Fitoterapia 81 (2010) 755–761

Contents lists available at ScienceDirect

Fitoterapia

journal homepage: www.elsevier.com/locate/fitote

Verbascoside isolated from *Tectona grandis* mediates gastric protection in rats via inhibiting proton pump activity

Neetu Singh [a], Nivedita Shukla [b], Pratibha Singh [a], Rolee Sharma [d], S.M. Rajendran [c], Rakesh Maurya [b], Gautam Palit [a,*]

[a] Division of Pharmacology, Central Drug Research Institute, CSIR, Lucknow - 226001, Uttar Pradesh, India
[b] Division of Medicinal and Process Chemistry, Central Drug Research Institute, CSIR, Lucknow - 226001, U.P, India
[c] Division of Botany, Central Drug Research Institute, CSIR, Lucknow - 226001, U.P, India
[d] Department of Biotechnology, Integral University, Lucknow - 226026, U.P, India

ARTICLE INFO

Article history:
Received 26 February 2010
Accepted in revised form 25 March 2010
Available online 11 April 2010

Keywords:
Gastric ulcer
Tectona grandis
H^+ K^+-ATPase
Verbascoside
Antisecretory

ABSTRACT

Evidences have suggested that *Tectona grandis* (TG) attenuates gastric mucosal injury; however its mechanism has not yet been established. The aim of present study was to evaluate the gastroprotective mechanism of ethanolic extract of TG (E-EtOH), butanolic fraction (Fr-Bu) and to identify its active constituents. Anti-ulcer activities were evaluated against cold restraint (CRU) and pyloric ligation (PL) induced gastric ulcer models and further confirmed through H^+ K^+-ATPase inhibitory activity. Cytoprotective activity was evaluated in alcohol (AL) induced gastric ulcer model and further through PGE_2 level. E-EtOH and Fr-Bu attenuated ulcer formation in CRU. Moreover E-EtOH and Fr-Bu displayed potent anti-secretory activity as evident through reduced free acidity and pepsin activity in PL, confirmed further by *in vitro* inhibition of H^+ K^+-ATPase activity. In addition cytoprotective potential of E-EtOH and Fr-Bu were apparent with protection in AL model, increased PGE_2 content and enhanced mucin level in PL. Phytochemical investigations of Fr-Bu yielded terpenoides and a phenolic glycoside, verbascoside. The anti-secretory mechanism of verbascoside mediated apparently through inhibition of H^+ K^+-ATPase with corresponding decrease in plasma gastrin level, is novel to our finding. Gastroprotection elicited by TG might be through proton pump inhibition and consequent augmentation of the defensive mechanism.

1. Introduction

Gastric ulcer is a very common global problem today. The pathophysiology of these ulcers involves an imbalance between offensive (acid, pepsin, and Helicobacter pylori) and defensive factors (mucin, prostaglandin, bicarbonate, nitric oxide and growth factors). Today, there are two main approaches for treating peptic ulcer. The first deals with reducing the production of gastric acid and the second with re-enforcing gastric mucosal protection [1]. Modern approach includes proton pump inhibitors, histamine receptor blockers, drugs affecting the mucosal barrier and prostaglandin analog but clinical evaluations of these drugs have shown incidences of relapse, side effects and drug interactions. These therapeutic limitations have provided impetus anti-ulcer drugs. This has also resulted in the extension of the search for novel molecules to medicinal plants that can offer better protection and have better safety profile.

Tectona grandis (TG), belonging to the family Verbenaceae is locally called as "Thekku maram" and commonly known as Indian teak. In traditional medicine, its leaf is extensively used for wound healing activity when administered orally [2], it is also beneficial in dyspepsia with burning of stomach [3]. Further reports have shown the presence of a napthaquinone lapachol, in TG roots to possess anti-ulcer [4] and nitric oxide

* Corresponding author. Tel.: +91 522 2612411 418x4303; fax: +91 522 2623405, +91 522 2623938.
E-mail addresses: singh_neetu2000@rediffmail.com (N. Singh), gpalitcdri@gmail.com (G. Palit).

0367-326X/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.fitote.2010.03.019

scavenging activity [5]. But the mechanism through which it offers gastroprotective effects remains unexplored.

The present study was undertaken to investigate the underlying mechanism of the anti-ulcer property of TG leaf extract and also to identify the active constituent responsible for these gastroprotective effects.

2. Materials and methods

2.1. Plant material

TG plant grows naturally in dry and moist deciduous forest of the Aravali range in Rajasthan, U.P., Orissa, Maharashtra and Tamil Nadu. The TG leaves were collected in the month of February from Sirumalai Tiruchirapalli (Tamil Nadu) and was identified by the Division of Botany, Central Drug Research Institute (CDRI), Lucknow. The voucher of this specimen is kept at the herbarium of CDRI (acquisition number 8276).

