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RESEARCH ARTICLE

Ulcer Protective and Spasmolytic Activity of Aqueous Extract of *Solanum nigrum* Leaves in Experimental Rats

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ABSTRACT

Solanum nigrum Linn. (solanaceae) is a widely growing and cultivated traditional medicinal plant mainly used for the treatment of gastric illness and mouth ulcer. Aim of the present study was to evaluate the activity of aqueous leaves extract of *Solanum nigrum* Linn on irritable bowel syndrome and gastric ulcers. Ulcer protective and anti spasmodic effect was investigated in cold restraint stress, aspirin induced and pyloric ligated ulcer models on experimental rats. The aqueous leaf extract of *Solanum nigrum* (SNALE) was given orally in two doses of 200mg/kg, and 400mg/kg. The dose of 400mg/kg significantly inhibited the gastric ulcer induced by all three models when compared with the serum parameters' like Pylorus ligated (PL), Aspirin induced ulcerogenesis (APL) and cold resistant stress induced ulcer (CRSU) with the standard drug omperazole. Anti spasmodic effect of *Solanum nigrum* was determined by rat ileum contractility. Presence of quercetin in the extract was responsible for the anti-spasmodic effect. The aqueous leaf extract of *Solanum nigrum* significantly ($p<0.001$) inhibited ulcer index, total and free acidity and significantly ($p<0.01$) shows gastroprotective in pylorus ligation and aspirin induced gastric ulcer models. This extract also significantly ($p<0.01$) shows spasmolytic effect in ileum contraction model. These results suggested that aqueous extract of *Solanum nigrum* leaves shows gastro protective, spasmolytic effect and anti-secretory effect.

KEYWORDS

Solanum nigrum Linn, Spasmolytic, anti-ulcer, albino Rats.

INTRODUCTION

Peptic ulcer disease embraces both gastric and duodenal ulcer and has been a major threat to the world's population over the past two centuries, with a high morbidity and substantial mortality[1].

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Epidemiological data for this disease and its complications have shown salient geographical variations in incidence and prevalence.

Development of ulcer disease and death from it has been associated with the birth of urbanization and was interpreted as a birth-cohort event with the peak of disease in those born during the late 19th century[1].

The management of ulcer disease and its complications remains a clinical challenge. Additionally non- steroidal anti inflammatory

drugs (NSAIDs) and low dose of aspirin are an increasingly important cause of ulcers and their complications even in H-pylori- negative patients[2]. Others rare causes of ulcer disease are in the absence of H-pylori, NSAID's and aspirin. *Solanum nigrum* Linn from solanaceae has been used in the traditional medicine for treating and managing the different types of gastric ulcers. Herbs are producing pharmacological effect without adverse effects in ayurvedic traditional medicinal system[3]. *Solanum nigrum* are used in folk medicines as antioxidant[4], antiulcerogenic and ulcer healing[5], to reduce fever, diarrhea, and ophthalmic troubles [6, 7].

MATERIALS AND METHOD

Drugs and Chemicals

Double distilled water (DDW), Aspirin, propanolol and glibenclamide (Glib) were purchased from Sigma laboratories. Omperazole was purchased from Dreedy, Topers reagent and sodium chloride (0.01 N) from Merck, Naloxone from Tolidaru (Iran) and other chemicals from Merck (Germany). All chemicals and extract were dissolved in the Tyrode solution and the total volume of all the solutions were added to the organ bath with less than 5% of the bath volume and CMC were used.

Plant Material

Solanum nigrum was collected from college area of Tirupur district, Tamil Nadu, India. The plant was identified and authenticated by Dr. G.V.S.Murthy Scientist F & Head of Office Botanical Survey of India, Southern Regional Centre Coimbatore 6416003.

Extraction and Phytochemical Evaluation

Mature leaves of *Solanum nigrum* were dried and coarsely powered. 200g of the powered material were extracted with double distilled water in soxhelt apparatus. The yield of the extract was found to be 7.5% w/w. The aqueous leaf extract of *Solanum nigrum* (SNALE) was subjected to the phytochemical screening and the same was used of pharmacological activity[8].

