

Full Length Research Paper

Evaluation of spasmolytic and analgesic activity of ethanolic extract of *Chenopodium album* Linn and its fractions

Mansoor Ahmad^{1*}, Omair Anwar Mohiuddin², Mehjabeen³, NOOR JAHAN², MUNIR Anwar¹, Salman Habib⁴, S. Mahboob Alam⁵ and Iftikhar Ahmed Baig¹

¹Department of Pharmacognosy, Research Institute of Pharmaceutical Sciences, University of Karachi, Karachi-75270, Pakistan.

²Dow College of Pharmacy, Dow University of Health sciences, Karachi-74200, Pakistan.

³Department of Pharmacology, Federal Urdu University of Arts, Science and Technology, Karachi-75300, Pakistan.

⁴Department of Nuclear Medicine, Karachi Institute of Radio Therapy and Nuclear Medicine, Karachi, Pakistan.

⁵Department of Pharmacology, Jinnah Postgraduate Medical Centre, Karachi, Pakistan.

Accepted 2 July, 2012

Chenopodium album Linn is commonly consumed as vegetable and has been traditionally used for the treatment of hepatic disorders and inflammation. Standardization of *C. album* was carried out by using ultraviolet (UV)-spectrophotometer, high performance liquid chromatography (HPLC), Fourier transforms near infrared (FT-NIR) and Fourier transform infrared (FTIR). The plant was extracted in ethanol and fractionated in ethyl acetate, chloroform, *n*-butanol and water. The crude extract and its fractions were tested *in vitro* on intestinal smooth muscles of rabbit. The crude extract exhibited a dose-dependent increase in relaxation of smooth muscles, starting from 5 mg/ml and maximum effect was found at 20 mg/ml (92.86%). All the fractions were administered to rabbit's intestine at 15 mg/ml dose. The ethyl acetate and chloroform fractions of *C. album* exhibited relaxation of the intestinal muscles (43.48 and 51.52%, respectively); whereas, *n*-butanol fraction of *C. album* produced strong relaxant effect (91.18%). The contractile effect was only observed in aqueous fraction (29.41%). Overall, the activity produced by *n*-butanol fraction was found to be highly significant (by statistical analysis). Analgesic effect of the crude extract was carried out by tail flick method in mice. Significant analgesic effect was observed at 500 mg/kg dose from 30 min up till 210 min.

Key words: *Chenopodium album* Linn, high performance liquid chromatography (HPLC), Fourier transform infrared (FTIR), Fourier transforms near infrared (FT-NIR) smooth muscles, analgesic activity.

INTRODUCTION

Chenopodium album Linn (family: Chenopodiaceae) is cultivated in gardens and agricultural land; it is distributed all over South East Asia. It is found in areas around Mumbai, Kashmir, Sikkim and throughout Pakistan (Baquer et al., 1989). *C. album* is commonly called 'white goose foot', whereas in Pakistan's local

language, it is called 'Bathua', which is a vegetable and consumed as a food product (Nadkarni, 1976).

The important constituents present in the plant that contribute to its nutritional value and pharmacological effects include, flavanols (Bylka and Kowalewski, 1997), carotene (Greca et al., 2004), vitamins A and C (Aliotta and Pollio, 1981), minerals including potash salts (Dahot and Soomro, 1997), and amides (Bernard et al., 1983).

The plant has laxative properties, it is also used in hepatic disorder and conditions due to enlarged spleen

*Corresponding author. E-mail; herbalist53@yahoo.com. Tel: +92 321 2006547. Fax: +92 21 9261340.

(Baquer et al., 1989; Nadkarni, 1976). It is used as diuretic, febrifuge, emollient for throat and chest, nutritive and thirst quenching agent (Khan et al., 1997). Methanolic extract of the plant has shown to produce significant anthelmintic activity (Jabbar et al., 2007). The seed extract of *C. album* has been reported to produce significant spermicidal activity (Kumar et al., 2007; Kumar et al., 2011). The plant extract has also been identified to contain free radical scavenging activity and might have some use in cancer treatment (Kumar and Kumar, 2009). The plant seeds have been used as an article of food in regions of South East Asia for a long time.

