

Antiulcerogenic Effect of *Carthamus oxycantha* M. Bieb (Asteraceae) in Mice and Rat Models

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Abstract.- The ulcer protective potential of methanolic extract of seeds of *Carthamus oxycantha* M. Bieb. (CO) was evaluated in different acute gastric ulceration models in experimental animals. The ulcer induced agents were aspirin and ethanol in swiss albino mice, whereas the pyloric ligation was performed in albino wister rats. The extract was administered orally at dosages of 200 and 400 mg/kg of body weight once daily for 7 days. The extract showed significant protection against ulcer in a dose-dependent manner in all acute gastric ulcer models (20.68 to 58.06% protection, $p < 0.01$ to $p < 0.001$). The results were compared with the standard reference drug omeprazole, that showed a significant ulcer protection index of 55.73-66.12%. Furthermore, administration of CO lead to a decrease in volume of gastric juice (3.98 ± 0.391 to 3.18 ± 0.287 ml), free acidity (73.2 ± 3.721 to 55 ± 5.496 Eq/l/100 gm), total acidity (118.2 ± 4.705 to 99.4 ± 6.532 mEq/l/100 gm) and increased value of pH (3.4 ± 0.253 to 4.18 ± 0.404). Animals treated with CO exhibited a low ulcer index as compared to control animals in three models. The antiulcer potential of CO, however was less than omeprazole. Administration of CO prevented the development of gastric mucosal lesion significantly and decreased the gastric toxicity produced by ulcerogenic agents. The results of our study indicated that CO possesses antiulcer potential which may be due to antioxidant components present in extract.

Key words: Ulceroprotective, *Carthamus oxycantha*, pyloric ligation.

INTRODUCTION

One of the most common gastric problem globally is peptic ulcer. The major reasons and causative agents of peptic ulcer are stress, infection caused by *Helicobacter pylori* (Kaleem *et al.*, 2013), hereditary predisposition, inadequate dietary habits and excessive intake of NSAIDs. The antiulcer drugs available function by inhibiting gastric acid secretion, blocking apoptosis and stimulating epithelial cell proliferation leading to effective healing. Synthetic drugs available are either histamine H₂-receptor blockers like ranitidine and famotidine or proton-pump inhibitors like omeprazole and lansoprazole control excessive gastric acid secretion and acid-related gastric problems. These drugs however are not without side-effects and problem may come back even after

costly treatment (Chan *et al.*, 2009). So there is a requirement to explore new antiulcerogenic alternatives from plant source, having fewer side effects. *Carthamus oxycantha* M. Bieb (Asteraceae) (CO) locally known as pholi and kandyari is a multi-purpose wild growing weed and is well-known due to tagged medicinal properties. Due to its spiny nature, it is not used as fodder for cattle. However it is used as anti-diabetic therapy by local healers. Further its seeds are used to make biofuel. It is also used to cure nocturnal enuresis, tinnitus and earache (Bukhsh *et al.*, 2012; Ullah *et al.*, 2014). CO is used by indigenous communities as antiulcer remedy, therefore, current study has been designed to authenticate the tagged antiulcer property of this medicinal plant (Ahmed *et al.*, 2015; Riaz *et al.*, 2013). Since many factors and mechanisms are implicated in the ulcerogenesis, different models have been employed in the present study to authenticate and to understand the possible mechanism of action of CO extract. The antiulcer activity of CO was evaluated by employing aspirin, alcohol and pylorus ligation ulcer models in mice and rat.

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0030-9923/2015/0002-0529 \$ 8.00/0

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MATERIALS AND METHODS

Animals

Healthy adult Swiss albino mice and adult albino wistar rats of either sex obtained from the animal house of Agriculture University, Faisalabad were used for the antiulcerogenic study. The animals were used for the experiments under the guide lines of the Institute of Laboratory Animals Resource, Commission on Life Sciences, National Research Council and approved by Ethical Committee of Government College University Faisalabad.

Preparation of extract

Carthamus oxycantha M. Bieb (CO) was collected from wheat fields of Layyah (Punjab, Pakistan) during March-April, 2012. The plant was authenticated by local botanist and voucher specimen no. LAH-31120131ML was preserved in the herbarium of Department of Botany, Punjab University Lahore, Pakistan for further studies. The plant was extracted as reported previously by our research group (Zia-ul-Haq *et al.*, 2013, 2014a,b). The crude extract was stored in air tight amber colored bottles at 4°C.

