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ANTIDEPRESSANT ACTIVITY OF *FICUS RELIGIOSA* L. FRUITS IN RODENTS

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ABSTRACT

Background of research

Ficus religiosa L. (Moraceae) has been indicated for a range of ailments including disorders of the central nervous system (CNS) like convulsion, anxiety and amnesia. Its fruits/figs have been reported for high serotonergic content. Moreover, depression is a common mental disorder of dysfunctional monoamines (serotonin, noradrenalin and dopamine) level in CNS.

Objective

The current study was aimed at investigating the antidepressant effects of hydro-alcoholic extract of *Ficus religiosa* L. fruits (EFR) in rodent models of depression.

Material and Method

The rodents were pre-treated orally with suspension (DMSO 1: CMC 9) of EFR for 7 days, daily at the doses of 30, 100 or 300 mg/kg/day and screened with validated depression models viz., force swim test (FST), tail suspension test (TST) and 5-hydroxytryptophan (5-HTP) induced head twitches. The antidepressant effect of EFR was compared to that of imipramine (15 mg/kg, p.o.) and fluoxetine (20 mg/kg, p.o.).

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Result and discussion

The EFR significantly ($p<0.05$) decreases the duration of immobility time in rats of both FST and TST in a dose dependent manner. Further, extract also shows dose dependent increase in head twitches in mice. The efficacy of tested EFR was found to be comparable to that of imipramine and fluoxetine.

Conclusion

Results of the study establish the potential of hydro-alcoholic extract of *Ficus religiosa* L. fruits (EFR) against behavioural depression. Furthermore, the EFR possess antidepressant activity possibly via modulating serotonergic neurotransmission.

Keywords: Moraceae, Force swim test, Tail suspension test, Serotonin.

INTRODUCTION

Depression is a devastating illness with an estimated lifetime prevalence of 17% in general population. It is a leading cause of disability worldwide, bringing about considerable loss of life by suicide, as well as being a risk factor for cardiovascular disease and a multitude of neurological disorders, including dementia (Hajszan et al., 2009). Almost 1 million lives are lost yearly due to suicide, which translates to 3000 suicide deaths every day. For every person who completes a suicide, 20 or more may attempt to end their life (WHO, 2012). Among people with major depression 75-85% has recurrent episodes (Mueller et al., 1999); 10-30% recovers incompletely and has persistent, residual depressive symptoms (Mann, 2005). However, currently a wide range of antidepressants drugs arebeing used, but due to clinical limitations and adverse effects the efficient and safe treatment against depression remains a matter of dispute (Tran et al., 2003). Herbs can be effective therapeutic alternatives for treatment of depressive disorders.In this course, *Withania somnifera* (Bhattacharya et al., 2000), *Bacopa monniera* (Sairam et al., 2002), St. John's wort extract (Kasper et al., 2006) and *Asparagus racemosus* (Singh et al., 2009) have been documented to have antidepressant potential.

Ficus religiosa Linn (Moraceae) is a variety of fig tree, commonly known as 'pipal' and/or 'bodhi tree'. Itgrows throughout India and widely cultivated in south-east Asia especially in vicinity of temples,due to being the most sacred tree to both Hindus and Buddhists (Sitaramam et al., 2009). Atharvaveda (sacred text of Hinduism) links it with the third heaven and discusses its medicinal properties along with Soma and Kustha (holy medicinal herbs). The therapeutic utilities of *F. religiosa* have been indicated in traditional

systems of medicine (ayurveda, siddha, unani and homeopathy etc.) to cure the disorders of central nervous system, endocrine system, gastrointestinal tract, reproductive system, respiratory system including infectious diseases as well (Singh et al., 2011). Literature search reveals that the stems, leaves, fruits, roots and barks of *F. religiosa* are often used in the treatment of nervous disorders (Khare, 2007), drug addiction (*bhang* and opium) (Vedavathy, 2003), unconsciousness (Singh, 1986) and also used as a nervine tonic (Sivarajan and Balachandran, 1996). Many validated pharmacological studies have reported the plant and/or its parts for having CNS related effects viz., anticonvulsant (Goel and Singh, 2013; Patil et al., 2011; Singh and Goel, 2009), anti-amnesic (Kaur et al., 2010), anti-cholinesterase (Vinutha et al., 2007) and anxiolytic (Ratnasooriya et al., 1998). The medicinal importance of the *F. religiosa* and/or its parts i.e., leaves, stem, roots, bark, seeds, fruits and latex is due to the presence of different chemical constituents like tannins, saponins, flavonoids, steroids, terpenoids, cardiac glycosides, fats, amino acids, serotonin (5-HT) and vitamins.

