

Evaluation of gastric anti-ulcer activity of fixed oil of *Ocimum basilicum* Linn. and its possible mechanism of action

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Fixed oil of *O. basilicum* was found to possess significant antiulcer activity against aspirin, indomethacin, alcohol, histamine, reserpine, serotonin and stress-induced ulceration in experimental animal models. Significant inhibition was also observed in aspirin-induced gastric ulceration and secretion in pylorus ligated rats. The lipooxygenase inhibiting, histamine antagonistic and antisecretory effects of the oil could probably contribute towards antiulcer activity. *O. basilicum* fixed oil may be considered to be a drug of natural origin which possesses both antiinflammatory and anti-ulcer activity.

In traditional Indian medicine, several plants and herbs have been used to treat gastrointestinal disorders, including gastric ulcers¹. The first systematically effective drug against gastric ulcer was carbenoxolone, discovered as a result of research on a commonly used indigenous plant, *Glycyrrhiza glabra*². Studies on cabbage previously employed as an antiulcer agent in folk medicine, has led to the development of gefarnate³ which acts by enhancing the gastric mucosal strength⁴. Banana fruit has also been found to inhibit peptic ulceration⁵. Barnaulov *et al.*⁶ have reported antiulcer activity of plants of Campanulaceae family in experimental models; Solon⁷ a plant flavanoid has been found to be effective against ulcer in experimental animals⁷. *Melia azedarach* has been observed to inhibit stress-induced ulcer in rats⁸.

In our earlier studies, the fixed oil of *Ocimum basilicum* L. (Labiatae) commonly known as "Kali Tulsi" was found to possess significant antiinflammatory⁹ and analgesic¹⁰ properties without any noticeable toxicity. The present study has therefore, been conducted to evaluate the antiulcer activity of fixed oil of *O. basilicum* using different *in vivo* ulcer models in rats and guinea pigs.

Materials and Methods

The dried seeds of *O. basilicum* collected from Maidan Garhi, New Delhi, India and authenticated by a resident botanist, Department of Genetics, ICAR, New Delhi, India were crushed and cold macerated in petroleum ether (40°-60°C) (M/s S.D. Fine Chemi-

cals Ltd., Bombay, India) for three days. The petroleum ether extract was evaporated and the oil was filtered. The fixed oil thus obtained was subjected to different studies on laboratory animals. Wistar strain of albino rats (140-200 g) and guinea pigs (300-400 g) supplied by M/s Lucky Zoological House, New Delhi were used in these experiments. The animals were kept under standard laboratory conditions and fed on a standard diet from M/s Lipton (India) Ltd., New Delhi and water was allowed *ad libitum*.

Aspirin-induced gastric lesions—Adult wistar strain of albino rats were fasted for 36 hr. with water *ad libitum* prior to the experiment and were divided into four groups of six animals each. Different groups of animals received either fixed oil of *O. basilicum* (1.0, 2.0 and 3.0 ml/kg) or control vehicle (Dist. water) intraperitoneally. After 30 min aspirin (M/s Reckitt and Colman of India Ltd.) (suspended in 1% carboxymethyl cellulose (M/s Central Drug House, New Delhi, India) in water (20 mg/ml) at a dose of 500 mg/kg was administered orally to all the animals and 4 hr later, the animals were sacrificed¹¹. The stomachs were removed, opened along the greater curvature to determine the ulcer index as given below:

Erosions	Score
1 mm or less	1
1 mm to 2 mm	2
more than 2 mm	3

The overall score was divided by a factor 10, which was designated as the ulcer index¹².

Indomethacin-induced gastric lesions—Twenty four hours fasted albino rats, divided into four groups of six animals each were given 20 mg/kg of indomethacin (M/s Indian Drugs and Pharmaceutical Ltd.) orally. Groups of animals were treated with fixed oil of *O. basilicum* (1.0, 2.0 and 3.0 ml/kg) or control vehicle (Dist. water) intraperitoneally 30 min prior to the indomethacin treatment. The rats were sacrificed 6 hr later¹³ and their stomachs removed and examined as before for ulcer index.

