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EVALUATION OF ANTI-INFLAMMATORY POTENTIAL OF PETAL EXTRACTS OF *CROCUS SATIVUS* "CASHMERIANUS"

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ABSTRACT

Different petal extracts of *Crocus sativus "*Cashmerianus*"* were examined for anti-inflammatory activity by *in-vitro* human red blood cell (HRBC) membrane stabilization method and *in-vivo* carrangeenan- induced rat paw edema. The petroleum ether, chloroform, ethyl acetate, methanol and aqueous extracts of petals showed 49.36%, 59.39%, 48.10%, 70.37% and 68.89% protection at the dose of 400mg/ml respectively by *in-vitro* method. However, in-vitro most promising effective chloroform, methanol and aqueous extracts were selected for *in-vivo* anti-inflammatory method. The *in-vivo* anti-inflammatory activity was found as 50%, 63.16% and 57.89% inhibition of paw volume after 5h of chloroform, methanol and aqueous extracts treated at the dose of 400mg/kg groups respectively. The potency of the petals extracts were compared with Diclofenac (10 mg/ml) for *in-vitro* and 10mg/kg for *in-vivo* model. The methanol and aqueous extract showed the most promising anti-inflammatory activity in membrane stabilizing action on human red blood cell membrane and reduction of edema in carrageenan induced rat paw edema model. The results of present and first time reporting this study on petals extracts of *Crocus sativus "*Cashmerianus*"* demonstrate that extracts of petals possess significant ($p<0.05$) anti-inflammatory potential.

Keywords: *Crocus sativus*, anti-inflammatory, carrageenan, Human red blood cell membrane stabilization method.

Introduction:

Crocus sativus L. (Iridaceae) is commonly has known as kesar (Hindi), avarakta (Sanskrit), zaffran (Kashmiri) and saffron (English). It comprises of the dried red stigma with a small portion of the yellowish style attached. It is cultivated in Azerbaijan, France, Greece, Iran, Italy, Spain, China, Israel, Morocco, Turkey, Egypt, Mexico and Kashmir in India (Zargari, 2007). The method for the cultivation of saffron contributes greatly to its high price. The value of saffron is determined by the existence of three main secondary metabolites: crocin, picrocrocin, and safranal (Negbi, 1999). It is used in folk medicine as antispasmodic, carminative, stomachic, expectorant, aphrodisiac, cardiotonic and stimulant (Abe *et al*., 2000). In traditional medicine this plant is utilized as an exhilarant and curative of anxiety (Salomi *et al*., 1991). It has been recently reported that ethanol extract of Saffron petals possesses antidepressant activity (Salomi *et al*., 1991). Phenolic compounds are likely to be the biologically active components of the petals (Karimi *et al*., 2001). Flavanoids and anthocyanins are among the phenolic compounds of this species (Garrido *et al*., 1987).

Inflammation is a normal protective response to tissue injury and involves a complex array of enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown and repair (Vane *et al*., 1995) which are aimed at host defense and usually activated in most disease conditions. The critical role of inappropriate inflammation is becoming accepted in many diseases that affect man, including cardiovascular diseases, autoimmune disorders, neurodegenerative conditions, infections and cancer (Mariotti *et al*., 2004).

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In appreciating the inflammatory process, it is important to understand the role of chemical mediators. These are the substances that tend to direct the inflammatory response. These inflammatory mediators come from plasma proteins or cells including mast cells, platelets, neutrophils and monocytes/macrophages. They are triggered by bacterial products or host proteins. Chemical mediators bind to specific receptors on target cells and can increase vascular permeability and neutrophil chemotaxis, stimulate smooth muscle contraction, have direct enzymatic activity, induce pain or mediate oxidative damage. Most mediators are short-lived but cause harmful effects. Examples of chemical mediators include vasoactive amines (histamine, serotonin), arachadonic acids (prostaglandins, leukotrienes) and cytokines (tumor necrosis factor and interleukin–1) (Smith *et al*., 2004). In this work the various extracts of the petals of *Crocus sativus "*Cashmerianus*"* of Kashmir valley were studied for its anti-inflammatory activities using both *In-vitro* and *In-vivo* models.