Depression is associated with decreased function in the serotonergic (dorsal and median raphe), noradrenergic (locus coeruleus) and dopaminergic (ventral tegmental area regions) (Gonzalez and Aston-Jones, 2008). According to the serotonergic hypothesis of major depression, a deficit in the 5-HT activity is the most important etiological factors for genesis of depression (Maes and Meltzer, 1995; Meltzer and Lowy, 1987). Further, hydro-alcoholic extract of *F. religiosa* fruits has enriched in 5-HT content and found to be effective against convulsions (Singh and Goel, 2009) and amnesia (Kaur et al., 2010) by modulating serotonergic pathway. Therefore,we hypothesized that hydro-alcoholic extract of *F. religiosa* fruits may have antidepressant potential.

In the view of above mentioned facts the present study was undertaken to investigate the antidepressant effects of *F. religiosa* L. fruits in a battery of rodent models of depression.

MATERIALS AND METHODS

Plant material and preparation of extract

The fruits (figs) of *Ficus religiosa* L. were collected in the month of November-December from Farrukhabad and were authenticated from Department of Botany, Mahatma Jyotiba Phule Rohilkhand University, Bareilly. Authenticated fruits were washed with water, shade-dried and ground to a moderately coarse powder. The powdered fruits were subjected to extraction by refluxing with 70% ethanol (w/v) in a Soxhlet extractor for 10-12 hours.

The resultant extract was evaporated to dryness using rotavapor and stored at 4°C (yield: 12%, w/w). The hydro-alcoholic extract of *F. religiosa* fruits (EFR) was reconstituted by dissolving it in di-methyl sulfoxide (DMSO) 10% (v/v) and then suspending the resultant solution in 0.3% CMC (carboxymethylcellulose) suspension (DMSO 1:CMC 9) freshly before use.

Drugs and chemicals

All standard chemicals used in this study were of analytical grade. Imipramine and fluoxetine were acquired from Torrent Pharmaceuticals Ltd. (Ahmadabad, India). DMSO, CMC and 5-hydroxytryptophan were purchased from HiMedia Laboratories Pvt. Ltd (Nashik, India).

Animals

Adult Wistar albino rats (150±10g) and Swiss albino mice (20±5g), of either sex, were employed in the present study. The animals were housed in polypropylene cages at an ambient temperature of 25°C±1°C and 45-55% RH, with a 12:12 h light/dark cycle. The animals had free access to commercial food pellets (standard laboratory rodent's chow) and water *ad libitum* unless stated otherwise. Experiments were conducted between 10:00 and 16:00 h. Animals were acclimatized for at least one week before using them for experiments and exposed only once to every experiment. All experimental procedures were in compliance with National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985) and approved by the Institutional Ethical Committee.

Acute toxicity study

A toxicity study of the EFR was performed according to OECD guideline no. 425. A limit test was performed initially using Wistar albino female rats (150±10g). Six rats were serially administered with a 2000mg/kg dose of extract prepared in water as recommended in the guideline. After administration of the dose, each animal was observed every hour for signs of toxicity and abnormality in behaviour up to the 48th hour. Subsequently daily observations were made for toxicity and mortality up to 14 days.

Drug Treatments

The animals were pre-treated orally with suspension (DMSO 1: CMC 9) of EFR for 7 days daily at the doses of 30, 100 or 300 mg/kg/day. All the experimental procedures were started on day 7, 1 h after drug administration. In case of FST (8 days), drug

administration was continued till the end of the experimental schedule. The dose range was selected based on the outcome of the acute toxicity study as well as literature search (Singh and Goel, 2009). Control animals were treated with equal volume of vehicle (DMSO 1: CMC 9 suspension). Imipramine (15 mg/kg, p.o.) and fluoxetine (20 mg/kg, p.o.), the reference drugs, were administered 1hr before experimentation.

Forced swimming test (FST)

The procedure was used as described by Porsolt et al. (1978) except that the water level was deeper. Swimming sessions were conducted by placing rats in individual glass cylinders (45 cm high×20 cm in diameter) containing (25±2 °C) water 38 cm deep, so rats could not support themselves by touching the bottom with their feet. Two swimming sessions were performed between 10:00 h and 16:00 h, an initial 15 min pre-test followed 24 h later by a 5 min test. Drugs were administered 1 h after the pre-test and 1 h before the test session. Following both swimming sessions, the rats were removed from the cylinders, dried with paper towels and placed in heated cages for 15 min, and then returned to their home cages (Kumar et al., 1999). The immobility (remained floating in water without struggling and making only those movements necessary to keep its head above water) period in seconds was measured live in each test session by a blind observer.

