Available online on 30.08.2019 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Research Article

Phytochemical Screening and Evaluation of Antiulcer Activity of Hydroalcoholic Extract of *Lilium Candidum* Flowers

Mithun Balmik*[1], Gaurav Dubey[1], Sailesh Kumar Ghauary[1], Prabhat Kumar Jain[2]

[1]RKDF, School of Pharmaceutical Sciences, Bhopal (M.P.)

[2]Scan Research Laboratories, Bhopal (M.P.)

ABSTRACT

Peptic disorders like gastro esophageal reflux disease, gastritis, peptic ulcer, duodenal ulcer etc., are the common in today's life style. This may be due to stressful life style or improper balance diet. The pathology behind these disorders may be discrepancy between offensive and defensive mechanisms either by excess secretion of acid and pepsin or diminished ability of the gastro-duodenal mucosal barrier to protect against stomach acid-pepsin secretion. Non-steroidal anti-inflammatory drugs (NSAIDs) are a class of the most commonly used medicines and proven to be effective for certain disorders. Some people use NSAIDs on daily basis for preventive purpose. But a variety of severe side effects can be induced by NSAIDs. Studies have shown that edible natural ingredients exhibit preventive benefit of gastric ulcer. Therefore present study was designed to evaluate antiulcer activity of hydroalcoholic extract of *Lilium candidum* flowers in rats. Qualitative analysis of various phytochemical constituents and quantitative analysis of total phenolics and flavonoids were determined by the well-known test protocol available in the literature. The *in vivo* anti-ulcer activity of hydroalcoholic extract was assessed against Aspirin-induced gastric ulcer in rats. Depending on the model, outcome measures were pH of gastric fluid and ulcer index as well as percent inhibition of ulcer index. Preliminary phytochemical screening revealed the presence of flavonoids, carbohydrates, saponins, polyphenols and amino acids. The total phenolics content of *L. candidum* extract was (1.039mg/100mg), followed by flavonoids (0.941mg/100mg) respectively. Further hydroalcoholic extract of 100 and 200mg/kg/p.o significantly ($p<0.01$) reduced the gastric pH, ulcer index in aspirin induced ulcer models in rats. The findings of this study confirmed that *L. candidum* extract has anti-ulcer pharmacologic activity due to one or more of the secondary metabolites present in it. Therefore, this study validates its anti-ulcer use in Indian folk medicine. Further investigations on isolation of specific phytochemicals and elucidating mechanisms of action are needed.

Keywords: *Lilium candidum,* Phytochemical constituents, Antiulcer, Non-steroidal anti-inflammatory drugs, Aspirin-induced gastric ulcer.

Article Info: Received 19 June 2019; Review Completed 17 Aug 2019; Accepted 20 Aug 2019; Available online 30 Aug 2019

Cite this article as:

Balmik M, Dubey G, Ghauary SK, Jain PK Phytochemical Screening and Evaluation of Antiulcer Activity of Hydroalcoholic Extract of *Lilium Candidum* Flowers, Journal of Drug Delivery and Therapeutics. 2019; 9(4-A):646-650
http://dx.doi.org/10.22270/jddt.v9i4-A.3545

***Address for Correspondence:**

Mithun Balmik, RKDF, School of Pharmaceutical Sciences, Bhopal (M.P.)

INTRODUCTION

Ulcers are an open sore of the skin or mucus membrane characterized by sloughing of inflamed dead tissue[1]. Ulcers are lesions on the surface of the skin or a mucous membrane characterized by a superficial loss of tissue. Ulcers are most common on the skin of the lower extremities and in the gastrointestinal tract, although they may be encountered at almost any site. There are many types of ulcer such as mouth ulcer, esophagus ulcer, peptic ulcer and genital ulcer. Of these peptic ulcer is seen among many people. The peptic ulcers are erosion of lining of stomach or the duodenum[2]. The two most common types of peptic ulcer are called gastric ulcer and duodenal ulcer. The name refers to the site of ulceration. A person may have both gastric and duodenal ulcers at the same time. Gastric ulcers are located in the stomach, characterized by pain; ulcers are common in older age group. Eating may increase pain rather than relieve pain. Other symptoms may include nausea, vomiting and weight loss. Although patients with gastric ulcers have normal or diminished acid production, yet ulcers may occur even in complete absence of acid[3]. Duodenal ulcers are found at the beginning of small intestine and are characterized by severe pain with burning sensation in upper abdomen that awakens patients from sleep. Generally, pain occurs when the stomach is empty and relieves after eating. A duodenal ulcer is more common in younger individuals and predominantly affects males. In the duodenum, ulcers may appear on both the anterior and posterior walls[4]. In some cases, peptic ulcer can be life threatening with symptoms like bloody stool, severe abdominal pain and cramps along with vomiting blood[5]. The pathophysiology of peptic ulcer disease involves an imbalance between offensive (acid, pepsin, and *Helicobacter pylori*) and defensive factors (mucin, prostaglandin, bicarbonate, nitric oxide and growth factors) [6]. Peptic ulcers are once believed to be caused by spicy food and stress; these have been found merely to be aggravating factors and the real causes have been found by research to