MATERIAL AND METHODS

Plant Material

The petals of *Crocus sativus "*Cashmerianus*"* were collected from Pampore area of Kashmir (J&K, India). *C. sativus* L. was properly identified by Dr. A.R. Naqshi, Taxonomist, Department of Pharmaceutical Sciences, University of Kashmir, Srinagar. The collected petals were shade dried and coarsely powdered. The coarse powder was subjected to continuous extraction in a soxhlet apparatus separately using petroleum ether, chloroform, ethyl acetate, methanol and water as solvents.

Animals

Albino rats (Wistar strain) of either sex (180-200 g) were obtained from the animal house of Indian Institute of Integrative Medicine (IIM), Jammu. Animals were kept under the laboratory conditions (25 ± 2°C, 12 h light). They were provided with standard rodent pellets diet. Food was withdrawn 12 h before the experimental work and water was provided *ad libitum*. After a 7 days of acclimatization period, animal were randomly selected for different experimental groups (6 animal/ group) and used for the *In vivo* determination of anti-inflammatory activity. This Institution is approved for carrying out animal studies (Approval No.801/03/ca/CPCSEA) and the protocol for the present study was approved by Institutional Animal Ethical Committee [Approval no. F-IAEC (Pharm. Sc.) APPROVAL/2011/02].

METHOD

In-vitro anti-inflammatory activity

The human red blood cell (HRBC) **membrane stabilization method** (Gandhisan *et al*, 1991)

The blood was collected from healthy human volunteer who had not taken any NSAIDS for 2 weeks prior to the experiment and mixed with equal volume of Alsever solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl) and centrifuged at 3,000 rpm. The packed cells were washed with isosaline and a 10% suspension was made. Various concentrations of extracts were prepared (200 and 400 µg/ml) using distilled water and to each concentration 1 ml of phosphate buffer, 2 ml hyposaline and 0.5 ml of HRBC suspension were added. It was incubated at 37^0C for 30 min and centrifuged at 3,000 rpm for 20 min. and the hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm. Diclofenac (50 and 100 µg/ml) was used as reference standard and a control was prepared by omitting the extracts.

The percentage of HRBC membrane stabilization or protection was calculated by using the following Formula,

$$\% \text{ Protection} = 100 - \frac{\text{Optical density of drug treated sample}}{\text{Optical density of control}} \times 100$$

Acute toxicity studies (Ecobichon, 1987): Acute toxicity study was carried out for different petals extract using Acute Toxic Class Method as described in OECD (Organization of Economic Co-operation and Development) Guidelines No.423. The petals extract was safe up to a dose of 4,000 mg/kg body weight, hence 200 mg/kg and 400 mg/kg were used as moderate dose for the evaluation.

***In-vivo* anti-inflammatory activity** (Ramprasath *et al*., 2004): Anti-Inflammatory activity was evaluated using the carrangeenan induced rat paw edema. The rats were divided into five groups (n = 6). Acute inflammation was produced by the subplantar administration of 50 µl of 1% carrageenan in normal saline in the right paw of each rat. Group I served as control, Group II, III,IV received chloroform extract, methanol extract, aqueous extract at the dose of 200and 400 mg/kg each separately and Group V served as standard (diclofenac 50 mg/kg) administered 1 h before the injection of carrageenan. The volume of the paw was measured 1 h before the injection and at 1, 3 and 5 hrs after the injection of carrageenan. Edema was expressed as the increment in paw thickness due to carrageenan administration.

Percent inhibition of edema volume between treated and control group was calculated as follows:

$$\text{Percent inhibition} = \frac{Vc - Vt}{Vc} \times 100$$

Where, Vc and Vt represent mean increase in paw volume in control and treated groups respectively.

Results

The results are reported in table 1. The petroleum ether, chloroform, ethyl acetate, methanol and aqueous extracts of the petals of *Crocus sativus "*Cashmerianus*"* were studied for *in-vitro* anti-inflammatory activity by HRBC membrane stabilization method. The anti inflammatory activity of the extracts was concentration dependent as the activity increased with corresponding increase in drug concentration. Among all the extracts, methanol extract at a concentration of 400 mg/ml showed 70.37% protection ($^{**}p < 0.01$) than of HRBC in hypotonic solution. All the results were compared with standard diclofenac at 10 mg/ml which showed 79.26% protection.