Alcohol-induced gastric lesions—Four groups of six adult albino rats were fasted for 24 hr following water *ad libitum*. *O. basilicum* fixed oil (1.0, 2.0 and 3.0 ml/kg) or control vehicle (Dist. water) were administered intraperitoneally. After 30 min, ulceration was induced by administration of 50 % ethanol (5 ml/kg) orally. The animals were sacrificed after 1 hr. of administration of ethanol¹⁴. The stomachs were removed, opened and sum of length of lesions (mm) was evaluated for "lesion-index" as described above.

Histamine-induced gastric ulcers in guinea pigs—Guinea pigs weighing 300-400 g were fasted for 36 hr. with water *ad libitum* prior to the experiment and were divided into two groups of six animals each. Gastric ulceration was induced by intraperitoneal administration of histamine acid phosphate (Sigma, USA) (50 mg. base). To protect the animals against histamine toxicity, 5 mg. of promethazine hydrochloride (M/s Rhone-Poulenc (India) Ltd., Bombay) was injected intraperitoneally to each animal 15 min prior and 15 min after histamine administration. The fixed oil of *O. basilicum* (3.0 ml/kg) or control vehicle (Dist. water) were given orally 45 min before histamine administration¹¹. The animals were sacrificed after 4 hr following histamine administration and the stomachs were dissected out to determine the degree of ulceration as described above.

Reserpine-induced gastric ulcers—This was done following the technique described by Parmar *et al*¹⁵. Adult albino rats were fasted for 24 hr. following water *ad libitum*. Reserpine (M/s Ciba Geigy Ltd. Bombay) (5 mg./kg) administered intramuscularly to four groups of six rats each. 30 min after the administration of fixed oil of *O. basilicum* (1.0, 2.0 and 3.0 ml/kg) or control vehicle (Dist. water) intraperitoneally. All the animals were sacrificed after 18 hr. their stomachs were removed, opened

along the greater curvature and sum of lengths (mm) of all lesions for each rat was used as "lesion-index".

Serotonin-induced gastric ulcers—Serotonin creatinine sulphate (Sigma, USA) (20 mg/kg) was administered subcutaneously to four groups of six rats (24 hr fasted). Fixed oil of *O. basilicum* (1.0, 2.0 and 3.0 ml/kg) or control vehicle (Dist. water) was administered intraperitoneally 30 min prior to serotonin injection. The animals were sacrificed after 18 hr, their stomachs were removed, and the ulcer index was determined as described earlier¹².

Stress-induced gastric ulcer—Four groups of six albino rats were fasted for 12 hr following water *ad libitum*. *O. basilicum* fixed oil (1.0, 2.0 and 3.0 ml/kg) or control vehicle (Dist. water) were administered intraperitoneally. After 30 min, the rats were immobilised in a stress cage and forced to remain in a cold chamber (4-6°C) for 3 hr¹¹. The animals were then sacrificed and sum of length of erosions was expressed as "lesion-index".

Aspirin-induced gastric ulcerations in pylorus ligated rats—Adult albino rats were fasted for 24 hr with water *ad libitum* prior to the experiment and were randomly divided into two groups of six animals each. The operative procedure for pylorus ligation adopted was that of Shay *et al*¹⁶. The animals were treated with *O. basilicum* fixed oil (3.0 ml/kg) or control vehicle (Dist. water) intraperitoneally 30 min prior to ligation. The rats were anaesthetized with ether and the abdomen was opened by a small midline incision below the xiphoid process. The pylorus was secured and ligated with silk sutures after which the abdominal wall was closed by sutures and animals were allowed to recover from the anaesthesia. Aspirin (100 mg/kg) suspended in 1% carboxymethylcellulose in water was given orally to all the rats 15 min after pylorus ligation. The animals were sacrificed after 7 hr. the stomachs were removed, opened and sum of length (nm) of all lesions in each rat was used as "lesion-index"¹⁷.

Gastric secretion in pylorus ligated rats—Two groups of six albino rats, fasted for 24 hr were made pylorus ligated as described earlier. *O. basilicum* fixed oil (3.0 ml/kg) or control vehicle (Dist. water) were administered intraperitoneally 30 min prior to pylorus ligation and 4 hr later, the animals were sacrificed and the abdomen was opened. The cardiac end of the stomach was ligated. The stomach was then removed and gastric juice was evacuated into a

centrifuge tube. After centrifugation (3,000 rpm, 10 min), the gastric contents of each animal were individually assayed for volume of gastric secretion, and the total acidity by titration with 0.01N sodium hydroxide using phenolphthalein as an indicator¹⁸. Ulcer index was also determined as described earlier.

