

Full Length Research Paper

## Anti-ulcerogenic activity of unsymmetrically substituted urea derivative (1-benzyl-3-(4-methylphenyl) urea)

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Gastric ulcers are mucosal lesions that result from an imbalance between aggressive factors such as acid and pepsin, and defensive mechanisms like gastric mucous, high mucosal blood flow and high mucosal turnover rate that work towards maintenance of mucosal integrity. The aetiology of gastric ulcers is not completely understood and continuous use of anti-ulcer agents leads to many side effects. In the present study, the unsymmetrically substituted urea derivative (1-benzyl-3-(4-methylphenyl) urea) was tested for the anti ulcerogenic activity of alcohol and aspirin induced ulcer models on rats. Ulcer index was calculated by histopathological studies. The test compound at a concentration of 50 and 100 mg/kg exhibited a protective effect on ulcer-induced models in a dose dependent manner, and was comparable with the standard drug ranitidine. These findings indicate that unsymmetrically substituted urea derivative has ulcer protective activity.

**Key words:** Alcohol, aspirin, gastric ulcer, necrosis, stomach.

### INTRODUCTION

Ulcers are the areas of degeneration and necrosis of gastro intestinal mucosa exposed to acid of the alimentary tract that is exposed to hydrochloric acid and pepsin. They occur most commonly (98 to 99%) in either the duodenum or the stomach in the ratio 4:1 (Harsh, 2009). Ulcers can occur in the stomach, where they are called gastric ulcers or they can occur in the first portion of the small intestine called duodenal ulcers. "Peptic Ulcer" is the term used to describe either or both of these two types of ulcer (Mahajan et al., 2009).

Ulcers occur due to imbalance between the protective factors and the aggressive factors (gastric mucosal integrity). The most often aggressive and protective

factors in the stomach are acid pepsin secretion, mucosal barrier, blood flow, cellular regeneration, prostaglandins and epidermal growth factors. Sometimes the gastric mucosa is continuously exposed to potentially injurious agents such as pepsin, bile acids, food ingredients, bacterial products (*Helicobacter pylori*) and drugs. However, other factors such as stress, smoking, nutritional deficiency and ingestion of non steroidal anti-inflammatory drugs all can increase the incidence of gastric ulcers. It is reported that prolonged anxiety, emotional stress, haemorrhagic surgical shock, burns and trauma are known to cause severe gastric irritations (Neetesh et al., 2010).

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## Test drug

Unsymmetrically substituted urea derivative (1-benzyl-3-(4-methylphenyl)urea) was used as test substance; urea derivatives had wide range of pharmacological activities. They show human immunodeficiency virus (HIV) protease inhibitor (Omar et al., 2012), anti bacterial, hypnotic, sedative (Vijey et al., 2011), anti depressant (Perveen et al., 2012), anti oxidant (Kitti et al., 2009) and anti proliferative activities (Khemkaran et al., 2011). The present study was undertaken to evaluate the anti-ulcerogenic activity of unsymmetrically substituted urea derivative by estimating the various biochemical parameters of ulcer induced models.

## MATERIALS AND METHODS

Figure 1 shows urea derivative (1-benzyl-3-(4-methylphenyl)urea) structure with molecular formula of  $C_{15}H_{16}NO_2$ , molecular weight of 240, DMSO solubility,  $R_f$  value of 0.6 (n-hexane: ethyl acetate - 1:1), and melting point at 246 to 250°C.

### Experimental animals

Wistar albino rats of either sex weighing 150 to 200 g were used in this study. Animals maintained under the standard conditions of temperature ( $24 \pm 2^\circ\text{C}$ ) and relative humidity ( $44 \pm 5\%$ ) with a 12:12 light:dark cycle (Gil et al., 2009; Naresh et al., 2012). Animals were given standard diet supplied by the Sainath agencies (Hyderabad) and water *ad libitum*. 24h before the experiment, animals were deprived of food but not water (Adit et al., 2009; Thirunavukkarasu et al., 2009; Bahuguna et al., 2009).

All procedures involving the animals were carried out under the Institute Animal Ethical Committee Approval (CPCSEA guidelines).

### Chemicals and drugs

Ethanol, aspirin, ranitidine, sodium carboxy methyl cellulose, urea derivatives, phenolphthalein, Topfer's reagent, and sodium hydroxide were used in the study.

