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# Anti-Inflammatory Activity of Methanolic Fraction of Ethanolic Extract of Crocus Sativus Stigmas in Rats and Mice

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**Abstract**
**Background:** *Extensive use of anti-inflammatory drugs is associated with undesirable side effects often limiting their use. Hence the search for indigenous safer analgesic agent is always going on. It was considered worthwhile to evaluate anti-inflammatory activity of Crocus sativusethanolic extract methanolic fraction (CSEEMF) in animal models.*
**Methodology:** *After obtaining permission from animal ethics committee, animals were divided into 5 groups of 6 animals each group like control, standard - Diclofenac 10mg/kg intra-peritoneal or Dexamethasone 0.5mg/kg orally, CSEEMF (200, 400 and 600 mg/kg). Anti- inflammatory activity of stigmas of Crocus sativus was evaluated by using carrageenan induced paw edema, cotton pellet granuloma, formaldehyde induced arthritis in Wistar albino rats and xylene induced ear edema model in Swiss albino mice.*
**Results:** *Statistical analysis was done by one way analysis of variance followed by Dunnett's test. $p<0.05$, $p<0.01$ and $p<0.001$ were considered as statistically significant. CSEEMF (200, 400 and 600 mg/kg) revealed anti-inflammatory activity in dose dependent manner in carrageenan induced paw edema model. In cotton pellet granuloma model, CSEEMF (400 and 600 mg/kg P.O) significantly decreased granuloma formation. In formaldehyde induced arthritis model, CSEEMF (200, 400 and 600 mg/kg, intra peritoneally) significantly lowered signs of arthritis. Statistically significant result was also obtained in xylene induced ear edema model with all three test doses however maximum inhibitory results were observed with 600mg/kg dose, when compared with control.*
**Conclusion:** *CSEEMF showed promising anti-inflammatory activity in dose dependent manner thus CSEEMF may be beneficial in treating inflammatory diseases.*
**Keywords:** *Anti-inflammatory activity, Crocus sativusethanolic extract methanol fraction, Carrageenan, Paw edema, Xylene.*

## Introduction

Inflammation is a standard protective response to tissue injury and it involves a complex array of enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown and repair[1]. It is a complex process, which is frequently associated with pain and involves manifestations such as the increase in vascular permeability, increase of protein denaturation and

membrane alterations[2]. Inflammation is a host defence mechanism to combat, resist and surmount the invading pathogens or the foreign particles. Inflammation occurs in three different phases, which involves different physiological and immunological mediators. The three phases are - acute phase which involves transient local vasodilatation and increased capillary permeability; sub-acute phase which involves infiltration of leucocytes and other phagocytic cells and chronic phase which involves degeneration of the affected tissue and fibrosis[3].

Based on statistical data, a high majority of patients are suffering from diseased conditions, pain, and inflammation, which hence proves to be a common grievance. Chronic inflammation is associated with certain severe disease like rheumatoid arthritis, Cohn's disease, type II diabetes, Alzheimer's disease, etc. Non-steroidal anti – inflammatory drugs (NSAIDs) and corticosteroids are commonly used for the treatment of inflammation-related diseases such as arthritis, asthma, and cardiovascular disease[4]. However, the long-term administration of NSAID may induce gastric intolerance, bone marrow depression, water and salt retention leading to reactions such as gastro-intestinal ulcers, bleeding, tolerance and dependence[5]. Even long term administration of corticosteroids can lead to Cushing habitus, osteoporosis, and susceptibility to infection, muscular weakness, growth retardation in children, posterior sub-capsular cataract and glaucoma etc[6]. So It is believed that current drugs available such as NSAIDS and corticosteroids are not useful in all cases of inflammatory disorders because of their side effects and potency as a result the search for other alternatives seems necessary and beneficial [7]. There is evidently a need to identify and develop new anti-inflammatory drugs with low side effects.

Hence, the search for safer anti-inflammatory drug is still on. According to World Health Organization (WHO) estimation, about 80% of the population in developing countries relies on herbal medicines at least for their primary health care. So, keeping in the mind this fact, herbal medicines derived from the plant extracts are being increasingly utilized to treat a wide variety of clinical diseases, though relatively little knowledge about their mode of action is available. A bibliographic survey showed that there is large number of reports on the anti – inflammatory activity of different Indian traditional plant [8].