Animals

Wistar albino rats of either sex (home bred) aged 7–8 weeks and weighing 150–200 g, were obtained from the Experimental Animal House, The Erode college of Pharmacy and Research Institute, Erode. The animals were fed standard diet chow and water *ad libitum* and were maintained under standard conditions of humidity, temperature and light. The rats were randomly assigned to different control and treatment groups. This study was approved by institutional animal ethical committee (PCOL/02/2013/IAEC/ECP), under the guidelines of CPSCEA (committee for the purpose of control and supervision of experimental or animals).

Dosage Schedule

Control animals are received only double distilled water (DDW). Experimental animals are received dried SNALE, suspended with 0.5% Carboxy Methyl Cellulose (CMC) using double distilled water in the doses of 200 mg 400 mg /kg and next group of animals are received anti ulcer drug omperazole (OMP) 10mg/kg and another group of animals received aspirin.

Experimental Design

The experimental animals were divided into four groups, each group comprising of six rats in all ulcer methods.

Anti ulcer Activity

Pylorus Ligated (Shay) Rats [9, 10, 11]

The animals were fasted for 36 h with access to water *ad libitum* before the pylorus was ligated under ether anesthesia, care being taken not to cause bleeding or to occlude blood vessels [9]. *Solanum nigrum* (200 and 400 mg/kg body weight) and OMP were administered immediately after pylorus ligation by intraperitoneally. The animals were sacrificed 6 h after the pylorus ligation. The stomach was excised carefully keeping the esophagus closed, opened along the greater curvature and the luminal contents were removed. The gastric contents were collected in a beaker and

centrifuged at 1000 rpm for 10 min. The samples were analyzed for gastric volume, pH, free and total acidity as the sample subjected to analysis for titratable acidity against 0.01N NaOH to pH 7. Each stomach was examined for lesions. The mucosa was flushed with saline and stomach pinned on a frog board and scored. The percentage inhibition of ulcer index was calculated [10].

1. Group 1 -Control animals are received only 0.5% CMC using double distilled water (DDW).
2. Group 2 - control group of animals are received anti ulcer drug omperazole OMP 10mg/kg/p.o [11]
3. Group 3 Experimental animals are received SNALE, suspended with 0.5% CMC using double distilled water in doses of 200 mg/kg /p.o.(prior treatment for 7 days) [11]
4. Group 4 - Experimental animals are received SNALE, suspended with 0.5% CMC using double distilled water in doses of 400 mg /kg /p.o. (prior treatment for 7 days)[11]

***Aspirin-induced Ulcerogenesis in Pylorus Ligated Rats*[12]**

In aspirin-induced ulcerogenesis in pylorus ligated rats, aspirin is given orally at a dose of 200 mg/kg in a suspension prepared in 0.5% CMC with water 1h prior to pyloric ligation (time interval between reference drugs and aspirin should be 1 hour) and the process described above was followed [11,12].

1. Group 1 -Control animals are received aspirin 200mg/kg[11].
2. Group 2 - Control group of animals are received anti ulcer drug omperazole OMP 10mg/kg/p.o [11]
3. Group 3 Experimental animals are received SNALE, suspended with 0.5% CMC using double distilled water in doses of 200 mg/kg /p.o.(prior treatment for 7 days) [11]
4. Group 4 - Experimental animals are received SNALE, suspended with 0.5% CMC using double distilled water in doses of 400 mg /kg /p.o. (prior treatment for 7 days)[11]

***Stress induced Ulcer by Cold Water Immersion Method* [11]**

The experimental rats were fasted 24h prior and a test sample is given by orally 1h before stress induction. Animals were immobilized in a stress cage and immersed to the level of the xiphold in a water bath at 23± 0.2 c for 4h. After 4h animals were removed and sacrificed[5]. The stomach was excised carefully keeping the esophagus closed, opened along the greater curvature and the luminal contents were removed. The ulcerative index and percentage of ulcer protection were calculated [11].