include bacterial infection (*Helicobacter pylori*) or reaction to various medications, particularly NSAIDS (non-steroidal anti-inflammatory drugs)[7]. *Helicobacter pylori*, NSAIDS drugs, emotional stress, alcohol abuse, and smoking are the principal etiological factors associated with peptic ulcer[8,9]. The Gram-negative bacterium *Helicobacter pylorus* remains present between the mucous layer and the gastric epithelium and is strategically designed to live within the aggressive environment of the stomach. Initially, *Helicobacter pylorus* resides in the antrum but over time migrates toward the more proximal segments of the stomach[10]. Peptic ulcer is one of the world's major gastrointestinal disorders and affecting 10% of the world population[11]. About 19 out of 20 peptic ulcers are duodenal. An estimated 15000 deaths occur each year as a consequence of peptic ulcer. Annual incidence estimates of peptic ulcer hemorrhage and perforation were 19.4-57 and 3.8-14 per 100,000 individuals, respectively. The average 7-day recurrence of hemorrhage was 13.9% and the average long-term recurrence of perforation was 12.2%[12]. In the Indian pharmaceutical industry, antacids and antiulcer drugs share 6.2 billion rupees and occupy 4.3% of the market share. In this modern era also 75-80% of the world populations still use herbal medicine mainly in developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body, and lesser side effects[13]. Histological studies revealed that these medicinal plants did not show any acute toxicity. Preliminary photochemical screening of this medicinal plant identified the presence of important secondary metabolites like flavonoids and tannins which are the active principles of antiulcer activity[14]. *Lilium candidum* L. (Liliaceae), the so called white Madonna lily, is well known in folk medicine and contains various biologically active compounds. Bulbs and flowers of this plant have been used for the treatment of ulcers, furuncles, finger ulcers, reddened skin, burns and injuries[15]. *L. candidum* has a high susceptibility to plant viruses and fungi[16]. This plant is successfully cultivated in Europe, USA and many other countries including Iraq for medical, ornamental and in perfumes purposes[17-19]. However, alkaloids like pyrroline and pyrrolidine may be enrolled in inducing significant oxidative stress and DNA damage, which lead to cell apoptosis or necrosis[20]. Hence, the objective of the present investigation is to evaluate the anti-ulcer activity of *L. candidum* against NASAID (Aspirin) induced gastric ulcer in Wister albino rat model.

MATERIALS AND METHODS

Plant material

Flowers of *L. candidum* were collected from local area of Bhopal (M.P.) in the month of January, 2019.

Chemicals and reagents

All the drugs, solvents and chemicals used in the study were of analytical grade. Ranitidine was obtained as a gift sample from Scan Research Lab, Bhopal, MP, India. All other chemicals e.g. Methanol, ether, formalin, sodium hydroxide, citric acid monohydrate, trichloroacetic acid, sodium nitrate, sodium potassium tartrate, ethylene diamine tetra acetic acid disodium salt were purchased from S. D. Fine Chemicals, Mumbai, India. Tris buffer, Topfer's reagent, Folin's Reagent and Phenolphthalein were purchased from Hi-Media Pvt. Ltd., Mumbai, India.

Extraction by maceration process

Flowers of *L. candidum* were shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether. The extraction was continued till the defatting of the material has been taken place. 50 gm of dried powdered rhizomes of *L. candidum* has been extracted with hydroalcoholic solvent (20:80) using maceration process for 48 hrs. The extracts were evaporated above their boiling points and stored in an air tight container free from any contamination until it was used. Finally the percentage yields were calculated of the dried extracts.

Phytochemical screening

Hydroalcoholic extract of *L. candidum* flowers was subjected to qualitative phytochemical investigation for the identification of the different phytoconstituents using standard tests and procedures[21,22].

