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PHARMACOLOGICAL AND PHYTOCHEMICAL EVALUATION OF ANTI-ULCEROGENIC POTENTIAL OF *SOLANUM NIGRUM*

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ABSTRACT

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Anti-ulcer activity of the *Solanum nigrum* L. extracts was studied in rats by using pylorus ligated ulcer model. Rantidine was used as the standard drug for comparison. The animals were sacrificed after 19 hrs after the ligation. Stomach was dissected out and contents were drained into tubes and were centrifuged at 1000 rpm for 10min and volume was noted. The PH of gastric juice was recorded using a PH meter. The contents were subjected for analysis of free and total acidity. The numbers of ulcers per stomach was noted and severity of ulcers scored. Then the blood samples were collected and subjected to estimation of serum calcium and alkaline phosphatase level. The results revealed that 200 and 400mg/kg dosages of *Solanum* leaf extracts showed significantly comparable anti ulcer-activity as that of Rantidine, showing an anti-ulcer activity of the *extracts*. Thus *Solanum nigrum* leaves may reduce the acidity of the gastric juice and also prevent the mucosal damage and ulcer formation.

INTRODUCTION: Peptic ulcers are open sores in the lining of stomach, esophagus, or duodenum. Peptic ulcer is one of the most common gastrointestinal disorders, causing high rate of morbidity. Ulcers are not caused by spicy food or stress¹ but it is caused by a type of bacteria called *Helicobacter pylori*. At least 70-90% of patients with gastric ulcers and 80-95% with duodenal ulcers are infected by *H. pylori* Microorganism which seems to be curative for the disease.

Several other causative factors are implicated in the pathogenesis of gastric ulcer including increased acid-pepsin secretion, impaired bicarbonate neutralization, impaired mucus secretion and precipitate lesions on the mucosal layer². There is a powerful association between peptic ulcers and infection of *Helicobacter*

pylori. There is a balance between the aggressive (i.e. acid, pepsin, active oxidants, *H. pylori*) and the mucosal protective (i.e. mucus, bicarbonate, prostaglandin's) factors in stomach. Medication is necessary to alleviate these symptoms and must be used to eradicate *H. pylori*. In addition to medications many lifestyle changes like diet and exercise are important. Certain herbs, acupuncture, or homeopathy might be helpful additions to usual medical care.

Treating peptic ulcer has been commonly targeted at either counteracting the aggressive factors or stimulating defensive one. *Solanum nigrum*, a plant herb is one of the traditional medicines with high nutraceutical properties due to its antiseptic, antidysenteric and antidiuretic properties and it has been used for the treatment of many skin diseases,

kidney disorders, non-communicable diseases and many other common ailments³. *Solanum nigrum* have been proved for its potential anti-ulcerative properties⁴.

MATERIALS AND METHODS: This study evaluated the anti-ulcer activity of *Solanum nigrum* L. extracts in rats by using pylorus ligated ulcer model and it was subjected to preliminary phytochemical studies for the identification of phytoconstituents.

Plant Material: Good quality *Solanum nigrum* leaves were purchased from Othakadai, Annanagar, local market from Madurai, Tamil Nadu, India. The purchased leaves were cleaned properly by removing the insect damaged leaves, deteriorated leaves, stems and unmaturing fruits and the good quality leaves were selected. The collected leaves were washed with tap water to removing adhering dust followed by distilled water, shade dried, and size reduced into small pieces.

Dried materials were coarsely powdered and macerated with alcohol and aqueous solvents for 72 hrs. The total aqueous extracts were also prepared separately by cold maceration method⁵. The final product was filtered, dried and the color and consistencies were taken. The dried extracts were subjected to routine chemical test for the presence of phyto constituents.

Selection of Animals: Albino rats of either sex weighing around 150-250g were taken from in-breed colony animals, which were housed in polypropylene cages and maintained under standard laboratory conditions of temperature at $24\pm 2^{\circ}\text{C}$. Commercial pellet diet supplied by Sankaralingam college of Pharmacy and water were provided *ad libitum*. The food was withdrawn 18 hours before the experiment but allowed free access of water. To avoid Coprophagy and fighting, the rats were fasted in wire-bottomed cages⁵. All animal experiments were carried out in accordance with the guidelines of "Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA)" (No.- B 6705/2011).