1. Group 1 -Control animals are received solvent 0.5% CMC 1h before water immersion stres .
2. Group 2 – Negative control group of animals are received anti ulcer drug omperazole OMP 10mg/kg/p.o 1h before water immersion stress[11.]
3. Group 3 Experimental animals are received SNALE, suspended with 0.5% CMC using double distilled water in doses of 200 mg/kg /p.o 1h before water immersion stress.
4. Group 4 - Experimental animals are received SNALE, suspended with 0.5% CMC using double distilled water in doses of 400 mg /kg /p.o1h before water immersion stress.

Anti Spasmodic Activity[14, 15]

Ileum Preparation

The rats (over night fasted) are sacrificed by a blow on the head & carotid bleeding. The abdomen is cut opened & the carcum is lifted to trace the ileo- coecal junction. A few cm long of the ileal portion is cut and removed & immediately it is placed in the watch glass containing tyrode solution. The mesentry is trimmed and with gentle care the contents of the ileum is cleared by pouring the tyrode solution into the lumen of the ileum. Almost care is taken to avoid any damage to the gut muscle. The ileum is cut into small segments of 2-3cm long, one piece of ileum of 2-3 cm long is taken

and the thread is tied to the top and bottom ends without closing the lumen. The tissue is mounted in the organ bath containing tyrode solution maintained at 32 – 35 ° c & bubbled without air. A tension of 0.5 gm is applied and the tissue is allowed to equilibrate for 30 min before adding drug to the organ bath[13]. Concentration dependent responses and plateau was recorded by using frontal writing lever. Contact times of 30 sec & 5 min time cycle are kept for proper recording of the responses. The SNALE (0.125- 2mg/ml) was added to the tissue bath respectively. After 30 min tissue incubations the effect of SNALE was studied with the involvement of some receptors (opioid receptors antagonists and β-adrenoceptors), and recorded the spasmolytic effect[14]. Furthermore, in (Ca2+-free) [15] and rich KCl (60 mM) [14, 15] Tyrode solution, the tissue was contracted by cumulative concentrations of CaCl2 (0.225-2.7 mM) before and after tissue incubation (3 min) with the extract (0.0125-0.1 mg/ml). In addition, the antispasmodic effect of the *SNALE* was evaluated after the tissue incubation (5 min) with the K+ channels blockers such as glibenclamide (10 µM) .At least 4 concentration dependent responses are recorded. The tracing is labeled, and the tracing is fixed with the help of fixing solution[15].

Statistical analysis

Values are expressed in terms of mean ± S.E.M. statistical analysis was carried by GraphPad InStat DTCG through one way ANOVA followed by Dunnett's test,. *, P < 0.05; ** P < 0.01, *** P < 0.01 was considered as a significant.

RESULTS AND DISCUSSION

Anti Ulcer Activity[12]

In the pylorus ligation induced ulcer model, it was observed that the treatment with SNALE (400 mg/kg) and OMP (10 mg/kg) significantly reduced the ulcer index in comparison with negative control group (p < 0.001). Whereas SNALE (200 mg/kg) treated animals did not show any significance when compared with the control group[16].

The results are shown inTable-1. However, the gastric volume, pH, free acidity and total acidity parameters and percentage of protection were significantly reduced when compared with the negative control group (p < 0.01). Treatment with SNALE (400 mg/kg) and OMP (10mg/kg) reduced significantly all the evaluated parameters. Hence, the low dose of the extract has not produced any anti-ulcer activity but it produced anti secretary activity; it was evidenced by comparing with the high dose of the extract (Table-1).Regarding the aspirin-induced ulcer model, it was observed that a significant reduction in ulcer index in animals treated with SNALE (400 mg/kg) and OMP (10mg/kg). Furthermore, reduced volume of gastric juice, total, free acidity and raised gastric pH were observed and shown in Table-2. However, SNALE aqueous extract (200 mg/kg) treated animals did not show any significance when compare with the control group [12]. The gastric volume and pH parameters were not significantly reduced in compare with the negative control group were observed and shown in Table -3.

