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Research Article

Assessment of the Extracts of *Centaurea tchihatcheffii* Fischer for Anti-inflammatory and Analgesic Activities in Animal Models

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Abstract

Purpose: *To evaluate the ethanol extracts of the flowers, leaves, and stems of Centaurea tchihatcheffii Fischer & C.A. Meyer (Asteraceae) for their anti-inflammatory and analgesic activities in male Swiss albino mice.*

Methods: *For the evaluation of anti-inflammatory activity, hind paw oedema was induced in the mice with carrageenan and prostaglandin-E_2 (PGE_2) and the mice received either 100 and 200 mg/kg body weight doses of the flower extract or 200 mg/kg body weight dose of the leaf and stem extracts. Furthermore, ear oedema was induced in other groups of mice with 12-O-tetradecanoyl-13-acetate (TPA) and then administered with 0.5 mg/ear dose of the extract of either of the three plant parts. In order to evaluate analgesic activity, p-benzoquinone-induced abdominal constriction test was used with 100, 200 and 400 mg/kg body weight doses of the flower or 200mg/kg body weight dose of the leaf and stem extracts administered. Indomethacin and acetylsalicylic acid were the reference drugs for anti-inflamatory and analgesic evaluations, respectively. Phytochemical screening of the flower extract was carried out by thin layer chromatography (TLC).*

Results: *The results of evaluation of the anti-inflammatory activities induced by carrageenan and PGE_2 showed that the flower extract diminished cyclo-oxygenase activitiy at the 200 mg/kg dose to the same level as the reference drug, indomethacin. However, no anti-inflamatory activity was seen in the TPA-induced ear oedema model. The extracts from all three parts of the plant showed analgesia in p-benzoquinone-induced abdominal constriction test. TLC analysis of the flower extract indicated the presence of sesquiterpen lactones, which may have been responsible for the analgesic activity.*

Conclusion: *Our results support the use of C. tchihatcheffii in traditional medicine in Turkey for their anti-inflammatory and analgesic properties.*

Keywords: *Centaurea tchihatcheffii, leaves, flowers, stems, anti-inflammatory, analgesic.*

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INTRODUCTION

The Mediteranean region is highly rich in *Centaurea* (Asteraceae) species. Although, more than 700 species are found in the region, only about 178 of them grow in Turkey[1]. Besides their attractive flowers, many of the *Centaurea* genuses have significant applications in traditional medicine. For instance, the dried flowers of *Centaurea cyanus* L. are used as a 5% infusion to ease the symptoms of diarrhoea, as well as to gain energy, increase appetite, and relieve chest tightness[2-3]. *Centaurea behen* L. is used for alleviating stomach problems and initiating menstruation[4] while *Centaurea calcitrapa* L. is used as a 2-6% infusion for the relief of fever and *Centaurea iberica* Trev. ex Spreng for relieving abdominal pains and as a remedy for insect and snake bites[4]. Also, most of the *Centaurea* species have been used individually or in combination with some plants for the folkloric treatment of diabetes, rheumatism and inflammation[5-6]. In addition to these traditional uses, the species have also found use for their choleretic, digestive,, diuretic, astringent, hypotensive, antipyretic, cytotoxic, and antibacterial effects[7-10].

In this study, the anti-inflammatory and analgesic properties of *C. tchihatcheffii* Fischer & C.A. Meyer have been evaluated using rodent models.

MATERIALS AND METHODS

Plant material and preparation of extracts

C. tchihatcheffii plant samples were collected from the Gölbasi district of Ankara during the month of May, 2007. The plant was authenticated by Prof. Dr. Mecit Vural of the Department of Biology, Faculty of Art and Science, Gazi University, Ankara, Turkey. A specimen of the original collection was placed in the herbarium of the Faculty of Pharmacy, Gazi University, Ankara, Turkey (Herbarium no. 2592).

The plant materials, comprising of leaves, flowers and stems were air-dried at room temperature and powdered by using a pestle and mortar. Thereafter, 10 g each of the leaves, flowers, and stems of *C. tchihatcheffii* was extracted separately with 3 x 200 ml 80% ethanol (EtOH) at room temperature overnight. In each case, the pooled extract was evaporated to dryness *in vacuo* using a rotary evaporator at 40 ºC to give a yield of 38.5 % (flowers), 39.2 % (leaves) and 35.0 % (stem).