Dai et al. (2002) studied the anti-inflammatory activity of *C. album*. In the study, antipruritic effect of the plant extract was examined in mice and the authors concluded that the plant can be used for the treatment of cutaneous pruritis. Ethanolic extract of *C. album* has been reported to produce strong analgesic activity, when administered in rats (Ibrahim et al., 2007). The plant has also been reported to produce sedative effect (Okuyama et al., 1993). *Chenopodium chilense* has been used traditionally in different parts of the world as a remedy for stomach ache; the activity of the plant on intestinal smooth muscles has also been studied in rats and some spasmolytic activity has been observed (Ibironke and Ajiboye, 2007).

Previous researches have indicated that *C. album* can produce anti-inflammatory and analgesic activity (Nedialkova et al., 2009). Some *Chenopodium* species are known to produce spasmolytic effect and *C. album* has often been reported in the folklore to be helpful in intestinal pains, but there is lack of scientific research to confirm the activity of *C. album* on smooth muscles. The current study was divided into two parts; initially, the plant extract was to be standardized by the help of analytical techniques including high performance liquid chromatography (HPLC) and then the pharmacological activity of the plant was to be studied. In the current study, the main aim was to establish the anti-spasmodic activity along with its possible mechanism of action and any possible analgesic activity of crude extract and fractions of *C. album*.

MATERIALS AND METHODS

Identification of the plant and extraction

C. album Linn was purchased from the local market in Karachi, Pakistan. The identification of plant was carried out by Prof. Dr. Mansoor Ahmad in the Department of Pharmacognosy at University of Karachi. The plant voucher specimens were deposited to the Department of Pharmacognosy (No: CA-2-2-03). The plant parts used for the study were seeds and leaves. The plant parts were shade dried at room temperature and then ground to powder form. The powdered plant material 0.685 kg was soaked in 8 L ethanol for 1 month; the crude extract was later filtered and then dried by evaporating ethanol at 40°C in a rotary evaporator under reduced pressure. The extraction

process yielded 20.38 g of crude drug. The crude extract was later fractionated in ethyl acetate, chloroform, *n*-butanol and water, for further experiments.

Standardization of the drug

For the purpose of ultraviolet (UV) spectrophotometry, the extract was diluted in ethanol and analyzed by Lambda-20 Perkin Elmer. Fourier transforms infrared (FTIR) and Fourier transform near infrared (FT-NIR) analysis was performed using the methods as described by Andreia et al. (2011, 2006), respectively.

HPLC was performed using Agilent 1100 series machine (Germany). The crude extract sample was prepared in 0.2 gm/ml concentration; water and methanol were used as mobile phase in the ratio of 633:367, respectively. The mobile phase was run through ODS-C18 column (4.6 × 250 mm) at 0.7 ml/min. Final detection of the eluate was done using UV detector at 225 and 325 nm (Liu et al., 2004).

Smooth muscles study

Rabbits (6 to 7 months old) weighing approximately 1.0 to 1.5 kg were purchased from Aga Khan University Hospital, animal house in Karachi and were kept at the animal house facility of Research Institute of Pharmaceutical Sciences, University of Karachi. The animals were sacrificed by a blow on the neck, abdomen was opened and intestine was removed. Approval for animal sacrifice was acquired from ethical review committee for animal handling at Research Institute of Pharmaceutical Sciences. The activity of plant extract and its fractions was tested on the rabbit intestine mounted on an organ bath according to the method described by Ahmad et al. (2012) and Anwar et al. (2011).

The crude plant extracted was administered to the rabbit intestine in different concentrations, including 1, 5, 10, 15, 20 and 25 mg/ml. Similarly, 15 mg/ml each of ethyl acetate, chloroform, *n*-butanol and aqueous fractions of *C. album* were also administered to the isolated rabbit intestine.

Analgesic activity testing by tail flick method

The method of Di Stasi et al. (1988) and Ahmad et al. (2011) was used for the determination of analgesic activity of crude plant extract. Mice were divided into five groups with each group containing five animals. Animals kept as control were administered with 10 ml/kg of 0.9% NaCl orally while test animals were administered plant extracts at 300 and 500 mg/kg dose orally. Both standard (diclofenac sodium) and test drug (plant extract) were administered orally to the mice and the first reading was taken at time zero (0) and then at 30, 60, 90, 120, 150, 180 and 210 min.

Statistical analysis

The data obtained from smooth muscle activity and analgesic effect study were statistically analyzed by Student's t-test ($P < 0.05$), using 'Graphpad software, Quick calcs online calculator for scientists'.