Anti Spasmodic Activity [14]

Spasmolytic activity of SNALE on the rat ileum contraction induced by KCl (60mM) and Ach (10µM) or Cch (10µM) and the presence of anti spasmodic drugs such as propanolol (1µM) and glibenclamide (10µM) added in different doses as shown in figure 1.It is observed that the Spasmolytic effect produced by SNALE on rat ileum contraction induced by Kcl and Ach or Cch does not show any variance compared to the treatment of rat ileum with propanolol, which acts as a β blockers as shown in figure 2. Further incubation of ileum preparation with glibenclamide act as an ATP- dependent potassium channel blockers does not attract the anti-spasmodic activity produced by SNALE. But it rather potentiated the SNALE spasmolytic effect shown in figure 3. Various concentrations of $Cacl_2$ to the calcium free and high potassium (60mM) tyrode solution caused tissue contractions vary between different doses. After incubation (3 min) with different dose concentration of SNALE (0.125-1mg/ml),

Table 1: Effect of aqueous leaf extract of *Solanum nigrum* linn *against* ulcer induced by pylorus ligated rat model (n=6)

Group	Gastric volume(ml)	Ulcer index	pH of gastric juice	Free acidity(meq/l)	Total acidity(meq/l)	Percentage inhibition
Control	1.9±0.14	4.25±0.21	1.53± 0.24	49.3±1.53	84.0±0.40	
Omperazole	0.88±0.10**	1.25±0.17***	3.2 ±0.35***	19.8±0.91***	57.7±2.26***	70.58
SNALE –I	1.6±0.23^{ns}	2.91±0.20***	1.96± 0.12^{ns}	36.8±0.57***	73.2±0.47***	31.37
SNALE – II	1.15±0.13*	1.83±0.16***	2.55±0.25*	31.1±0.64***	68.4±0.47***	56.86

Values are expressed in terms of mean ± S.E.M. statistical analysis was carried by GraphPad InStat DTCG through one way ANOVA, followed by Dunnett's *t*-test. *, P < 0.05; **, P < 0.01, *** P < 0.01 was considered as a significant.

Table 2: Effect of aqueous leaf extract of *Solanum nigrum linn* in aspirin –induced ulcerogenesis in pylorus ligated rat (n=6)

Group	Gastric volume(ml)	Ulcer index	pH of gastric juice	Free acidity(meq/l)	Total acidity(meq/l)	Percentage inhibition
Control	1.96±0.17	4.83±0.10	1.22±0.09	56.2±0.75	91.0 ±1.29	
Omperazole	0.91±0.06***	1.50±0.22***	2.65±0.06***	24.0±0.67***	58.8±0.85***	68.96
SNALE – I	1.65±0.08^{ns}	3.16±0.16***	1.60±0.13^{ns}	50.9±0.68***	81.6±0.49***	34.48
SNALE – II	1.31±0.15*	2.33±0.10***	1.95±0.17**	37.8±1.09***	71.7±0.85***	51.72

Values are expressed in terms of mean ± S.E.M. statistical analysis was carried by GraphPad InStat DTCG through one way ANOVA., followed by Dunnett's *t*-test *, P < 0.05; **, P < 0.01, *** P < 0.01 was considered as a significant.

Table 3: Effect of aqueous leaf extract of *Solanum nigrum* linn in stress induced ulcer in water immersion method in rat (n=6)

Group	Gastric volume(ml)	Ulcer index	pH of gastric juice	Free acidity(meq/l)	Total acidity(meq/l)	Percentage inhibition
Control	2.05±0.13	3.16±0.16	1.78±0.24	56.9±0.74	85.4±0.71	
Omperazole	0.96±0.06** *	1.08±0.20***	3.83±0.29***	25.3±0.61*	66.1±0.76***	65.78
SNALE – I	1.61±0.12^{ns}	2.25±0.17***	2.68±0.15***	46.7±0.77*	78.8±2.06***	28.94
SNALE - II	1.1±0.1***	1.5±0.12***	3.33±0.12**	35.0±0.56*	72.9±1.98***	52.63

Values are expressed in terms of mean ± S.E.M. statistical analysis was carried by GraphPad InStat DTCG through one way ANOVA., followed by Dunnett's *t*-test *, P < 0.05; **, P < 0.01, *** P < 0.01 was considered as a significant.