So, keeping all the risk and benefit aspects of the presently ongoing molecules in the mind, Crocus sativus, herbal moiety, was brought into consideration. *Crocus sativus* from Iridaceae family is an autumn-flowering plant with permanent underground stem bases called bulbs or corms[9]. Among 85 species belonging to the *Crocus* genus, saffron is the most fascinating and intriguing species. Saffron is the dried stigma (tiny thread like strands) of the Crocus sativus also called the "fall flower". It is world's most expensive spice by weight used in industry, with many different uses as drug, textile dye and culinary adjunct. It is mainly valued as food additive for tasting, flavouring and colouring, as well as for its therapeutic properties. Saffron is cultivated in countries such as Iran, Spain, Italy, Switzerland and India [10]. The saffron and its coloured components carotenoids (Crocine) constitute a high range of medicinal value. The extracts of crocus sativus appear to have anti-tumour[11], radical scavenger[12] activity. It also prevents renal toxicity induced by cisplastin[13], anticonvulsant[14] and positive effect on learning and memory [15,16]. Hence, the use and the demand for saffron have been on a stark rise in the recent years as a result of the promotion in newly launched products.

Many parts of Crocus sativus are believed to be chief targets that can be researched on, and keeping the previous research work positive result outcomes into consideration[17] and the use of such information in medicinal plant research for drug development has again received considerable interest in the media and in some segments of the scientific community. So the present research work was designed with the goal to explore the anti-inflammatory activities of methanolic fraction of

ethanolic extract of Crocus sativus (CSEEMF) in animal models.

## Materials and Methods

### A) Preparation of extract

I) Flower collection and stigma identification:

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

II) Photochemical Screening

Crocus stigmas revealed the presence of Glycosides, alkaloids, resins, saponins and steroids.

III) Extraction and fractionation of stigmas:

65 grams of crocus sativus were subjected to cold maceration process for three consecutive days using 95% ethanol with periodic shaking.

IV) Preparation of Fractions:

The extract obtained was subjected to fractionation with different solvents under Descending column chromatography.

V) Final yield derived was subjected for column chromatography for fractionation with Methanol, Petroleum benzine, chloroform and Distilled water according to their increasing polarity. Thin layer chromatography technique was performed for the determination of various active constituents[18].

**B) Chemicals and Drugs** used were carrageenan (a light yellow colour powder) and it was purchased from Sigma life science which was dissolved with normal saline for administration. Formaldehyde (37-41% w/v liquid) was purchased from Lobachemie and xylene (colourless liquid) was taken from the science company. Acetone (485 micro liters) and xylene (15 micro liters) were mixed to make 500 microliter. All the chemicals were of analytical grade. Drugs - Dexamethasone (cadila) [0.5mg tab] was dissolved in 10ml normal saline, diclofenac (Reactin 50) [100 gm] dissolved in 1ml, were purchased commercially.

**C) Animals:** Wistar albino rats of either sex weighting 150 to 250 grams were randomly selected from central animal facility and Swiss albino mice weighting between 25-30 grams were selected. Animals were randomised in the group of 5 with 6 rats in each group, at an ambient temperature of 25˚c with ad libitum access to food and water. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC).

**D) Pilot study** on Crocus sativus ethanol extract methanolic fraction (CSEEMF) in different doses 15mg/kg, 25mg/kg, 50mg/kg, 100mg/kg, 200mg/kg was done to estimate desired dose for the study. Since therapeutic effects was observed at 200mg/kg dose. The study was designed with initial dose of 200mg/kg followed by 400 and 600mg/kg.

**E) Experimental models for evaluating anti-inflammatory activity**.

a) **Carrageenan induced rat paw edema model**: This test was conducted as per the method described by Winter et al[19]. The test is based on the principle to evaluate acute anti inflimmatory activity in rats by creating inflammatory models with carrageenan a sulphated polysaccride a phologistic agent which shows signs and symptoms of inflammation. Wistar Albino Rats weighting between 150 -200 gm were selected and marked, randomised into 5 groups with 6 animals in each with proper cage labelling. The group I served as control and received normal saline intra peritoneally at the dose of 10ml/kg, whereas group II served as standard received Diclofenac (10mg/kg) intra peritoneally, group III, IV, V received CSEEMF at a dose of 200,400and 600mg/kg intra peritoneally. Test drug was given 1 hour before injection of 0.1ml of 1% carrageenan in normal saline into the sub plantar surface of right hind paw of each rat aseptically. Edema was expressed as the increment in paw thickness due to carrageenan administration. The paw edema was measured by digital plethysmometer (ml) at 0, 1, 2, 3, 4, and 6 hours after the injection of carrageenan. The paw edema was obtained in all the animals at given point of time of each group and mean