Acute Oral Toxicity Studies: A safe dose of the extract was determined by acute oral toxic class method of Organization of Economic Co-Operation and Development (OECD) as per 423 guidelines⁶. The animals were divided into 4 groups of each. The first

group served as a solvent control, the second group was administered with Rantidine 2mg/kg p.o⁶. The remaining two groups were treated with the extracts of *Solanum nigrum* at 200 and 400mg/kg p.o respectively. The following groups of animals were used

1. Group I: Control
2. Group II: Standard Ranitidine 2mg/kg p.o.
3. Group III: Solanum extract (200mg/kg)
4. Group IV: Solanum extract (400mg/kg)

Experimental Procedure:

Pylorus Ligation Model: The test samples were administered to animals in 5 ml/kg as suspensions oral treatment were carried out 1 and 0.5 hour before pyloric ligation, respectively. After 18 hours of fasting, ulcer induction was undertaken⁷. The rats were quickly and mildly anaesthetized with chloroform and the abdomen was cut open through a midline incision. The pylorus was secured and ligated with silk sutures, after which the wound was closed and the animals were allowed to recover from anesthesia. After ligation of the pylorus, drinking water was withheld and the gastric examinations were under taken 19 hours after pylorus ligation.

The animals were sacrificed with an overdose of chloroform and the stomachs were opened along the greater curvature, rinsed with saline to remove gastric contents and blood clots and examined by a magnifier lens (x5) to assess the formation of ulcer. Using 3 M transpore surgical tapes assessed ulcer area, which was fixed on a light and transparent sheet and the ulcer area was measured for each stomach. The number of erosions formed on glandular portion of stomach was counted and each was given a severity rating on a 1-3 scale.

The overall total divided by a factor of 10 was designated as the ulcer index for that stomach. The gastric contents were collected through the esophagus. The mucous was then washed and extent of ulceration was scored. Blood sample were withdrawn from the marginal vein and subjected to estimation of serum alkaline Phosphatase and serum calcium.

Total acidity of Gastric Juice: An aliquot of 1ml of gastric juice was taken in to a 50 ml conical flask and two drops of phenolphthalein indicator was added and titrated with 0.01N NaOH until a permanent pink color was established. The volume of 0.01N NaOH consumed was noted, total acidity was calculated and expressed as meq/l⁸.

Estimation of Serum Alkaline Phosphatase and Calcium: Alkaline phosphatase is an enzyme capable of catalyzing the hydrolysis of various phosphates esters at alkaline pH. Alkaline phosphatase activity has been reported to be increased in the bone diseases liver diseases and gastro intestinal lumen. The releases of this enzyme have been suggested with various model

TABLE 1: EFFECT OF RANTIDINE, EXTRACTS OF *SOLANUM NIGRUM* ON GASTRIC SECRETION, pH AND ULCER SCORE IN PYLORUS LIGATED RATS

Group No.	Drug & Dose (mg/kg)	pH	Ulcer scores	Gastric volume
I	Control	2.56 ± 0.25	1.5 ± 0.4	5.1 ± 0.74
II	Rantidine (50mg/kg)	4.82 ± 1.32 *	0.25 ± 0.29 **	2.65 ± 0.39 **
III	Solanum Extract 200mg/kg	4.53 ± 1.07 *	0.63 ± 0.48 *	3.25 ± 0.71 **
IV	Solanum Extract 400mg/kg	4.65 ± 0.58 *	0.38 ± 0.48 **	3.02 ± 0.41 **

Solanum extracts showed (3.25 and 3.02 ml) high significant equipotent effects as that of Ranitidine (2.65 ml) in reducing the gastric juice volume and also showed elevation in pH indicating their capacity to reduce the acidity of the gastric juice.

The mean pH value of Solanum extracts at 400mg/kg was 4.65 which showed almost equipotent as that of Rantidine (4.82). The severity of the Gastric ulceration in pylorus ligation method as assessed is based on the mean ulcer index.

The mean ulcer index in control group was 1.5. The Solanum extract at 200 mg/kg and 400 mg/kg exhibited mean ulcer index of 0.63±0.48 and 0.38±0.48 respectively, which is almost significantly comparable to that of Rantidine (0.25±0.29) and proved its protection effect against ulcer formation due to pylorus ligation (**Fig. 1**).

In control group the mean gastric juice was 5.1 ± 0.74 ml and Rantidine (50mg/kg), decreased the mean gastric volume (2.65±0.39 ml). The data revealed that Solanum extract at 200mg/kg and 400mg/kg decreased the mean gastric juice volume significantly to 3.25±0.71 and 3.02±0.71 ml respectively.

of the gastrointestinal ulceration⁹. Alkaline phosphatase activity was determined by using the method of kind and king (1954)¹⁰. Serum calcium was estimated by Ortho Cresolphthelin Complexone (OCPC) method

Statistical analysis: The statistical analysis of all the results was carried out using one-way ANOVA followed by Dunnett's test and all the results obtained in the study were compared with the vehicle control group.

RESULTS AND DISCUSSION: The results of anti-ulcer activity of the extracts of *Solanum nigrum* on pylorus ligation induced gastric ulcer in rats are summarized in **Table 1 and 2**.

All the test extracts showed the decrease in gastric juice volume on comparison to control group and indicated their anti-secretory mechanism.