The spasmogenic of $CaCl_2$ was attenuated significantly as shown in figure 4.

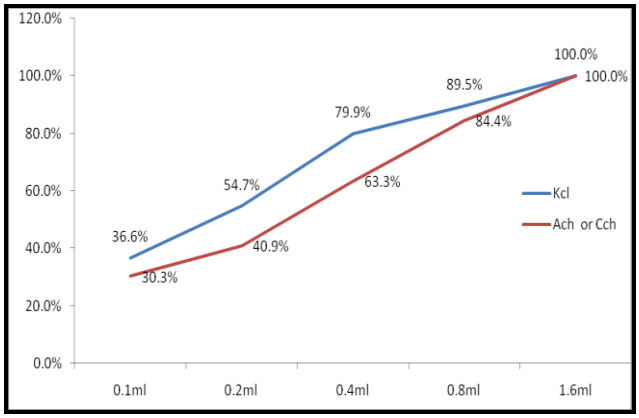

Figure 1: Spasmolytic effect of aqueous extract of *Solanum nigrum* leaves induced by KCl & Ach or Cch

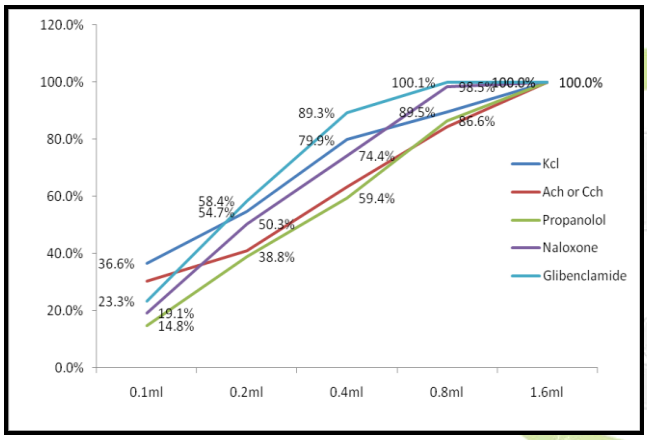

Figure 2: Spasmolytic Effects of *Solanum nigrum* Induced by KCl & Ach or Cch with presence of (Naloxone, Glibenclamide & Propanolol)

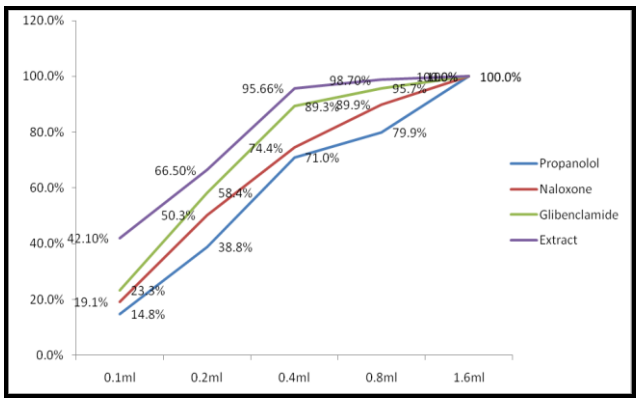

Figure 3: Spasmolytic Effects of aqueous extract *Solanum nigrum* leaves Induced by KCl & Ach or Cch in presence of only (Naloxone, Glibenclamide & Propanolol)