2.2. Phytochemical screening

Shed dried leaves of *Tectona grandis* (1.0 kg) were extracted 4 times with ethanol at room temperature. The ethanolic extract (E-EtOH) thus obtained was concentrated under vacuum (yield: 9.09%). Further, E-EtOH was suspended in water and partitioned with equal volume of butanol, resulting in 38 g of butanolic fraction (Fr-Bu) after solvent elimination. Thereafter, Fr-Bu was fractionated and analyzed by TLC on Silica Gel 60 (Merck) using $CHCl_3$: MeOH (9:1 and 8:2) as mobile phase, one sprayed with $FeCl_3$ and other with anysaldehyde reagent, both TLCs were visualized under UV light (254–363 nm). They were screened and the presence of phenolic glycosides and terpenoids were detected. Repeated column chromatography of Fr-Bu yielded betulinic acid and ursolic acid [6], β-sitosterol [7], β-sitosterol-D-glucoside [8,9] and verbascoside [10,11], which were characterized by 1D and 2D NMR experiments at 300 MHz. Verbascoside (Fig. 1) isolated is being reported for the first time from this plant, it has been earlier reported from *Verbascum sinuatum* [12].

2.3. Experimental animals

Sprague–Dawley (SD) rats of either sex, weighing 180–200 g were obtained from National Animal Laboratory Centre of our Institute and kept in environmentally controlled rooms (25 ± 2 °C, 12 h light and dark cycle). Animals were fed with standard laboratory food pellets and water was provided ad libitum. Experimental protocols were approved by our Institutional Ethical Committee which follows guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) and complies with international norms of INSA (Indian National Science Academy).

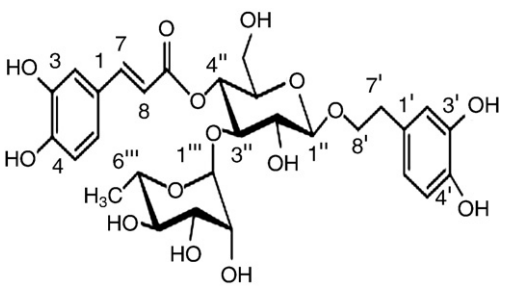

Fig. 1. Structure of verbascoside.

2.4. Treatment schedule

E-EtOH, Fr-Bu, verbascoside and standard anti-ulcer drug omeprazole (Omz) (Sigma Chemicals, USA), sucralfate (SUC) (Meranani pharmaceutical, India) drugs were prepared in 1% sodium carboxymethylcellulose (CMC) suspension as vehicle and administered orally 45 min prior to exposure to ulcerogens to the animals at a volume of 1 ml/200 g of body weight. All animals were deprived of food for 16 h before ulcerogens exposure and were divided into four groups, each group comprising of six animals. Control group of animals were treated with vehicle similar to experimental groups.

3. Anti-ulcer studies

3.1. Cold restraint induced gastric ulcer (CRU)

Animals of different experimental group were subjected to cold and restraint stress after 45 min of treatment with graded doses of E-EtOH (125, 250 and 500 mg/kg, p.o.), Fr-Bu (20, 40 and 80 mg/kg, p.o.) and Omz (10 mg/kg, p.o.). All the animals were immobilized in a restraint cages and kept at 4 °C in an environmental chamber for 2 h [13]. The animals were then sacrificed and stomachs were observed under Magnascope for ulcers and scored.

3.2. Pyloric ligation induced ulcer model (PL)

Pyloric ligation was done [14] under chloral hydrate anesthesia (300 mg/kg, i.p.). After 45 min pretreatment with E-EtOH (250 mg/kg, p.o.), Fr-Bu (40 mg/kg, p.o.), verbascoside (40 mg/kg, p.o.) and Omz (10 mg/kg, p.o.), pyloric end of the stomach was ligated and abdomen was closed by suturing. After 4 h of surgery, rats were sacrificed and the stomach was dissected out and the accumulated gastric juice was collected. Ulcers were also scored after examining the dissected stomach under Magnascope.

3.3. Gastric secretion study

Free and total acidity were measured from the collected gastric juice by titrating against 0.01 N NaOH, using phenolphthalein as indicator and expressed in terms of μequiv./ml [15]. Peptic activity was determined by measuring the amount of liberated tyrosine by the action of pepsin on hemoglobin as substrate and expressed in terms of units/ml [16]. Mucin level in gastric juice was quantified with a fluorometric assay and expressed as μg of mucin/ml of gastric juice [17].

3.4. Alcohol induced gastric ulcers in rats (AL)

Gastric ulcer was induced in rats by administering absolute alcohol (1 ml/200 g, 1 h) [18]. The E-EtOH (250 mg/kg, p.o.), Fr-Bu (40 mg/kg, p.o.), verbascoside (40 mg/kg, p.o.) and

SUC (500 mg/kg, p.o.) were administered 45 min before alcohol treatment. After 1 h, the animals were sacrificed and stomachs were excised to observe the gastric lesions and were measured using Biovis image analyzer software.

