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The effective study of Aqueous extract of *Crocus sativus* Linn. in Electrical Induced Convulsants in rats.

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ABSTRACT

To evaluate the anti-convulsion activity of *Crocus sativus* linn. aqueous extract in electrical induced epilepsy models. To compare the anti convulsant activity of crocus sativus linn. with phenytion .After obtaining Institutional Ethical Committee approval, Wistar albino rats (150-200g) of either sex were randomly divided into 5 groups of 6 animals each. Dried powder of crocus sativus linn. was boiled with distilled water, cooled, filtered, placed on hotplate for complete evaporation, finally weighed and stored. The control group, test group and standard drugs group received saline, crocus sativus linn. extract (200,400 & 800 mg/kg), phenytoin (25 mg/kg) respectively by oral feeding. The anti-convulsent effect was assessed by maximal electrical shock (MES) in rats. In electrical induced epilepsy models It implies that saffron 400mg/kg (groupIV) and 800mg/kg(groupV) significantly ($p<0.001$) delays the onset and significantly ($p<0.001$) decreased the duration of tonic hind limb extension of MES induced convulsions compared to that of control. The current study demonstrates statistically significant anti-convulsant activity of crocus sativus linn.

Keywords: *Crocus sativus* Linn., anti-convulsant, MES.

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INTRODUCTION

Crocus sativus L. (Iridaceae), commonly known as saffron[4]. In Ayurveda, saffron is used to treat epilepsy, chronic diseases such as asthma and arthritis, depressant, cold and coughs. Ayurvedic medicines containing saffron are used to treat acne and several skin diseases. A paste of the spice can be used as a dressing for bruises and superficial sores. Ancient texts on Ayurveda have information about the herb's use as an aphrodisiac. It is largely used as an indigenous medicine across India[5-6].Recent pharmacological studies has demonstrated that saffron extract has hypolipidemic effect as well as learning or memory-improving properties[7-8]. In view of the need for safe and effective herbal antiepileptic and anti depressant, the present study was undertaken to evaluate the anticonvulsant and anti depressant effect of aqueous extract of crocus sativus linn. Chemical studies have shown that Crocus sativus contains constituents such as crocin, crocetin safranal and picrocrocin[9-11]. Among the constituents of saffron extract, crocetin is mainly responsible for the above pharmacological activities.

MATERIALS AND METHODS

Preparation of Aqueous Extract:

Stigmas were collected from KING INDO AGRO PRODUCTS PVT.LTD, from Srinagar Kashmir, dried in shadow, and subsequently grounded. The plant was authenticated for their correct botanical identity by the chief botanist. .For the preparation of the aqueous extract, In the maceration method, 3g of stigma was macerated in 400ml of distil water (80%, v/v) for 3 days. The mixture will be subsequently filtered and concentrated at 35ºc. The extract was preserved in deep freezer in air tight container.

Animals:

Wistar albino rats (150-200g) of either sex were procured from the central animal house of the Institute. They were housed in standard polypropylene cages and were kept under controlled room temperature at 25 ± 2^0C in a 12h light /dark cycle. Animals were given dry pellets and water ad libitum. The animals were accustomed during the day time to new environment for at least 2 days prior to the experiment. Institutional Animal Ethics Committee approval was taken prior to the start of study. The ethical guidelines for the investigation of animals used in experiments were followed in all tests.

MES Induced Seizures:

MES seizures was electrically induced by means of an Electro convulsiometer 150mA current is delivered transauricularly (ear clips) for 0.2sec. This current intensity elicited complete tonic hind

limb extension (THE) in animals. For measuring various parameters, rats were placed in a clear rectangular plastic cage with an open top, permitting full view of the animals' motor responses to the seizures. Later each animal was then individually observed for 2 hours to study convulsive effects on general behavior. Suppression of tonic hind limb extension was taken as a measure of efficacy of the drugs in this test. Anti convulsive drugs abolishes or reduces the duration of time of tonic hind limb extension phase of MES. After 30 mins of administering respective drugs of those particular groups, MES was induced. Onset and duration of time for tonic hind limb extension (THE) was noted and compared the in all groups.

Statistical Analysis:

The data was collected in case record forms. Then they are entered into excel spreadsheet 2007. Statistical analysis was performed using Microsoft Excel-2007 and Sigma Graph pad prism version-4 USA. Data was described as Mean ± Standard deviation, and Percentages. Percentage change as compared to control was analyzed. ANOVA followed by Tukeys multiple comparison tests was used for analysis of data between the four. For all inferential statistical tests a two tailed P value of 0.05 considered significant.