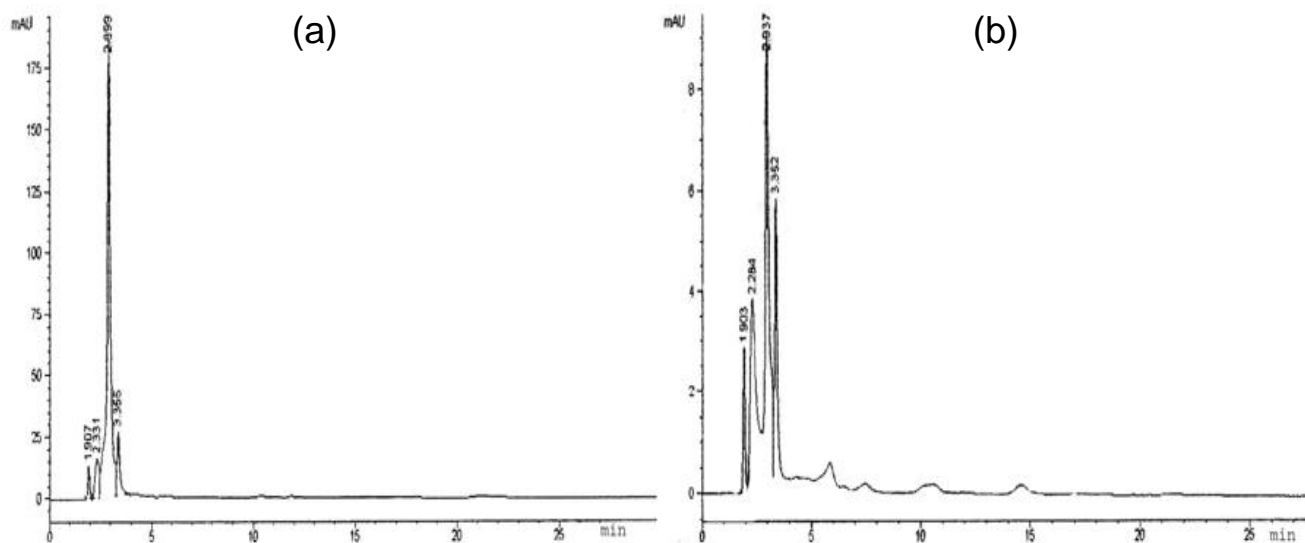
RESULTS

The extraction of plant constituents by maceration

Table 1. Standardization of crude *C. album* extract.

Peak	UV – peaks (nm)	FTIR peak (cm ⁻¹)	FT-NIR peak (cm ⁻¹)	HPLC 225 nm (min)	HPLC 325 nm (min)
Peak 1	233.99	3315.21	6861.11	1.907	1.903
Peak 2	-	2945.65	5791.66	2.331	2.284
Peak 3	-	1641.30	5194.44	2.899	2.937
Peak 4	-	1043.47	5805.56	3.355	3.352
Peak 5	-	-	-	4458.33	-

The table shows the wavelength and frequency at which UV, FTIR and FT-NIR peaks appeared. The retention time of different constituents whose peaks appeared after HPLC are also mentioned.

**Figure 1.** HPLC spectra of *C. album*: (a), 225 nm; (b), 325 nm.

yielded 2.97% crude drug, which was used for pharmacological studies. The plant extract was standardized using UV, FTIR, FT-NIR spectrophotometry and HPLC; the results obtained are shown in Table 1 and Figures 1 and 2. The standardization data can be used in the future for the identification of this plant. The drug displayed dose-dependent relaxant effect; at lower doses slight relaxant effect was observed, but the effect is increased with the increase in dose. The highest relaxant effect was observed at 20 mg/kg dose, which was 92.86%, but there was a decrease in the relaxant activity at 25 mg/kg dose (61.29%). During the analysis of fractions of the plant crude extract, ethyl acetate and chloroform fractions were observed to produce spasmolytic effect on intestinal smooth muscles of the rabbit. The spasmolytic effect produced by ethyl acetate and chloroform was found to be 43.48 and 51.52%, respectively. *n*-butanol fraction displayed highly significant spasmolytic activity that was calculated to be 91.18%. Aqueous fraction of the plant extract caused extract was co-administered with standard drugs

including acetylcholine, adrenaline, histamine and atropine to understand the mechanism of action of the drug.