3.5. Measurement of ulcer index

Ulcers formed in stomach of CRU and pyloric ligated rats were scored according to the arbitrary scoring system [19] and graded as following: (i) Shedding of epithelium = 10; (ii) Petechial and frank hemorrhages = 20; (iii) one or two ulcers = 30; (iv) more than two ulcers = 40; and (v) Perforated ulcers = 50. In AL model the length of the lesions were measured using Biovis image analyzer software and summated to give a total lesion score.

4. In vitro assay of H^+ K^+-ATPase activity

H^+ K^+-ATPase activity was analyzed in gastric microsomes isolated from rat stomach [20] by measuring the inorganic phosphate released after hydrolysis of ATP. For the enzyme assay, gastric microsomes incubated with or without different concentrations of E-EtOH, Fr-Bu and verbascoside as well as standard drug Omz for 10 min at 37 °C, were added to an assay buffer containing (in mM) 150 KCl, 10 PIPES, 1 $MgSO_4$, 5 Mg ATP, 1 EGTA and 0.1 ouabain, at pH 7.2 and 10 μg/ml valinomycin, 2.5 μg/ml oligomycin. The reaction was carried out at 37 °C for 20 min and was stopped by adding 10% ice-cold trichloroacetic acid. After centrifugation (2000 *g* for 1 min), inorganic phosphate release was determined from the resulting supernatant spectrophotometrically at 310 nm wavelength [21] and expressed as μM/hr/mg protein.

5. Gastrin measurement

In order to determine the gastrin levels in plasma, blood was collected by cardiac puncture, centrifuged, and the plasma was analyzed for gastrin levels using rat gastrin I enzyme immunoassay kit (assay designs, Hines Drive Ann Arbor, USA) following the manufacturer's instructions. The results were expressed as pg/ml.

6. PGE_2 estimation

For measurement of COX activity, PGE_2 was determined in mucosal tissue samples obtained from sham, control and treatment groups. Briefly, mucosa was scrapped and rapidly rinsed with ice-cold saline. The tissue was weighed and homogenized in 10 volumes of phosphate buffer (0.1 M, pH-7.4) containing 1 mM EDTA and 10 μM indomethacin. The homogenate was centrifuged (10,000 rpm, 10 min, 4 °C), and the supernatant was processed for PGE_2 estimation using the Biotrak enzyme immunosorbent assay kit (Amersham Biosciences, Piscataway, NJ), following the manufacturer's instructions. Results were expressed as pg PGE_2/mg protein.

7. Statistical analysis

All values shown in the figures and tables represent the means ± S.E.M. IC_{50} values with 95% confidence limits were estimated using Maximum Likelihord Iterative Procedure [22]. Statistical analysis was performed with Prism version 3.0 software using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. $P<0.05$ was considered to be statistically significant.

8. Results

8.1. Anti-ulcer effect of E-EtOH against acute gastric ulcer models

In our pilot study, graded doses of E-EtOH of TG leaves (125, 250 and 500 mg/kg) showed protection of 41.65%, 66.77% ($P<0.01$) and 68.73% ($P<0.01$) respectively whereas standard drug, Omz (10 mg/kg, p.o.) showed a protection of 77.73% ($P<0.01$) as compared to control against CRU model. Based on these results 250 mg/kg p.o. was taken as the effective dose and selected for further studies. In PL induced gastric ulcer model, E-EtOH (250 mg/kg) and Omz (10 mg/kg, p.o.) exhibited 61.02% ($P<0.01$) and 69.42% ($P<0.01$) protection respectively. Moreover, E-EtOH significantly reduced free acidity and pepsin activity accompanied by increased mucin level. Results of gastric acid study are presented in Table 1. Pretreatment of animals with E-EtOH (250 mg/kg, p.o.) also exerted 78.11% ($P<0.01$) protection against gastric mucosal damage induced by AL. SUC (500 mg/kg, p.o.), the standard drug used in AL model exhibited only 62.72% ($P<0.05$) protection in comparison to control. The results are graphically represented in Fig. 2.

8.2. Effect of Fr-Bu on anti-ulcer activity

Phytochemical analysis through fractionation studies of E-EtOH of TG leaves yielded Fr-Bu (38 g). Fr-Bu (20, 40 and 80 mg/kg, p.o.) and Omz (10 mg/kg, p.o.) exerted 33.30%, 69.70% ($P<0.01$), 70.73% ($P<0.01$) and 77.73% ($P<0.01$) protection respectively in CRU model as compared to control.