Drugs and chemicals

Carrageenan (Sigma, St. Louis, Missouri, USA), PGE_2 (Fluka Chemie AG), 12-*O*-tetradecanoyl-13-acetate (TPA) (Sigma-Aldrich, St.Louis, USA), *p*-benzoquinone (Merck), indomethacin (Bayer AG), acetylsalicylic acid (Bayer AG), sodium carboxymethylcellulose (Aldrich Steinheim, Germany), absolute ethanol (Merck) were used as drugs and/or chemicals.

The plant extracts were suspended in a mixture of distilled H_2O and 0.5% sodium carboxymethyl cellulose (CMC) and given to the test animals by mouth. The control group received the same treatment as the test groups except that the drug was replaced with an appropriate volume of vehicle. Indomethacin (10 mg/kg and 0.5 mg/ear) and acetylsalicylic acid (ASA; 200 mg/kg) in 0.5% CMC were used as reference drugs.

Animals

Male Swiss albino mice (20-25 g) were purchased from the animal breeding laboratories of Refik Saydam Central Institute of Health, Ankara, Turkey. The animals were left for two days, to acclimatize to ambient conditions, maintained on standard pellet diet and given water *ad libitum*. The food was withdrawn on the day before the experiment; however, they were allowed free access to water. Throughout the experiments, the animals were handled according to the prescribed ethical guidelines of Gazi

University Ethical Council Project Number: G.Ü.ET-05.004 for the care of laboratory animals.

In preliminary experiments, 200mg/kg dose extract of either the flowers, leaves or stems were tested on the animals. At this dose, the leaf and stem extracts were not effective except for the flower extract. Therefore, a dose - response was established by administering half and twice the above dose, respectively.

Anti-inflammatory tests

Carrageenan-induced hind paw oedema model

Carrageenan-induced hind paw oedema model was used for the determination of anti-inflammatory activity[11]. Six animals were used for each extract dose, as well as the control and reference groups. For flower extract,, 100 and 200 mg/kg doses were administered while for the stem and leaf extract, only 200 mg/kg dose was given one hour after oral administration of the extract/drug or vehicle, the subplantar tissue of right hind paw of each mouse was injected (Hamilton® microsyringe) with 25 µl of 20 mg/ml of freshly prepared carrageenan in physiological saline (0.9% NaCl). For control purposes, 25 µl of saline was injected into subplantar tissue of left hind paw. Thereafter, paw edema was measured at 1.5 h interval for 6 h. The difference of thickness in footpad was measured with calipers (Ozaki Co., Tokyo, Japan). Indomethacin (10 mg/kg) was used as the reference drug.

PGE_2-induced hind paw oedema model

PGE_2-induced hind paw edema model was also used for the determination of anti-inflammatory activity following the method described by Kasahara et al[12]. Six animals per group were were given either an extract, control and reference drug (indomethacin, 10 mg/kg). The extract dose was 200 mg/kg for each of the plant extract. One hour after oral administration of an extract, drug or vehicle (control), each mouse received 5 µl of freshly prepared suspension of PGE_2 (1 mg/ml) in Tyrode's solution by injection into the subplantar tissue of the right hind paw except that for control, 5 µl of Tyrode's solution was injected into the left hind paw. Thereafter, paw edema was measured at 15 min interval for 75 min. The difference in the thickness of the footpad was measured as earlier described.

TPA-induced mouse ear oedema

Each mouse received 20 µl of 125 µg/ml of TPA dissolved in 70% EtOH[13]. Six animals were used for each group, namely, each of the extracts, control and reference groups. For the extracts and reference drug, 0.5 mg/ear dose was applied by a pipette to both anterior and posterior surfaces of the right ear. The same volume of solvent (70% EtOH) was applied to the left ear which served as control. Anti-inflammatory activity was determined by two different methods. First, the thickness of each ear was measured 4 h after induction of inflammation with calipers and the level of oedema was expressed as the difference in thickness between the right and left ears. Inhibition (%) was expressed as a reduction in thickness with respect to the control. In the second method, the animals were killed under deep ether anesthesia 4 h after drug/extract administration. Discs of 6 mm diameter were removed from each ear and weighed. The swelling was estimated as the difference in weight between the discs cut from the right and left ears, which was expressed as increase in ear thickness.

