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EVALUATION OF ANTIINFLAMMATORY ACTIVITY OF WATER FRACTION FROM ETHANOLIC EXTRACT OF CROCUS SATIVUS STIGMAS IN RATS

Pharmacology

Dr. Heenu Dhar Assistant Professor, Department of Pharmacology, FMHS SGT University, Gurugram.

Dr. Shalinder Koul* Assistant Professor, Department of Surgery, FMHS SGT University, Gurugram. *Corresponding Author

ABSTRACT

Background: Crocus sativus l (Iridaceae) is geographically rarely distributed flower whose stigmas are used in managing inflammatory disorders. The current study was done to evaluate antiinflimmatory activity of water fraction from ethanolic extract of crocus sativus stigmas. **Material and Methods:** The study was performed by using Wistar albino rats (150-250 gm). Crocus sativus ethanolic extract water fraction (CSEEWF) was prepared by cold maceration extraction procedure followed by column chromatography for fraction separation. The results obtained from CSEEWF were tabulated for anti-inflammatory activity using carrageenan induced paw edema method (acute) and Cotton wool granuloma (subacute) method . **Results:** (ANOVA) was applied for Statistical analysis followed by Dunnett's test $p<0.01$ and $p<0.001$ was considered as statistically significant. The CSEE -WF (200, 400 & 600 mg/kg) revealed antiinflammatory activity in dose reliant form in carrageenan induced paw edema model significantly for CSEE-WF (200, 400 & 600 mg/kg, intraperitoneally and Cotton wool granuloma model (200, 400 & 600 mg/kg) for subacute inflammatory diseases. **Conclusion:** CSEE-WF demonstrates promising anti-inflammatory activity results in dose reliant manner. In both acute and subacute inflammatory models and thus indicate that CSEE-WF may be useful in treating inflammatory diseases.

KEYWORDS

Crocus sativus ethanolic extract water fraction, Anti-inflammatory activity, Carrageenan, Paw edema

INTRODUCTION

Vast majority of patients are diagnosed with disease conditions like Arthritis and inflammation. Inflammation is said to be host defence method to fight, oppose, and surmount the invading pathogens or the foreign particles. Non-steroidal anti-inflammatory drugs (NSAIDs) are largest class of drugs used for pain and inflammation[1].

Along with most prototypical health ailments in the globe for acute and chronic inflammatory disorders, even though quite a lot of agents are known to take care of inflammatory diseases, their long-lasting intake frequently leads to unfavourable situations such as gastric ulcers, Allergic reactions, Liver and Kidney problems, Leg Swelling, bone marrow depression. So there is undoubtedly a call to, identify, categorize, and develop novel anti-inflammatory molecules with diminutive side effects. Inflammation is said to be a standard protective response to tissue injury and it engages a complex array of enzyme activation mediator discharge, fluid extravasations cell migration, tissue breakdown and repair [2]. Furthermore, a state which can be positioned as an intermediate between chronic and acute inflammation, that show signs of some of the characteristics of each, is referred to as sub-acute inflammation [3].

NSAIDs are well thought-out to be the most clinically suitable and essential medicines that are highly functional for the management of inflammation-related diseases such as arthritis, asthma, and cardiovascular disease[4]. Long-standing administration of NSAID may provoke and set off reactions such as gastro-intestinal ulcers, bleeding, Kidney problems tolerance and dependence[5].

Saffron, the dried stigmas of Crocus sativus Linn. (Fam: Iridaceae) is the popular spice used around the world With permanent underground stem bases called bulbs or corms[6] It is used in the folk medicine for various purposes such as an aphrodisiac, antispasmodic and expectorant. The saffron and its coloured carotenoid pigments (Crocin) constitute a high range of medicinal value like Anticancer[7,8] anti-Psoriasis[9] Antitumour,[10]

radical scavenger[11], Nephroprotective[12], anticonvulsant[13] along with positive effect on learning and memory[14, 15].