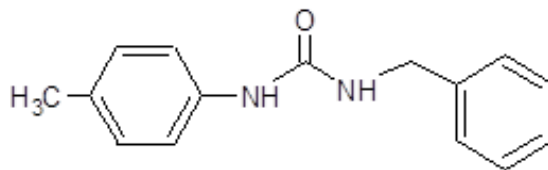
### Acute toxicity studies

Toxicity studies of the test substance were carried out in albino mice of either sex weighing 20 to 30 g.  $LD_{50}$  test drug was found to be safe up to 1000 mg/kg peritoneally (P.O).

### Anti ulcer activity

#### Alcohol induced ulcers

Animals were divided into five groups of six animals (Rasika et al., 2010; Suthar et al., 2007). Group I served as control received distilled water. Group II received ulcer inducing agent, that is, ethanol 99.8% (1 ml/animal, P.O). Group III received standard drug ranitidine aqueous solution (20 mg/kg, P.O). Group IV animals were administered test drug in 1% sodium carboxymethylcellulose (CMC) at the dose of 50 mg/kg (P.O) and Group V animals received test drug in 1% sodium CMC at the dose of 100 mg/kg (P.O), respectively. Standard and test groups were administered ethanol



**Figure 1.** Urea derivative (1-benzyl-3-(4-methylphenyl)urea) structure.

99.8% in 1 ml/animal orally after the administration of the standard and test drugs, respectively. Animals were sacrificed after 1 h (Deshpande et al., 2003; Ubaka et al., 2012) by the ether anaesthesia. Stomach was removed and incised along the greater curvature. Ulcer index was determined and histopathological studies were performed.

### Aspirin induced ulcers

Animals were divided into five groups of six animals. Group I served as control and received distilled water. Group II received ulcer inducing agent, that is, aspirin 200 mg/kg (P.O). Group III received standard drug ranitidine aqueous solution of 20 mg/kg (P.O). Group IV animals were administered test drug in 1% sodium CMC at the dose of 50 mg/kg (P.O) and Group V animals received test drug in 1% sodium CMC at the dose of 100 mg/kg (P.O), respectively. Standard and test groups were administered aspirin at the dose of 200 mg/kg orally after the administration of the standard and test drugs, respectively. Animals were sacrificed after 4 h by the ether anaesthesia and stomach was removed and incised along the greater curvature. Ulcer index was determined and histopathological studies were performed (Khaja et al., 2011; Papiya et al., 2008).

### Ulcer index

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

where UI is Ulcer index, UN is the average of the number of ulcer per animal, US is the average of severity score, and UP is the percentage of animal with ulcer (Nagajananeyulu et al., 2012).

### Ulcer scores

The ulcer scores are 0=No lesion, 1=1–3 Small lesions ( $\leq 10$  mm length), 2=1–3 Large lesions ( $\geq 10$  mm length), 3=1–3 Thickened lesions, 4=More than 3 small lesions, and 5=More than 3 large lesions, 6=More than 3 thickened lesions (Galati et al., 2001).

Percentage protection was calculated using the formula (Jhansi et al., 2010; Vinod et al., 2010):

$$\text{Percentage protection} = \frac{(\text{Ulcer index}) \text{ Control} - (\text{Ulcer index}) \text{ Test}}{(\text{Ulcer Index}) \text{ Control}}$$

### Histopathological studies

Gastric tissue samples from each group were fixed in 10% formalin for 24 h. The formalin fixed specimens were embedded in paraffin and section (3 to 5  $\mu\text{m}$ ) and stained with haematoxylin and eosin dye. The histochemical sections were evaluated by light microscopy.

### Estimation of free acidity and total acidity

One millilitre of gastric juice was pipetted into a 100 ml conical flask; 2 or 3 drops of Topfer's reagent was added and titrated with 0.01 N sodium hydroxide until all traces of red colour disappears and the colour of the solution turns to yellowish orange. The volume of alkali added was noted. This volume corresponds to free acidity, then 2 or 3 drops of phenolphthalein solution was added and titration was continued until a definite red tinge appears. Again the total volume of alkali added was noted, now this volume corresponds to total acidity (Venkat et al., 2011).

Acidity = Volume of NaOH × Normality of NaOH × 100/0.1 meq/L

### Statistical analysis

Statistical analysis was carried out using analysis of variance (ANOVA) by Dunnet's multiple comparison test using graph pad prism software version 5. P values < 0.05 were considered significant.

