

In Vitro and In Vivo Evaluation of Gastro-protective activity of chloroform extract of *Ocimum basilicum* Linn. seeds

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Abstract: The gastro-protective activity of chloroform extract of *Ocimum basilicum* L. seeds was investigated in in-vitro: acid neutralizing capacity and H^+/K^+ - ATPase inhibition activity and in in-vivo: NSAIDs (Aspirin) induced and stress (water immersion) induced gastric ulcer models in experimental rats at a dose of (200 & 400mg/kg b.w p.o). The common parameter determined was ulcer index and Ranitidine (50mg/kg b.w p.o) and Misoprostol (100mcg/kg b.w p.o) were used as standard drugs. In acid neutralizing capacity, the ANC value of standard Gelusil [aluminium hydroxide (250mg)+magnesium hydroxide (250mg)] was 12.3 while the chloroform extract showed 81.6, 41.6 and 19.5 for three concentrations i.e., 200, 400 and 800 mg respectively. In H^+/K^+ - ATPase enzyme inhibition activity, there was dose dependent inhibition of enzyme by Omeprazole and extract at various concentrations of 20, 40, 60, 80 and 100 µg, suggesting that the *Ocimum basilicum* seeds extract was significantly able to inhibit enzyme H^+/K^+ - ATPase. Results for in-vivo study showed that the chloroform seeds extract of *Ocimum basilicum* L. exhibited significant and dose-dependent gastro-protective activity in the ulcer models. Maximum percentage ulcer protection of chloroform seeds extract of *Ocimum basilicum* L. at a dose of 400mg/kg for Aspirin induced mucosal damage and stress (water immersion) induced gastric ulcer models were found to be 76.7% and 58.9% respectively. Results of our study suggest that chloroform seeds extract of *Ocimum basilicum* L. possess gastro-protective property which may be due to the presence of flavonoids in the extract as it has astringent, anti-secretory, cytoprotective and antioxidant properties.

Keywords: *Ocimum basilicum*, Acid neutralizing capacity, H^+/K^+ ATPase inhibition activity, NSAIDs (Aspirin), Stress (water immersion), Ulcer index, Gastro-protective activity, Flavonoids

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I. Introduction

Peptic ulcer disease is characterized by the imbalance between gastric offensive factors like acid, pepsin secretion, lipid per-oxidation, nitric oxide and defensive mucosal factors like mucin secretion, mucosal cell shedding, glycoprotein, proliferation & antioxidant enzyme like catalase, superoxide dismutase & glutathione levels.[1] *Ocimum basilicum* Linn. is also called as Sweet basil, it is an autogamous, aromatic and annual herb. The chemical constituent of *Ocimum basilicum* are linalol, methylchavicol, methylcinnamate, and linolen, omega 3 fatty acids. Essential oil is also found in sweet basil like eucalyptol, α -terpineol, eugenol β -elemene, α -bergamotene, α -guaiene, germacrene, cubenol, etc. Basil is well-known for its folk medicinal value and is accepted officially in a number of countries. The leaves and flowers of basil are used as a tonic and vermifuge, and basil tea is good for treating dysentery, nausea and flatulence. The plant is effective in treatment of stomach problems, fever, cough, gout and given internally to treat cystitis, nephritis and in internal piles. Reported pharmacological activities are analgesic and anti-inflammatory activity, hypoglycemic and hepatoprotective activity, anti-hyperlipidemic and anti-ulcerative, cardio protective and stimulant activity etc.[2]

The main aim of the study was to evaluate Gastroprotective activity of *Ocimum basilicum* seeds extract in in-vitro and in-vivo models.

II. Materials And Methods

2.1 Collection and Authentication of Plants material

The plant materials were collected from the area of chevella and were authenticated by L.RASINGAM, Scientist In-charge of BOTANICAL SURVEY OF INDIA, Deccan regional center, Hyderabad, Telangana and voucher specimen number is 1013.