Treatment schedule

CO extract (200 mg/kg and 400 mg/kg) and omeprazole (OMZ) were diluted in 1% sodium carboxy-methylcellulose (CMC, 1%) and administered orally once per day for seven days. Each animal was weighted daily and dose was adjusted regarding to the weight.

Antiulcer studies

Three models were used for antiulcer studies, *i.e.*, aspirin, alcohol and pylorus ligation ulcer models.

Aspirin-induced ulcers (ASP)

Aspirin (200 mg/kg) was used for the induction of ulcers and was administered orally (PO) with the aid of round tip cannula (Akhtar and Ahmad, 1995). The aspirin was given after 45 min of CO methanolic extract and OMZ treatment. After 4 h, cervical dislocation method was used for euthanication of animals. The stomach was taken

out and cut along the greater curvature. Tissue samples of stomach were dehydrated and fixed in paraffin and stained with hematoxylin and eosin for histopathology (Raghavendran *et al.*, 2011). The ulcers were scored for the measurement of gastric lesions with the help of microscope.

Alcohol-induced ulcers (AL)

Absolute ethanol was given PO at the dose of 1mg/200 g after 45 min of methanolic extract of CO and standard drug treatment (Dharmani *et al.*, 2005). After 1 h of ethanol administration, the animals were sacrificed by cervical dislocation. The stomach was taken out and the ulcer was scored microscopically for the measurement of gastric lesions (Malairajan *et al.*, 2007).

Pylorus ligation-induced ulcers (PL)

Adult wistar albino rats (180-200 ± 25 g) of either sex were divided into four groups of five animals each. Pyloric ligation was done after 45 min of CO extract and OMZ administration. The animals were anesthetized with light chloroform. The abdomen was cut and ligation of pylorus was performed with a silk surgical suture. The stomach was then replaced in the abdomen and the wall was sutured with silk surgical threads. The animals were kept alive and allowed to recover from anaesthesia for a period of 4 h. After 4 h, animals were euthanized by cervical dislocation. Stomach was cut along the greater curvature and gastric juice was collected in the tubes. Ulcer index was calculated as a mean ulcer value of each animal. Scoring for ulcer was done according to the method of Malairajan *et al.* (2007) whereby normal colored stomach, red colored, spot ulcered, haemorrhagic streak, ulcer and perforation were allotted scores of 0, 0.5, 1, 1.5, 2 and 3 respectively. The percentage of inhibition of ulcer was measured by following formula as follows:

$$\text{Inhibition of ulcer (\%)} = \frac{\text{Control mean ulcer index} - \text{Test mean ulcer index}}{\text{Control mean ulcer index}} \times 100$$

Gastric secretion study

Gastric juice (1 ml) was taken in conical flask (100 ml) and diluted with distilled water (10 ml). Topfer's reagent (2-3 drops) was poured in conical flask. It was titrated against NaOH (0.01 N) until the

color of solution turned to yellowish orange and all traces of red color disappeared. The volume of NaOH was noted and this measured to free acidity (Patil *et al.*, 2010). For the determination of total acidity phenolphthalein (2-3 drops) was poured in to the conical flask and again titrated against NaOH, until a clear red tinge reappeared. The volume of added NaOH was noted. The final volume used determined the total acidity. Acidity was calculated as described previously (Sivaraman and Muralidharan, 2011).

Statistical analysis

Values were expressed as mean \pm SEM and data was assessed by one way analysis of variance (ANOVA). The value of $P < 0.05$ was considered to be significant.

RESULTS AND DISCUSSION

The methanolic extract of CO showed a significant gastric ulcer protection effect at doses of 200-400 mg/kg when administered once daily for 7 days against gastric ulcer induced by aspirin (ASP), ethanol (AL) and pyloric ligation (PL). CO showed a dose dependent decrease in terms of ulcer index. Aspirin damages mucosa by its direct irritant effect leading to increased gastric acid secretion, resulting in excessive production of products of lipoxygenase pathway like leuko-trienes and interfering prostaglandin synthesis. The methanolic extract of CO showed significant values of mean ulcer index in aspirin induced ulcers. It is evident from results that two doses of CO, *i.e.* 200 and 400 mg/kg showed percentage protection of 34.42% and 49.18%, respectively (Table I).

