn* extract by its two doses in our study, revealed significant reduction in the acid output and volume of gastric secretion as related to the control group. Thus, the results of the present study indicated that *Hibiscus esculentus linn* extract may play a role

in facilitating and enhancing gastric mucosal defensive factors. As the preliminary phytochemical analysis of the *Hibiscus esculentus linn* extract used in this study revealed the presence of flavonoids, tannins and alkaloids. In this regard, there are several reports which are consistent with our results where they reported that flavonoids have shown cytoprotective and anti-secretory properties in different experimental models of gastric ulcer (Takase *et al*., 1994; Martin *et al.*, 1998; Sumbul *et al.*, 2011). These findings have contributed in explaining the anti-ulcer activity observed for the extract of *Hibiscus esculentus linn*. Nowadays, flavonoids have aroused a considerable interest because of their potential beneficial effects on human health where they have been reported to possess antiviral, anti-allergic, antiplatelet, anti-inflammatory, antitumor and antioxidant activities (Burler and Miranda, 2003). Besides, there is a considerable correlation between the antioxidant and the antiulcer activity of flavonoids (Galati *et al.*, 2003). Several researches have shown and confirmed the efficacy of several plant extracts for the treatment of gastroduodenal disease. The anti-ulcerogenic effect of some of these plants is related to their flavonoid content, micronutrients activity, organic and inorganic compounds (Okasha *et al.,* 2008; Jayakumari *et al.,* 2012).

Tannins were also screened to be present in *Hibiscus esculentus linn* and are reported also to possess gastroprotective and antiulcer effects. Tannins isolated from *S. cumini* have a significant gastroprotective property (Ramirez and Rao, 2003). Tannins have protein precipitating and vasoconstriction properties. They may precipitate microproteins on the site of ulcer resulting in the formation of an impermeable protective layer over the lining to prevent absorption of toxic substances and resist the attack of proteolytic enzymes, thus acting as an anti-ulcer agent (John-Africa *et al.,* 2012). It has been shown that all of the effective gastroprotective plant extracts have contained tannins and/or flavonoid (Mota *et al.,* 2009).

Thus, it can be concluded by the analysis of the obtained data that the anti-ulcer (preventive) activity of the *Hibiscus esculentus linn* extract against indomethacin – induced mucosal damage is not through its effect on the different parameters of gastric secretion, where indomethacin showed a similar effect. On the other hand, we can relate the preventive effect of the extract to be due to the presence of flavonoids and tannins as reported by Czinner *et al.* (2001) and Ramirez and Rao (2003).

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