was calculated. The difference between mean paw edema at 0 and 1hour, 0 and 2 hour, 0 and 3hour, 0 and 4 hour, 0 and 6 hour expressed the actual edema. This was calculated for each rat in each group which was labelled as mean increment in paw edema. Groups and treatment schedule was as follows

| Group | Treatment | Dose |
|---|---|---|
| I | Normal saline | 10ml/kg intra-peritoneal = Control |
| II | Diclofenac | 10mg/kg intra-peritoneal = Standard |
| III | CSEEMF | 200mg/kg dissolved in normal saline - intra-peritoneal |
| IV | CSEEMF | 400mg/kg dissolved in normal saline - intra-peritoneal |
| IV | CSEEMF | 600mg/kg dissolved in normal saline - intra-peritoneal |

Percentage inhibition of paw edema formation was taken as an index of acute anti-inflammatory activity. It was calculated by:

The percentage inhibition of edema = 100x {1-(Vt / Vc)} Where VC = Mean increment in right hind paw edema in control group

Vt= Mean increment in right hind paw edema in drug treated group

**b) Cotton Pellet induced granuloma animal model** - Method of winter and Porter [20] was used to study sub-acute inflammation activity of the drug. Wistar Albino Rats of either sex weighting between 150 -200 gm were selected and marked, randomised into 5 groups with 6 animals in each with proper marking. In all the groups the axillary skin was shaved and disinfected with 70% ethanol two sterile cotton pellets weighting 20mg were implanted subcutaneously in both axillae of each rat. Animals were marked properly. Dosing of drugs was started on the same day of implantation of cotton pellets and then was done every day at sharp 9 am. Normal saline (10ml/kg p.o.) was given in control group while standard group received drug Dexamethasone 0.5mg/kg i.p and test group received CSEEMF 200mg/kg, 400mg/kg, 600mg/kg i.p which was continued for 7 days along with free access to water and food ad libitum later the animals were sacrificed on the 8 day and the cotton pellets with granulation tissue were removed surgically, cleaned of the extraneous tissue and the pellets were weighted immediately for wet weight after that pellets were dried in a hot air oven to a constant temperature of 55˚c for 24 hours and the dry granuloma weight was determined. Dry weight of cotton pellet was obtained at a given point of time in each animal and the mean was calculated. This mean was labelled as mean dry weight in (mg) of that group.The mean weight of the cotton pellet before implantation was subtracted from the mean weight of the dried granuloma pellets. That increment in the mean dry weight of the pellet was taken as a measure of granuloma formation.

Groups and treatment schedule was as follows

| Group | Treatment | Dose |
|---|---|---|
| I | Normal saline | 10ml/kg orally = Control |
| II | Dexamethasone | Standard = Dexamethasone 0.5mg/kg orally |
| III | CSEEMF | 200mg/kg dissolved in normal saline – orally |
| IV | CSEEMF | 400mg/kg dissolved in normal saline – orally |
| IV | CSEEMF | 600mg/kg dissolved in normal saline – orally |

Percent anti granuloma activity was noted as 100x (1-Wt/Wc)

Where Wt= Increment in mean dry weight of granuloma in drug treated groups and WC = Increment in mean dry weight of granuloma in control group.

**c) Formaldehyde induced arthritis model of Seyle** – The chronic inflammatory model is based on the principle to induce an inflammatory reaction with formaldehyde at the site of injection, which has been used for studying inflammation quantitatively in the rat foot. Seyle first described the effect of injected formaldehyde in the rat foot as an arthritic reaction[21]. Wistar Albino rats weighting between 150 -200gm were randomly selected for 5 groups with 6 animals in each group. Animals were marked properly and then drug dosing was started on the same day of the formaldehyde injection at sharp

9am with standard drug diclofenac 10mg/kg i.p and test drug CSEEMF 200mg/kg, 400mg/kg,600mg/kg i.p for 10 days. The edema was produced 45 minutes later by subaponeurotic injection of 0.1ml of 2% formaldehyde in the right hind paws of the each rat on the first day and the same injection was repeated on third day. Edema was expressed as the increment in paw thickness due to formaldehyde administration. Paw edema was obtained in all the animals at given point of time of each group and mean was calculated. Paw edema was measured by digital plethysmometer (ml) on day 0, 1, 3, and 10. The difference between mean paw edema at 0 and 1 day, 0 and 3 days and 0 and 10 day expressed the actual edema. This was calculated for each rat in each group which was labelled as mean increment in paw edema.