Aspirin produced gastric hyperacidity as evidenced by increased acid content of gastric juice and decreased pH of gastric juice. Omeprazole also showed antiulcer activity by reducing gastric acid output. The inhibition may be due to antioxidant constituents present in CO extract that antagonize the effect of aspirin. The extract in dose dependent manners caused the protection of gastric mucosa against necrotizing agents, enhancing mucosal resistance and potentiation of defensive factors of gastric mucosa. The protective effect of CO may be due to blockage of aspirin-produced direct irritation

effects, lipoxygenase-inhibitory potential and increased mucus secretion thus diminishing aspirin-induced gastric lesions.

In ethanol model, ulcers are produced because of disruption of superficial epithelial cells especially the mucosal mast cells resulting in secretion of vaso-active mediators like histamine thus damaging the gastric mucosa (Miller and Henagan, 1984) and uncontrolled generation of free radicals thereby increasing lipid peroxidation, which damages cell and cell membrane (Mizui *et al.*, 1987). The CO protected the gastric mucosa significantly against ethanol challenge as shown by decreased lesion index as compared to control group suggesting its potent cytoprotective effect (Table II).

The CO extract at doses of 200 mg/kg and 400 mg/kg of body weight depicted a significant percentage protection of 20.68% and 44.82% respectively. The results were compared to control pre-treated with omeprazole showed the protection index of 65.5%. It indicates that a concomitant increase in sulfhydryl compounds (Szabo *et al.*, 1981) or prostaglandins (Pihan *et al.*, 1986) has contributed in protecting the stomach from ethanol injury. The flavonoids are believed to be responsible for antiulcer effects. It is well-known that pylorus ligation-induced-ulcers are due to the rupture of gastric mucosal barrier and autodigestion of the gastric mucosa (Sairam *et al.*, 2002). Pylorus ligation in ulcerated control group had produced ulcer in all animals and the mean ulcer index was 6.2 ± 0.58 indicating the ulcerogenic effect (Table III).

Ulcers were observed in CO pretreated animals also. However, a significant dose-dependent reduction was observed in CO-pretreated animals at 200 mg/kg (UI, 3.2 ± 0.489) and 400 mg/kg (UI, 2.6 ± 0.678). In other words, gastroprotection observed was 48.38% and 58.06% at 200 and 400 mg/kg, respectively when compared with ulcerated control. OMZ treated (20 mg/kg) animals also exhibited ulcers. However, ulcer index (2.1 ± 0.400) showed significant reduction as compared with ulcerated control and showed 66.12% gastroprotection. Various bioactive constituents like flavonoids, glycosides, tannins and triterpenoids present in CO may be responsible for this antiulcer power of CO extract.

Table I.- Effect of CO in aspirin induced ulcer.

S.No	Treatment	Dose	Body weight of animals	Mean ulcer Index \pm SEM	% protection
I	Control	Placebo	26.54 \pm 2.21	0	---
II	Aspirin	200 mg/kg, orally	25.84 \pm 0.85	6.1 \pm 0.87	---
III	Omeprazole and aspirin	20 mg/kg, I.P	26.96 \pm 0.56	2.7 \pm 0.73***	55.73
IV	Extract and aspirin	200 mg/kg, P.O	28.14 \pm 1.17	4.8 \pm 1.14***	34.42
V	Extract and aspirin	400 mg/kg, P.O	30.12 \pm 1.17	3.1 \pm 0.78***	49.18

Values are expressed as mean \pm SEM, n = 7 mice in each group. *Significant (p < 0.05), **highly significant (p < 0.01), ***Very significant (p < 0.001)

Table II.- Effect of CO in alcohol induced ulcer.

S.No	Treatment	Dose administered	Body weight of animals	Mean ulcer Index \pm SEM	% protection
I	Control	Placebo	29.02 \pm 0.58	0	---
II	Alcohol	1 mg/200 g, orally	30.69 \pm 1.26	5.8 \pm 0.49	---
III	Omeprazole and alcohol	20 mg/kg, I/P	28.38 \pm 0.84	2 \pm 0.69***	65.50
IV	Extract and alcohol	200 mg/kg, P.O	28.68 \pm 1.04	4.9 \pm 0.92***	20.68
V	Extract and alcohol	400 mg/kg, P.O	27.06 \pm 0.54	3.2 \pm 0.80***	44.82

Values are expressed as mean \pm SEM, n = 7 mice in each group. *Significant (p < 0.05), **Highly significant (p < 0.01), ***Very significant (p < 0.001)

Table III.- Effect CO in pyloric ligation model.