## RESULTS

Acute toxicity studies of urea derivatives did not exhibit any signs of toxicity up to 1 g/kg body weight. Since no mortality of the animals was found at high dose. Hence, 50 and 100 mg/kg dose of the test drug selected for evaluation of anti-ulcer activity.

### Ethanol-induced ulcer

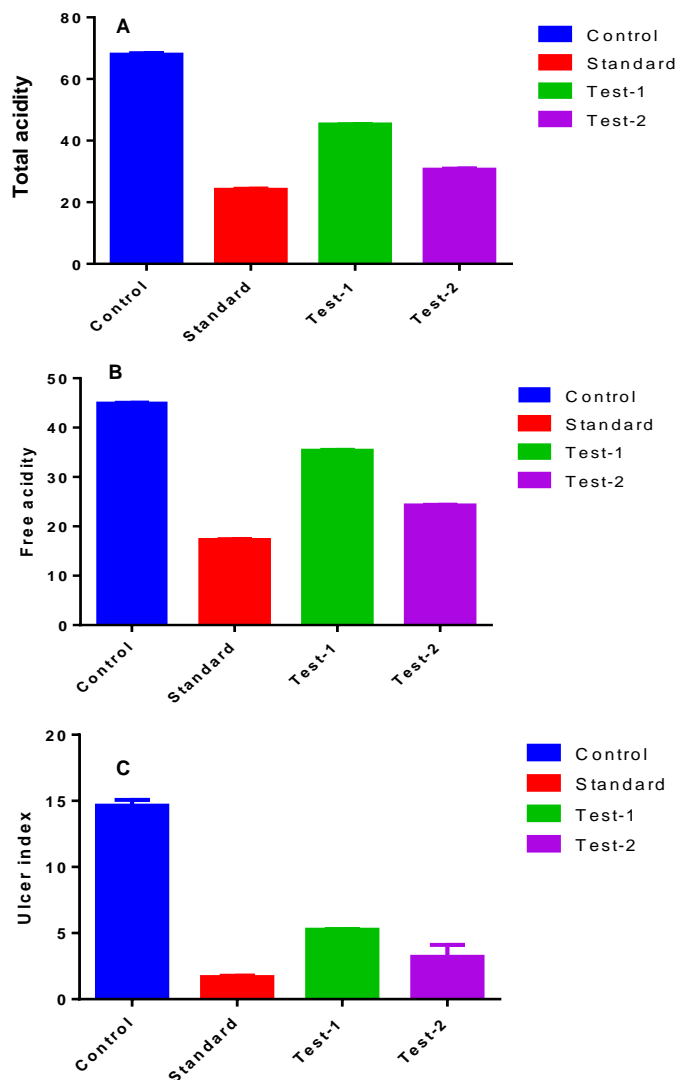
In Table 1 and Figure 2, ulcer inhibition was evident in all treatment of the urea derivative (1-benzyl-3-(4-methylphenyl)urea) compared to the negative control. However, statistically significant ulcer inhibition (64 and 78.1%,  $P < 0.05$ ,  $P = 0.001$ ) could be seen only at doses of 50 and 100 mg/kg. The protection from ulcer was dose dependent even as ulcer was produced in all rats in this model.

### Aspirin-induced ulcer

Urea derivative (1-benzyl-3-(4-methylphenyl)urea) at all the doses provided protection from ulcer and the protection was dose dependent. The urea derivative at doses of 50 and 100 mg/kg provided statistically significant protection (70 and 84.4%,  $P < 0.05$ ,  $P = 0.003$ ) when compared with the negative control (Table 2; Figure 3).