Analgesic activity of the plant extract was tested in mice using tail flick method. Significant analgesic effect was observed in mice, and when compared with diclofenac sodium, which is a well known analgesic drug, the plant extract exhibited potent analgesic effect.

DISCUSSION

C. album Linn crude extract was diluted to 1, 5, 10, 15, 20 and 25 mg/ml (Table 2). The crude extract produced biphasic responses in rabbit's ileum (intestinal smooth muscles). At the dose of 1 mg, there was negligible relaxant activity whereas at the dose of 5 mg initially, there was a relaxant effect that increased till 20 mg/kg dose (92.86%). The doses of 10, 15 and 20 mg displayed similar typical effect with the slight variation, whereas, at the dose of 25 mg, there was a

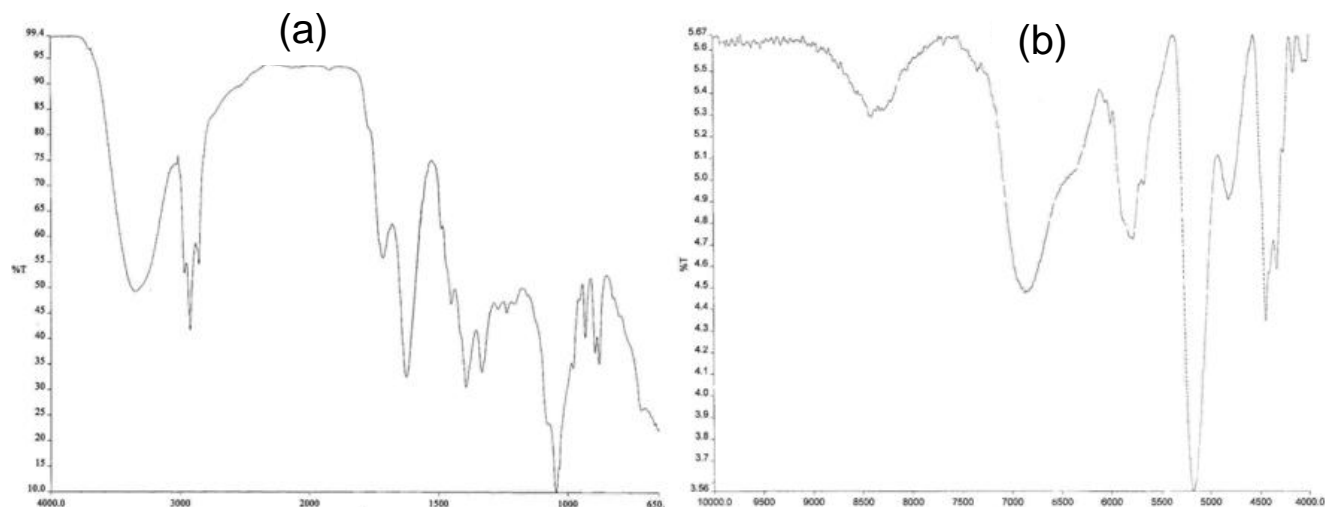


Figure 2. (a), FTIR spectra of *C. album*; (b), FT-NIR spectra of *C. album*.

Table 2. Dose related response of crude extract of *C. album* on isolated rabbit's intestine.

Dose (mg/ml)	Control (cm)	Treated (cm)	Response (%)	t- value
1	0.970 ± 0.0300	0.867 ± 0.0333	10.65	4.232
5	0.867 ± 0.0333	0.267** ± 0.0333	69.23	14.425
10	1.033 ± 0.0333	0.200** ± 0.0577	80.65	15.417
15	0.867 ± 0.0333	0.067** ± 0.0333	92.31	15.839
20	0.933 ± 0.0333	0.067** ± 0.0667	92.86	11.628
25	1.033 ± 0.0333	0.400** ± 0.0577	61.29	13.617

The results are expressed in Mean ± S.E.M. *, Significant at $p < 0.05$; **, highly significant at $p < 0.05$.

contraction of the smooth muscles (29.41%). The plant significant decrease in the relaxant effect (61.29%) of the drug (Table 2). To further elucidate these effects, the crude extract was subjected to fractionation. All the fractions were administered at the dose of 15 mg/ml. The ethyl acetate fraction of *C. album* caused relaxation of the intestinal muscles; the chloroform fraction also exhibited the same effect, whereas the *n*-butanol fraction of *C. album* displayed strong relaxing effect. Contractile effect was only observed when aqueous fraction was tested in rabbit's intestine. Overall, *n*-butanol and aqueous fraction produced significant activity on the smooth muscles (Table 3).