Results and Discussion

Antiulcer effects of *O. basilicum* fixed oil against various ulcerogens in different animal models are shown in Table 1. The fixed oil of *O. basilicum* at the dose level of 1.0, 2.0 and 3.0 ml/kg (ip) significantly reduced the ulcer index in dose dependent manner, both in aspirin-induced (500 mg/kg, po) and indomethacin-induced (20 mg/kg, po) gastric lesions in rats.

Non-steroidal anti-inflammatory drugs (NSAIDs) like aspirin and indomethacin are known to induce gastric ulceration. The reason being attributed principally to inhibition of biosynthesis of "cytoprotective prostaglandins" e.g. PGE's and PGI₂ (by inhibition of cyclooxygenase pathway of arachidonic acid metabolism) resulting in overproduction of leukotrienes and other products of 5-lipoxygenase pathway¹⁹. Hence, the protective action of *O. basilicum* fixed oil against aspirin and indomethacin-induced gastric lesions could possibly be due to its 5-lipoxygenase inhibitory effect.

The fixed oil offered significant protection against 50% ethanol-induced (500 mg/kg, po) ulceration at 1.0, 2.0 and 3.0 ml/kg (ip) dose levels in rats as well as histamine-induced (500 mg base ip) gastric ulcer in guinea pigs at 3.0 ml/kg (po) dose level. Similarly in reserpine (5 mg/kg, im), serotonin (10 mg/kg, s.c.) and stress-induced ulcer models in rats also fixed oil of *O. basilicum* at the dose level of 1.0, 2.0 and 3.0 ml/kg (ip) significantly suppressed the development of ulcers in dose dependent manner.

Ethanol-induced gastric lesion formation may be due to stasis in gastric blood flow which contributes to the development of the haemorrhage and necrotic aspects of tissue injury²⁰. It has also been reported that leukotriene antagonist and 5-lipoxygenase inhibitors are capable of inhibiting alcohol and NSAIDs-induced gastric ulceration in rats²¹, so the protection afforded by the fixed oil of *O. basilicum* against alcohol and NSAID's-induced gastric ulceration could also be due to inhibition of 5-lipoxygenase pathway or leukotriene antagonistic activity.

Histamine-induced gastric ulceration is recognised to be mediated through both enhanced gastric acid secretion and vasospastic action of histamine²². Hence, it appears that *O. basilicum* fixed oil has suppressed the histamine-induced vasospastic effect and gastric secretion. Administration of aspirin (100 mg/kg, po) into pylorus ligated rats induced severe ulceration in the gastric mucosa. Pretreatment with *O. basilicum* fixed oil (3.0 ml/kg, ip) markedly reduced the lesion index. The fixed oil (3.0 ml/kg, ip) also significantly reduced the ulcer index, gastric volume and total acidity in pylorus ligated rats. The antisecretory effect of the oil is demonstrated in the experiment on pylorus ligated rats where the fixed oil has been found to inhibit both gastric output and total acidity (Table 2).

Reserpine-induced gastric ulceration has been attributed to the degranulation of the gastric mast cells and consequent liberation of histamine which is believed to be cholinergically mediated²³. So, the antiulcer effect of *O. basilicum* fixed oil could be due to histamine-antagonistic and anticholinergic effects of the oil.

Serotonin-induced ulceration is believed to arise from a disturbance of gastric mucosal micro-circulation and the oil appears to improve the same. Since, the development of ulcers by serotonin and reserpine usually takes about 18 hr, it may also be inferred that the oil has a sustained effect.

Stress-induced ulcers are probably mediated by histamine release with enhancement in acid secretion and a reduction in mucus production. Moreover, stress-induced ulcers can be prevented partially or entirely by vagotomy and vagal overactivity has been suggested to be the principal factor in stress-induced ulceration²⁴. Accordingly, the protective action of *O. basilicum* fixed oil against stress-induced ulceration could be due to its histamine antagonistic, anticholinergic and antisecretory effects.