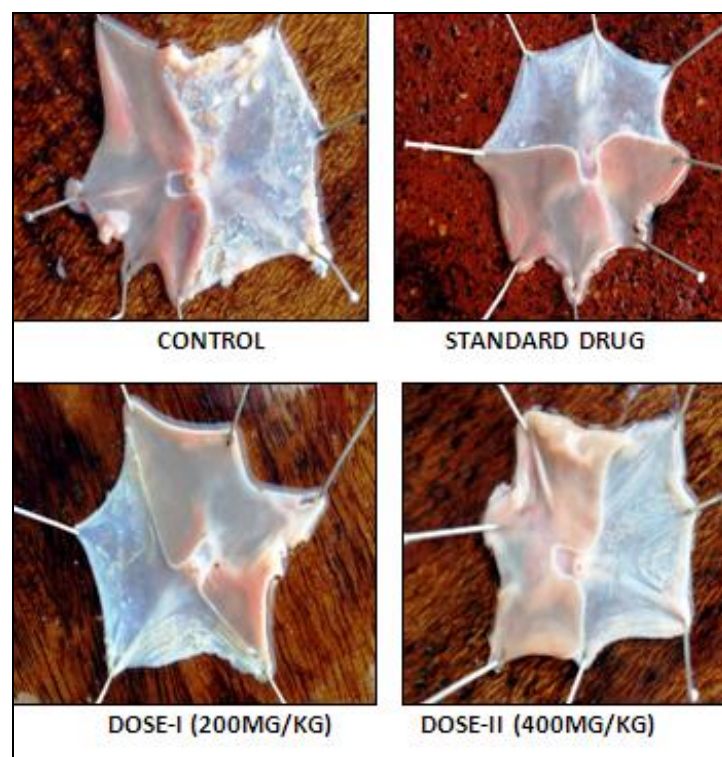


FIGURE 1: ANTIULCER ACTIVITY OF *SOLANUM NIGRUM* ON PYLORUS LIGATED (ALBINO) RATS

TABLE 2: EFFECT OF RANITIDINE, EXTRACTS OF *SOLANUM NIGRUM* ON ACIDITY AND PHOSPHATASE LEVELS IN PYLORUS LIGATED RATS

Group No.	Drug & Dose (mg/kg)	Total Acidity (mEq/L/100g)	Free acidity (mEq/L/100g)	Alkaline phosphatase
I	Control	41.75 ± 8.65	40.5 ± 5.8	43.64 ± 5.30
II	Ranitidine (50mg/kg)	16.75 ± 4.57 **	12.0 ± 2.16 **	25.69 ± 3.84 **
III	Solanum Extract 200mg/kg	22.75 ± 2.63 **	16.25 ± 3.59 **	30.36 ± 5.71 **
IV	Solanum Extract 400mg/kg	17.50 ± 5.80 **	14.75 ± 0.96 **	28.46 ± 2.17 **

One way Anova, Dunnett's 't' test * - P<0.05; ** - P<0.01

The test Solanum extracts at 200mg/kg and 400mg/kg (16.25 ± 3.59 and 14.75 ± 0.96 mEq/l) decreased the Gastric free acidity when compared to Ranitidine (12.0 ± 2.16 mEq/l) showing equipotent effect in reducing the Gastric free acidity.

Also, Solanum extract at 200mg/kg and 400mg/kg decreased total acidity (22.75 ± 2.63 and 17.50 ± 5.80 mEq/l), which showed significant equipotent effect as that of Ranitidine (16.75 ± 4.57 mEq/l). The ulcer curative ratio of Solanum extract at 200mg/kg (50%) was comparable to that of standard Ranitidine (80%). The ulcer curative ratio of Solanum extract at 400mg/kg (80%) was same as that of standard Ranitidine (80%). The serum calcium level was 7.14 mg/dl in control, 9.27 mg/dl in Ranitidine drug, 12.97 mg/dl in Solanum extract at 200mg/kg and 17.25 mg/dl in Solanum extract at 400mg/kg. The increase in alkaline Phosphatase level was observed for test extracts as 30.36 mg/dl and 28.46 mg/dl in 200mg/kg and 400mg/kg Solanum extracts when compared to control as 25.69 mg/dl.

From the above results, it can be inferred that, Solanum extracts showed marked anti ulcer activity in pylorus ligation induced gastric ulcer model in a dose dependent manner. Solanum extracts at 200 mg/kg and 400 mg/kg reduced the gastric volume, free acidity, total acidity and ulcer index thus showing the anti-secretory mechanism involved in the extracts for their anti-ulcerogenic activity.

Thus Solanum leaves exhibited significant anti ulcer activity and almost equipotent effect as that of Ranitidine and these results offer pharmacological

evidence and support on the folkloric use of *Solanum nigrum* leaves as an anti ulcer agent ^{11, 12}.

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