Analgesic test

p-Benzoquinone - induced abdominal constriction test[14] was performed on mice in order to determine analgesic activity. Six animals were used for each of the following groups: each extract, control (distilled water) and reference drug (ASA, 200 mg/kg). For the extracts, the doses administered were 100, 200, 400 mg/kg doses (flower) and 200

mg/kg (leaf and stem). One hour after oral administration of an extract, drug, or reference drug, the mice were given intraperitoneal injections of 0.1 ml/10 g body weight of 2.5% w/v of solution of *p*-benzoquinone (PBQ) in distilled water. The mice were then observed individually 5 min after PBQ injection for the number of abdominal contractions (writhing movements) for a period of 15 min.

The mice were killed under deep ether anesthesia and the stomach of each mouse was removed, opened through the greater curvature and examined under a dissecting microscope for lesions or bleedings.

Statistical analysis of data

Data obtained from animal experiments were expressed as mean ± S.E.M (standard error of the mean). Statistical differences between the treated and the control groups were evaluated by ANOVA and Students-Newman-Keuls post-hoc tests. $p<0.05$ was considered significant.

RESULT

Table 1 shows the dose - response data of the extracts with regard to *p*-benzoquinone-induced abdominal constriction). All the animals died within 2 h following the administration of 400 mg/kg dose of the flower extract but no effect was observed at 100 mg/kg dose. Consequently, 200 mg/kg dose was selected for further experiments. Although the leaf and stem extracts did not exert any meaningful analgesic effect, the flower extract (200 mg/kg dose) significantly inhibited (35.3%) abdominal constriction in the mice. Furthermore, no ulceration was observed in stomach of the animals following the administration of the flower extract.

Flower extracts displayed significant inhibition (24.8-34.0%) at dose of 200 mg/kg also in carrageenan-induced hind paw oedema compared to the other extracts (Table 2). In this regard the inhibitory effect of the flower extract (34%) was close to that of indomethacin (38.7%) after 270 min.

The results of the anti-inflammatory effect of the extracts on hind-paw oedema induced by prostaglandin E_2 are indicated in Table 3. This time measurements were kept between 0-60 min. with 15 min intervals. The extract of flowers considerably inhibited (23.2 - 27.2 % inhibition) hind-paw oedema while the leaf and stem extracts did not demonstrate a notable activity (Table 3). Maximum inhibition (27.2 %) by the flower extract was observed after 45 min which decreased thereafter. The reference drug (indomethacin) presented a similar trend, peak at 38% inhibition after 45 min.

As shown in Table 4, while indomethacin exhibited 74.5% inhibition on swelling thickness and 38.9% inhibition on weight edema, the flower extract displayed only 20.6 and 12% inhibition, respectively, thus indicating that the plant parts did not show remarkable anti-inflammatory activity in the TPA-induced experimental model.

DISCUSSION

Subcutaneous injection of carrageenan into the rat paw produces plasma extravasation and inflammation that are characterized by elevated tissue water and plasma protein exudation with neutrophil extravasation, in addition to increased metabolism of arachidonic acid by both cyclo-oxygenase and lipoxygenase enzyme pathways[15-16]. There are biphasic effects in carrageenan-induced oedema. The first phase begins immediately after injection and diminishes in 1 h. The second phase begins in an hour and remains through 3 h [17]. It is suggested that the early hyperemia of carrageenan-induced oedema results from the release of histamine and serotonin[18]. On the other hand, the delayed phase of carrageenan-induced oedema results mainly from the potentiating effect of prostaglandins on mediator release, particularly bradykinin. Hydrocortisone and

Table 1: Effect of the extracts of various parts of *C. tchihatcheffii* on p-benzoquinone-induced writhings in mice

Extract/ Drug	Dose (mg/kg)	Number of writhings ± SEM	Inhibition ratio (%)	Ratio of ulceration
Control	-	42.2 ± 4.8		0/6
Leaf	200	44.3 ± 3.3	-	2/6
	100	39.3 ± 2.7	6.9	0/6
Flower	200	27.3 ± 2.4**	35.3	0/6
	400	45.2 ± 1.4	----	[a]
Stem	200	52.3 ± 1.9	----	1/6
ASA	200	19.3 ± 2.4***	54.3	4/6

**:p<0.05. **:p<0.01. ***:p<0.001 (compared to control, n = 6)*
[a] The animals died in 2 h; therefore, ratio of ulceration could not be determined

Table 2: Effect of the extracts of different parts of *C. tchihatcheffii* on carrageenan-induced paw edema in mice