RESULTS AND DISCUSSION

The present study was aimed at evaluating the anti epileptic and anti depressant properties of saffron in comparison with standard drugs using animal models.

Saffron is a perennial stem less herb, indigenous ingredients of ayurvedic medicine. It is mainly used for its memory enhancing property, as anticonvulsant, antiaxiety, and anti depressant and for treatment of insomnia.

MES Induced Seizers:

The mean time for onset of THE in the control (group I) was 1.5(0.11) seconds and with saffron 200mg/kg (group III), 400mg/kg (group IV) and 800mg/kg (group V) it was 3.0 (0.19), 4.9(0.20) and 5.1(0.25) seconds respectively. While phenytoin completely abolished the It implies that saffron 400mg/kg (group IV) and 800mg/kg (group V) significantly ($p<0.001$) delays the onset of MES induced convulsions compared to that of control. Saffron 200mg/kg (group III) has less significant action on onset of THE.

The time duration of THE in control (group I) was 7.69 (0.19) seconds, with saffron 200mg/kg (group III) ,400mg/kg (group IV) and 800 mg/kg (group V) it was 7.01(0.15), 5.6(011) and 5.6(0.11) seconds respectively. Saffron 400mg/kg (group IV) and 800mg/kg(group V) significantly ($p<0.001$) decreased the duration of tonic hind limb extension compared to that of

control. Shown in the table 1 & graph-1. Saffron having dose dependent anti convulsant effect on MES induced convulsions.

All the doses saffron decreased the onset of Tonic Hind Limb Extension (THE) as compared to control in a dose depended manner but significantly decreased the duration of THE compared to that of control in a dose dependent manner.

Maximal electric shock induced convulsions is a best suitable test for evaluating anti-epileptic properties of drugs, because it is the best-validated preclinical test that predicts drugs effective against generalized seizures of the tonic–clonic (grand mal) type.

In our study, saffron showed significant anti epileptic activity with 400 and 800 mg/kg compared to control group but has less activity when compared to that of standard drug phenytoin. At low dose i.e.200mg/kg, saffron showed antiepileptic property but it was less significant as compared to control.

MES-induced tonic extension can be blocked by drugs that inhibit voltage dependent Na + channels, such as Phenytoin, Carbamazepine, and Valproate (Macdonald and Kelly, 1995; Rogawski and Porter, 1990; White, 1997) and drugs that enhance GABA-A receptor-mediated inhibitory neurotransmission, such as Benzodiazepines, Phenobarbital and Valproate (Macdonald and Kelly, 1995; Rogawski and Porter, 1990; White, 1997).

As MES is suppressed by drugs that enhance GABA-A receptor-mediated inhibitory neurotransmission the probable mechanism of saffron to show activity in MES induced convulsion is through GABA A mediated neurotransmission.

The two main constituents of saffron which include safronal and crocin are responsible for the anticonvulsant activity of saffron in MES induced convulsions.

Pushpa kumari B et al (2010) showed that aqueous extract of saffron decreased the tonic hind limb extension by nearly half the extension time in control. [34.]

Hosseinzadeh H and Khosravan V. (2002) showed that the ethanol and aqueous extract of saffron considerably increased the seizure threshold in the experimental model of generalized tonic-clonic seizures. As saffron contains these two as main constituents which are having action on MES induced convulsions saffron can also decrease the duration of THE. The MES model has served to identify antiepileptic drugs that are functionally similar to phenytoin and most of these compounds display the same ability to inactivate voltage dependent Na+ channels in a use dependent fashion. Saffron may also inactivate voltage dependent Na+ channels. [9-11]

The effects of aqueous and methanol extract of *Crocus sativus* L. stigmas (CSS) were studied for examination of in vitro antioxidant properties and its effect on abeta (1-40) fibrillogenesis. The

water: methanol (50:50, v/v) extract of *Crocus sativus* stigmas possesses good antioxidant properties.