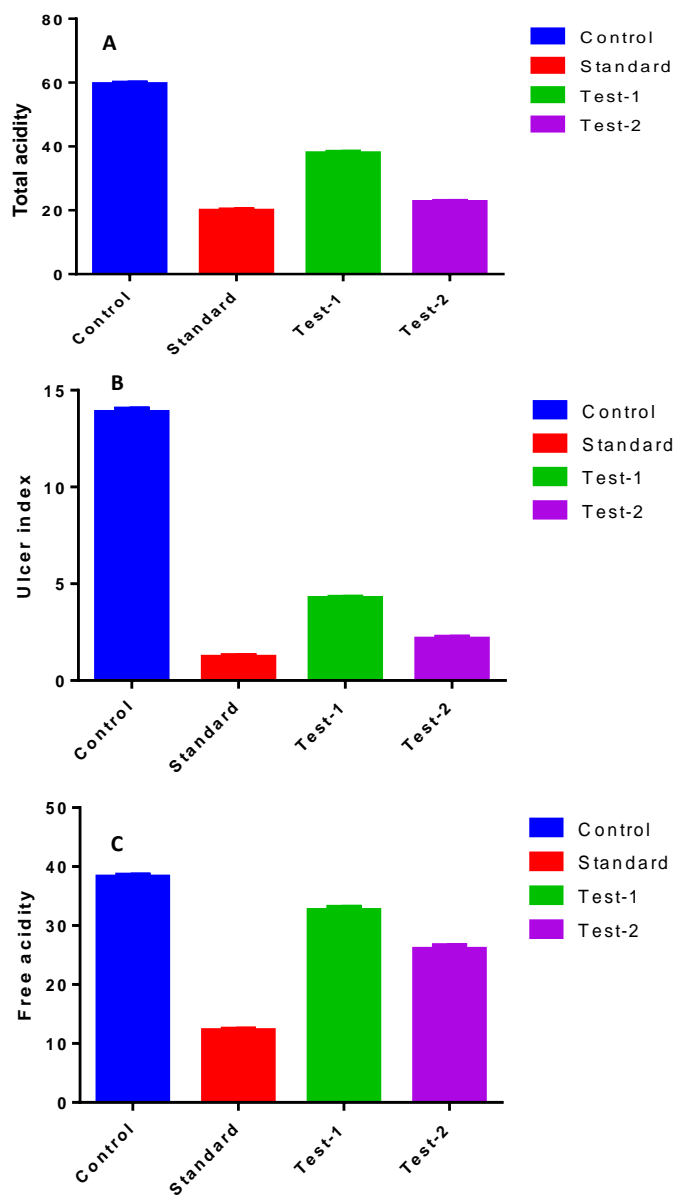
## DISCUSSION

Ethanol-induced gastric ulcers serve as a common ulcerogenic agent. Ethanol is metabolized in the body and releases superoxide anion and hydroperoxy free radicals. It has been found that oxygen derived free radicals are implicated in the mechanism of acute and chronic



**Figure 2.** (A) Total acidity (alcohol induced ulcers), (B) free acidity (alcohol induced ulcers), (C) ulcer index (alcohol induced ulcers).

ulceration in the stomach. There are various mechanisms involved in the ulcer production in different experimental models. Many experimental evidences have shown that antioxidants significantly strengthen the gastric walls and protect tissue from oxidative damage. Furthermore, gastric acid secretion now accepted to play an important role in the formation of gastric ulcer (Wasman et al., 2010). Urea derivatives have significant protective effect on the gastric mucosa against ethanol challenge as shown shown by reduced values of total acidity, free acidity, and ulcer index as compared to the control group suggesting its potent cytoprotective effect. Non-steroidal anti-inflammatory drugs (NSAIDs) like aspirin causes gastric mucosal damage by decreasing prostaglandin levels through inhibition of prostaglandin synthesis. Prostaglandins are protective agents for the gastric mucosa. They produce excess mucous and the bicarbonate ions, which



**Figure 3.** (A) Total acidity (aspirin induced ulcers), (B) free acidity (aspirin induced ulcers), (C) ulcer index (aspirin induced ulcers).

**Table 1.** Efficacy of urea derivative (Test 1 & 2) in Alcohol induced ulcers.

Group	Dose (P.O)	Total acidity (meq/L)	Free acidity (meq/L)	Ulcer index (mm length)	Protection (%)
Control (Alcohol)	99.8% 1 ml/animal	393.43±4.3	272.075±0.946	14.65±0.42	-
Standard (Ranitidine)	20 mg/kg	102.3±1.16***	85.275±1.55***	1.67±0.09***	88.56
Test-1	50 mg/kg	244.92±1.17*	197.47±1.136**	5.26±0.04*	64
Test-2	100 mg/kg	186.875±0.93**	94.6±1.52***	3.2±0.912**	78.1

Data expressed mean ± S.D.(n = 6). Statistical comparison was performed by using ANOVA coupled with Dunnet's multiple comparison test. \*P<0.05, \*\*P<0.01, \*\*\*P<0.001 were consider statistically significant when compared to control group.