Total phenol determination

The total phenolic content was determined using the method of Olufunmiso et al [23]. A volume of 2 ml of flowers of *L. candidum* extracts or standard was mixed with 5 ml of Folin Ciocalteau reagent (previously diluted with distilled water 1:10 v/v) and 4 ml (75g/l) of sodium carbonate. The mixture was allowed to stand for 15 min under room temperature. The blue colour developed was read at 765 nm using UV/visible spectrophotometer. The total phenolic content was calculated from the standard graph of gallic acid and the results were expressed as gallic acid equivalent (mg/g).

Total flavonoids determination

The total flavonoid content was determined using the method of Olufunmiso et al[23]. 1 ml of 2% $AlCl_3$ methanolic solution was added to 1 ml of extract or standard and allowed to stand for 60 min at room temperature; the absorbance of the reaction mixture was measured at 420 nm using UV/visible spectrophotometer The content of flavonoids was calculated using standard graph of quercetin and the results were expressed as quercetin equivalent (mg/g).

Animals

Wistar rats (150-200 gm) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55–65%). Rats received standard rodent chow and water *ad libitum*. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate group (n=6) of rats was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

Acute toxicity test

Preliminary experiments were carried out on rats (n=6). Hydroalcoholic flower extract of *L. candidum* were administered orally in different doses to find out the range of doses which cause zero and 100 % mortality of animals. Acute oral toxicity was conducted according to the method of Organization for Economic Co-operation and Development (OECD)[24]. Animals were kept fasting providing only water, Hydroalcoholic flower extract of *L. candidum* were given p.o. in doses of 500, 1000 and 2000 mg/kg/p.o. administered orally for 14 days of six groups of rats (n=6) and the animals were kept under observation for mortality as well as any behavioral changes for evaluation of a possible anti-ulcer effect.

Experimental designs

Aspirin-induced gastric ulcer

Group –1: Control

Group –2: Ranitidine (Standard)

Group –3: Hydroalcoholic flower extract of *L. candidum* (100mg/kg, p.o.)

Group –4: Hydroalcoholic flower extract of *L. candidum* (200mg/kg, p.o.)

The animals were fasted for 24 h prior to the experiment. Under anesthesia, ulcers were induced by applying aspirin (500 mg/kg. p.o.) over the anterior serosal surface of the stomach for 60 seconds. The animals were treated with Ranitidine (50 mg/kg, p.o.), low dose of hydroalcoholic flower extract of *L. candidum* (100 m/kg p.o.) or high dose of hydroalcoholic flower extract of *L. candidum* (200 m/kg p.o.) [Once daily, for 5 days after the induction of ulcer, while the control group received only the vehicle. The rats were sacrificed on the 5th day, the stomachs removed and cut open along the greater curvature[25].

The ulcer index was determined using the formula:

Ulcer index = 10/X

Where X = Total mucosal area/Total ulcerated area.

Based on their intensity, the ulcers were given scores as follows: 0 = no ulcer, 1 = superficial mucosal erosion, 2 = deep ulcer or transmural necrosis, 3 = perforated or penetrated ulcer.

RESULTS AND DISCUSSIONS

The crude extracts so obtained after the maceration process, extracts was further concentrated on water bath for evaporate the solvents completely to obtain the actual yield of extraction. The yield of *L. candidum* extracts was 2.6 %w/w. The results of preliminary phytochemical screening of hydroalcoholic extract of *L. candidum* flowers are shown in Table 1. The extract showed the presence of polyphenolic compounds, saponins, flavonoids and alkaloids. The content of total phenolic compounds (TPC) was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve:

$Y = Y = 0.042X+0.002$, $R^2= 0.999$

Where X is the gallic acid equivalent (GAE) and Y is the absorbance.

Total flavonoids content was calculated as quercetin equivalent (mg/g) using the equation based on the calibration curve:

$Y=0.040X + 0.009$, $R^2=0.999$,

Where X is the absorbance and Y is the quercetin equivalent (QE). Results was shown in Table 2 and Figure 1& 2

Table 1 Result of phytochemical screening of extracts of *L. candidum*

S. No.	Constituents	Hydroalcoholic extract
1.	Alkaloids	+
2.	Glycosides	-
3.	Flavonoids	+
4.	Saponins	+
5.	Phenolics	+
6.	Amino Acids	-
7.	Carbohydrate	-
8.	Proteins	-
9.	Diterpenes	-

Table 2 Total phenolic and total flavonoid content of flowers extract of *L. candidum*