2.2 Extraction and Phytochemical Screening

The seeds of plant *Ocimum basilicum* were collected and it was cleaned from dust and then 500gms of coarsely powdered material was subjected to extraction in chloroform and kept for maceration for 48 hrs. The material thus obtained was evaporated on water bath and concentrated. Standard methods,² were used for preliminary phytochemical screening to know the nature of phytoconstituents present.

2.3 Drugs and chemicals

Gelusil [Aluminium Hydroxide (250mg)+Magnesium hydroxide (250mg)], Omeprazole, HCl, Sodium hydroxide, Aspirin (200mg/kg b.w p.o), Ranitidine (50mg/kg b.w p.o), Misoprostol (100mcg/kg b.w p.o).

2.4 Experimental animals

The experimental protocol was approved by Institutional Animal Ethics Committee, Central Animal House (Registration No.1864/PO/Re/S/16/CPCSEA), Male albino rats weighing approximately 150-200gms were used for antiulcer activity and female wistar rats were used for acute toxicity study. The experimental animals were maintained under standard laboratory conditions, 12hours light/dark cycle under controlled temperature. All animals were acclimatized to the laboratory environment for atleast one week and they were given standard pellet diet and free access to water before the commencement of experiment.

2.5 Acute Toxicity Study (OECD guidelines 425)

The test was performed as per the OECD guidelines 425(Up and Down procedure), nulliparous and non-pregnant female albino wistar rats were randomly selected. Animals were kept under standard conditions for two to three days. Limit test was performed at 2000mg/kg b.w.p.o. as single dose and rats were kept without food for three to four hours prior to dosing but had access to water *ad libitum*. The animals were closely observed for first 30minutes, then for four hours for any behavioral changes. Food was provided after one to two hours of dosing. The animals were than observed upto 48hours for mortality. If mortality, then the second animal received reduced dose.[3]

2.6 In-vitro Evaluation of Gastroprotective activity of chloroform seed extract of *Ocimum basilicum* Linn.

2.6.1 Acid Neutralizing Capacity

The acid neutralizing capacity value for the chloroform extract of *Ocimum basilicum* seeds (200mg, 400mg, and 800mg) was compared with the standard antacid Gelusil[Aluminiumhydroxide(250mg)+magnesium hydroxide (250mg)]. To the 5ml quantity of this mixture, water was added to make up the total volume 70ml and then mixed for one minute. After that, 30ml of 1.0N HCl was added into standard and test preparation and stirred for 15minutes, drops of phenolphthalein solution was added and mixed. The excess HCl was immediately titrated with 0.5N Sodium hydroxide solution drop wise until a pink color is attained.[4,5]

The moles of acid neutralized is calculated by,

$$\text{Moles of acid neutralized} = (\text{vol. of HCl} \times \text{Normality of HCl}) - (\text{vol. Of NaOH} \times \text{Normality of NaOH})$$

Acid neutralizing capacity(ANC)
per gram of antacid =

moles of HCl neutralized

Grams of Antacid/Extract

2.6.2 H⁺/K⁺-ATPase Inhibition Activity

Preparation of H⁺/K⁺-ATPase Enzyme:

To prepare H⁺/K⁺-ATPase enzyme sample, fresh sheep stomach was obtained from a local slaughterhouse of Hyderabad. The stomach was cut opened, the mucosa at gastric fundus was cut-off, and the inner layer was scraped out for parietal cells. Thus obtained cells were homogenized in 16 mM Tris buffer (pH 7.4) containing 10% Triton X-100 and centrifuged at 6000 g for 10 min. The supernatant (enzyme extract) was used to determine the H⁺/K⁺-ATPase inhibition. Protein content of the cell extract was determined according to Bradford's method using the BSA as standard.[6]

Assessment of H⁺/K⁺-ATPase inhibition:

The reaction mixture containing 0.1 ml of enzyme extract (300 µg) and plant extract at different concentrations was pre-incubated for 60 min at 37°C. The reaction was initiated by adding substrate 2 mM ATP (200 µL), in addition to this 2 mM MgCl₂ (200 µL) and 10 mM KCl (200 µL) was added. After 30 min of incubation at 37°C, the reaction was stopped by the addition of assay mixture containing 4.5% ammonium molybdate and 60% perchloric acid followed by centrifugation at 2000 g for 10 min and inorganic phosphate released was measured spectrophotometrically at 660 nm by following Fiske-Subbarow method. Briefly, to the 1 ml of supernatant, 4 ml of millipore water, 1 ml of 2.5% ammonium molybdate, 0.4 ml of ANSA was added

and allowed to stand for 10 min at room temperature. Absorbance of released inorganic phosphate was measured at 660 nm.[6] Enzyme activity was calculated as micromoles of Pi released per hour at various doses (0-100 µg) of chloroform extract of *Ocimum basilicum*. Results were compared with the known antiulcer PPA inhibitor drug omeprazole and expressed as Mean ± SEM.

Percentage of enzyme inhibition was calculated using the formula:

$$\text{Percentage of inhibition} = \left[\frac{\text{Activity}_{(\text{control})} - \text{Activity}_{(\text{test})}}{\text{Activity}_{(\text{control})}} \right] \times 100.$$

2.7 In-vivo Evaluation of Gastroprotective activity of chloroform seed extract of *Ocimum basilicum* Linn.

2.7.1 NSAIDs (Aspirin) induced gastric ulcer in rats:

24 Male Albino rats weighing approximately upto 150-200gms were divided in to 4 groups with 6 in each. The animals were fasted for 24 hours. Group 1 (Control) received 20% Tween80, Group 2 was treated with standard drug Misoprostol (100mcg/kg b.w.) in 20% Tween 80 p.o and groups 3 and 4 was treated with chloroform extract of *Ocimum basilicum* extract of seeds in a dose of 200mg/kg b.w p.o and 400mg/kg b.w p.o. respectively in 20% Tween80. After 30 minutes, gastric ulcer was induced by administrating Aspirin (200mg/kg b.w p.o) in 20% Tween80 to all groups. After 4 hours, the rats were sacrificed using anesthetic ether and their stomachs was dissected out and they were opened along greater curvature for the determination of gastric lesions. Ulcer index was calculated by equation (1) noting the number of ulcers per animal and severity was scored by observing the ulcers microscopically with the help of hand lens (10x), percentage protection was calculated by equation (2). Scoring of ulcer was made as follows:[7]

Normal coloured stomach : 0

Red colouration : 0.5

Spot ulcer : 1

Hemorrhagic streaks : 1.5

Ulcers ≥ 3 but ≤ 5 : 2

Ulcers >5 : 3

Calculation of ulcer Index

$$\text{UI} = (\text{UN} + \text{US} + \text{UP}) \times 10^{-1} \text{ ----- (1)}$$

UI = Ulcer Index

UN = Average of number of ulcer per animal

US = Average of severity score

UP = Percentage of animal with ulcer

And percentage protection was observed by using the formula:

$$\% \text{ Protection} = \frac{(\text{Ulcer index})_{\text{Control}} - (\text{Ulcer index})_{\text{Test}}}{(\text{Ulcer index})_{\text{Control}}} \times 100 \text{ ----- (2)}$$

2.7.2 Stress(Water Immersion) Induced Ulcer in Rats:

24 Male Albino rats 150-200gm were divided in to 4 groups with 6 in each. Stress induced ulcers was induced by placing the rats in glass cylinder (height 45cm, diameter 35cm) containing water up to 35cm maintained at 35°C for 3 hrs for forced swimming. Animals were fasted for 24 hours prior to the experiment. Group 1 was considered as Control group and received 20% Tween 80. Group 2 received standard drug Ranitidine (50mg/kg b.w p.o) in 20%Tween80 and group 3 and 4 were treated with chloroform extract of *Ocimum basilicum* seeds in a dose of 200mg/kg b.w p.o and 400mg/kg b.w p.o. respectively in 20% Tween80. After the drug treatment, all animals of the groups were placed in water individually and allowed to swim for 3 hours. Then the animals were dissected and stomach was removed. Stomach was opened along the greater curvature, ulcer index and % protection was calculated by equation (1) & (2) respectively. Scoring of ulcer is made as follows:[7]

Normal coloured stomach : 0

Red colouration : 0.5

Spot ulcer : 1

Hemorrhagic streaks : 1.5

Ulcers ≥ 3 but ≤ 5 : 2

Ulcers >5 : 3

2.8 STATISTICAL ANALYSIS:

The data was analyzed using one-way analysis of variance (ANOVA) followed by Tukey test. All values are expressed as Mean ± SEM (n=6). *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 compared with control and analysed by one way ANOVA followed by Tukey test.