**Table 2.** Efficacy of urea derivative (Test 1 and 2) in aspirin induced ulcers.

Group	Dose (mg/kg, p.o)	Total acidity (meq/L)	Free acidity (meq/L)	Ulcer index (mm length)	Protection (%)
Control (Aspirin)	200	276.25±1.948	173.647±0.66	13.87±0.17	-
Standard (Ranitidine)	20	84.57±1.298***	74.2±1.290***	1.21±0.085***	91.2
Test-1	50	150.4±1.18*	143.425±0.573*	4.25±0.075**	69.9
Test-2	100	104.575±0.51**	84.35±0.525***	2.15±0.108***	84.4

Data expressed mean ± SEM (n = 6). Statistical comparison was performed using ANOVA coupled with Dunnet's multiple comparison test.\*P<0.05, \*\*P<0.01, \*\*\*P<0.001 were consider statistically significant when compared with the control group.

protects the gastric mucosa from ulcer inducers. Inhibitions of prostaglandin synthesis by aspirin coincide with the earlier stages of damage to the cell membrane of mucosal, parietal and endothelial cells (Donnelly et al., 1977). So, the possible mechanism of antiulcer action of urea derivative may be the reduction of the acid secretion. In this study, it was observed that urea derivative provides significant anti-ulcer activity against gastric ulcers in rats. Aspirin decreased the concentrations of all the individual carbohydrates and also the carbohydrate to protein ratio; however, a similar decrease in carbohydrate/protein ratio and of individual carbohydrates has been earlier reported in the non-dialyzable and lyophilized fractions of the mucus in aspirin-treated rats. These results tend to confirm that aspirin-like drugs cause ulceration by affecting the mucosal barrier and the carbohydrate/protein ratio of the gastric juice is a good index of the mucus barrier. The urea derivative at doses 50 and 100 mg/kg produced a significant reduction in gastric ulcer when compared with the negative control (aspirin) and with the ranitidine (standard) (Menguy et al., 1965).

## Conclusion

In the present investigation, unsymmetrically substituted urea derivative (1-benzyl-3-(4-methylphenyl)urea) showed significant anti-ulcer activity at two different doses 50 and 100 mg/kg in ethanol and aspirin induced ulcer in rats. Test drug showed significant reduction in the total acidity, free acidity, and ulcer index values of treated groups. It also showed significant protection as compared to standard. However, more experimentation on human and animals and experimental analysis are required for a definitive conclusion.

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## REFERENCES

- Bahuguna Y, Patil K, Rawat MSM, Jalalpure S, Uniyal S (2009). Antiulcer activity of *Melia azedarach* Linn in aspirin induced and pylorus ligated rats. *J. Pharm. Res.* 2:1456-1459.
- Chaturvedi A, Bhawani G, Agarwal PK, Goel S, Singh A, Goel RK (2009). Ulcer healing properties of ethanolic extract of *Eugenia jambolana* seed in diabetic rats: study on gastric mucosal defensive factors. *Indian J. Physiol. Pharmacol.* 53:16-24.
- Deshpande SS, Shah GB, Parmar NS (2003). Antiulcer activity of tephrosiapurpurea in rats. *Indian J. Pharmacol.* 35:168-172.
- Donnelly MT, Hawkey CJ (1997) COX-2 inhibitors—a new generation of safer NSAIDs? *Aliment Pharmacol Ther.* 11:227-236.
- Galati EM, Monforte MT, Tripodo MM, d'Aquino A, Mondello MR Galati EM, Monforte MT, Tripodo MM, Aquino A, Mondello R (2001). Antiulcer activity of *Opuntia ficusindica* (L.) Mill. (Cactaceae): Ultrastructural study. *J. Eethanoanopharmacol.* 76:1-9.
- Gil NS, Garg M, Basal R, Sood S, Muthuraman A, Bali M, Sharma PD (2009). Evaluation of anti oxidant and antiulcer potential of *Cucumis sativum* L. Seed extract in rats. *Asian J. Clin. Nutr.* 1:131-138.
- Harsh M (2009). Textbook of pathology, Sixth edition. Jaypee Publishers, New Delhi. pp. 549-555.
- Jhansi RM, Mohana LS, Saravana KS (2010). Evaluation of Anti-Ulcer Activity of Methanol Extract of *Dioscorea oppositifolia* Tubers in Adult Wistar Rats. *Int.ernational J. Preclin. Pharm. Res.* 1:19-24.
- Khaja Z, Mangamoori LN, MunaAbid, Mohammed I (2011). Evaluation of antiulcer activity of Boswellia serrata bark extracts using aspirin induced ulcer model in albino rats. *J. Med. Allied Sci.* 1:14-20.
- Khemkaran A, Sanmati K.J, Bina G (2011). Synthesis and Evaluation of some 1, 3 disubstituted urea derivative for Antiproliferative activity. *Int.ernational J. Pharm. Res. Dev.* 2:209-218.
- Kitti A, Apilak W, Virapong P (2009). Facile Synthesis of *N*-Aroylurea Analogs as Potential Antioxidants. *Eur. J. Sci. Res.* 31:510-518.
- Mahajan N, Sakarkar D, Sanghai D (2009). Evaluation of anti-ulcer potential of leaves of *Jasminum grandiflorum* L. *Int. J. Pharm. Sci.* 1:247-249.
- Menguy R, Masters, YF (1965). Effect of aspirin on gastric mucous secretion. *Surgery Gynecol. Obstet.* 120:92-98.
- Naganjaneyulu R, Ashok Kumar C.K, Avinash KG, Deena D et al (2012). Antiulcer activity of viscumarticulatum burm f. (viscaceae): *Int. J. Innov. Pharm. Res.* 2:139-143.
- Nair V, Arjuman A, Gopalakrishna HN, Dorababu P, Mirshad PV, Bhargavan D, Chatterji D Vinod Nair, Albina A, Gopalakrishna HN, Dorababu P et al (2010). Evaluation of the anti-ulcer activity of NR-ANX-C (a polyherbal formulation) in aspirin & pyloric ligation induced gastric ulcers in albino rats. *Indian J. Med. Res.* 132:218-223.
- Naresh SG, Manoj B (2012). Evaluation of antioxidant, anti ulcer activity of 9-beta-methyl-19-norlanosta-5-ene Type glycosides from *Cucumis sativus* seeds. *Res. J. Med. Plants* 6:309-317.