S. No.	Solvents→ Bioactive compound↓	Hydroalcoholic extract
	Flowers of *L. candidum*	
1.	Total Phenol (Gallic acid equivalent (GAE) mg/100mg)	1.039
2.	Total flavonoid (Quercetin equivalent (QE) mg/100mg)	0.941

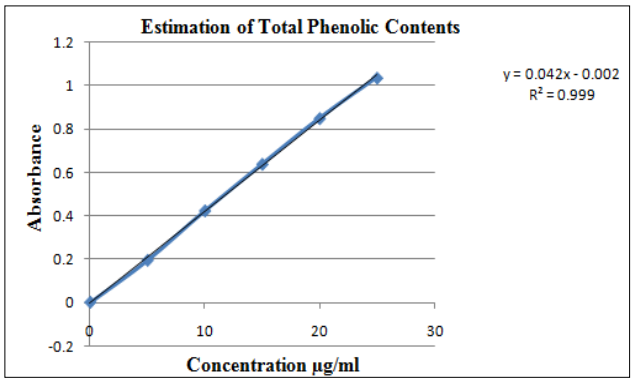

Figure 1: Graph of estimation of total phenolic content

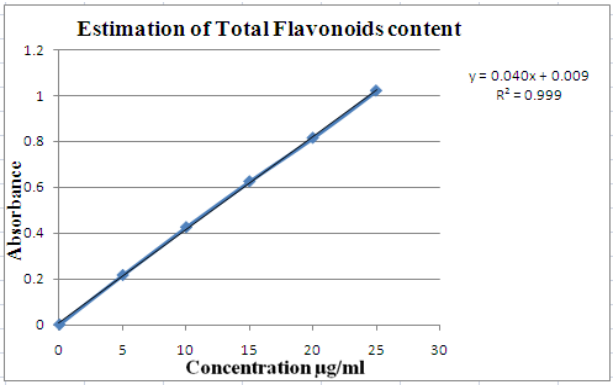

Figure 2: Graph of estimation of total flavonoid content

The acute oral toxicity study was done according to the OECD 425 guidelines. No adverse changes and mortality were observed in animals, which orally received hydroalcoholic extract (2000 mg/kg) of *L. candidum* rhizomes. This indicates that 2000 mg/kg is maximum safe dose. So 1/10th and 1/20th *i.e.* 200 and 100 mg/kg of body weight, of the maximum safe dose were selected for studying *in vivo* anti-ulcer effects. Aspirin induced ulcer was used to study the effect of hydroalcoholic extract of *L. candidum* flowers on gastric acid secretion and mucus secretion. Hydroalcoholic flower extract of *L. candidum* revealed that it has significant anti-ulcer activity. Usually, NSAIDs and

corticosteroids are widely used in clinical practice as anti-inflammatory agents. With the exception of newer highly selective COX-2 inhibitors, NSAID's and corticosteroids produce significant gastric irritation resulting in gastritis and gastric ulceration, especially on long-term treatment. Present study revealed that Hydroalcoholic flower extract of *L. candidum* has ulcer protective properties. Previous studies showed its potent anti-inflammatory activity. Therefore, it can be consider as an ideal substitute for conventional NSAIDs and glucocorticoid. Further studies have to be conducted to explain precisely the mechanism of action of this drug. Hydroalcoholic flower extract of *L. candidum* has an antiulcer effect. It increased healing of aspirin induced ulcer. The hydroalcoholic extract of *L. candidum* and Ranitidine significantly decreased the ulcer index and significantly enhance the pH; this suggests that it having an anti-secretory effect. Aspirin induced ulcer control rats shown perforated ulcer. There is a dose-dependent increase in anti-ulcer effect of hydroalcoholic flower extract of *L. candidum* (Table & Figure 3, 4).

Table 3 Anti-ulcerogenic effect of hydroalcoholic flower extract of *L. candidum* against ulcerogenic agents in rats (Ulcer index)

Treatment and dose	Aspirin
Control	3.80 ± 8.0
Ranitidine (50 mg/kg, p.o.)	1.80 ± 8.0***
L. candidum (100 mg/kg, p.o.)	2.15 ± 8.0**
L. candidum (200 mg/kg, p.o.)	1.76 ±8.0***

Values are expressed as mean±S.E.M. (n = 6).Percent inhibition calculated as compared to control group.***P < 0.001, ** P < 0.01, * P < 0.05 (One-way ANOVA followed by Tukey's post hoc test).