III. Results

3.1 Extraction & phytochemical analysis

The weight of extract was found to be 13.96 gms and its percentage yield was 2.79%. The results of preliminary phytochemical analysis of chloroform extract of *Ocimum basilicum* Linn. seeds showed the presence of carbohydrates, proteins, phenols, flavonoids, alkaloids, glycosides and saponins.

3.2 Acute toxicity study:

The chloroform extract of *Ocimum basilicum* seeds was found to be safe at the maximum tested dose of 2000mg/kg body weight by oral route. After 48 hours, animals was found well tolerated and there were no mortality and no sign of toxicity. Thus, 1/10th and 1/5th of maximum tolerated dose i.e., 200mg/kg b.w.p.o and 400mg/kg b.w.p.o was taken for the treatment.

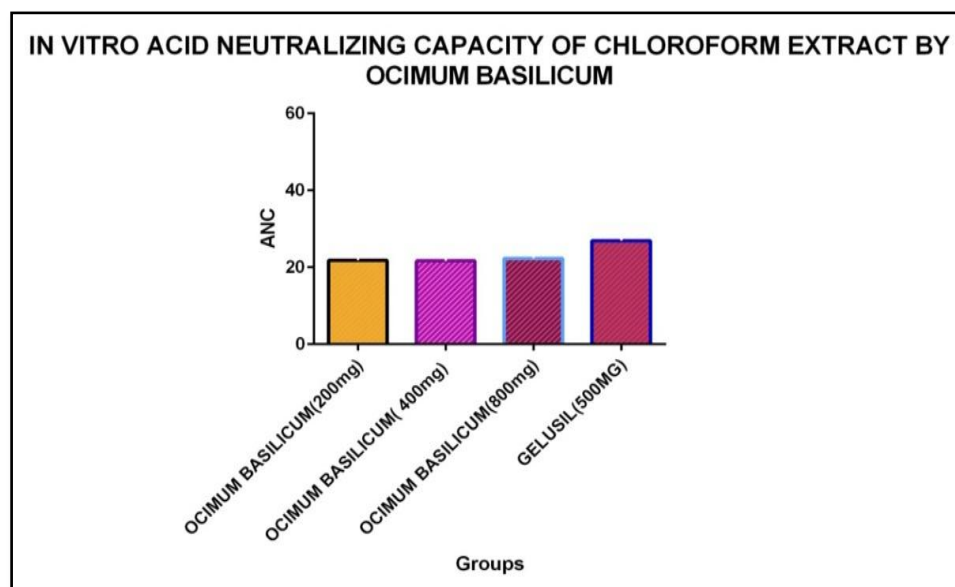
3.3 In-vitro Evaluation of Gastroprotective activity of chloroform seed extract of *Ocimum basilicum* Linn.

3.3.1 Acid Neutralizing Capacity

The neutralizing effect of the chloroform extract of *Ocimum basilicum* seeds was studied for three concentration (200mg, 400mg, 800mg) and standard Aluminiumhydroxide(250mg) and Magnesium hydroxide(250mg).The results obtained envisage that the extract at concentration 200mg, 400mg and 800mg showed significant reduction in acid neutralizing capacity i.e., 81.6, 41.6 and 19.5 respectively as compared to standard which is 12.3. The extract at concentration 800 mg was found toneutralize acid moresignificantly as compared to standard. The results are tabulated in table no. 1 and represented by graph no 1.