Groups and treatment schedule was as follows

Percentage inhibition of inflammation was calculated by (1- D/Cx100)

Where D represents the percentage difference in paw volume after test were administered to the rats and C represents the percentage difference of volume in the control group

**d) Xylene induced ear edema**[22] – This method is based on the principle to detect the inflammatory/anti-inflammatory potential effects of histamine ligands in a model of acute skin inflammation induced by local application of croton oil.Swiss albino Mice were randomly divided in group of five with 6 Swiss albino mice (25 – 30 gm) in each group. Dosing of the animals was started at 10 a.m. Thirty minutes after admistration of test drug i.e. CSEEMF 200,400,600mg/kg i.p. in respective groups and standard drug i.e. diclofenac 10mg/kg i.p., xylene (0.01ml which was prepared by diluting 485microlitre acetone with 15µl of xylene to make 500 µl) was administered to the anterior and posterior surfaces of the right ear and left ear was left untreated. Two hours after xylene application, mice were sacrificed and both ears removed. Circular sections were taken using a cork borer with a diameter of 7mm and weighed. Ear weight was obtained in all the animals at given point of time of each group and mean was calculated. The weight difference caused by the xylene was measured by subtracting the mean weight of untreated left ear section from that of mean weight treated right ear section.

Groups and treatment schedule was as follows

| Group | Treatment | Dose |
|---|---|---|
| I | Normal saline | 10ml/kg orally = vehicle group |
| II | Diclofenac | 10mg/kg intra-peritoneal = Standard |
| III | CSEEMF | 200mg/kg dissolved in normal saline - intra-peritoneal |
| IV | CSEEMF | 400mg/kg dissolved in normal saline - intra-peritoneal |
| IV | CSEEMF | 600mg/kg dissolved in normal saline - intra-peritoneal |

Statistics- The data was expressed as the mean ± SD. Statistical analysis was done by one way ANOVA

| Group | Treatment | Dose |
|---|---|---|
| I | Normal saline | 10ml/kg orally = Control |
| II | Diclofenac | 10mg/kg intra-peritoneal = Standard |
| III | CSEEMF | 200mg/kg dissolved in normal saline - intra-peritoneal |
| IV | CSEEMF | 400mg/kg dissolved in normal saline - intra-peritoneal |
| IV | CSEEMF | 600mg/kg dissolved in normal saline - intra-peritoneal |

followed by Dunnett's test. The probability of $P<0.05$, $p<0.01$and $p<0.001$ was considered statistically significant. For the statistical analysis IBM (SPSS version 20) was used.

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## Results

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

Animals of group II, III, IV were treated with the different doses of CSEEMF as a test drug, 1 hour prior to carrageenan injection. The results reported in Table 1/Fig 1 reveal that methanolic fraction of ethanolic extract of crocus sativus 200mg/kg, 400mg/kg and 600mg/kg and Diclofenac 10mg/kg significantly inhibited carrageenan induced rat paw edema at the end of six hours when compared with the control in a dose dependent manner. At the

interval of 6 hours, the percentage inhibition of test group at the dose of 600mg/kg was comparable standard group.

**Table 1:** Effect of CSEEMF on carrageenan induced mean paw edema in rats at different time intervals

| | | Mean paw edema | | | | |
|---|---|---|---|---|---|---|
| **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 i.p** | 0.81± 0.08 | 0.97± 0.11 | 0.97 ± 0.13 | 0.97 ± 0.10*** | 0.92± 0.13*** | 0.91± 0.08*** |
| **CSEEMF200 mg/kg ip** | 0.92± 0.20 | 1.02 ± 0.11 | 1.15 ± 0.11 | 1.41 ± 0.19 | 1.46* ± 0.15 | 1.31 ± 0.14** |
| **CSEEMF400 mg/kg i.p** | 0.92 ± 0.11 | 0.99 ± 0.14 | 1.49 ± 0.26 | 1.23 ± 0.27 | 1.18 ± 0.24* | 1.145 ± 0.18** |
| **CSEEMF 600mg/kgi.p** | 0.94 ± 0.12 | 1.04 ± 0.12 | 1.21 ± 0.22 | 1.40 ± 0.17 | 1.38 ± 0.17** | 1.266 ±0.28** |

Results are given as Mean±SD. of six animals in each group. Vehicle control group is compared with rest of the treated groups.

Significance at **p<0.01 and highly significant at *** p<0.001 when compared to vehicle control using one way ANOVA.