Hence, on the basis of above, it could be concluded that fixed oil of *O. basilicum* possesses significant antiulcer activity which may be due to lipoxygenase inhibitory, histamine antagonistic and antisecretory effects of the oil. However, further studies are required to evaluate the exact mechanism of action. We have already reported that the fixed oil of *O. basilicum* possesses significant antiinflammatory and analgesic activities without any noticeable toxicity. A drug that possesses both antiinflammatory and

Table I—Effect of *O. basilicum* fixed oil on aspirin, indomethacin, 50% ethanol, reserpine, serotonin, stress induced gastric lesions/ulcers in rats and histamine induced ulcers in guinea pig

[Values are mean $\pm$ SE from 6 animals in each group. Figures in parentheses are percentage inhibition]

Test treatment (Dose ip)	Aspirin (500 mg/kg po) induced gastric lesions in rats. Ulcer index (mm)	Indomethacin (20mg/kg po) induced gastric lesions in rats. Ulcer index (mm)	50% Ethanol (5ml/kg po) induced gastric lesion in rats. Lesion index (mm)	Reserpine (5mg/kg im.) induced gastric ulcers in rats Lesion index (mm)	Serotonin (20mg/kg s.c.) induced gastric ulcers in rats. Ulcer index (mm)	Stress induced gastric ulcers in rats. Lesion index (mm)	Histamine (50mg base ip) induced gastric ulceration in guinea pigs. Ulcer index (mm)
Distilled water (control) (2.0 ml/kg)	4.12 $\pm$ 0.25	1.70 $\pm$ 0.16	3.88 $\pm$ 0.26	8.70 $\pm$ 0.80	1.42 $\pm$ 0.16	9.32 $\pm$ 0.70	*4.40 $\pm$ 0.20
<i>O. basilicum</i> fixed oil (1.0 ml/kg)	2.60 $\pm$ 0.30** (36.90)	1.20 $\pm$ 0.12* (29.42)	2.72 $\pm$ 0.32* (29.90)	5.92 $\pm$ 0.36* (31.96)	0.88 $\pm$ 0.12* (38.03)	2.90 $\pm$ 0.16* (68.89)	-
<i>O. basilicum</i> fixed oil (2.0 ml/kg)	2.32 $\pm$ 0.20** (43.69)	0.80 $\pm$ 0.12** (52.95)	2.40 $\pm$ 0.26** (38.15)	5.66 $\pm$ 0.30* (45.14)	0.72 $\pm$ 0.14** (49.30)	1.86 $\pm$ 0.10** (80.05)	-
<i>O. basilicum</i> fixed oil (3.0 ml/kg)	1.28 $\pm$ 0.08** (68.94)	0.66 $\pm$ 0.08** (61.18)	1.72 $\pm$ 0.22** (55.68)	3.82 $\pm$ 0.30** (56.10)	0.32 $\pm$ 0.07** (77.47)	1.281 $\pm$ 0.08** (86.27)	*1.66 $\pm$ 0.16* (62.28)

▲ Control vehicle (i.e. Distilled water) and *O. basilicum* fixed oil were given orally at a dose of 3.0 ml / kg

* $P < 0.05$, ** $P < 0.01$

Table 2—Effect of *O. basilicum* fixed oil on gastric secretion and aspirin induced ulceration in pylorus ligated rats[Values are mean $\pm$ SE from 6 animals in each group. Figures in parentheses are percentage inhibition]

Test treatment (dose i.p.)	Gastric secretion and ulcer index in pylorus ligated rats			Aspirin (100mg/kg po) induced gastric lesion in pylorus ligated rats. Lesion index (mm)
	Volume (gastric juices) (ml/100g)	Total acidity (meq/L)	Ulcer index (mm)	
Distilled water (control) (3.0 ml/kg)	5.32 $\pm$ 0.26	138.00 $\pm$ 6.32	2.28 $\pm$ 0.16	4.20 $\pm$ 0.24
<i>O. basilicum</i> fixed oil (3.0 ml/kg)	3.32 $\pm$ 0.22* (37.60)	84.00 $\pm$ 4.12* (39.14)	0.62 $\pm$ 0.12** (72.81)	1.94 $\pm$ 0.18** (53.81)

* $P < 0.05$, ** $P < 0.01$

antiulcer activity is of great therapeutic importance as most of the antiinflammatory drugs used in modern day medicine are ulcerogenic. Further studies on the properties of the fixed oil are in progress.

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