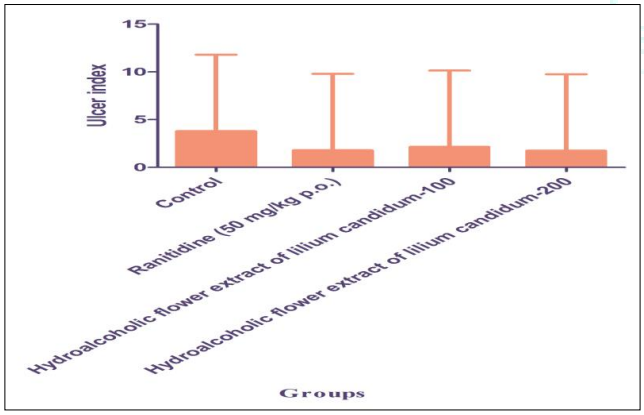

Figure 3: Anti-ulcerogenic effect of hydroalcoholic flower extract of *L. candidum* against ulcerogenic agents in rats (Ulcer index)

Table 4 Anti-ulcerogenic effect of Hydroalcoholic flower extract of *L. candidum* against ulcerogenic agents in rats (PH)

Treatment and dose	Aspirin
Control	1.20 ± 5.0
Ranitidine (50 mg/kg, p.o.)	6.40 ± 5.0***
L. candidum (100 mg/kg, p.o.)	5.20 ± 5.0*
L. candidum (200 mg/kg, p.o.)	6.10 ± 5.0***

Values are expressed as mean±S.E.M. (n = 6). Percent inhibition calculated as compared to control group.***P < 0.001, ** P < 0.01, * P < 0.05 (One-way ANOVA followed by Tukey's post hoc test).

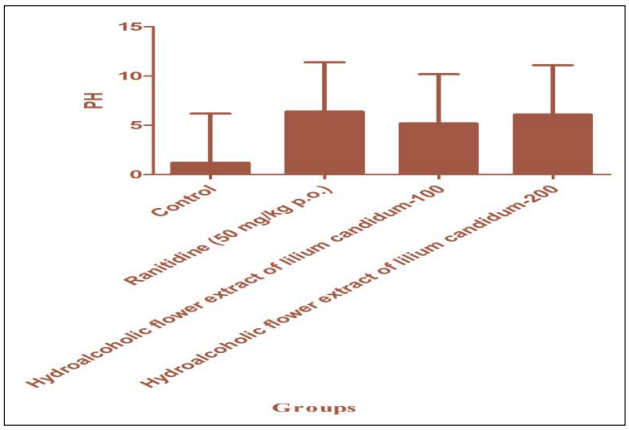

Figure 4: Anti-ulcerogenic effect of Hydroalcoholic flower extract of *L. candidum* against ulcerogenic agents in rats (PH)

CONCLUSION

The preliminary phytochemical investigation of hydroalcoholic extract of *L. candidum* flowers showed the presence of polyphenolic compounds, saponins, flavonoids and alkaloids. Hydroalcoholic extract was screened for acute oral toxicity and was found to be non toxic. Hydroalcoholic extract of *L. candidum* flowers possesses significant anti-ulcer activity. In conclusion, our results showed that the anti-ulcer activity of the extract was a result of the probable gastric ulcer healing mechanism (anti-secretory, cytoprotective and the antioxidant properties) of its active phytoconstituents. These findings suggest the potential for use of *L. candidum* as an adjuvant in the treatment of gastric ulcer. Further, studies are needed for the isolation of active constituents responsible for the anti-ulcer activity and to elucidate the exact mechanism of action in gastric ulcer healing.

ACKNOWLEDGMENTS

The authors would like to thank the Mr. Prabhat Kumar Jain, Geeta Parkhe and All supporting staff of Scan Research Laboratories, Bhopal (M.P.) who helped in the experiments during research work.