Novel anti-inflammatory and analgesic drugs that do not have adverse reactions are being tracked all over the world as convinced alternatives.. The research revolving around the herbal resources of alleged should be considered as a beneficial and logically practical strategy in pursuit for forthcoming analgesic and anti-inflammatory drugs[16] .Hence with this prospective into consideration the present research work was designed.

MATERIAL AND METHODS

A) Extract Preparation

I) Crocus Sativus stigma gathering and stigma recognition

Freshly procured *crocus Sativus stigmas* from Kashmir were dried under shade and authincated by Dr.J.K Dhar (quality analyst) IIIM Jammu India.

II) Phytochemical Screening

Crocus stigmas revealed the presence of Flavonoids, glycosides, Phenols, alkaloids, resins, Saponins and steroids.

III) Extraction process of stigmas:

65 grams of crocus sativus stigmas were subjected for extraction by cold maceration process for three successive days using 95% ethanol with periodic shaking after every1 hours.

IV) Preparation of Fractions By chromatography:

The final extract attained was separated Under column chromatography. Different solvents **like** Methanol, Petroleum benzine, chloroform and Distilled water were used according to their increasing polarity, thin layer chromatography technique was carried out for the determination of various active constituents

B) Chemicals and Drugs used: Diclofenac (Reactin 50), carrageenan (Sigma), Dexamethasone from (cadila) and all chemicals were of analytical grade.

C) Experimental Animals: Albino rats of either sex weighting between 150 to 250 grams were randomised. Experimental animals were selected from central animal facility in the group of 5 with 6 albino rats in each group, at an ambient temperature of 25+ with ad libitum access to food and water. The experimental protocol was approved by the Institutional Animal Care and Use Committee (IACUC).

D) A pilot and toxicity study of (CSEEWF) in different doses 15mg/kg, 25mg/kg, 50mg/kg 100mg/kg 200mg/kg was done to estimate dose for the study.

E) Anti-inflammatory activity Experimental models

a) Carrageenan Induced rat paw edema: This test was performed as per the method described by Winter *et al* [17]. Rats were randomised in 5 groups of 6 animals in each. Group I control: was given: 2ml 0.9% normal saline orally Group II was administered: standard drug Diclofenac 10mg/kg intraperitoneally while group III, IV, V were given test drug in the doses of 200,400,600mg/kg i.p respectively. The labelled Albino rats were pre-treated with drugs by intraperitoneal route 1 hour before aseptically injecting 0.1ml of 1% carrageenan into the sub plantar surface of right hind paw. Paw edema was determined by plethysmograph at the duration of 0, 1, 2, 3, 4, and 6 hours . The

difference between 0 and 1, 0 and 2, 0 and 3, 0 and 4 , 0 and 6 , hours signifies the actual edema.

Percentage inhibition against edema formation was taken as an index of acute anti-inflammatory activity.
It was calculated by:**The percentage inhibition of edema = 100x (1-V_t / V_c)**
Where V_c = paw edema control group
V_t = paw edema drug treated group

b) Cotton Pellet induced granuloma Model - winter and Porter[18] Technique was used, for evaluating chronic anti-inflammatory activity in both axilla of each rat two sterile cotton pellets weighting 20mg were implanted by subcutaneous route. Rats were administered the individual drugs Dexamethasone 10mg/kg by oral route and CSEEWF 200,400,600mg/kg orally daily for 7 days along with free access to water and food ad libitum later on rats were sacrificed on day 8 the cotton pellets along with granulation tissue were taken away surgically, along with extraneous tissue was cleaned followed by hot air oven drying to a constant weight and the dry granuloma weight was determined.

Dry weight of granuloma i.e. (amount of actual granulation tissue formed) was Tabulated by following formula

Percent anti granuloma activity was calculated as 100x (1-W_t/W_c)
Where W_t = dry weight of granuloma in drug administered groups and W_c = dry weight of granuloma in drug untreated control group inflamed ear

STATISTICS

Statistical analysis was performed by one way ANOVA method followed by Dunnett's test.
The probability of $p<0.01$and $p<0.001$ was considered statistically significant. For the statistical analysis.