S.No	Group	Dose administered	Mean ulcer Index \pm SEM	% protection
I	Control (Aspirin)	1 ml/kg, I.P	6.2 \pm 0.58	---
II	Omeprazole	20 mg/kg, I.P	2.1 \pm 0.40***	66.12
III	Extract	200 mg/kg, P.O.	3.2 \pm 0.48***	48.38
IV	Extract	400 mg/kg, P.O.	2.6 \pm 0.67***	58.06

Values are expressed as mean \pm SEM, n = 5 mice in each group. *Significant (p < 0.05), **Highly significant (p < 0.01), ***Very significant (p < 0.001)

Table IV.- Effect of CO on gastric secretions.

S.No	Groups	Dose administered	Body weight (gm)	Parameters			
				Volume of gastric juice (ml)	Free acidity (Eq/l/100 gm)	Total Acidity (mEq/l/100 gm)	pH
I	Control	1 ml/kg, I.P	184 \pm 5.09	4.86 \pm 0.27**	85 \pm 6.30***	141.6 \pm 4.31***	2.38 \pm 0.25***
II	Omeprazole	20 mg/kg, I.P	186 \pm 6.78	2.16 \pm 0.33**	44 \pm 3.56***	87.4 \pm 8.91***	5.34 \pm 0.39***
III	Extract	200 mg/kg, P.O.	184 \pm 5.09	3.98 \pm 0.39**	73.2 \pm 3.72***	118.2 \pm 4.70***	3.4 \pm 0.25***
IV	Extract	400 mg/kg, P.O.	190 \pm 4.47	3.18 \pm 0.28**	55 \pm 5.49***	99.4 \pm 6.53***	4.18 \pm 0.40

Values are expressed as mean \pm SEM, n = 5 mice in each group. *Significant (p < 0.05), **Highly significant (p < 0.01), ***Very significant (p < 0.001)

Effect on gastric secretion

The biochemical parameters like gastric pH, gastric volume, total acidity as well as free acidity were also measured to assess the effect of CO

administration on them. The treatment with test extract reverted the biochemical changes if any of ulcerogenic animals to normal levels and this change was dose dependent. The parameters

observed were mean gastric content volume (4.86 ± 0.27), pH (2.38 ± 0.25), free acidity (85 ± 6.30) and total acidity (141.6 ± 4.31) and pepsin content (5.56 ± 0.69) as shown in Table IV. The disruption of mucosal barrier and digestion of gastric mucosa observed in pylorus-ligation model are due to excessive acid secretion (Bafna and Balaraman, 2004). CO extract exhibited antiulcer potential by decreasing the gastric juice quantity and ulcer lesions, and by increasing pH. This antisecretory effect of CO may be due to increased HCO_3^- secretion (Table IV).