Extract/ Drug	Dose (mg/kg)	90 min		180 min		270 min		360 min	
		Swell. thick. ± SEM	Inh. %	Swell. thick. ± SEM	Inh. %	Swell. thick. ± SEM	Inh. %	Swell. thick. ± SEM	Inh. %
Control		43.2 ± 3.9	0	55.2 ± 3.5	-	60.5 ± 2.3	-	64.3 ± 4.5	
Leaf	200	40.8 ± 3.7	5.6	49.8 ± 2.3	9.8	54.8 ± 3.1	9.4	60.2 ± 3.1	6.4
Flower	100	41.7 ± 4.3	3.5	51.6 ± 4.9	6.5	59.1 ± 3.6	2.3	61.2 ± 4.7	4.8
	200	34.7 ± 3.2	19.7	41.5 ± 2.1	24.8*	39.9 ± 4.1	34.0**	44.2 ± 2.2	31.3**
Stem	200	40.5 ± 4.5	6.3	51.2 ± 3.2	7.2	58.2 ± 4.9	3.8	55.3 ± 3.4	13.9
Indomethacin	10	34.0 ± 3.1	21.3	35.3 ± 1.9	36.1**	37.1 ± 2.1	38.7***	39.9 ± 1.8	37.9***

Swell. thick.= Swelling thickness (x10^{-2} mm); SEM = standard error mean; Inh. = Inhibiton.
**:p<0.05. **:p<0.01. ***:p<0.001 (compared to control, n = 6)*

Table 3: Effect of the extracts of *C. tchihatcheffii* on PGE_2-induced paw edema in mice

Extract/ Drug	Dose (mg/kg)	0 min		15 min		30 min		45 min		60 min		75 min	
		Swell. thick. ± SEM	Inh. %	Swell. thick. ± SEM	Inh. %	Swell. thick. ± SEM	Inh. %	Swell. thick. ± SEM	Inh. %	Swell. thick. ± SEM	Inh. %	Swell. thick. ± SEM	Inh. %
Control		1.5±0.9	-	11.4±1.5	-	20.3±1.9	-	27.6±2.3	-	25.9±2.5	-	19.7±1.6	-
Leaf	200	1.6±0.7	-	15.8±1.1	-	24.7±1.4	-	29.5±1.7	-	31.7±2.4	-	27.6±1.8	-
Flower	200	1.5±0.8	-	13.1±1.3	-	15.6±1.1	23.2*	20.1±1.4	27.2*	20.1±1.2	19.7	17.6±1.4	10.7
Stem	200	1.7±0.9	-	10.2±1.4	10.5	19.7±1.6	2.9	29.2±2.3	-	27.6±2.7	-	20.3±2.1	-
Indome-thacin	10	1.5±0.6	-	9.8±1.3	14.0	13.9±1.4	31.5**	17.1±1.3	38.0***	20.4±1.1	21.2*	16.8±1.5	14.7

Swell. thick.= Swelling thickness (x10^{-2} mm); SEM = standard error mean, Inh. = Inhibition.
**:p<0.05. **:p<0.01. ***:p<0.001 (compared to control, n = 6);*

Table 4: Effect of the extracts of *C. tchihatcheffii* against TPA-induced ear edema in mice

Extract	Dose (mg/ear)	Swelling thickness (µm) ± S.E.M	Inhibition %	Weight edema (mg) ± S.E.M	Inhibition %
Control	-	211.9 ± 40.7		31.6 ± 7.3	
Folia	0.5	199.4 ± 28.9	5.9	35.1 ± 6.5	---
Flowers	0.5	168.2 ± 31.5	20.6	27.8 ± 5.1	12.0
Stem	0.5	236.7 ± 45.2	---	37.5 ± 8.3	---
Indomethacin	0.5	54.1 ± 23.0	74.5***	19.3 ± 5.2	38.9***

:p<0.05.:p<0.01. ***:p<0.001 ; S.E.M: standard error mean*

some NSAIDs inhibit strongly the second phase of carrageenan-induced oedema[19]. Flowers of *C. tchihatcheffii* seemed effective in both phases of acute inflammation. Therefore, *C. tchihatcheffii* may block prostaglandin and/or bradykinin release, and histamine and/or serotonin.

Although the TPA model has limited selectivity in determining the possible mechanism of action, it is appropriate for assessing anti-inflammatory effects in a first stage trial. It has been established that this agent exerts its inflammatory effect through protein kinase C activation with the subsequent cytosolic phospholipase A_2 stimulation, AA mobilization, and biosynthesis of prostaglandins and leukotrienes[20].