The main carotenoid constituent is trans-crocin-4 and digentibiosyl ester of crocetin. This inhibited abeta fibrillogenesis in the human brain. [12-13]

Pushpa kumari B et al showed that there was decreased in the duration of each seizure in the saffron-treated group in uncontrolled epilepsies in humans. They assumed that there may be an interaction with the endogenous opioid system which will protect individuals from seizures.[14]

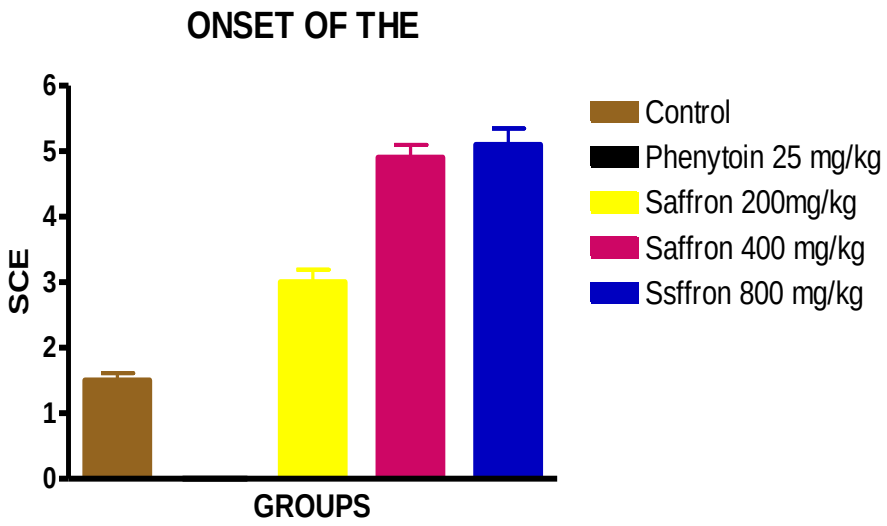
The earlier studies proved that saffron increases the Na + /K + , Mg 2+ and Ca 2+ -ATPases in rat brain This effect may contribute to its ability to block MES-induced tonic extension by maintaining the ionic equilibrium, but still it needs further studies.

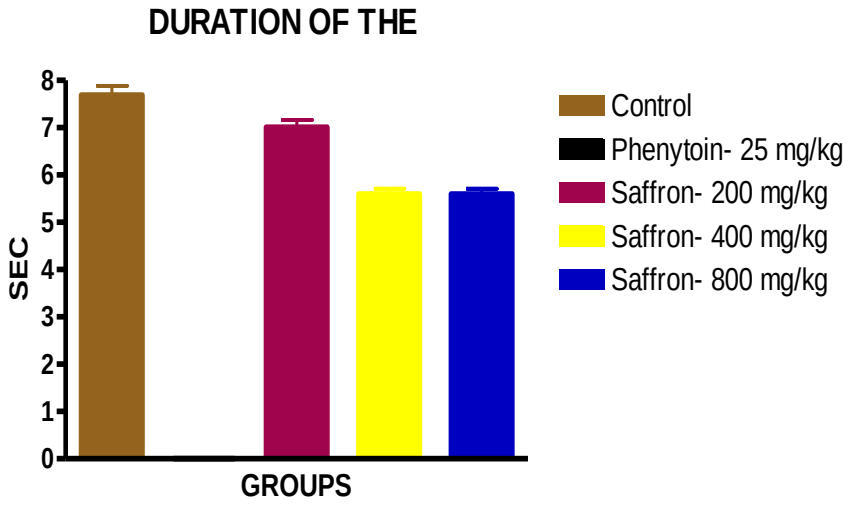
Table 1: Shows the effect of saffron on Maximal Electro Shock [MES] induced convulsions in rats. (n=6)

S.no	Group & Dose (mg/kg i.p)	Onset of tonic Hind Limb Extension (THE) phase in secs in mean (SD)	Duration of tonic Hind Limb Extension (THE) in secs in mean (SD).
1.	Control(Normal saline 0.5 ml)	1.5(0.11)	7.69(0.19)
2.	Phenytoin- 25 mg/kg	0***	0***
3.	Saffron- 200 mg/kg	3.0(0.19)**	7.01(0.15)**
4.	Saffron- 400 mg/kg	4.9(0.20)***	5.6(0.11)***
5.	Saffron- 800 mg/kg	5.1(0.25)***	5.6(0.11)***

*p<0.01, ** p<0.001 compared to control group

One way ANOVA followed by Newman-Keuls comparison test





Graph 1: Shows the effect of saffron on Maximal Electro Shock [MES] induced convulsions in rats. (As per table '1')

CONCLUSION:

Thus, the preliminary data of the present investigation provide some evidence for the effectiveness of saffron in the treatment of seizures claimed in the Ayurvedic system of medicine.

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