RESULTS

A) Anti-inflammatory activity Carrageenan- Induced Acute Inflammatory Model

Wistar albino rats from groups II, III, IV were administered with the different doses of CSEEWF as a test drug was administered 1 hour earlier to carrageenan injection. Paw volume was Determined at "0 hour" and then at the durations of 1, 2, 3, 4 and 6 hour respectively after carrageenan administration.

Raise in paw edema was calculated as the difference in paw volume at the durations of 0 and 1 2 3 4 and 6 hours respectively. The results represented in Table 1/Fig 1 reveal that water fraction of ethanolic extract from crocus sativus 200mg, 400mg and 600mg and Diclofenac 10mg/kg considerably suppressed carrageenan induced rat paw edema at the finish of six hours when compared with the control in a dose dependent manner. Diclofenac from 55.71 to 87.11 at 4-hour duration. The gap of 6 hours the percentage inhibition was not raised much in test group 600mg/kg dose and standard group. While CSEEMF 200mg/kg and 400mg/kg demonstrated maximum reserve at 6 hours duration.

Table 1: Effect of CSEEWF on carrageenan induced paw edema in rats at different Time intervals.

		Mean paw edema (ml)				
Drugs	**At 0 hr**	**At 1 hr**	**2 hr**	**At 3 hr**	**At 4 hr**	**At 6 hr**
Control 10ml/kg NS i.p.	0.85± 0.38	1.03 ± 0.25	1.2 ± 0.28	1.4166 ± 0.19	1.7166 ± 0.19	1.6333 ± 0.18
Diclofenac10 mg/kg	0.81± 0.08	0.97± 0.11	0.97 ± 0.13	0.97 ± 0.10***	0.92± 0.13***	0.91± 0.08***
CSEEWF200 mg/kg	0.99± 0.11	1.1± 0.08*	1.28± 0.09*	1.41± 0.09*	1.38± 0.17*	1.25± 0.25*
CSEEWF400 mg/kg	0.96± 0.12	1.06± 0.08*	1.21± 0.11*	1.36± 0.15*	1.35± 0.15*	1.20± 0.14**
CSEEWF 600mg/kg	0.99± 0.19	1.09± 0.16*	1.27± 0.88*	1.41± 0.09*	1.38± 0.17*	1.25± 0.25***

Results are represented as Mean±SD. Vehicle control group is compared with rest of the treated groups. Significance at *$p<0.05$, **$p<0.01$ and highly significant at *** $p<0.001$ when compared to vehicle control using one way ANOVA.

Table 2: Percentage inhibition of paw edema in rats at different time intervals

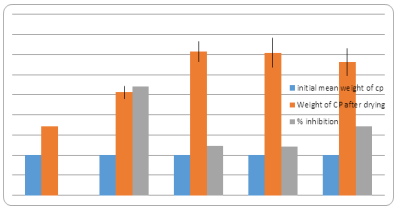
Drugs	At 1 hr	2hr	3hr	4hr	6hr
Diclofenac 10mg/kg i.p	16.36	55.71	72.94	87.11	88.08
CSEEWF 200mg/kg ip	39.81	16.66	24.10	54.45	66.88
CSEEWF 400mg/kg i.p	43.51	28.57	29.00	55.42	66.46
CSEEWF 600mg/kgi.p	42.59	19.04	30.05	38.95	72

Table: 3 - Effect of CSEEWF in Cotton pellet granuloma (sub acute inflammation) model and percentage inhibition