* H represents hours and their effect after 1, 2,3,4,6, hour interval

**Table 2:** Effect of CSEEMF on carrageenan induced mean increment in paw edema in rats at different time intervals.

| | Mean increment in paw edema | | | | |
|---|---|---|---|---|---|
| **Drugs** | **At 1 hr** | **2 hr** | **At 3 hr** | **At 4 hr** | **At 6 hr** |
| **Control 10ml/kg NS i.p.** | 0.18±0.14 | 0.35 ± 0.13 | 0.56 ± 0.33 | 0.86 ± 0.37 | 0.78 ± 0.33 |
| **Diclofenac 10mg/kg i.p** | 0.15± 0.11 | 0.16 ± 0.08 | 0.15 ± 0.08*** | 0.11± 0.09*** | 0.09± 0.05*** |
| **CSEEMF 200mg/kg ip** | 0.10 ± 0.12 | 0.22 ± 0.12 | 0.48 ± 0.33 | 0.39 ± 0.20 | 0.40 ± 0.20 |
| **CSEEMF 400mg/kg i.p** | 0.07 ± 0.07 | 0.57 ± 0.27 | 0.30 ± 0.31 | 0.25 ± 0.30* | 0.22 ± 0.20** |
| **CSEEMF 600mg/kgi.p** | 0.09 ± 0.03 | 0.27 ± 0.19 | 0.46 ± 0.20 | 0.44 ± 0.17** | 0.32 ±0.24** |

Results are given as Mean±SD. of six animals in each group. Vehicle control group is compared with rest of the treated groups.

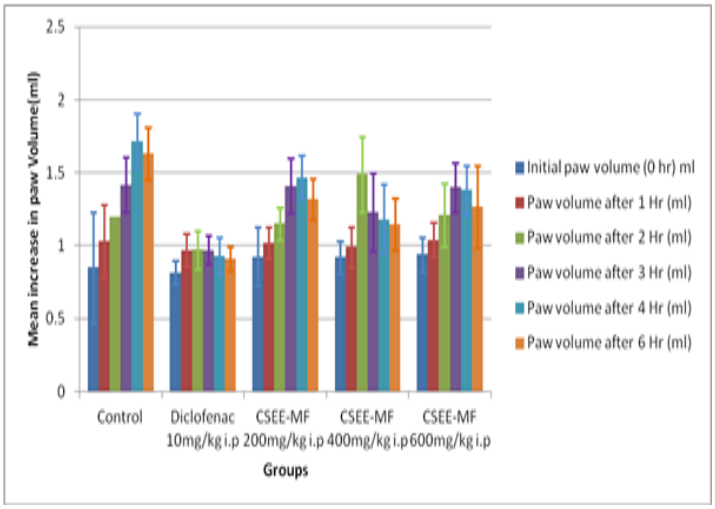
Significance at **p<0.01 and highly significant at *** p<0.001 when compared to vehicle control using one way ANOVA.

*H represents hours and their effect after 1, 2,3,4,6, hour interval

**Table 3:** Percentage inhibition of paw edema in rats at different time intervals

| **Drugs** | **At 1 hr** | **2 hr** | **At 3 hr** | **At 4 hr** | **At 6 hr** |
|---|---|---|---|---|---|
| **Diclofenac10mg/kg i.p** | 16.36 | 55.71 | 72.94 | 87.11 | 88.08 |
| **CSEEMF 200mg/kg ip** | 44.54 | 35.71 | 43.38 | 54.80 | 49.99 |
| **CSEEMF 400mg/kg i.p** | 46.54 | 21.42 | 18.73 | 49.03 | 58.51 |
| **CSEEMF 600mg/kgi.p** | 61.81 | 40.05 | 45.58 | 70.19 | 71.91 |

**Figure 1:** Effect of CSEEMF on carrageenan induced Mean paw edema in rats at different time intervals




Results are given as Mean±SD. of six animals in each group. Vehicle control group is compared with rest of the treated groups.

Significance at **p<0.01 and highly significant at ***p<0.001 when compared to vehicle control using one way ANOVA.