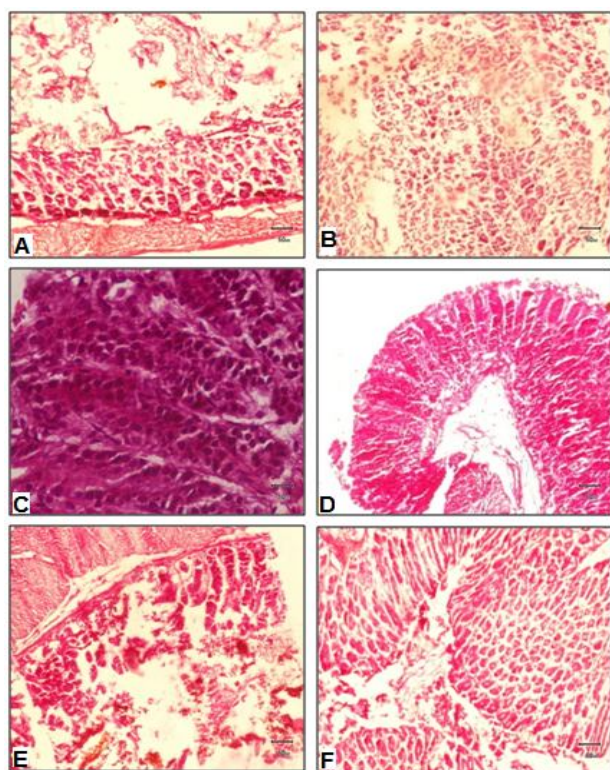


Fig. 1. Histological structure of gastric mucosa of albino Wister rats treated with aspirin alone (A), ethanol alone (B), inflammatory cells in animals treated with methanolic extract (200 mg) with aspirin (C). Slugging and degeneration of mucosa in animals treated with methanolic extract (200 mg) with ethanol (D). Mild inflammatory cells in animals treated with methanolic extract (400 mg) with ethanol (E). Appearance of normal tissues in animals treated with extract (400 mg) with aspirin (F).

The significant decrease of gastric secretions and reduction of ulcer formation by CO after pylorus ligation indicates that cytoprotective potential of CO may be due to direct reduction of gastric secretion. The antiulcer activity of CO however, was less than that of standard drug. Various bioactive constituents present in crude extract like terpenoids, glycosides and saponins are believed to possess the antiulcer activity. These bioactive constituents present in CO might have the ability to protect against ulceration. The antiulcer effect was further confirmed histological study.

Histopathology of ulcer models

Histopathological analysis of all ulcer models and extract treatment are given in the Figure 1. In histological study, it was observed that CO pretreatment preserved the functional cytoarchitecture of the entire gastric mucosa. It is evident that CO treatment maintained and even regenerated gastric mucosa in the damaged regions. These findings confirm the cytoprotective nature of CO. The microscopic observations showed normal appearance of gastric mucosa in control group (A). ASP-PL induced rats (B) epithelial disruption identified as marker of ulcer formation was apparent in standard drug treated rats. The glandular organization, maintenance of mucosal structure, epithelialisation and decreased size of ulcer-crater in CO pretreated rats distinguished CO pretreated rats. The results indicated that CO pretreatment markedly ameliorated the biochemical and pathological changes of ASP induced gastric ulceration in rats.

CONCLUSION

The results of study showed that the methanolic extract of seeds of CO in a dose dependent order caused an inhibition or protection of induced ulcer models. It is suggested that CO exerts its ulcer protection potential by decreasing gastric acid secretion and ulcer and by increasing the mucin contents and glycoprotein levels thereby maintaining the mucosal epithelium. The protection to the gastric mucosa against ulceration may be because of antisecretory and cytoprotective property of CO. Further study is needed to isolate active components responsible for this activity.