Table 1: Effect of Chloroform Extract of *Ocimum basilicum* on Acid Neutralizing Capacity

Test substance with Concentration	Vol. of NaOH consumed(ml)	mEq of acid consumed	ANC per gram of antacid
200mg/kg OB	27.33	16.33	81.6
400mg/kg OB	26.33	16.66	41.6
800mg/kg OB	29.33	15.33	19.5
Gelusil [Aluminium hydroxide(250mg)+ Magnesium hydroxide(250mg)]	47.63	6.18	12.3



Graph 1: Effect of Chloroform Extract of *Ocimum basilicum* on Acid Neutralizing Capacity

3.3.2 H⁺/K⁺ - ATPase Inhibition Activity

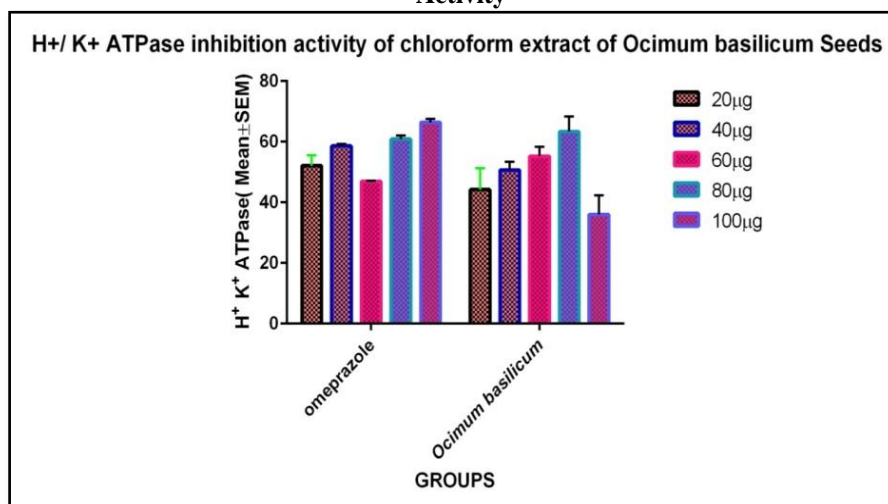
The H⁺/ K⁺ ATPase inhibition activity chloroform extract of *Ocimum basilicum* seeds at various concentration (20µg, 40µg, 60µg, 80µg, 100µg) was compared with Omeprazole as standard. The extract significantly showed activity in a dose dependent manner. At a concentration of 80µg/ml the extract and omeprazole showed 63.37% and 60.89%. The results are shown in table 2 and represented by graph 2.

Table 2: Effect of Chloroform Extract of *Ocimum basilicum* on In Vitro H⁺/K⁺ - ATPase Inhibition Activity

Concentration	Percentage Inhibition	
	Standard Omeprazole	Extract <i>Ocimum basilicum</i>
20µg	52.09±3.40	44.20±7.09
40µg	58.62±0.67	50.67±2.67
60µg	46.89±.2	5.21±3.12
80µg	60.89±1.13*	63.37±4.93*
100µg	66.36±1.22**	36.02±6.26

Values are expressed as Mean±SEM (n=6). *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 compared Standard Vs. Extract and analyzed by one way ANOVA followed by Tukey test.

Graph 2: Effect of Chloroform Extract of *Ocimum basilicum* on *In Vitro* H⁺/K⁺ - ATPase Inhibition Activity