*H represents hours and their effect after 1,

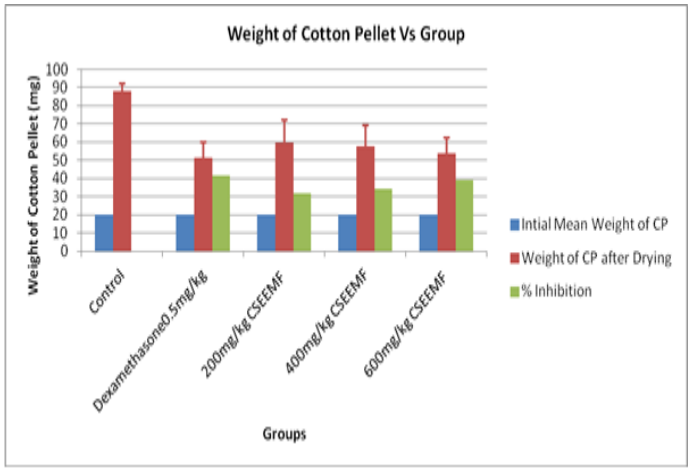
**B. Sub-acuteanti-inflammatory activity (Cotton Pellet induced granuloma):** CSEEMF treated group with 600 mg/kg and standard drug dexamethasone 0.5mg/kg mg/kg results were highly significant (p<0.001) whereas treatment with CSEEMF 200 mg/kg and 400 mg/kg were significant (p<0.01). The percentage inhibition of CSEEMF at the dose of 400 mg/kg (34.49%) was comparable with the standard drug (41.66%) (Table 4/Fig 2).

**Table: 4** Effect of CSEEMF in Cotton pellet granuloma *(sub-acute inflammation)* model and percentage inhibition

| Group | Treatment | Mean Weight of cotton pellet after drying(mg) | Mean Weight increment of cotton pellet after drying | % inhibition |
|---|---|---|---|---|
| I | Control Normal saline10ml/kg P.O | **87.92** ±4.46 | 67.92 ±12.62 | - |
| II | Dexamethasone 0.5mg/kg PO | **51.28625** ± 3.15*** | 31.28 ±8.92 | **41.66** |
| III | 200mg/kg CSEEMF P.0 | **59.8475** ± 4.48** | 39.84 ±12.68 | **31.92** |
| IV | 400mg/kg CSEEMF P.0 | **57.59375** ± 4.23** | 37.59 ±11.99 | **34.49** |
| V | 600mg/kg CSEEMF P.0 | **53.565** ± 3.24*** | 33.56 ± 9.17 | **39.07** |

Values are expressed 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 <0.001 and ** represent p < 0.01 compare to control

**Figure 2:** Effect of CSEEMF in Cotton pellet granuloma *(sub-acute inflammation)* model and percentage inhibition




**C) Effect of CSEEMF for Mean paw edema in (formaldehyde induced arthritis) in rats.**

Inflammation was produced by subaponeurotic injection of 0.1ml of 2% formaldehyde in the right hind paws of the rats on the first and the third day. The animals were treated daily with the test drug CSEEMF 200,400,600mg/kg i.p and diclofenac 10mg/kg i.p as a standard for 10 days. All CSEEMF doses (200,400,600 mg/kg body weight) showed significant reduction in formaldehyde induced rat paw edema compared to controls, with maximum effect produced by dose of 600mg/kg on day 1 of admission followed by dose of 400mg/kg of CSEMF on day 10. Meanwhile, 10 mg/kg Diclofenac significantly inhibited formaldehyde induced rat paw edema compared to controls by 96.46%. (Table 5/Fig 3)

**Table 5:** Effect of CSEEMF for mean paw edema (formaldehyde induced arthritis) in rats

| Drugs | Mean Paw Edema | | | |
|---|---|---|---|---|
| | Day 0 | Day 1 | Day 3 | Day 10 |
| **NS 10ml/kg p.o.** | 0.73 ±0.05 | 0.98 ±0.14 | 1.17 ±0.20 | 1.53 ±0.12 |
| **Diclofenac 10mg/kg i.p.** | 0.43 ±0.07 | 0.47 ±0.07*** | 0.51 ±0.08*** | 0.46 ±0.08*** |
| **200 mg/kg CSEEMF i.p.** | 0.52 ±0.11 | 0.54 ±0.10*** | 0.69 ±0.06*** | 0.64 ±0.06*** |
| **400mg/kg CSEEMF i.p.** | 0.52 ±0.07 | 0.61 ±0.05*** | 0.68 ±0.10*** | 0.59 ±0.06*** |
| **600mg/kg CSEEMF i.p.** | 0.48 ±0.04 | 0.52 ±0.09*** | 0.66 ±0.100*** | 0.54 ±0.11*** |

Results are given as Mean±SEM. of six animals in each group. Vehicle control group is compared with rest of the treated groups.
Significance at and highly significant at ** p<0.001 when compared to vehicle control using one way ANOVA.

