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Anti-Ulcer Activity of Tuber Extracts of *Solanum tuberosum* (Solanaceae) in Rats

Original research article/Review

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Abstract *Solanum tuberosum* (Solanaceae) is a plant species widespread throughout India and world. *S. tuberosum* contains starch, sugar (glucose, sucrose and fructose), cellulose (10–20%), crude fibre, pectin substances (0.7–1.5% of dry weight), hemicelluloses (1%), fat (1.1%) and vitamin C. The proteins of *S. tuberosum* tuber comprised of about 60–70% globulin and 20–40% glutelin with no albumin. For external use, the grated raw *S. tuberosum* is applied locally in cases of arthritis, itching, neuralgia and mild burns. Therefore, the present study was aimed to assess the potential of *S. tuberosum* for the treatment of ulcers. Ranitidine is used as a standard referenceto evaluate anti-ulcer activity in models such as pylorus ligation model and stress-induced ulcers by cold water immersion. When alcoholic extract of tubers of *S. tuberosum* (AETST) and aqueous extract of tubers of *S. tuberosum* (AQETST) were subjected for LD50 study at the dose level of 2,000 mg/kg body weight. Preliminary phytochemical investigations revealed the presence of tannins, carbohydrates, sterols, flavonoids, glycosides, alkaloids and triterpenes in both the extracts. The dose was selected as low (100 mg/kg), medium (200 mg/kg) and high (400 mg/kg), and the doses of both the extracts significantly reduced the ulcer ($P < 0.05$*, 0.01** and 0.001***). The present study revealed that both the AETST and AQETST possessed anti-ulcer activity. Phytochemical constituents such as tannins, flavonoids and triterpenes have already been reported for their anti-ulcer activity. These phytochemical constituents were present in both the extracts and, hence, responsible for the observed activity.

Keywords *Pylorus ligation model – Stress-induced ulcers by cold water immersion – Ranitidine*

INTRODUCTION

The *Solanum* genus contains approximately 1,500 species distributed all over the world. Plants of this genus are distributed in the tropical and subtropical regions and an estimated 1,000–1,100 species ofthe genus are found in South America regions. Owing to the large number of species in this genus, the family was named Solanaceae. Many species of the genus areknown for its economic importance, such as tomato (*Solanum lycopersicum*), eggplant (*Solanum melongena*) and potato (*Solanum tuberosum*), and some are used in folk and traditional medicine, such as *Solanum americanum*; used in the treatment of gastric ulcer, bladderspasm and joint pains; and used as an effective vermifuge. Phytochemical constituent such as tannins, flavonoids and triterpenes have been already reported for their anti-ulcer activity.

The *S. tuberosum* is a starchy, tuberous and perennial plant of the Solanaceae family (also known as the nightshades). The tubers are used as anti-ulcer, anti-gout, anti-inflammatory, anti-arthritic, diuretic and anti-scurvy and to increase milk in lactating mothers.For external use, the grated raw *S. tuberosum*is applied locally in cases of arthritis, itching, neuralgia and mild burns (Umamaheswari *et al*., 2007; Deshpande *et al*., 2003). As the plant *S. tuberosum* has not explored to significant extent, and based on the background of available information of the plant, the present work was designed with the following objectives. First, to prepare various extracts (alcoholic and aqueous) with tubers of *S. tuberosum* by successive extraction technique and analyse the extracts for the phytochemical constituents; second,

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to establish the pharmacological profile (anti-ulcer) of the extracts of tubers of *S. tuberosum*.

MATERIALS AND METHODS

Experimental animals

Albino rats (Wistar strain) of either sex weighing between 120 and 200 g and albino mice weighing between 18 and 22 g were procured from the National Centre for Laboratory Animal Sciences, c/o Shri. Venkateswara Enterprises, Bengaluru, for experimental purpose and the animals were acclimatised for seven days under standard husbandry condition:

Room temperature– 26 ± 2°C
Relative humidity– 45–55%
Light/dark cycle– 12:12 h

The animals were fed with synthetic standard pellet diet purchased from Amrut Laboratories and Pranav Agro industries Ltd. Sangli, Maharashtra, India, and water was allowed *ad libitum* under strict hygienic conditions. All the animal studies were performed in accordance to Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines no. 425 and Institutional Animal Ethical Committee of V.L. College of Pharmacy, Raichur (Karnataka), with CPCSEA Registration Number 557/02/e/CPCSEA.