Further, experiments were carried out on the intestinal smooth muscles, by administering fractions of crude drug along with the standard drugs including acetylcholine, adrenaline, histamine and atropine (Figures 3 to 4).

Figure 3 shows the effect of *n*-butanol fraction of *C. album* pre-treated with acetylcholine 1×10^{-2} M. The *n*-butanol fraction overcame the effect of acetylcholine, it neutralized the effect of acetylcholine and normal activity of the tissue was restored. Figure 3 also shows

the effect of *n*-butanol fraction of *C. album* when post-treated with acetylcholine 1×10^{-4} M, histamine 1×10^{-2} M and adrenaline 1×10^{-2} M. The *n*-butanol fraction did not allow acetylcholine to produce full response when administered after the *n*-butanol fraction. This result indicates possible involvement of muscarinic receptors, since the drug inhibited acetylcholine and induced contraction. The *n*-butanol fraction did not inhibit the effect of histamine significantly. In the case of *n*-butanol fraction of *C. album* post-treated with adrenaline, no cumulative inhibitory effect was observed. Figure 4 shows the effect of ethyl acetate fraction of *C. album* on intestinal smooth muscles when pre-treated with acetylcholine 1×10^{-4} M and post-treated with atropine in 1×10^{-2} M. Treatment of the smooth muscles with ethyl acetate fraction, which were pretreated with acetylcholine, did not allow acetylcholine to produce its full effect and antagonized its effect. The effect of ethyl acetate fraction was totally inhibited when post-treated with atropine, whereas the aqueous fraction antagonized the effect of atropine. In Figure 4, no contractile response was seen in the aqueous fraction treated with adrenaline. These results

Table 3. Effect of different fractions of *C. album* extract on isolated rabbit's intestine.

Fraction	Dose (mg/ml)	Control (cm)	Treated (cm)	Response (%)	t- value
Ethyl acetate	15	0.767 ± 0.033	0.433 ± 0.033	43.48	8.485
Chloroform	15	1.100 ± 0.058	0.533 ± 0.033	51.52	13.883
<i>n</i> -butanol	15	1.133 ± 0.088	0.100** ± 0.058	91.18	12.090
Aqueous	15	1.133 ± 0.133	1.467 ± 0.088	-29.41	-5.421

The results are expressed in Mean ± S.E.M. *, Significant at p < 0.05; **, highly significant at p < 0.05.