Effects of petal extracts on carrageenan-induced paw edema in rats

The results are reported in table 2. The anti-inflammatory effects of petal extracts were evaluated by carrageenan-induced paw edema in rats that was used an acute model of inflammation. The *in-vivo* data of the experiment have been statistically analyzed by ANOVA. Treatment with different extract showed significant difference on paw edema inhibition when compared with control group exhibited a potent inhibition on paw edema at 1 hour and 5 hours, respectively (Table.2). Therefore, we suggested the on-set time of paw edema inhibition from the methanol extract was more quickly than chloroform extract. The data (Table.2) demonstrated that methanol extract has more anti-inflammatory activity than chloroform and aqueous extract in vivo.

Table 1. *In-vitro* anti- inflammatory activity of petal extracts of *Crocus sativus* "Cashmerianus"

S.No.	Groups	Concentration (mg/ml)	(%Protection) Mean±S.E.M
1	Control	-	-
2	Petroleum ether extract	200 400	44.00 ±2.21 49.63 ±1.65
3	Chloroform extract	200 400	51.31 ±1.67 59.39 ±1.70
4	Ethyl acetate extract	200 400	41.08 ±1.00 48.10 ± 2.28
5	Methanol extract	200 400	67.29 ±2.3 70.37±1.27[a]
6	Aqueous extract	200 400	63.70 ±1.31 68.89 ±1.23[a]
7	Diclofenac	10	79.26 ±1.40[a]

The results were expressed as mean˙ ± S.E.M¨ [n=6], a ($p< 0.05$).

Table 2. *In vivo* anti-inflammatory activity of petal extracts of *Crocus sativus* "cashmerianus" on carrageenan induced paw edema in rats

Group	Drug mg/kg	Mean paw volume (ml) at time (hours)			% Inhibition after at time (hours)		
		1	3	5	1	3	5
Control	0.5% CMC	0.19 ± 0.04	0.32 ± 0.01	0.38 ± 0.02	-	-	-
Chloroform extract	200	0.18 ± 0.06	0.19 ± 0.02	0.21 ± 0.03	05.26	40.62	44.74
	400	0.17 ± 0.03	0.18 ± 0.04	0.19 ± 0.05	10.53	43.75	50.00
Methanol extract	200	0.17 ± 0.02	0.16 ± 0.01	0.15 ± 0.02	10.52	50.02	60.53
	400	0.16 ± 0.04	0.15 ± 0.04	0.14 ± 0.05[b]	15.79	53.13	63.16[a]
Aqueous extract	200	0.17 ± 0.02	0.18 ± 0.03	0.18 ± 0.04	10.53	43.75	52.63
	400	0.16 ± 0.03	0.17 ± 0.02	0.16 ± 0.03	15.78	46.88	57.89
Diclofenac	10	0.16 ± 0.02	0.13 ± 0.02	0.11 ± 0.02[b]	15.79	59.38	71.05[a]

Values are expressed as mean ± SEM. n=6 animals/ group. Statistical evaluation by one- way ANOVA followed by Dunnett's *t* – test; Symbols statistical significance: b ($p < 0.05$), a ($p< 0.01$).

Discussion

Crocus sativus "Cashmerianus" petal extracts exhibited membrane stabilization effect by inhibiting hypotonicity induced lysis of erythrocyte membrane. The erythrocyte membrane is analogous to the lysosomal membrane (Chou, 1997) and its stabilization implies that the extract may as well stabilize lysosomal membranes. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extra cellular release (Murugasan *et al*., 1981). Some of the NSAIDs are known to posses membrane stabilization properties which may contribute to the potency of their anti-inflammatory effect. Though the exact mechanism of the membrane stabilization by the extract is not known yet; hypotonicity-induced hemolysis may arise from shrinkage of the cells due to osmotic loss of intracellular electrolyte and fluid components. The extract may inhibit the processes, which may stimulate or enhance the efflux of these intracellular component (Rajendran *et al*., 2008) Methanol extract showed significant *in-vitro* anti-inflammatory activity as compared to standard. The methanol extract showed significant anti-inflammatory activity (70.37 %) at the dose of 400 mg/ml. The results of the *in-vivo* evaluation correlated well with the in vitro results with the methanolic extract showing the most promising results. Hence it can be concluded that the petals of *Crocus sativus* "Cashmerianus" have statistically significant anti-inflammatory activity. Further studies are going on to isolate the compound/s responsible for the activity.

Statistical analysis

The data's were expressed as mean ± SEM, statistical analysis was performed by one way ANOVA followed by Dunnett's *t* – test, p values <0.05 were considered as significant.