**Table 6:** Effect of CSEEMF for mean increment in paw edema (formaldehyde induced arthritis) in rats

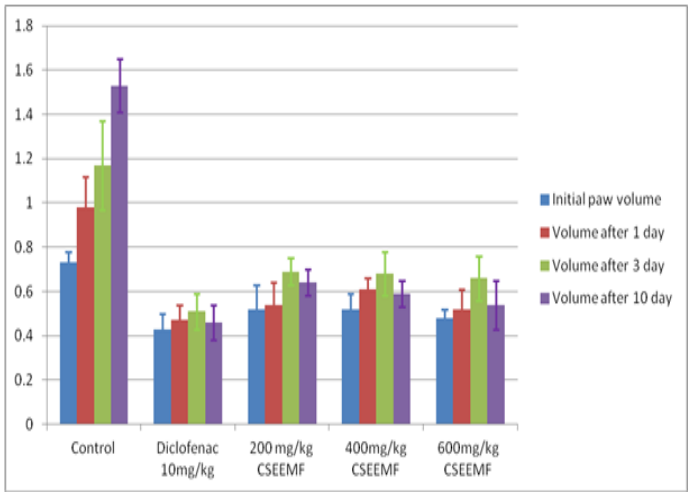
| Drugs | Mean increment in Paw Edema | | |
|---|---|---|---|
| | Day 1 | Day 3 | Day 10 |
| **NS 10ml/kg p.o.** | 0.25 ±0.13 | 0.43 ±0.19 | 0.8 ±0.12 |
| **Diclofenac 10mg/kg i.p.** | 0.03 ±0.008*** | 0.07 ±0.01*** | 0.02 ±0.01*** |
| **200 mg/kg CSEEMF i.p.** | 0.02 ±0.02*** | 0.17 ±0.08*** | 0.12 ±0.09*** |
| **400mg/kg CSEEMF i.p.** | 0.09 ±0.10*** | 0.16 ±0.13*** | 0.007 ±0.07*** |
| **600mg/kg CSEEMF i.p.** | 0.0 3±0.02*** | 0.17 ±0.12*** | 0.05 ±0.04*** |

Results are given as Mean±SD. of six animals in each group. Vehicle control group is compared with rest of the treated groups. Highly significant at *** p<0.001 when compared to vehicle control using one way ANOVA

**Table 7:** Percentage inhibition of paw edema in rats at different time intervals Formaldehyde induced arthritis model

| Drugs | Day 1 | Day 3 | Day 10 |
|---|---|---|---|
| **Diclofenac 10mg/kg i.p** | 86 | 81.92 | 96.46 |
| **CSEEMF 200mg/kg i.p** | 90 | 59.55 | 84.16 |
| **CSEEMF 400mg/kg i.p** | 62 | 62.69 | 90.83 |
| **CSEEMF 600mg/kgi.p** | 86.67 | 60 | 93.33 |

**Figure 3:** Effect of CSEEMF on chronic inflammation (formaldehyde induced arthritis) in rats




**D. Xylene induced ear edema-** The CSEEMF, at the doses of 200,400,600mg/kg showed statistically significant reduction in ear edema when compared to disease control group. Among all other test groups maximum percentage inhibition of 37.19 was obtained with 600mg/kg group.

**Table 8:** Effect of CSEEMF of ethanolic extract of crocus sativus in xylene induced ear edema

| Group | Dose | Route | Right ear edema eight (mg) | Left Ear weight (mg) | Mean increment in weight (mg) | % of inhibition |
|---|---|---|---|---|---|---|
| NS(vehicle control ) | 10ml/kg | PO | 75.66 ± 4.65 | 56.16 ±3.18 | 19.49 ±3.25 | - |
| Diclofenac | 10 mg/kg | I.P | 68.5 ±1.87*** | 59.73 ±4.79 | 11.21 ±3.77*** | 42.48 |
| CSEEMF | 200 mg/kg | I.P | 42.5 ±1.87** | 29.09 ±5.44 | 13.40 ±5.32** | 31.14 |
| CSEEMF | 400 mg/kg | I.P | 52.5 ±1.87*** | 39.29 ±3.73 | 13.20 ±2.07*** | 32.27 |
| CSEEMF | 600mg/kg | I.P | 60.5 ±1.87*** | 49.08 ±3.89 | 11.41 ±2.81*** | 37.19 |

Values are expressed as mean±SD (n=6) One Way ANOVA F-statistic= 7.39 df=4, 35 Significance = *** represent p <0.001 and ** represent p < 0.01 compare to control group.