- Neetesh KJ, Namrata S, Pushpendra K, Navneet G, Garud A, Tonpay SD (2010). Pharmacological screening of anti ulcer agents-A Review. *Int. J. Pharm. Sci. Res.* 9:29-37.
- Omar D, Mohammad J (2012). Exploring QSARs for Inhibitory Activity of Cyclic Urea and Non-peptide-Cyclic Cyanoguanidine Derivatives HIV-1 Protease Inhibitors by Artificial Neural Network. *Adv. Chem. Eng. Sci.* 2:82-100.
- Papiya MM, Sasmal D, Aruvindi N (2008). Anti ulcerogenic and antioxidant activity of *Coccinia grandis* (Linn) Voigt leaves on aspirin induced ulcers. *Nat. Prod. Radiance* 7:15-18.
- Perveen S, Mustafa S, Khan MA, Dar A, Khan KM, Voelter W, Perveen S, Mustafa S, Khan MA, Dar A *et al* (2012). Substituted urea derivatives: a potent class of antidepressant agents. *Med. Chem.* 8:330-336.
- Rasika DB, Mahendra A.G, Sneha J. A, Subodh C. Pal (2010). Anti-ulcer activity of the ethanol extract of leaves of *Sesbania grandiflora* (Linn.). *Int. J. Pharm. Pharm. Sci.* 2:206-208.
- Suthar PB, Jain SM, Mehata AA, Santhani DD (2007). Study of anti ulcer activity of sodium metavanadate on aspirin and pylorus ligation induced gastric ulcers in rats. *Indian J. Pharm. Sci.* 67:454-456.
- Thirunavukkarasu P, Ramkumar L, Ramanathan T (2009). Anti-ulcer Activity of *Excoecaria agallocha* bark on NSAID-induced Gastric Ulcer in Albino Rats. *Glob. J. Pharmacol.* 3:123-126.
- Venkat RN, Kola V, Sowmya U, Jayapal RG, Anirudh K (2011). Evaluation of anti-ulcer activity of *Momordica charantia* in rats. *Int. J. Pharm. Biol. Sci.* 1:1-16.
- Vijey AM, Prem SM, Shiny G, Rakhi C (2011). Synthesis, Antimicrobial and Sedative Hypnotic Activity of Cinnamoyl Ureas. *Int. J. Pharm. Technol. Res.* 1:99-103.
- Wasman QS, Mahmood AA, Salehuddin H, Zahra AA, Salmah I (2010). Cytoprotective activities of *Polygonum minus* aqueous leaf extract on ethanol-induced gastric ulcer in rats. *J. Med. Plants Res.* 4:2658-2665.