Group	Treatment	Mean ± SD	Weight increment of cotton pellet after drying	% inhibition
I	Control 0.9% Normal saline 2ml	87.92±4.46	67.92±12.62	-
II	Dexamethasone0.5mg /kg	51.28±8.92 **	31.28±8.92	**41.67**
III	200mg/kg CSEEWF P.0	71.39 ± 5.10**	51.15±5.43	**24.69**
IV	400mg/kg CSEEWF P.0	70.88 ± 7.27**	50.88±7.27	**25.08**
V	600mg/kg CSEEWF P.0	66.29 ± 6.79***	44.63±7.69	**34.29**

values are represented as mean±SD. Statistical analysis by one way ANOVA followed by Dunnett's test (F statistic=12.29 df=4,35) Symbols for Statistical Significance = ** represent $p<0.01$ and * represent $p<0.05$ compare to control

Figure: 1 Effect of CSEEWF in Cotton pellet granuloma (sub acute inflammation) model and percentage inhibition



DISCUSSION:

Acute inflammation takes place because of immediate reactions to damage that further results in outcomes such as vascular and exudative changes. Neutrophils and macrophages are engaged in chronic inflammation, which signifies a long-term reaction with inflammatory stimulus regarded by nonstop conscription of mononuclear leukocytes, monocytes and lymphocytes. The harmful stimuli keeps on frequently extending from several weeks or months resulting to trigger proliferative as compared to exudative out flux reactions. Macrophages and lymphocytes are mainly associated in chronic inflammations, sometimes plasma cells also add up for the same reason.

Crocus sativus stigma is a triploid sterile flowering plant made up of a unique and distinctively pungent, honey-like essence and intense odour due to the presence of glycoside constituents[19].

The major central chemical constituent in saffron can be viewed as crocin, Picrocrocin and safranal.[20] These components are majorly held responsible for saffron colour, flavour and odour. [21] Picrocrocin ($C_{16}H_{26}O_7$) is liable for bitter taste and fragrance of saffron by proceeding for this substance to hydrolysis and dehydratation; it is likely to obtain an aldehyde safranal [22]. Safranal also appears during the dehydration, manipulation, and storage of the fresh product[23]. Safranal has molecular formula $C_{10}H_{14}O$, which corresponds to 2, 6, 6-trimethyl-1, 3-cyclohexadiene-1-carboxaldehyde.

Subplanter injection of carrageenan in the hind paw of a rat is regarded as an established oedemogen, it works in two phases in the first phase release of histamine and serotonin takes place subsequently second phase is their wherein a kinin like substance is released. Moreover, in the third step lastly, the liberation of prostaglandins takes place. In the present study, all the three doses of extract Fraction significantly reduced paw edema [Table1/Figure1]. The maximum inhibition

wherein was observed is CSEEWF 400mg/kg 77.44 at 24. Standard drug diclofenac 10mg/kg revealed inhibition by 87.44% at the end of 24 hours Cotton wool granuloma model for proliferative changes were prioritized which leads to granuloma formation because of cotton pellet implantation. In the post-observation phase, the newly formed connective tissue is rendered visible after drying the cotton pellette. Studies on *crocus sativus* water fraction as anti-inflammatory and antinociceptive agents are extremely uncommon and rare, and therefore the present study has attempted to emphasize on these lacunae and portray a promising endeavour in the field of research as the postulation of theories on crocus sativus.

Similar studies like Zulfiquar ali[23] and Simone Li Puma [24] on the same herbal moiety was carried out Further studies, by applying chronic inflammatory models of inflammation depict CSEEWF (at the doses employed), significantly inhibited formaldehyde mediated arthritis in rats. Thereby, suggesting the possible anti-arthritic activity of the same.

CONCLUSION

Thus, the present study explored the potent anti-inflammatory results of ethanolic extract water fraction from the stigmas of crocus sativus on acute and Sub-acute inflammation. However, the mechanism of action of extract fractions needs to be investigated in more depth. Moreover, further studies can be planned on the foundation of dose alteration of extracts and combination of existing drugs can planned with other herbal medicines to get more officious results for the benefit of the society.

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