## Discussion

Inflammation can be classified as acute and chronic. Acute inflammation which is a short time process characterised by classical signs of inflammation swelling, redness, pain heat, and loss of function occurs as long as injurious stimulus is present and ceases once the stimulus has been removed .The process of acute inflammation is initiated by the blood vessels local to the injurious tissue. Chronic inflammation is a pathological condition characterized by concurrent active inflammation tissue destruction and attempts at repair chronically inflamed tissue is characterized by the infiltration of mononuclear immune cells (monocytes, macrophages, lymphocytes and plasma cells are majorly involved in chronic inflammation and at times plasma cells also add up for the same , injurious stimuli persist often for longer durations amounting to several weeks or months leading to proliferative, rather than exudative reactions.[23]

Commercial crocus sativus is extracted from the dried stigmas of the saffron flower which is a triploid sterile plant and is composed of a unique and distinctively pungent, honey-like essence and aroma identified due to its glycoside constituents [24]. The most central compounds in saffron can be observed as crocin, picocrocin and safranal, responsible for saffron colour, flavour and aroma respectively, Picrocrocin (C16H26O7) is responsible for the bitter taste and aroma of saffron by submitting this substance to hydrolysis and dehydratation, it is possible to obtain safranal[25]. Safranal also appears during the dehydration, manipulation, and storage of the fresh product[26] Safranal has the molecular formula C10H14O, which corresponds to 2, 6, 6-trimethyl-1, 3-cyclohexadiene-1-carboxaldehyde [27].

The inhibition of carrageenan induced acute inflammation in rats is an established model for evaluating antiinflimmatory drugs (Headley pm 1985). Carrageenan induced acute inflammation is related to neutrophil derived free radicals. It works in two phases; the first phase represents the release of histamine and serotonin followed by second phase wherein a kinin like substance is released.

Lastly, the third phase is the stage that signifies the release of prostaglandins. In this study, all three extract Fraction doses significantly inhibited paw edema [Table1/Figure1]. However maximum inhibition of paw edema was observed by the CSEEMF at the dose of 600mg/kg at6 hr and even standard drug diclofenac (10mg/kg) showed maximum inhibition of paw edema at the end of 6 hours.

Cotton pellet granuloma, a sub-acute inflammatory study involves infiltration of macrophages, neutrophils and proliferation of fibroblasts (Grover,1990). The significant anti-inflammatory effect of the extract fraction in cotton-pellet induced granuloma suggests its efficacy in inhibiting inflammation in a dose dependent manner. Treatment with standard drug Dexamethasone 0.5mg/kg PO and CSEEMF 600mg/kg were more significant ($p<0.001$) whereas treatment with CSEEMF 200 mg/kg and CSEEMF 400 mg/kg were less significant ($p<0.01$). The percentage inhibition of CSEEMF at the dose of 600mg/kg (52.84%) was comparable with the standard Dexamethasone 0.5mg/kg (59.98%) (Table 4).

For proliferative changes cotton wool granuloma method was prioritized leading to granuloma formation due to cotton pellet implantation. In the post-observation phase, the newly formed connective tissue is rendered visible after drying the cotton pellette.

Formaldehyde induced model for chronic inflammation indicates that CSEEMF (at the doses employed), significantly inhibited edema in rats. Inhibition of inflammation induced by this model is one of the most suitable test procedures to screen anti-arthritic and anti-inflammatory agents in rats as it closely resembles human arthritis. Formalin induced arthritis is a model used for the evaluation of an agent with probable anti proliferative activity. CSEEMF inhibited this model of inflammation in dose dependent manner which indicates its anti-proliferative and anti-arthritic activities.

Signs of acute inflammation such as severe vasodilation and edematous changes of skin are known to be caused by Xylene. Such histopathologicalchanges leads to increased thickness of ear tissues. In the present study, it is noted that the Crocus SativusEthnolic Extract Methonolic fraction inhibited the xylene-induced increases in ear weight in a dose related manner. This inhibition capacity of the CSEEMF signifies the anti-inflammatory efficacy of the CSEEMF for the fact that it reduced vasodilation and improved edematous condition.

**Conclusion**

Studies on crocus sativus methanolic fraction as anti-inflammatory agents are extremely rare and uncommon, and hence, 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 Hosseinzadeh H[(28)] with the resemblances of claiming the same herbal medical plant for anti inflimmatory activity. Whereas the variations in the studied were also observed at the same time on the basis of dose selection as well as the extract fraction utilized for the study.

The present study explored the potential anti-inflammatory effect of ethanolic extracts methanolic fraction from the stigmas of crocus sativus on acute, sub-acute and chronic inflammation. The mechanism of action of fractioned extract of Crocus sativus at molecular level needs to be explored in more depth. Moreover, further studied can be designed on the basis of dose modification of fractioned extract and combination with other herbal formulations to asses anti- inflammatory effect

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