In contrast with the results obtained in the carrageenan-induced hind paw oedema model, the flower extract failed to inhibit ear oedema. This may be due to the different mechanisms of action of the phlogistic agents or to the fact that the systemic route of administration failed to achieve adequate concentration of the compounds at the site of inflammation.

In a earlier study, 2-methylpropanoate and 2-methyl-2-propenoate of 11, 13-dehydro-melitensin, were isolated from the aerial parts of *C. chilensis*. The mixture of both substances exhibited anti-inflammatory activity in the carrageenan-induced paw edema assay[21]. A 20 % infusion, administered orally at a dose of 4 ml/kg, exhibited a mild inflammatory activity on laboratory animals while 400 and 600 mg/kg doses of the methanol extract with propylene-glycol as the solvent produced 29 and 49% inhibition, respectively. Naproxen-sodium (4.3 mg/kg), used as a reference, showed 58% inhibitions. According to these results, the extracts of *C. chilensis* were at least as effective as other synthetic inflammatory compounds due to the presence of sesquiterpene lactones (notably α-methylene-γ-lactone) but less effective than naproxen.

The presence of orally effective anti-inflammatory sesquiterpene lactones clarifies the traditional use of these plants to heal inflammatory diseases[18]. These results are valuable since *C. chilensis* is from the same family as *C. tchihatcheffii* and might contain the compounds. Our unpublished chromatographic studies on the flower extracts of *C. tchihatcheffii* show that it contains sesquiterpene lactones.

Garbacki et al studied the anti-inflammatory properties of polysaccharides extracted from *C. cyanus* flower heads[22]. These polysaccharides were found to be primarily composed of galacturonic acid, arabinose, glucose, rhamnose and galactose. Fractions, soluble only in water, as well as those soluble in both water and ethanol were effective against carrageen-induced oedema; however, the fraction, which was insoluble in ethanol, was more effective. This fraction showed 69% inhibition of carrageen-induced oedema at a

dose of 60 mg/kg and 52% inhibition of croton oil-induced edema at 800 µg/ear topical administration[22].

A study has asserted that the anti-inflammatory effect of the compound, centaureidin, is the result of inhibition of the activities related to lipoxygenases and cyclo-oxygenase in the path[7]. Another study has demonstrated the anti-inflammatory effects of *C. hierapolitana*, *C. calolepis* and *C. cadmea in vitro*. The extracts of these plants exhibited strong anti-inflammatory activities by inhibiting their effects on the activation of the transcription factor, NF-kappa B [23]. These authors also declared that a good number of *Centaurea* species frequently contain sesquiterpene lactone derivatives and flavonoid derivatives and thus their activity might come from the effects of these phytochemicals.

CONCLUSION

The analgesic and anti-inflammatory activities of leaf and stem extracts of *C. tchihatcheffii* were found to be statistically insignificant but the flower extract showed these significant biological activities without inducing any apparent acute toxicity as well as gastric damage at a dose of 200 mg/kg body weight. This suggests that *C. tchihatcheffii* may reduce the risk of inflammation-related diseases and thus supports the folkloric of most of the *Centaurea* species for inflammatory conditions. However, further studies need to be conducted in order to clarify which constituent(s) of the extract is/are responsible for these activities.