Determination of acute toxicity(LD_{50})

The acute toxicity of AETST and AQETST was assessed in albino mice of either sex weighing between 18 and 22 g who were maintained under standard husbandry conditions. The animals were fasted 3 h before the experiment, and "up-and-down" (CPCSEA guideline no. 425) method was adopted for toxicity studies. Animals were administered with single dose of extracts and observed for its mortality during 48-h study period (short-term) toxicity. On the basis of the short-term toxicity profile of the extracts, the doses of the next animals were determined as per as CPCSEA guidelines no. 425. All the animals were observed for long-term toxicity (seven days) and then 1/5th, 1/10th and 1/20th of the maximum dose tested for LD_{50} of the individual extract was taken as effective dose $ED_{50}$and were used throughout the experimental studies (Umamaheswari *et al*., 2007).

Experimental Procedure

Albino rats weighing between 150 and 200 g were divided into eight groups having six rats per group. They were fasted in individual cages for 24 h before study. Group A served as normal control, which was given with vehicle only. Group B with standard drug, groups C, D, E and F, G, H were administered with low, medium and high doses of AETST and AQETST, respectively. The various groups were treated with vehicle/extracts 30 min before pylorus ligation (Umamaheswari *et al*., 2007; Gregory *et al*., 2009; Khandare *et al.,* 2009; Khare, 2007; Brodie, 1968; Bhatnagar *et al*., 2005; Singh & Majumdar, 1999).

The abdomen was opened, the pylorus was ligated and sutured under light ether anaesthesia. Subsequent to 4 h post ligation, all the animals were sacrificed with excess of anaesthetic ether and the stomachs were dissected out. Gastric juice was collected into tubes and centrifuged at 1,000 rpm for 10 min and volume was recorded. The pH of the gastric juice was recorded by using pH meter (Systronic India, Mumbai). The gastric content was subjected for analysis of free and total acidity. The glandular portion of the stomach was opened along the greater curvature and the severity of hemorrhagic erosions in the acid secreting mucosa was assessed on a scale of 0 to 3.The ulcer index was determined by the following formula:

Ui = UN+US+UP×10-1

where Uiis ulcer index
UN = average of number of ulcers per animal
US = average of severity score
UP = percentage of animals with ulcers

Mean ulcer score for each animal is expressed as ulcer index. The percentage ulcer protection was calculated using the formula

Percentage ulcer protection = Uc–Ut/Uc× 100 (better write it using equation)

where
Ut = ulcer index of treated group
Uc = ulcer index of the control group

Determination of free and total acidity

The gastric juice (1 ml) was drawn into a 100-ml conical flask, two or three drops of Topfer's reagent was added and titrated with 0.01 N sodium hydroxide until all traces of red colour disappeared and the colour of the solution was yellowish orange. The volume of alkali added was noted. This volume corresponds to free acidity. Then two or three drops of phenolphthalein solution was added and titration was continued until a definite red tinge appeared. Again, the total volume of alkali added was noted, now this volume corresponds to total acidity.

Acidity was calculated by using the formula (Deshpande *et al*., 2003; Rajkapoor *et al*., 2002).

$$\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Normality of NaOH} \times 100}{0.1} \text{ mEq/lt/100 g}$$

Albino rats of either sex weighing between 150 and 200 g were divided into eight groups of six rats in each. Group A served as normal control, which was administered with vehicle only. Group B was administered with standard drug. Groups C, D, E and F, G, H administered with low, medium and high doses of AETST and AQETST respectively. After 30 min of oral administration of the vehicle/standard drug/extracts, rats were placed vertically in individual restraint cages in cold water maintained at 22°C for 1 h. Then, they were taken out, dried and injected with 30 mg/kg evans blue (i.v.) via the tail vein. Ten minutes later, they are sacrificed using ether and their stomachs were removed. Formal-saline (2% v/v) was then injected into the entirely ligated stomachs and were kept overnight. The next day, the stomachs were opened along the greater curvature, washed in warm water and examined for ulcers microscopically with the help of hand lens (10×). Mean ulcer score for each animal was expressed as ulcer index (Vogel & Vogel, 2002).

Statistical analysis

All results were expressed as mean ± SEM from six animals. Statistical difference in mean was analysed using one-way ANOVA (analysis of variance) followed by post hoc test (Dunnett's't' test). P< 0.05*, 0.01** and 0.001*** was considered as statistically significant.