3.4 In-vivo Evaluation of Gastroprotective activity of chloroform seed extract of *Ocimum basilicum* Linn.

3.4.1 NSAIDs (Aspirin) induced gastric ulcer in rats:

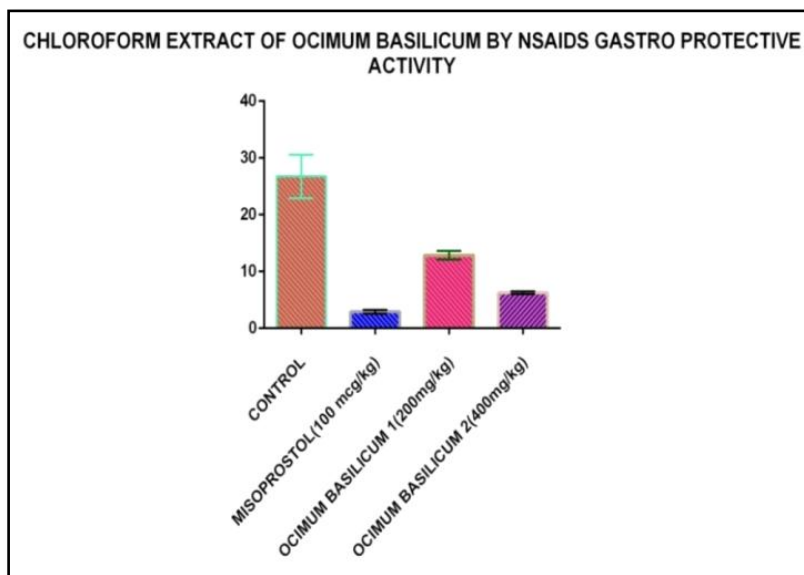
Ulcer index decreased significantly in Misoprostol(100µg/kg) treated group upto 2.88±0.31 (**P<0.01) compared to control group in which the value was 26.70 ± 3.84. The value of ulcer index reduced significantly in the group treated with chloroform extract of *Ocimum basilicum* seeds to 12.84±0.753 and 6.21±0.22(*P<0.05)at a doses of 200 & 400 mg/kg b.w. p.o respectively when compared to control group. The result was significant at a dose of 400 mg/kg b.w. when compared to control group and the percentage protection was 76.7% as compared 89.1% in misoprostol treated group. The results have been tabulated in table no.3 and represented by graph no.3.

Table 3: Effect of Chloroform extract of *Ocimum basilicum* Seeds on ulcer index in NSAIDs(Aspirin) induced gastric ulcers in rats

Treatment	Dose	Ulcer Index (Mean ± SEM)	% Protection
Control	(1 ml/kg b.w) p.o	26.70 ± 3.84	--
Misoprostol	(100mcg/kg b.w.) p.o	2.88 ± 0.31**	89.1%
<i>Ocimum basilicum</i> 1	(200mg/kg b.w.) p.o	12.84 ± 0.75 ^{ns}	51.6%
<i>Ocimum basilicum</i> 2	(400mg/kg b.w.) p.o	6.21 ± 0.22*	76.7%

Values are expressed as Mean±SEM (n=6). *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 compared Standard Vs. Extract and analyzed by one way ANOVA followed by Tukey test.

Graph 3: Effect of Chloroform extract of *Ocimum basilicum* Seeds on ulcer index in NSAIDs(Aspirin) induced gastric ulcers in rats



3.4.2 Stress(Water Immersion) Induced Ulcer in Rats:

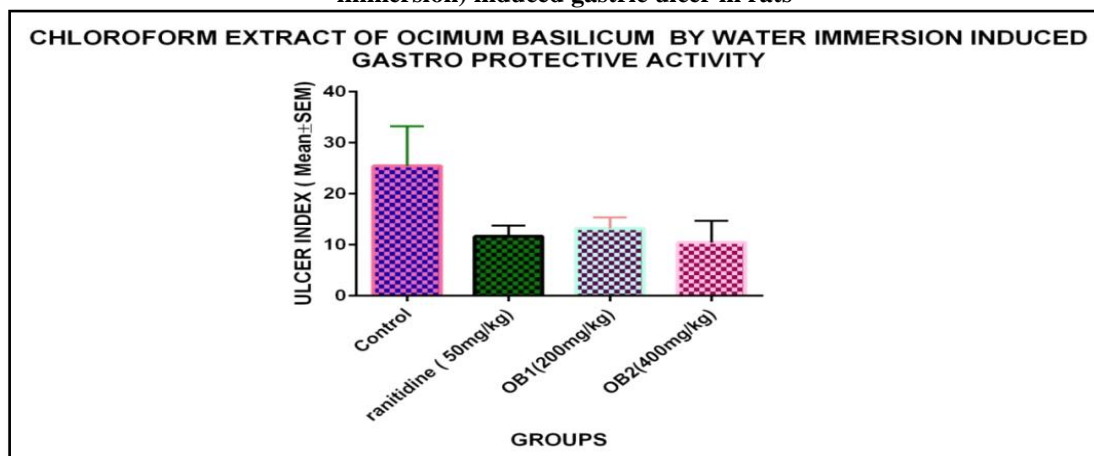
Ulcer index in Ranitidine (50mg/kg b.w. p.o.) treated group decreased significantly upto 11.66 ± 0.85 (* $P < 0.05$) compared to control group in which the value was 25.45 ± 3.17 . The value of ulcer index was reduced significantly in the group treated with chloroform extract of *Ocimum basilicum* seeds to 13.23 ± 0.872 (* $P < 0.05$) and 10.45 ± 1.73 (* $P < 0.05$) at doses of 200 & 400 mg/kg b.w. p.o respectively as compared to control group. The result was significant at a dose of 400 mg/kg b.w. when compared to control group and the percentage protection was found to be 58.9% as compared to 54.1% in Ranitidine treated group. The result have been tabulated in table no.4 and represented by graph no.4.