REFERENCES

- AHMED, S., GUL, S., ZIA-UL-HAQ, M., RIAZ, M. AND MOGA, M., 2015. Anti-inflammatory effects of cyclooxygenase-2 inhibitors in rabbits. *Pakistan J. Zool.*, **47**: 209-216.
- AKHTAR, A.H. AND AHMAD, K.U., 1995. Anti-ulcerogenic evaluation of the methanolic extracts of some indigenous medicinal plants of Pakistan in aspirin-ulcerated rats. *J. Ethnopharmacol.*, **46**: 1-6.
- BAFNA, P.A. AND BALARAMAN, R., 2004. Antiulcer and antioxidant activity of Normacid, a herbomineral formulation. *Indian J. exp. Biol.*, **42**: 674-680.
- BUKSH, E., MALIK, S. A., AHMAD, S.A. AND ERUM, S., 2012. Hepatoprotective and hepatocurative properties of alcoholic extract of *Carthamus oxyacantha* seeds. *Afr. J. Pl. Sci.*, **8**:34-41.
- CHAN, F.K., SUNG, J.J., SUEN, R., LEE, Y.T., WU, J.C., LEUNG, W.K., CHAN, H.L., LAI, A.C., LAU, J.Y., NG, E.K. AND CHUNG, S.C., 1998. Does eradication of *Helicobacter pylori* impair healing of nonsteroidal anti-inflammatory drug associated bleeding peptic ulcers? A prospective randomized study. *Aliment. Pharmacol. Ther.*, **12**: 1201-1206.
- DHARMANI, P., KUCHIBHOTLA, V.K., MAURYA, R., SRIVASTAVA, S., SHARMA, S. AND PALIT, G., 2004. Evaluation of anti-ulcerogenic and ulcer-healing properties of *Ocimum sanctum* Linn. *J. Ethnopharmacol.*, **93**: 197-206.
- KALEEM, W.A., NISAR, M., QAYUM, M., ZIA-UL-HAQ, M., CHOUDHARY, M.I. AND ERCISLI, S., 2013. Urease inhibitory potential of *Zizyphus oxyphylla* Edgew extracts and compounds. *Turk. J. med. Sci.*, **43**:497-500.
- MALAIRAJAN, P., GOPALAKRISHNAN, G., NARASIMHAN, S., VENI, K. AND KAVIMANI, S., 2007. Anti-ulcer activity of crude alcoholic extract of *Toona ciliata* Roemer (heart wood). *J. Ethnopharmacol.*, **110**: 348-351.
- MILLER, T.A. AND HENAGAN, B., 1984. Indomethacin decreases resistance of gastric barrier to disruption by alcohol. *Dig. Dis. Sci.*, **29**: 141-149.
- MIZUI, T., SATO, H., HIROSE, F. AND DOTEUCHI, M., 1987. Effect of antiperoxidative drugs on gastric damage induced by ethanol in rats. *Life Sci.*, **41**: 755-763.
- PATIL, P.H., PATIL, J.Y., MAHALE, J.N., PATEL, J.B. AND SURANA, S.J., 2010. Evaluation of antiulcer activity of the terpenoid fraction from the leaves of *Thespesia populnea* (L) (Malvaceae) in albino rats. *Res. J. Pharm. biol. Chem. Sci.*, **1**: 495-513.
- PIHAN, G., MAJZOUBI, D., HAUDENSCHILD, C., TRIER, J.S. AND SZABO, S., 1986. Early microcirculatory stasis in acute gastric mucosal injury in the rat and prevention by 16,6 dimethyl prostaglandin E2 or sodium thiosulfate. *Gastroenterology*, **91**: 1415-1426.
- RAGHAVENDRAN, H.R.B., SRINIVASAN, P. AND REKHA, S., 2011. Immunomodulatory activity of fucoidan against aspirin-induced gastric mucosal damage in rats. *Int. Immunopharmacol.*, **11**: 157-163.
- RIAZ, M., RAHMAN, N. AND ZIA-UL-HAQ, M., 2013. Anthelmintic and insecticidal activities of *Verbascum thapsus* L. *Pakistan J. Zool.*, **45**: 1593-1598.
- SAIRAM, K., RAO, C.V., BABU, D.M., AGRAWAL, V.K. AND GOEL, R.K., 2002. Antiulcerogenic activity of methanolic extract of *Emblca officinalis*. *J. Ethnopharm.*, **82**: 1-9.
- SIVARAMAN, D. AND MURALIDHARAN, P., 2011. Cytoprotective effect of *Morinda tinctoria* Roxb. against surgical and chemical factor induced gastric and duodenal ulcers in rats. *Ulcer*, **20**: 2-9.
- SZABO, S., TRIER, J.S. AND FRANKEL, P.W., 1981. Sulfhydryl compounds may mediate gastric cytoprotection. *Science*, **214**: 200-202.
- ULLAH, S., KHAN, M.R., SHAH, N.A., SHAH, S.A., MAJID, M., AND FAROOQ, M. A., 2014. Ethnomedicinal plant use value in the Lakki Marwat District of Pakistan. *J. Ethnopharm.*, **158**: 412-422.
- ZIA-UL-HAQ, M., AHMAD, S., AMAROWICZ, R. AND DEFEO, V., 2013. Antioxidant activity of the extracts of some cowpea (*Vigna unguiculata* (L) Walp.) cultivars commonly consumed in Pakistan. *Molecules*, **18**: 2005-2017.
- ZIA-UL-HAQ, M., RIAZ, M., DEFEO, V., JAAFAR, H.Z.E., AND MOGA, M., 2014a. *Rubus fruticosus* L.: constituents, biological activities and health related uses. *Molecules*, **19**: 10998-11029.
- ZIA-UL-HAQ, M., AHMAD, S., BUKHARI, S.A., AMAROWICZ, R., ERCISLI, S. AND JAAFAR, H.Z.E., 2014b. Compositional studies and biological activities of some mash bean (*Vigna mungo* (L.) Hepper) cultivars commonly consumed in Pakistan. *Biol. Res.*, **47**:23-31.

(Received 16 September 2014, revised 12 January 2014)