RESULTS

In pylorus-ligation-induced ulcer model in rats (positive control), a significant increase in ulcer number (5.00 ± 0.51), ulcer score (2.41 ± 0.27) and ulcer index (10.74) were noted. In the same model a significant increase in volume of gastric juice (6.70 ± 0.10 ml), free acidity (31.33 ± 1.15 mEq/l) and total acidity (82.33 ± 1.89 mEq/l) were noted.

The group of rats administered with standard drug ranitidine (30 mg/kg) has significantly reduced ulcer number (0.33 ± 0.21), ulcer score (0.50 ± 0.12), ulcer index (3.41), volume of gastric juice (4.28 ± 0.13 ml), free acidity (17.33 ± 0.66 mEq/l), total acidity (43.50 ± 1.17 mEq/l) and the ulcers were inhibited by 68.24 %.

AETST with low, medium and high dose treatment showed a significant decrease in ulcer number (3.66 ± 0.42, 1.33 ± 0.33 and 0.83 ± 0.16), ulcer score (2.33 ± 0.21, 1.58 ± 0.08 and 0.83 ± 0.10), ulcer index (10.59, 10.29 and 8.49), volume of gastric juice (6.03 ± 0.07 ml, 5.01 ± 0.07 ml and 4.75 ± 0.07 ml), free acidity (25.00 ± 0.85, 22.33 ± 0.66 and 20.83 ± 1.01 mEq/l), total acidity (59.67 ± 1.05, 47.33 ± 2.01and 46.67 ± 0.80 mEq/l) and the ulcers were inhibited by 1.39%, 4.18% and 20.94 %, respectively.

AQETST with low, medium and high dose treatment showed a significant decrease in ulcer number (3.33 ± 0.21, 1.16 ± 0.16 and 0.66 ± 0.21), ulcer score (2.08 ± 0.20, 1.33 ± 0.10 and 0.75 ± 0.11), ulcer index (10.54, 10.24 and 6.80), volume of gastric juice (5.71 ± 0.11 ml, 4.85 ± 0.05 ml and 4.56 ± 0.12 ml), free acidity (23.83 ± 1.35, 21.83 ± 0.65 and 20.00 ± 0.73 mEq/l), total acidity (58.00 ± 1.39, 46.67 ± 1.99 and 46.00 ± 0.96 mEq/l) and the ulcers were inhibited by 1.86%, 4.65% and 36.68 %, respectively.

The order of potency in anti-ulcer activity was ranitidine >AQETST>AETST. The results are shown in Figs. 1–3and Table 1.

In stress-induced ulcer model in rats (positive control), a significant increase in ulcer number (4.67 ± 0.33), ulcer score (2.67±0.21), ulcer index (10.73), volume of gastric juice(6.77 ± 0.09 ml), free acidity (33.33 ± 0.66 mEq/l) and total acidity (84.83 ± 1.19 mEq/l) were noted.

The group of rats treated with standard drug ranitidine (30 mg/kg) has significantly reduced ulcer number (0.50 ± 0.22), ulcer score (0.75 ± 0.11), ulcer index (5.12), volume of gastric juice (4.38 ± 0.10 ml), free acidity (19.00 ± 0.68 mEq/l), total acidity (45.67 ± 0.91 mEq/l) and the ulcers were inhibited by 52.28%.

AETST with low, medium and high dose treatment showed a significant decrease in ulcer number (3.50 ± 0.22, 1.67 ± 0.21 and 0.83 ± 0.16), ulcer score (2.50 ± 0.22, 1.83 ± 0.10 and 1.08 ± 0.08), ulcer index (10.60, 10.35 and 8.52), volume of gastric juice (6.20 ± 0.05, 5.15 ± 0.04 and 4.88 ± 0.06 ml), free acidity (26.83 ± 0.60, 24.50 ± 0.88 and 22.67 ± 0.71 mEq/l), total acidity (61.67 ± 0.95, 49.00 ± 1.15 and 48.67 ± 0.61 mEq/l) and the ulcers were inhibited by 1.21%, 3.54% and 20.59% respectively.