CONCLUSION

The methanol extracts exhibited membrane stabilization effect by inhibiting hypotonicity induced lysis of erythrocyte membrane. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bacterial enzymes and proteases which cause further tissue inflammation and damage (Murugasan et al., 1981). Since petal extracts showed significant *in-vitro* anti-inflammatory activity it was selected for the evaluation of *in-vivo* anti-inflammatory activity by carrageenan induced paw edema model in rats. The methanol extract showed significant anti-inflammatory activity (63.16%) at the dose of 400 µg/ml while the standard diclofenac showed 71.05% inhibition of edema at dose of 10mg/kg. On the basis of the above results it can be concluded that the methanol extract posses significant anti-inflammatory activity studied by *in-vitro* and *in vivo* models.

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References

Abdullaev FI. Cancer chemopreventive and tumoricidal properties of saffron (*Crocus sativus L.*). *Exp Biol Med*, 227, 2002, 20-25.

Abe K, Saito H. Effects of saffon and its constituent crocin on learning behavior and long-term potentiation. *Phytother Res*, 14, 2000, 149-52.

Chou CT. The anti-inflammatory effect of *Tripterygium wilfordii* Hook F on adjuvant-induced paw edema in rats and inflammatory mediators release. *Phytother. Res*., 11, 1997, 152-54.

Ecobichon DJ. The basis of toxicology testing. CRC Press, New York, 2nd ed.,1997, 43-60.

Gandhisan R, Thamaraichelvan A, Baburaj. Antiinflammatory action of *Lannea coromandelica* HRBC membrane stabilization. *Fitotherapia*, 62, 1991, 82-83.

Garrido-Pol JL, Diez de Bethencourt C, Revilla E. Flavonoids composition of hydrolyzed petal extracts of *Crocus sativus*. *Anal Brometal*, 39, 1987, 69-77.

Isao k, Ikuyo KH. Flavonols from saffron flowers, tyrosinase activity and inhibition mechanism. *J. Agric. Food Chem*, 47, 1999, 4121-4125.

Karimi G, Hosseinzadeh H, Khaleghpanah P. Study of antidepressant effect of aqueous and ethanol extract of *Crocus sativus* in mice. *Iranian J. Basic Sciences*, 4, 2001, 186-190.

Mariotti A. A primer on inflammation. Compend Cont. Edu. Dent., 25 (7) (Suppl. 1), 2004, 7-15.

Mirheidar H. Maarif-e Ghiahi, Farhang-e Eslami Press, Tehran, 1994, 341-345.

Murugasan N, Vember S, Damodharan C. Studies on erythrocyte membrane IV. In vitro haemolytic activity of Oleander extract. Toxicol. Lett, 8, 1981, 33-38.

Negbi M. Saffron cultivation: past, present and future prospects. Saffron *Crocus sativus* L. Amsterdam: *Harwood Academic Publishers*: 1999, 1-19.

Rajendran Vadivu, Lakshmi KS. In vitro and in vivo anti inflammatory activity of leaves of *Symplocos cochinchinensis* (Lour) Moore ssp Laurina. *Bangladesh J. Pharmacol*, 3, 2008, 121-124.

Ramprasath VR, Shanthi P, Sachdanandam P. Anti-inflammatory effect of *Semecarpus anacardium* Linn. Nut extract in acute and chronic inflammatory conditions. *Biol. Pharm. Bull*, 27, 2004, 2028–2031.

Salomi MJ, Nair SC, Panikkar KR. Inhibitory effects of *Nigella sativa* and *Crocus sativus* on chemical carcinogenesis in mice. *Nutr. Cancer*, 16, 1991, 67-72.

Smith GR, Sotiris M. Cancer, inflammation and the AT1 and AT2 receptors. *Journal of Inflammation*, 1, 2004, 3.

Vane JR, Botting RM. New insight into the mode of action of anti-inflammatory drugs. Inflamm Res., 44, 1995, 1-10.

Xuabin N. Research progresses on the saffron crocus (*Crocus sativus*). *Zhongcaoyao*, 23(2), 1992, 100-107.

Xue XH. Cultivation of *Crocus sativus. Chung Yao Tung Pao*: 7(4): 1982, 3-4.

Zargari A. Medicinal Plant. Tehran: Tehran University Press; 2, 1990, 574.

Zargari, A, Medicinal Plants. Tehran University Press, Tehran; 4, 1993, 574-578.