Table 1
Effect of E-EtOH (250 mg/kg), Fr-Bu (40 mg/kg) and verbascoside (40 mg/kg) of *Tectona grandis* and Omeprazole on free acidity, total acidity, pepsin and mucin contents in pyloric ligation model ($n = 6$ in each group).

Treatment	Free acid μequiv./ml	Total acid μequiv./ml	Peptic activity units/ml	Mucin μg/ml
Control	43.825 ± 3.22	31.975 ± 4.686	23.842 ± 3.519	84.47 ± 17.92
E-EtOH(250 mg/kg)	32.2 ± 3.988*	29.825 ± 6.205	16.1032 ± 2.348*	400.9 ± 29.74**
Fr-Bu(40 mg/kg)	27.86 ± 2.780*	28.236 ± 2.316	11.628 ± 1.168**	389.06 ± 26.806**
Verbascoside(40 mg/kg)	28.31 ± 1.24*	23.03 ± 4.56*	18.35 ± 0.16*	107.27 ± 7.68
Omeprazole(10 mg/kg)	23.33 ± 1.284**	18.295 ± 1.309**	9.6944 ± 1.151**	234.6 ± 9.918*

Data expressed as mean ± S.E.M. Statistical analysis was done by One Way ANOVA followed by Dunnett's Multiple Comparison Test. *Statistically significant at $P<0.05$ and **$P<0.01$, in comparison to control.

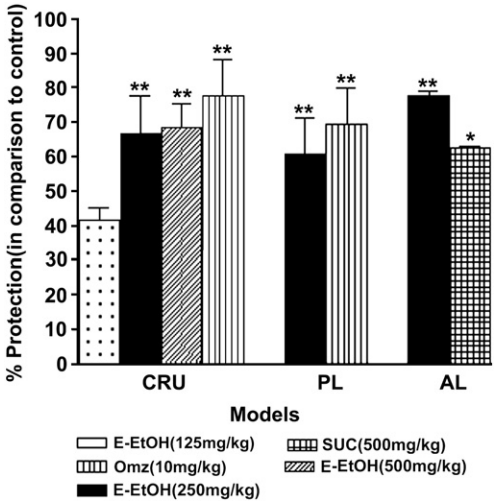

Fig. 2. Effect of E-EtOH of TG and standard drug (Omz and SUC) on percentage of ulcer against cold restraint, pyloric ligation and alcohol induced ulcer in rats. Data expressed as mean % protection ± S.E.M. Statistical analysis was done by One Way ANOVA followed by Dunnett's Multiple Comparison Test. *Statistically significant at $P<0.05$ and **$P<0.01$, in comparison to control. $n=6$ in each group.

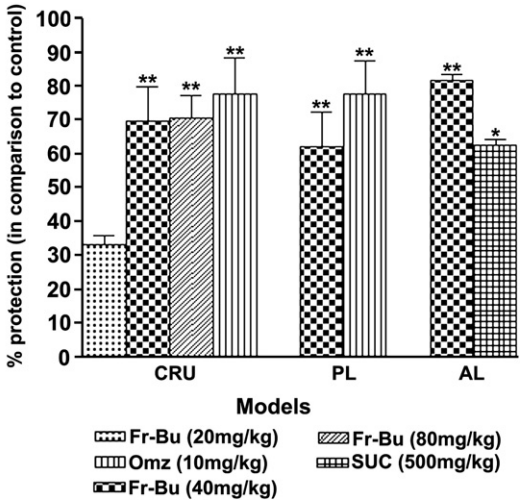

Fig. 3. Effect of Fr-Bu of TG and standard drug (Omz and SUC) against cold restraint, pyloric ligation and alcohol induced ulcer in rats. Data expressed as mean % protection ± S.E.M. Statistical analysis was done by One Way ANOVA followed by Dunnett's Multiple Comparison Test. *Statistically significant at $P<0.05$ and **$P<0.01$, in comparison to control. $n=6$ in each group.

These results indicated 40 mg/kg p.o as the effective dose and thus selected for further studies. In PL model, 62.02% ($P<0.01$) protection was observed with Fr-Bu (40 mg/kg, p.o.) as compared to control. Omz (10 mg/kg, p.o.) showed percentage protection of 69.42% ($P<0.01$) in PL model. Furthermore, results with gastric secretion study summarized in Table 1 presented that Fr-Bu (40 mg/kg, p.o.) potently reduced free acidity and pepsin and upregulates mucin level. Fr-Bu when tested against AL induced gastric ulcer model, exhibited 81.65% ($P<0.01$) protection whereas standard drug SUC (500 mg/kg, p.o.) showed only 62.72% ($P<0.05$) protection as compared to control. The results are graphically represented in Fig. 3.

8.3. Phytochemical analysis of Fr-Bu

The butanolic fraction (Fr-Bu) weighted 38 g and was determined to be essentially composed of triterpenoids such as betulinic acid (2.50%), ursolic acid (2.70%), β-sitosterol (1.94%), β-sitosterol-D-glucosidase (2.02%) and a phenolic glycoside verbascoside (28%).