AQETST with low, medium and high dose treatment showed a significant decrease in ulcer number (3.17 ± 0.16, 1.50 ± 0.22 and 0.83 ± 0.16), ulcer score (2.50 ± 0.22, 1.67 ± 0.10 and 0.91 ± 0.08), ulcer index (10.56, 10.31 and 8.50), volume of gastric juice (6.02 ± 0.07, 4.95 ± 0.07 and 4.77 ± 0.07 ml), free acidity (25.67 ± 0.84, 23.33 ± 0.76 and 21.50 ± 0.76 mEq/l), total acidity (60.33 ± 0.55, 47.83 ± 1.57 and 47.17 ± 0.94 mEq/l) and the ulcers were inhibited by 1.58, 3.91 and 20.78 % respectively.

The order of potency in anti-ulcer activity was ranitidine >AQETST>AETST.

DISCUSSION

Peptic ulcer is a conglomerate of most common heterogeneous disorders, present as a crater in the lining of the gastrointestinal tract (GIT) mucosa because of acid, pepsin, bile acid, pancreatic enzyme and bacteria. It is due to an imbalance between aggressive (acid and pepsin) and defensive (bicarbonates, mucin, etc.) factors. Peptic ulcer disease also occurs due to administration of nonsteroidal anti-inflammatory drugs (NSAIDs), stress, *Helicobacter pylori* or pathological condition such as Zollinger-Ellison syndrome. NSAIDs cause erosions, petechiae, type C gastritis and ulceration in combination with interference of ulcer

Table 1. Anti-ulcer effects of AETST and AQETST in different ulcers models in rats

		Pylorus ligation model					Stress-induced ulcer model				
Groups	**Treatment**	**Ulcer Number**	**Ulcer Score**	**Incidence of Ulcers (%)**	**Ulcer Index**	**Inhibition of Ulcers (%)**	**Ulcer Number**	**Ulcer score**	**Incidence of Ulcers (%)**	**Ulcer Index**	**Inhibition of Ulcers (%)**
Control	vehicle 10 ml/kg p.o	**5.00** ±0.51	**2.41** ±0.27	100	10.74	-	**4.67** ±0.33	**2.67** ±0.21	100	10.73	--
Standard	Ranitidine 30 mg/kg	**0.33** ±0.21***	**0.50** ±0.12***	33.33	3.41	68.24	**0.50** ±0.22***	**0.75** ±0.11***	50	5.12	52.28
AETST	100 mg/kg p.o	**3.66** ±0.42*	**2.33** ±0.21ns	100	10.59	1.39	**3.50** ±0.22**	**2.50** ±0.22 ns	100	10.60	1.21
AETST	200 mg/kg p.o	**1.33** ±0.33***	**1.58** ±0.08**	100	10.29	4.18	**1.67** ±0.21***	**1.83** ±0.10**	100	10.35	3.54
AETST	400 mg/kg p.o	**0.83** ±0.16***	**0.83** ±0.10***	83.33	8.49	20.94	**0.83** ±0.16***	**1.08** ±0.08***	83.33	8.52	20.59
AQETST	100 mg/kg p.o	**3.33** ±0.21**	**2.08** ±0.20ns	100	10.54	1.86	**3.17** ±0.16***	**2.50** ±0.22 ns	100	10.56	1.58
AQETST	200 mg/kg p.o	**1.16** ±0.16***	**1.33** ±0.10***	100	10.24	4.65	**1.50** ±0.22***	**1.67** ±0.10***	100	10.31	3.91
AQETST	400 mg/kg p.o	**0.66** ±0.21***	**0.75** ±0.11***	66.66	6.80	36.68	**0.83** ±0.16***	**0.91** ±0.08***	83.33	8.50	20.78

n = 6, Significant at P < 0.05, 0.01** and 0.001***, ns = not significant.*

AETST, alcoholic extract of tuber of S. tuberosum; AQETST, aqueous extract of tuber of S. tuberosum.

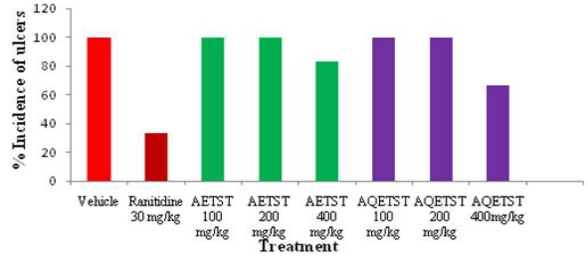

Figure 1. Anti-ulcer activity of AETST and AQETST in pylorus-ligation-induced ulcer model in rats