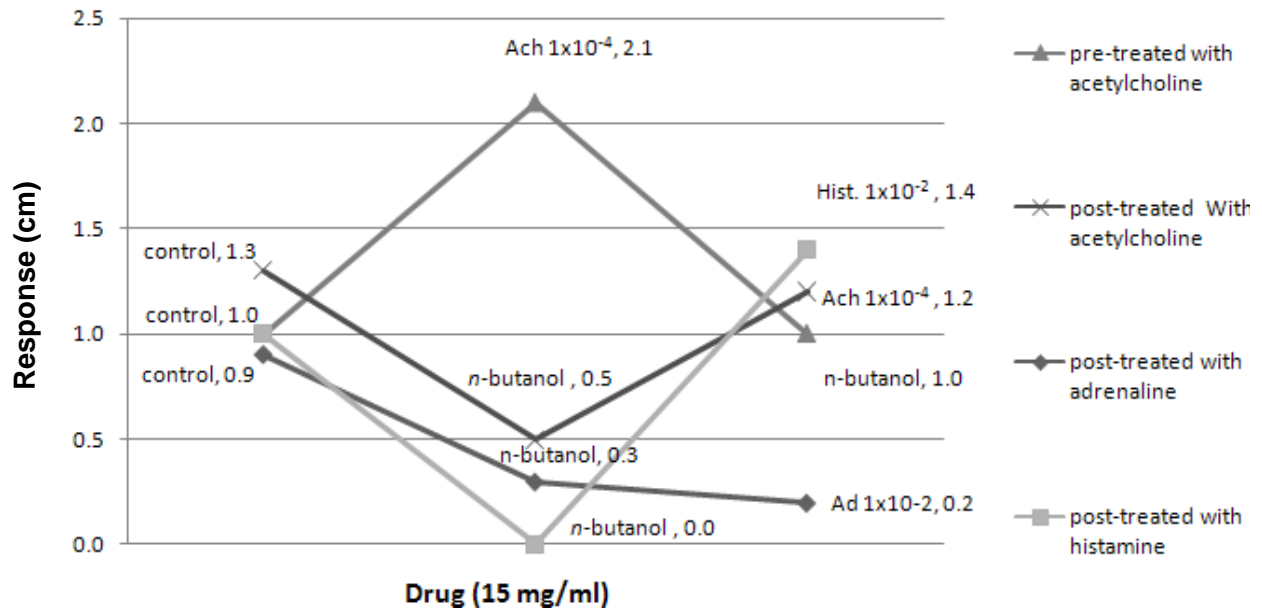


Figure 3. Effect of *n*-butanol fraction of *C. album* on rabbit's intestine pre and post-treated with standard drugs.

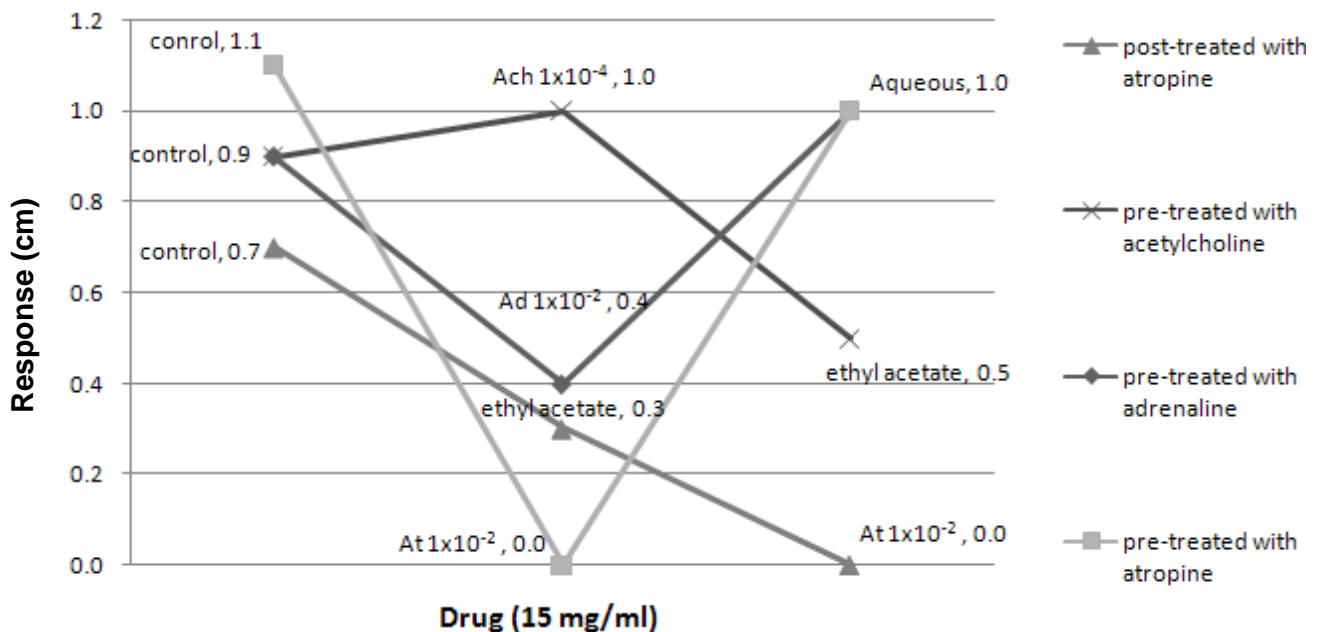


Figure 4. Effect of aqueous and ethyl acetate fraction of *C. album* on rabbit's intestine pre and post-treated with standard drugs.

Table 4. Analgesic activity of *C. album* (showing the tail flick time after treatment with diclofenac sodium and *C. album* extract at 300 and 500 mg/kg dose).