8.4. Characterization of verbascoside

Compound verbascoside was obtained as amorphous powder. It confirmed to ferric chloride and Fiegel colour tests for phenolic glycosides. The IR spectrum showed absorption bands at 3394 cm^{-1} for hydroxyl group, 1701 cm^{-1} for conjugated ester, and 1596, 1525 cm^{-1} for aromatic rings. UV spectrum showed absorption band at 204, 216 and 332 nm, which indicated for phenolic glycoside. The FAB mass spectrum of verbascoside contained a peak at m/z 625 $[M+H]^+$ corresponding to the molecular formula of $C_{29}H_{36}O_{15}$. This information was further supported by ^1H and ^{13}C NMR spectra (Table 2).

8.5. Gastro-protective effect of verbascoside

Anti-ulcer activity of verbascoside was observed against pyloric ligation induced ulcer in rats where it showed protection of 58.3% ($P<0.01$) and omeprazole showed 69.42% ($P<0.01$) protection. Its antisecretory effect was evaluated by estimating free and total acidity of gastric juice and by

Table 2
NMR data for verbascoside in DMSO-d^6.

Position	δ_H (J in Hz)	δ_C
1	–	125.5
2	7.02 (*s*)	116.3
3	–	145.5
4	–	148.4
5	6.76 (*brs*)	113.5
6	6.97 (*brs*)	121.4
7	7.45 (*d*, $J=15.6$)	145.0
8	6.19 (*d*, $J=15.6$)	119.5
9	–	165.7
1′	–	129.1
2′	6.62 (*s*)	115.4
3′	–	145.0
4′	–	145.5
5′	6.48 (*d*, $J=6.9$)	114.6
6′	6.62 (*brs*)	115.7
7′	2.69 (*m*)	35.0
8′	3.88 (*m*)	70.2
1″	4.34 (*d*, $J=7.5$)	102.2
2″	3.63–3.10 (*m*)	74.7
3″	3.70 (*t*, $J=9.6$)	79.1
4″	4.70 (*t*, $J=9.6$)	69.1
5″	3.63–3.10 (*m*)	74.5
6″	3.63–3.10 (*m*)	60.7
1‴	5.02 (*brs*)	101.2
2‴	3.63–3.10 (*m*)	70.5
3‴	3.63–3.10 (*m*)	70.3
4‴	3.63–3.10 (*m*)	71.6
5‴	3.63–3.10 (*m*)	68.7
6‴	0.95 (*d*, $J=5.7$)	18.1

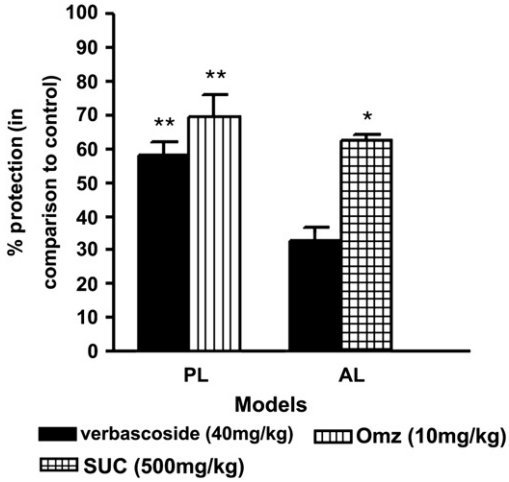

Fig. 4. Effect of Verbascoside and standard drug (Omz and SUC) against pyloric ligation and alcohol induced gastric ulcer in rats.

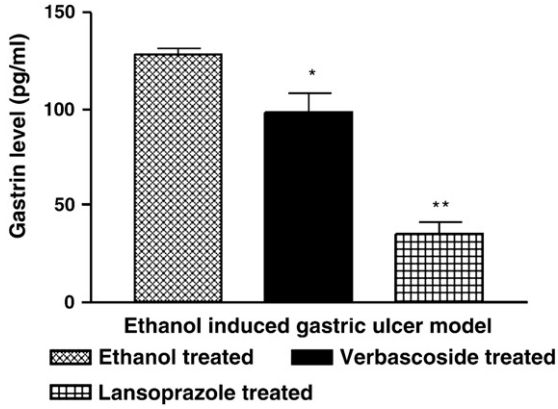

Fig. 5. Effect of Verbascoside on plasma gastrin hormone concentration in ethanol induced ulcer model. Results are expressed as mean ± S.E.M. ($n = 6$). *Statistically significant at $P<0.05$ and **$P<0.01$, in comparison to control. $n = 6$ in each group.

estimating the activity of pepsin and mucin as shown in Table 1. Though verbascoside did not show significant effect on defensive factors like mucin secretion, it significantly reduces free acidity (35.4%, $P<0.05$), total acidity (27.97%, $P<0.05$) and peptic activity (23.03%, $P<0.05$), which was comparable with standard drug omeprazole in comparison to control. Verbascoside exhibited only 33.02% protection when tested against AL induced gastric ulcer model, whereas standard drug SUC (500 mg/kg, p.o.) showed 62.72% ($P<0.05$) protection as compared to control. The results are illustrated in Fig. 4.