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REFERENCES

1. Seçmen Ö, Gemici Y, Görk G, Bekat L, Leblebici E. Systematics of Seed Plants. Bornova, İzmir, Turkey, Aegean University Press, 1995, pp 301-396, (In Turkish).
2. Wagenitz G. Centaurea L. In: Flora of Turkey and The East Aegean Islands, Ed. Davis, P.H V, Edinburgh, Edinburgh University Press, 1975, pp 465-586.
3. Baytop T. Therapy with Medicinal Plants in Turkey (Past and Present), 2nd Edition, Istanbul, Nobel Tıp Basımevi, 1999, pp.316.
4. Erol MK, Tuzlacı, E. Plants used in folk medicine in the region of Eğirdir (Isparta) "XI.th Meeting on plant drug active constituents' Ed. Coşkun, M, Ankara University Pharmacy Faculty No:75, Ankara, Ankara Universitesi Basımevi, 1997, pp. 466-475.
5. Chucla MT, Lamela M, Gato A, Cadavid I. Centaurea corcubionensis: A study of its hypoglycemic activity in rats. Planta Med, 1988; 107-109.
6. Talhouk RS, El-Jouni W, Baalbaki R, Gali-Muhtasib H, Kogan J, Talhouk AN. Anti-inflammatory bio-activities in water extract of Centaurea ainetensis. J Med Plants Res, 2008; 2:24-33.
7. Orallo F, Lamela M, Camina M, Uriatre E, Calleja M. Preliminary study of the potential vasodilator effects on rat aorta of centaurein and centaureidin, two flavonoids from Centaurea corcubionensis. Planta Med, 1998; 64:116-119.
8. Barrero AF, Herrador MM, Arteaga P, Cabrera E, Rodriguez-Garcia I, Garcia-Moreno M, Gravalos DG. Cytotoxic activity of flavonoids from Carthamus arborescens, Ononis natrix ssp. ramosissima and Centaurea malacitana. Fitoterapia, 1997; 68:281-283.
9. Gürkan E, Sarıoğlu I, Oksüz S. Cytotoxicity assay of some plants from Asteraceae. Fitoterapia, 1998; 69:81-82.
10. Wei HX, Gao WY, Tian YK, Guan YK, Huang MH, Cheng DL. New eudesmane sesquiterpene and thiophene derivatives from the roots of Rhaponticum uniflorum. Pharmazie, 1997; 52:245-247.
11. Yeşilada E., Küpeli E. Berberis crategina DC. Root exhibits potent anti-inflammatory, analgesic and febrifuge effects in mice and rats. J Ethnopharmacol, 2002; 79: 237-248.
12. Kasahara Y, Hikino H, Tsurufiji S, Watanabe M, Ohuchi K. Antiinflammatory actions of ephedrines in acute inflammations. Planta Med, 1985; 51:325-331.
13. De Young LM, Kheifets JB, Ballaron SJ, Young JM. Edema and cell infiltration in the phorbol ester treated mouse ear are temporally separate and can be differently modulated by pharmacologic agents. Agents and Action, 1989; 26:335-341.
14. Okun R, Liddon SC, Lasagnal L. The effect of aggregation, electric shock and adrenergic bloking drugs on inhibition of the "writhing

syndrome". J Pharmacol Exp Ther, 1963; 139:107-109.

15. *Szolcsanyi J, Helyes Z, Oroszi G, Nemeth J, Pinter E. Release of somatostatin and its role in the mediation of the anti-inflammatory effect induced by antidromic stimulation of sensory fibres of rat sciatic nerve. Br J Pharmacol, 1998; 123:936-942*
16. *Gamache DA, Povlishock JT, Ellis EF. Carrageenan induced brain inflammation, characterization of the model. J Neurosurg, 1986; 65:679-685.*
17. *Garcia-Pastor P, Randazzo A., Gomez-Paloma L, Alcaraz MJ, Paya M. Effects of petrosaspongiolide M, a novel phospholipase A2 inhibitor, on acute and chronic inflammation. J Pharmacol Exp Ther, 1999; 289:166-172.*
18. *Negrete R, Backhouse N, Cajıgal I, Delporte C, Cassels BK, Breitmaier E, Eckhardt G. Two new antiinflamatory elemanolides from Centaurea chilensis. J Ethnopharmacol, 1993; 40:149-153.*
19. *Kulkarni SK, Mehta AK, Kunchandy J. Anti-inflammatory actions of clonidine, guanfacine and B-HT 920 against various inflammagen-induced acute paw oedema in rats. Arch Int Pharmacodyn Ther, 1986; 279:324-334.*
20. *Nishizuka Y. The role of protein kinase C in cell surface signal transduction and tumour promotion. Nature, 1988; 334:661-665.*
21. *Negrete R, Backhouse N, Avendano S, San Martin A. Dehydrocostus lactone and 8β-hydroxydehydrocostus lactone in Centaurea chilensis Hook and Arn. Planta Med, 1984; 18:226-232.*
22. *Garbacki N, Gloaguen V, Damas J, Bodart P, Tits M, Angenot L. Anti-inflamatory and immunological effects of Centaurea cyanus flower-heads. J Ethnopharmacol, 1999; 68:235-241.*
23. *Karamenderes C, Konyalioglu S, Khan S, Khan I. Total phenolic contents, free radical scavenging activities and inhibitory effects on the activation of NF-kappa B of eight Centaurea L. species. Phytother Res, 2007; 21:488-491.*