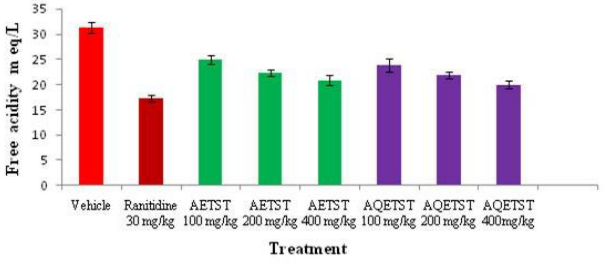

Figure 2. Anti-ulcer activity of AETST and AQETST on free acidity in pylorus-ligation-induced ulcers in rats

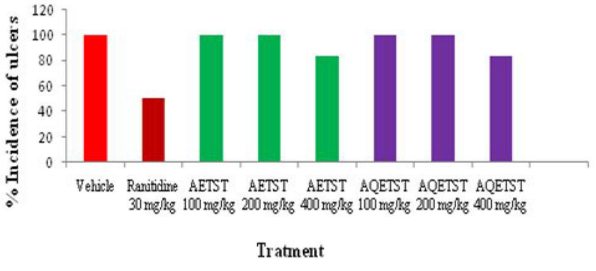

Figure 3. Anti-ulcer activity of AETST and AQETST in stress-induced ulcer model in rats

healing. Further, they also induce damage of the mucosa with imbalance between aggressive and defensive factors. Though a very good number of anti-ulcers drugs such as antisecretory drugs, H_2receptor antagonists, proton pump inhibitors, antimuscarinic, cytoprotectants and prostaglandins analogues are available, the side effects associated with these drugs limit their use. Many herbal drugs from Ayurveda of Indian traditional system of medicine are advocated for the management of peptic ulcer. Herbal medicines used as whole plant powders/extracts from different parts are now a day's considered as safe medication for the treatment of a number of diseases as it is a general notion that plant based drugs are safer without any side effects.

Though the presently available anti-ulcer drugs have remarkable effects in ulcer therapy, the efficacy is still incomplete, as there are a number of incidences of relapse with adverse effects and drug–drug interaction were reported with the therapy. Hence, there is a need for ideal anti-ulcer drugs with extended action from herbal source with a calibre of better protection and low incidence of relapse of ulcers (Thirunavukkarasu *et al*., 2009; Gupta *et al*., 2010; Raju *et al*., 2009).

Stress-induced ulcer are caused by the release of histamine accompanied with an increase in acid secretion and reduction in mucus production. Stress stimulates adenohypophysial axis and causes the release of endogenous opiates and also produces severe gastric erosion by the activation of central vagal discharge that release endogenous opiates that causes mucosal congestion by peripheral mechanism to develop gastric ulcers. Both the extracts have significantly reduced gastric secretion and thus prevented gastric mucosa from the development of ulcers. Several studies reported that gastroduodenal protection by prostaglandins is due to increase in mucosal resistant and decrease in aggressive factors such as acid and pepsin(Raju et al., 2009; Yelken *et al*., 1999; Mota *et al*., 2000; Jain & Surana, 2009; Devaraj *et al*., 2007; Kurian, 2007).

In Indian system of medicine, a very good number of herbs are reported to produce anti-ulcer activity. Hence, in the present study, a plant by name S. *tuberosum* has considered to evaluate its anti-ulcer activities scientifically. This alcohol and aqueous extracts prepared from the tubers of the plant were tested against different ulcer models and inflammatory models in rats.

In pylorus-ligation-induced ulcer model, ulcers are resulted owing to the accumulation of acid at the pyloric end that causes ulcers. Both the extracts AETST and AQETST significantly reduced ulcers by decreasing gastric volume and increasing the pH, thereby reducing the severity of ulcers,that is, ulcers number and ulcers index.

CONCLUSION

Preliminary phytochemical evaluation of both AETST and AQETST revealed the presence of tannins, carbohydrate, sterols, flavonoids, glycosides, alkaloids and triterpenes in both the extracts. Acute oral toxicity studies recorded no mortality with either of the extracts even at the dose level of 2,000 mg/kg body weight.

Anti-ulcer activity have been confirmed with both the extracts in experimental animals, with different ulcer models. In ulcer, both the extracts at low, medium and high doses produced a significant anti-ulcer activities ($P < 0.05$*, 0.01** and 0.001***). Phytochemical constituents such as tannins, flavonoids and triterpenes are already reported for their anti-ulcer activity and both the extracts contained the above mentioned constituents. Hence, these can be accounted for the observed anti-ulcer activity in rats.

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