8.6. Effect of E-EtOH, Fr-Bu, verbascoside and omeprazole on H^+ K^+-ATPase activity

E-EtOH (200–600 μg/ml), Fr-Bu (10–100 μg/ml) and verbascoside (10–100 μg/ml) inhibited the gastric H^+ K^+-ATPase activity in comparison to control with an IC_{50} value of 499.3 μg/ml, 69.03 μg/ml and 60.98 μg/ml respectively. Omz (10–50 μg/ml) used as positive control reduced the enzyme activity with an IC_{50} value of 30.24 μg/ml (Table 3).

8.7. Effect of verbascoside on gastrin hormone concentration

The concentration of gastrin hormone in the plasma of ulcer control group (ethanol treated) was 128.3 ± 2.8 pg/ml. Pretreatment with verbascoside (40 mg/kg) significantly ($P<0.05$) reduced the plasma gastrin level (98.6 ± 9.6 pg/ml) in comparison to control. Lansoprazole (30 mg/kg) used as reference drug gives 35.4 ± 5.6 pg/ml ($P<0.01$) plasma gastrin level (Fig. 5).

8.8. Effect of E-EtOH, Fr-Bu, verbascoside and omeprazole on PGE_2 level

The PGE_2 generation in the ulcer control group was 2533.489 ± 707.0 pg/mg tissue protein. The PGE_2 value of E-EtOH, Fr-Bu, verbascoside and omeprazole treated group was found to be 3993.003 ± 838.6 ($P<0.05$), 3859.319 ± 639.2 ($P<0.05$), 2748.315 ± 214.4, 4553.415 ± 504.8 ($P<0.05$) respectively (Table 4).

9. Discussion

Earlier studies have shown that TG exerts anti-ulcer activity in experimental gastric ulcer models [23] but the mechanism of actions through which it mediates gastroprotective effects were not elucidated. In our study, we explored the mechanism of actions involved in the gastroprotective effects of TG.

In the present study, we demonstrated that E-EtOH and Fr-Bu of TG displayed anti-ulcer activities, as evident by significant protection in different gastric ulcer models, reduced gastric secretions through proton pump inhibition, increased PGE_2 level and enhanced gastric mucin content. E-EtOH was also observed to be beneficial in healing of chronic

Table 3
Effect of E-EtOH, Fr-Bu and verbascoside of *Tectona grandis* and standard drug Omeprazole on H^+ K^+-ATPase isolated from rat gastric microsomes. Data expressed as mean ± S.E.M. of experiments performed in triplicates ($n = 3$ in each group).

Treatment (μg/ml)	Mean % inhibition	H^+ K^+-ATPase inhibition IC50 (μg/ml)	95% confidence limit of IC50 Lower limit	Upper limit
Control				
E-EtOH(200–600 μg/ml)	61.53 ± 10.03	499.36 μg/ml	490.28	503.03
Fr-Bu(10–100 μg/ml)	69.00 ± 10.64	69.03 μg/ml	63.02	76.09
Verbascoside(10–100 μg/ml)	81.98 ± 9.457	60.98 μg/ml	55.46	67.06
Omeprazole(10–50 μg/ml)	80.07 ± 11.59	30.24 μg/ml	27.52	33.25

Table 4
Effect of E-EtOH, Fr-Bu and verbascoside of *Tectona grandis* and standard drug Omeprazole on PGE_2 level.

Group	Prostaglandin PGE_2 (pg/mg protein)
Control	2533.489 ± 707.0
E-EtOH	3993.003 ± 838.6*
Fr-Bu	3859.319 ± 639.2*
Verbascoside	2748.315 ± 214.4
Omeprazole	4553.415 ± 504.8*

Data expressed as mean ± S.E.M. Statistical analysis was done by One Way ANOVA followed by Dunnett's Multiple Comparison Test. *Statistically significant at $P<0.05$, in comparison to control ($n=6$ in each group).

gastric ulcers. Moreover, the proton pump inhibitory activity of E-EtOH and Fr-Bu may be attributed to the presence of verbascoside.