Activity	Time (min)								
	0	30	60	90	120	150	180	210	240
Control	2.98 ± 0.44	1.8 ± 0.37	2.4 ± 0.24	1.8 ± 0.24	2.2 ± 0.20	2.2 ± 0.20	2.2 ± 0.20	2.6 ± 0.24	2.6 ± 0.24
Diclofenac-Na (50 mg/kg)	1.97 ± 0.13	2.6* ± 0.24	3.6* ± 0.25	4.12** ± 0.10	4.06** ± 0.10	3.3 ± 0.20	2.3 ± 0.20	2.1 ± 0.10	2.1 ± 0.10
Treated (300mg/kg)	1.2 ± 0.20	2.70 ± 0.20	3 ± 0.16	3.6* ± 0.24	3.9* ± 0.24	3.5* ± 0.22	3.4* ± 0.24	2.6 ± 0.19	2.2 ± 0.20
Treated (500 mg/kg)	1.4 ± 0.24	4.4** ± 0.24	4.6** ± 0.24	4.4** ± 0.24	4.2** ± 0.20	3.8* ± 0.20	3.7* ± 0.20	4.7** ± 0.30	3.4* ± 0.24

Results are expressed as Mean ± SEM. *, Significant at $p < 0.05$; **, highly significant at $p < 0.05$.

show that adrenergic receptors may also be involved in producing the response of drug.

Since, both contraction and relaxation of the intestinal muscle was observed in the experiments performed, therefore, there is an increased possibility of the involvement of more than one physiological receptors for the production of response to the drug. The overall results indicate that there is a strong possibility of the involvement of both adrenergic and muscarinic receptors and the mix effect of the crude extract on intestinal smooth muscles could be due to this reason. In crude extract, the contractile effect is dominant, due to which this the drug produces laxative effect on the intestinal muscles. However, the fractions of *C. album* exhibited relaxant and contractile action, due to which it is clear that more than one active principal are responsible for the pharmacological activity of the plant extract on smooth muscles. Previous literature indicates the use of this drug for throat and chest as an emollient (Nedialkova et al., 2009), which is possible when the drug interacts with the adrenergic receptor. Therefore, there is a strong possibility that the drug acts through adrenergic receptors.

Analgesic activity was assessed in mice by tail flicking, the results were found significant for the crude extract (Table 4). The analgesic effect observed at 300 mg/kg dose was not highly

significant, whereas at the dose of 500 mg/kg; the onset of action was achieved with in 30 min which persisted till 210 min showing strong analgesic effect. The analgesic effect of the crude extract was found to be similar to that produced by diclofenac sodium up till 2 h, but the crude drug (500 mg/kg) exhibited prolonged analgesic activity which was significant up till 4 h.

The standardization of plant extract carried out in the present study can be useful for the identification of the plant in the future. The results obtained from the study conducted strongly suggest that the plant possesses potent spasmolytic activity. The spasmolytic effect of crude drug was dose-dependent and increased with increasing dose. The *n*-butanol fraction exhibited maximum relaxant effect on smooth muscles; further studies on the *n*-butanol fraction of *C. album* can be useful for the determination of active principle responsible for the spasmolytic effect. The aqueous fraction displayed slight contraction of the smooth muscles, but the effect was not found to be significant. Strong analgesic activity was observed in mice for a prolonged period of time. The results obtained from the current study strongly suggest that *C. album* can be a good candidate for the development of a therapeutic drug for the treatment of muscle spasm and pain.

REFERENCES

- Ahmad M, Mehjabeen M, Jahan N (2011). Determination of LD₅₀ and ED₅₀ by dose response relationship and assessment of toxicological and non toxicological behaviour of *Ipomoea hederacea*. J. Pharm. Res. 4(4):1176-1178.
- Ahmad M, Muhammad N, Mehjabeen Jahan, Ahmad M, Obaidullah M, Jan SU (2012). Spasmolytic effects of *Scrophularia nodosa* extracton isolated rabbit intestine. Pak. J. Pharm. Sci. 25(1): 267-275.
- Aliotta G, Pollio A (1981). Vitamin A and C contents in some edible wild plants in Italy. Riv. Ital. EPPOS. 63(1):47-48.
- Andreia A, Bunaciu HY, Aboul-Enein, Şerban F (2006). FT-IR Spectrophotometric analysis of acetylsalicylic acid and its pharmaceutical formulations. Can. J. Anal. Sci. Spectrophotomet. 51(5):253-259.
- Andreia M, Mawsheng C, Jeemeng L, Wing H, Joshua L, Pamela C, Miguel E (2011). Combining multivariate analysis and monosaccharide composition modeling to identify plant cell wall variations by Fourier Transform Near Infrared spectroscopy. Plant Method. 7:26.
- Anwar M, Ahmad M, Mehjabeen, Jahan N, Mohiuddin OA, Qureshi M (2011). Phytopharmacological assessment of medicinal properties of *Psoralea corylifolia*. Afr. J. Pharm. Pharmacol. 5(23):2627-2638.
- Baquer SR (1989). Medicinal and Poisonous Plants of Pakistan. Printas Karachi: Karachi. 278:368, 103.
- Bernard M, Drost-Karbowska K, Kowalewski Z (1983). Basic compounds of white pigweed (*Chenopodium album*). Acta. Pol. Pharm. 40(5- 6):691-692.
- Bylka W, Kowalewski Z (1997). Flavonoids in *Chenopodium album* L. and *Chenopodium opulifolium* L. Herba. Pol. 43(3):208-213.
- Dahot MU, Soomro ZH (1997). Proximate composition, mineral and vitamin content of *Chenopodium album*. Sci. Int. (Lahore). 9(4):405-407.