REFERENCES

1. Chan FKL, Graham DY. Review article: prevention of non-steroidal anti-inflammatory drug gastrointestinal complications-review and recommendations based on risk assessment, Alimentary Pharmacology and Therapeutics 2004; 19(10): 1051-1061.
2. Debjit B, Chiranjib C, Tripathi KK, Sampath Kumar KP. Recent trends of treatment and medication peptic ulcerative disorder, International Journal of Pharm Tech Research 2010;2 (1): 970-980.
3. Vyawahare NS, Deshmukh VV, Godkari MR, Kagathara VG. Plants with anti-ulcer activity. Pharmacognosy Review 2009; 3:108-115.
4. Brooks FP. The pathophysiology of peptic ulcer disease. Digestive Diseases and Sciences 1985; 30(11): 15S-29S.
5. http://www.betttermedicine.com/article/peptic-ulcer-1/symptoms, October 2011.
6. Hoogerwerf WA, Pasricha PJ. Agents used for control of gastric acidity and treatment of peptic ulcers and gastro esophageal reflux diseaseedition, McGraw-Hill, New York, NY, USA, 10th edition, 2001 pp. 1005-19.
7. Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. The Lancet 1984; 1 (8390): 1311-1315.
8. Malfertheiner P, Chan FK, McColl KE. Peptic ulcer disease. The Lancet 2009; 374 (9699): 1449-1461.
9. Nagar H, Tiwari P, Jain DK, Chandel HS. Evaluation of anti-ulcer activity of stem bark extract of aphanmixis polystachya

in experimental rats. Ind J Pharm Edu Res 2012; 46 (3): 222-227.
10. Kasper DL, Braunwald E, Hauser SL, Jameson JL, Fauci AS, Lengo DL. Principles of Internal Medicine, McGraw-HillMedical Publishing Division, New York, NY, USA, 16th edition, 2005 pp. 221-222.
11. Zapata-Colindres JC, Zepeda-Gomez S, Montano-Loza A, Vasquez-Ballesteros E, de Jesus Villalobos J, Valdovinos-Andraca F. The association of helicobacter pylori infection and nonsteroidal anti-inflammatory drugs in peptic ulcer disease. Canadian Journal of Gastroenterology 2006; 20(4): 277-280.
12. Lau JY, Sung J, Hill C, Henderson C, Howden CW, Metz DC. Systematic review of the epidemiology of complicated peptic ulcer disease: incidence, recurrence, risk factors and mortality. Digestion 2011; 84(2): 102-113.
13. Kumar R. A review on medicinal plants for peptic ulcer. Scholar Research Library. Der Pharmacia Lettre 2011; 3(3): 414-420.
14. Patel G, Jain S. Antiulcer activity of Neriutn indicum in rats. Research Journal of Pharmacology 2010; 4 (3): 66-68.
15. Mimaki Y et al. Steroidal saponins from the bulbs of Lilium candidum. Phytochemistry 1999; 51: 567-573.
16. Prosceivicius J et al. Screening of hybrid progeny derived from Lilium candidum L. Sci Work Lithuanian Inst Hortic Lithuanian Univ Agric 2007; 26, 187-193.
17. Fazil O, Haci T, Ozlem A. the anatomy and morphology of the medicinal plant, *Lilium candidum* L. (liliaceae), distributed in marmara region of turkey). Pak. J. Bot 2012 ; 44(4): 1185-1192.
18. Khawar KM, Çöçü S, Parmaksiz I, Sarihan EO, Sancak C, Özcan S. Mass proliferation of Madona Lilly (*Lilium candidum* L.) under in vitro conditions. Pak. J. Bot 2005; 37(2): 243-248.
19. Saifullah K, Sheeba N, Mariam R, Naheed K, Asma N, Bushra S. Cultivation of Lilies (Lilium regale) for commercialization in Pakistan. Pak J Bot 2010; 42(2): 1103-1113.
20. Rakba N, Loyer P, Gilot D, Delcros JG, Glaise D, Baret P, Pierre JL, Lescoat G. Antiproliferative and apoptotic effects of O-Trensox, a new synthetic iron chelator, on differentiated human hepatoma cell lines. Carcinogenesis 2000 ; 21, 943-951.
21. Khandelwal KR. Practical pharmacognosy techniques and experiments. Nirali Prakashan: India; 2005.
22. Evans WC. Trease and evans pharmacognosy. WB Saunders: New York; 2005.
23. Olufunmiso OO, Afolayan AJ. Phenolic content and antioxidant property of the bark extract of Ziziphus mucronata willd. Subsp. mucronata willd, BMC Complement Alternative Medicine, 2011; 11:130.
24. Guideline Document on Acute oral Toxicity Testing, Series on Testing and Assessment No. 423. Paris: Organization for Economic Co-Operation and Development, OECD Environment, Health and Safety Publications; 1996. Available from: http//www.oecd.org/ehs.
25. Desai JK, Goyal, RK, Parmar NS. Review Article Pathogenesis of Peptic ulcer diseases and current trends in therapy. Indian J. Physiology and Pharmacology 1997; 41: 03-15.