We performed a dose dependent (125, 250 and 500 mg/kg p.o body weight) anti-ulcer study of E-EtOH in CRU model. CRU is a well-accepted model for the induction of gastric ulcers where peripheral sympathetic activation and increased acid secretion plays an important role [24]. E-EtOH exhibited significant protection in a dose dependent manner against CRU model, with maximum protection observed at 250 mg/kg, p.o. Hence, this dose was considered for further studies in all other models. Moreover, acute toxicity studies on TG leaf extract have shown it to be safe up to a dose of 2 g/kg p.o. body weight of rat [2]. Thus, the selected dose of 250 mg/kg was considered to be safe.

Gastric acid plays a central role in ulcer induction through pyloric ligation model [14]. In this model, gastric mucosal auto-digestion *via* increased gastric acid secretion and pepsin activity was believed to propagate the ulcer formation [25]. Significant inhibition of free acidity and pepsin level was observed with E-EtOH (250 mg/kg) in this model, reflecting its potent anti-secretory activity *in vivo*.

Furthermore, for the identification of the phytochemical constituents responsible for the above mentioned anti-ulcer effect, E-EtOH was fractionated into Fr-Bu and tested in different gastric ulcer models. Graded doses of Fr-Bu exerted anti-ulcer effect in the CRU model, offering maximum protection at much lower dose 40 mg/kg than E-EtOH (250 mg/kg) indicating concentration of active constituents in this fraction. 40 mg/kg dose of Fr-Bu was chosen to perform further studies. Gastric secretion study with above dose of Fr-Bu also reflected the anti-secretory mode of action, as it reduced free acidity, pepsin level and ulcer incidences in pyloric ligated rats.

While secretion of gastric acid is regulated by activation of several stimulatory receptors such as Histamine H_2, muscarinic M_3 and gastrin CCK_2 receptors located on the basolateral membrane of parietal cells, the ultimate target in the anti-secretory pathway is H^+ K^+-ATPase (proton pump). This proton pump is a membrane bound enzyme that catalyses H^+ transport at the expense of ATP hydrolysis. Thus the inhibition or the blockade of H^+ K^+-ATPase may account for suppressed acid secretion observed in the *in vivo* studies. The results obtained with gastric microsomes isolated from rat stomach showed that E-EtOH and Fr-Bu potently inhibited the H^+ K^+-ATPase activity, the inhibition elicited by Fr-Bu is even more effective than E-EtOH and was comparable to the positive control, Omeprazole, there by suggesting that E-EtOH and Fr-Bu might be imparting anti-ulcer activity through decrease in acid secretion via proton pump inhibition.

Moreover E-EtOH and Fr-Bu exerted a protective effect against ethanol-induced gastric lesions in contrast to standard drug, sucralfate. Since ethanol directly causes mucosal damage by inhibiting the release of mucosal prostaglandins [26] and depressing the gastric defensive mechanisms [27], these agents appear to augment the gastric mucosal defense. The gastroprotective effect of E-EtOH and Fr-Bu could be attributed to its mucoprotective activity as evident through upregulated mucin level in PL model.

Further exploration of Fr-Bu for its chemical constituents demonstrated the presence of terpenoids such as betulinic acid, ursolic acid, β-sitosterol, β-sitosterol-D-glucoside and phenolic glycoside, verbascoside. The terpenoides isolated from Fr-Bu have been reported to associate with various biological activities including anti-ulcer. For instance, β-sitosterol and β-sitosterol-D-glucoside have been reported as anti-ulcerative agents in cold stress and acetic acid induced ulcer models [28]. Betulinic acid was demonstrated to possess free radical scavenging, anti-inflammatory and immunomodulatory activities [29]. Moreover, ursolic acid derivatives were elucidated by Farina et al. 1998 [30] to have anti-ulcer activity. Thus, the cytoprotective potential of the fraction might be attributed to the presence of terpenoids. Unlike these terpenoides the gastroprotective mechanism of the phenolic glycoside verbascoside is not well explored. Thus, we investigated the anti-ulcer activity of verbascoside both *in vivo* and *in vitro*. It was tested in ethanol induced gastric ulcer model and further checked for PGE_2 level in order to elucidate its cytoprotective activity. Verbascoside showed neither significant gastric mucosal protection nor increase in PGE_2 content suggestive of its role other than cytoprotection. Verbascoside was then tested for gastric secretion studies using pylorus ligated rats, where it did not significantly upregulate mucin level, but it significantly reduced the ulcer index, free, total acidity and pepsin level respectively. It was then checked for *in vitro* H^+ K^+-ATPase activity in isolated gastric microsomes from rat stomach where it inhibited the proton pump activity with an IC_{50} comparable to that observed with Fr-Bu signifying that the anti-ulcer effect of verbascoside was unrelated to increased mucin secretion but mediated through reduction of gastric acid secretion.