- Dai Y, Ye WC, Wang ZT, Matsuda H, Kubo M, But PP (2002). Antipruritic and antinociceptive effects of *Chenopodium album* L in mice. *J. Ethnopharmacol.* 81(2):245-50.
- Di LC, Costa M, Mendacoli SJL, Kirizawa M, Gomes C, Trollin G (1988). Screening in mice of some medicinal plants used for analgesic purposes in the state of Sao Paulo. *J. Ethnopharmacol.* 24: 205-211.
- Greca MD, Di Marino C, Zarrelli A, D'Abrosca B (2004). Isolation and phytotoxicity of apocarotenoids from *Chenopodium album*. *J. Nat. Prod.* 67(9):1492-1495.
- Ibironke GF, Ajiboye KI (2007). Studies on the anti-inflammatory and analgesic properties of *Chenopodium ambrosioides* leaf extract in rats. *Int. J. Pharmacol.* 3(1):111-115.
- Ibrahim LF, Kwashty SA, Baiuomy AR, Shabana MM, El-Eraky WI, El-Negoumy SI (2007). A comparative study of the flavonoids and some biological activities of two *Chenopodium* species. *Chem. Nat. Compd.* 43(1):24-28.
- Jabbar A, Zaman MA, Iqbal Z, Yasmeen M, Shamim A (2007). Anthelmintic activity of *Chenopodium album* (L.) and *Caesalpinia crista* (L.) against trichostrongylid nematodes of sheep. *J. ethnopharmacol.* 114(1):86-91.
- Khan UG, Saeed A, Alam MT (1997). *Indusynic Medicine*, Univ. Karachi Press, Karachi, Pakistan. pp. 591.
- Kumar S, Kumar D (2009). Antioxidant and free radical scavenging activities of edible weeds. *Afr. J. Food Agric. Nutr. Dev.* 9(5):1174-1190.
- Kumar S, Biswas S, Banerjee S, Mondal NB (2011). Evaluation of safety margins of *Chenopodium album* seed decoction: 14-daysubacute toxicity and microbicidal activity studies. *Reprod. Biol. Endocrinol.* 9:102.
- Kumar S, Biswas S, Banerjee S, Mandal D, Roy HN, Chakraborty S, Kabir SN, Banerjee S, Mondal NB (2007). *Chenopodium album* seed extract: a potent sperm-immobilizing agent both *in vitro* and *in vivo*. *Contraception.* 75(1):71-78.
- Liu R, Li A, Sun A, Kong L (2004). Preparative isolation and purification of psoralen and isopsoralen from *Psoralea corylifolia* by high-speed counter-current chromatography. *J. Chromatogr.* 1057:225-228.
- Nadkarni KM (1976). *The Indian Materia Medica*. Popular Prakashan, Bombay.
- Nedialkova ZK, Nedialkov PT, Nikolov SD (2009). The genus *Chenopodium*: Phytochem. *Ethnopharmacol. and pharmacol. Pharmacogn. Rev.* 3(6):280-306.
- Okuyama E, Umeyama K, Saito Y, Yamazaki M, Satake M (1993). Ascaridole as a pharmacologically active principle of Paico, a medicinal Peruvian plant. *Chem. Pharm. Bull.* 41(7):1309-1311.