Table 4: Effect of Chloroform extract of *Ocimum basilicum* Seeds on Ulcer Index in Stress (water immersion) induced gastric ulcer in rats

Treatment	Dose	Ulcer Index (Mean ± SEM)	% Protection
Control	(1ml/kg b.w.)p.o	25.45 ± 3.177	--
Ranitidine	(50mg/kg b.w.)p.o	11.66 ± 0.859*	54.1%
<i>Ocimum basilicum</i> 1	(200mg/kg b.w.) p.o	13.23 ± 0.872*	48.0%
<i>Ocimum basilicum</i> 2	(400mg/kg b.w.)p.o	10.45 ± 1.733*	58.9%

Values are expressed as Mean±SEM (n=6). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ compared Standard Vs. Extract and analyzed by one way ANOVA followed by Tukey test.

Graph 4: Effect of Chloroform extract of *Ocimum basilicum* Seeds on Ulcer Index in Stress (water immersion) induced gastric ulcer in rats



IV. Discussion

The study was designed to evaluate the gastroprotective activity of chloroform extract of *Ocimum basilicum* seeds using *in vitro* (acid neutralizing capacity and H^+/K^+ - ATPase inhibition activity) and *in vivo* (NSAIDs (Aspirin) and Stress (water immersion) induced gastric ulcers) models which operate by distinct mechanisms of ulcerogenesis. Stomach acid contains hydrochloric acid which aids in food digestion. Excess stomach acid produces a condition known as acid indigestion or acid reflux. The acid neutralizing capacity (ANC) of an antacid is the amount of acid that it can neutralize and is measured by back titration process. In ANC, the chloroform extract of *Ocimum basilicum* seeds at 800mg concentration showed significant reduction in ANC of 19.5. H^+/K^+ - ATPase is a key enzyme in inducing acidity; it is located on apical secretory membrane of parietal cells. In H^+/K^+ -ATPase inhibition activity, the extract showed maximum percentage inhibition of 63.37% at 80 μ g concentration. In gastric ulcer model induced by Aspirin (200mg/kg b.w p.o), the percentage protection was found to be 51.6% and 76.7% at doses 200 mg/kg b.w. and 400 mg/kg b.w. respectively as compared to standard Misoprostol (100 μ g/kg b.w.) with 89.1% and in the stress(water immersion) induced ulcer model the percentage protection was found to be 48.0% and 58.9% at doses 200 mg/kg b.w. and 400 mg/kg b.w. respectively as compared to standard Ranitidine (50mg/kg b.w.) with 54.1%.

Thus, the results obtained in the present investigation is suggestive that chloroform extract of *Ocimum basilicum* Linn. seeds may possess antacid, antisecretory, antiulcer property which may be due to presence of flavonoids in the extract. However, further studies are required to establish its exact mode of action and the active principles involved in it antiulcer effect.

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