Further, to substantiate the antisecretory potential of verbascoside, its effect on plasma concentration of gastrin hormone in ulcerated rats was determined. Gastrin hormone is a known modulator of gastric acid secretion [31] which stimulates the parietal cell to hypersecrete acid, resulting in the development of gastric ulcer. Verbascoside significantly decreases the gastrin secretion in ethanol induced ulcer model which further confirmed its antisecretory potential. Thus, the antisecretory activity of verbascoside appears to be mainly related to the inhibition of H^+ K^+-ATPase activity and suppression of gastrin release.

Conclusively, the present study demonstrated that TG and its active butanolic fraction impart gastroprotection via inhibition of H^+ K^+-ATPase (proton pump) activity and simultaneous strengthening of mucosal defense mechanism. The H^+ K^+-ATPase inhibitory activity of TG and its fraction was apparently attributed to the presence of its major

constituent i.e. verbascoside which seemingly regulate acid secretion via inhibiting gastrin hormone release. Thus, verbascoside of TG could act as a potent therapeutic agent against gastric ulcer disease.

Acknowledgements

This work was carried out under an ICMR funded project and gratefully acknowledges ICMR New Delhi, for providing financial support. Authors are thankful to Mrs. Shibani Sen Gupta for her technical support.

References

[1] Hoogerwerf WA, Pasricha PJ. In: Brunton LL, Lazo JS, Parker KL, editors. The Pharmacological Basis of Therapeutics. New York: Mc Graw Hill; 2006. p. 967–81.
[2] Majumdar M, Nayeem N, Kamath JV, Asad M. Pak J Pharmacol Sci 2007;20:120.
[3] Shah CS, Qadry JS. A text book of Pharmacognosy. 11th ed. New Delhi (India): B.S. Shah Prakashan; 1995.
[4] Goel RK, Pathak NK, Biswas M, Pandey VB, Sanyal AK. J Pharm Pharmacol 1987;39:138.
[5] Jagetia GC, Baliga MS. J Med Food 2004;7:343.
[6] Mahato SB, Kundu AP. Phytochemistry 1994;37:1517.
[7] Greca MD, Monaco P, Previtera L. J Nat Prod 1990;53(6):1430.
[8] Ahmed W, Ahmed Z, Malik A. Phytochemistry 1992;31(11):4038.
[9] Iribarren AM, Pomilo AB. Phytochemistry 1984;23:2087.
[10] Andary CC, Wylde C, Laffite G, Privat I, Winternitz. Phytochemistry 1982;21:1123.
[11] Calis I, Basaran AA, Saracoglu I, Sticher O, Ruedi P. Phytochemistry 1990;29:1253.
[12] Scarpati ML, Monache D. Ann Chim 1963;53:356.
[13] Suleyman H, Demirezer LO, Buyukokuroglu ME, Akcay MF, Gepdiremen A, Banoglu ZN, et al. Phytother Res 2001;15:625.
[14] Shay M, Kamarov SA, Fels D, Meraaze D, Grueinstein H, Siplet H. Gastroenterology 1945;5:43.
[15] Anoop A, Jegadeesan M. J Ethnopharmacol 2003;84:149.
[16] Debnath PK, Gode KD, Das DG, Sanyal AK. Br J Pharmacol 1974;51:213.
[17] Crowther RS, Wetmore RF. Anal Biochem 1987;163:170.
[18] Suleyman H, Demirezer LO, Kuruuzum-Uz A. Pharmazie 2004;59:147.
[19] Srivastava SK, Nath C, Gupta MB, Vrat S, Sinha JN, Dhawan KN, et al. Pharmacol Res 1991;23:81.
[20] Berglindh T. Methods Enzymol 1990;192:93.
[21] Sanui H. Anal Biochem 1974;60:489.
[22] Finney DJ. A statistical treatment of the sigmoidal response curve. 2nd ed. New York London: Cambridge Univ. Press; 1952. p. 318.
[23] Pandey BL, Goel RK, Pathak NK, Biswas M, Das PK. Indian J Med Res 1982;76:89 Suppl.
[24] Djahanguiri B, Taubin HL, Landsburg L. J Pharmacol Exp Ther 1973;184: 163.
[25] Goel RK, Bhattacharya SK. Indian J Exp Biol 1991;29:701.
[26] Miller TA, Henagan JM. Dig Dis Sci 1984;29:141.
[6] Farina C, Pinza M, Pifferi G. Farmaco 1984;53:22.
[27] Kinoshita M, Noto T, Tamaki H. Biol Pharm Bull 1995;18:223.
[28] Xiao M, Yang Z, Jiu M, You J, Xiao R. Hua Xi Yi Ke Da Xue Xue Bao 1992;23:98.
[29] Liby K, Honda T, Williams CR, Risingsong R, Royce DB, Suh N, et al. Mol Cancer Ther 2007;6:2113.
[30] Farina C, Pinza M, Pifferi G. Farmaco 1998;53:22–32.
[31] Walsh JH (Ed.). Gastrin Raven Press, New York 1993; pp. 273.