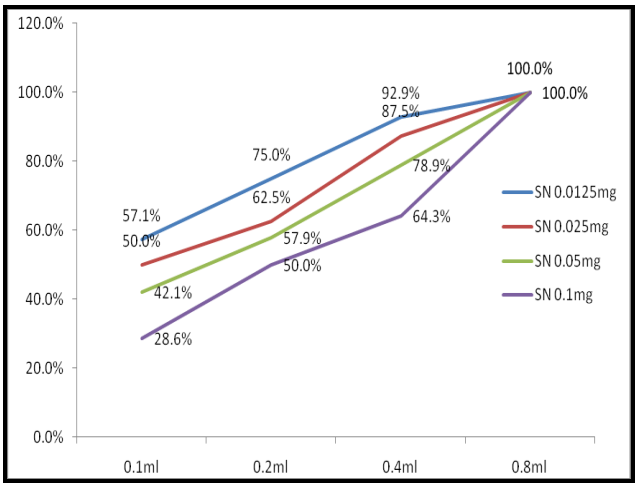

Figure 4: Spasmolytic Effects of aqueous extract *Solanum nigrum* leaves Induced by $CaCl_2$ in presence of extract in different doses (0.0125-0.1mg/ml)

Gastric mucosal injury is caused by an impairment of balance between the offensive and defensive factors due to an increase in either factor or interaction between the two factors, as in the balance theory proposed[17]. There were various causes of potentiation of the offensive factor and reduction of the defensive factor. Extracts of *Solanum nigrum* significantly reduced the formation of gastric lesions in rats induced by Pylorus ligated and aspirin-induced ulcerogenesis. A dose dependent response on the intensity of gastric ulceration was noted. Anti-inflammatory agents such as aspirin can reduce gastric cyclooxygenase activity and decrease endogenous prostaglandin levels and increase the acid secretion. These agents break the mucosal barrier, provoke an increase in gastric mucosal permeability to H+ and Na+ ions and a drop in the transmucosal potential difference, and induce the formation of erosions and ulcers. There is mounting evidence that an increase of certain endogenous prostaglandins can enhance gastric mucosal resistance against ulcerogenic substances such as anti-inflammatory agents[18]. In this present work denote that, the SNALE was also able to produce a significant reduction of the gastric mucosal damage induced by aspirin (Table 2). Our results also show a significant reduction in content of gastric volume and acidity after aspirin administration. Pretreatment of rats with

SNALE significantly prevent ulcer index in the above selected models. The significant reduction in basal gastric secretion and complete inhibition of ulcers by SNALE after pylorus ligation suggest that, the cytoprotective mechanism of action of the extract on gastric mucosa may involve direct reduction of gastric acid, pepsin and increase mucus secretion though one or more of the possible mechanisms discussed[19]. Moreover, gastric acid is an important factor for the genesis of ulceration of pylorus ligation ulcer in rats[16]. Gastric acid secretion is regulated by many factors including anxiety effect in the CNS, vagal activity, cholinergic, histaminergic and gastrinergic neurotransmissions, the activities of various post-synaptic receptors and the proton pump [20]. The current data clearly demonstrated that, at the very least, SNALE dose-dependently inhibited the aggressive factor, gastric acid secretion. The anti-ulcerogenic effect of the extract may be related to its anti-secretory action since acid is a major factor in the development of peptic ulcer. However, certain anti-ulcer drugs increase the amount of gastric mucus secretion in gastric mucosa[21]. In the results of anti secretary parameter investigation, the gastric mucosa of rats revealed that the pretreatment of SNALE can absolutely inhibited the aspirin induced hemorrhagic necrosis of rat stomach. Those substances act as a (Kcl or Cch) receptors operating spasmogen, in the same route and elevates calcium via calcium channel[13]. The extract inhibits the influx of calcium without affecting the intracellular calcium routes. It seems therefore that the SNALE reduced the rat ileum contraction via blocking these channels[14]. This suggestion is supported by the extract was produced spasmolytic effect induced by KCl induced contraction. In Ca^{2+} free and high potassium tyrode solution, the ileum has been depolarized by K^{+}. In addition of KCl added to tissue bath to induce contraction[14,15]. The cholinergic receptor antagonist activity of SNALE did not attenuate. SNALE inhibited both KCl or Cch induced contraction.

CONCLUSION

The oral administration of the *Solanum nigrum* aqueous leaf extract produced a significant antiulcer activity without any apparent toxicological effects, which supports the use of *solanum nigrum* aqueous leaf extract for drug induced ulcer condition and spasmolytic effect. Further experiments and detailed phytochemical analyses could be explained the mechanism involved in anti-ulcer and anti-spasmodic activity.

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