Tail suspension test (TST)

A rat was hung on a wire in an upside down posture so that its nostrils just touch the water surface in a container. After initial vigorous movements, the rat assumes an immobile posture and the period of immobility during a 5 min observation period were noted (Kumar et al., 1999).

5-hydroxytryptaphan (5-HTP)-induced head twitches in mice

Mice were treated with 5-HTP (100 mg/kg i.p.) and the numbers of head twitch performed by each mice was counted by staggering method using three 2 min periods (19-21 min), (23-25 min), (27-29 min) after 5-HTP administration. 5-HTP was administered 1 h after 7 day pre-treatment with drug and head twitches were scored live by a blind observer (Schreiber et al., 1995).

Statistical analysis

Data of all experiments were expressed as Mean ± Standard Error of Mean (SEM) of six animals in each group. The data were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests using the GraphPad

Prism 5. The level of significance was determined in comparison with the control group. Statistical significance was accepted for $p<0.05$.

RESULTS

Acute toxicity study

In the acute toxicity study, neither death nor any observable neurobehavioral effects were observed in the limit test. Due to the lack of observable toxicity, no LD50 determination was carried out.

FST

The effect of EFR on the duration of immobility time (sec.) in the behaviour despair test is depicted in figure 1. One-way ANOVA followed by post-hoc analysis revealed that all dose levels of EFR and imipramine (IMP) treated groups were significantly different ($p<0.05$) from the vehicle treated group. Figure 1, also shows that EFR dose dependently decreases the duration of immobility time comparable to that of IMP.

TST

Likewise FST, all treated doses of EFR and IMP were found significant ($p<0.05$) in decreasing the immobility time (sec.) in TST. Figure 2 reveals the dose dependent effect of EFR in reducing the immobility time, similar to IMP (standard drug). Decrease in the immobility time is an indicative of antidepressant effect.

5-HTP induced head twitching

Figure 3 illustrate the effect of EFR and fluoxetine (FLT) on 5-HTP (100 mg/kg i.p.) induced head twitching in mice. One way ANOVA shows significant ($p<0.05$) difference between the treatment groups when compared with control group. Post hoc analysis reveals the dose dependent effect of EFR in increasing the number of head twitches (N). Further, 300 mg/kg of EFR shows effect equivalent to FLT, suggesting modulation of serotonin neurotransmission by the EFR.

DISCUSSION

Depression is a significant contributor to the global burden of disease and affects people in all communities across the world. Today, depression is estimated to affect 350 million people. Depressive disorders often start at a young age; they reduce people's functioning and often are recurring. For these reasons, depression is the leading cause of disability worldwide in terms of total years lost due to disability. The demand for curbing depression and other mental health conditions is on the rise globally. A recent World Health

Assembly called on the World Health Organization and its member states to take action in this direction (WHO, 2012). During the last 50 years there has been major progress in drug treatment for depression. Many different animal models of behavioural despair have been proposed and evaluated on the basis of different criteria. Among all the models FST and TST are considered as major and recognizable paradigms for screening antidepressant agents (Kulkarni and Dhir, 2007).

FST was proposed by Porsolt et al. (1978), suggested that rodents forced to swim in a restricted space from which they cannot escape exhibit a characteristic immobility. The immobility in this test reflected either a failure of persistence in escape directed behaviour (i.e. behavioural despair) or the development of passive behaviour that disengages the animal from active forms of coping with stressful stimuli, and this behaviour is sensitive for antidepressants (Redrobe and Bourin, 1999). Antidepressant agents reduce the amount of immobility, or delay its onset, and increase or prolong active escape behaviours displayed during the FST (Vogel and Vogel, 1997). FST is considered relatively quick, simple and most widely used test to screen antidepressant activity of drugs. Interestingly, EFR effectively increases the escaping activity by reducing the immobility time in the challenged animals, suggesting its potential against depressive state.

Apart from FST paradigms, the TST is an additional measure for assessing antidepressant activity. TST is based on the observation that rodents after initial escape oriented movements develop an immobile posture when suspended by tail (Vogel and Vogel, 1997). The immobility displayed by rodents when subjected to an unavoidable and inescapable stress, has been hypothesized as reflect of depressive disorders. TST is a relatively rapid and sensitive test to detect the antidepressant effects. This is perceived as one of the shortcomings associated with its use, as it is in contrast with the observation that chronic treatments with antidepressants are usually required as a prerequisite for full clinical recovery in humans (Cryan et al., 2005).The result of TST also reveals the antidepressant potential of EFR in dose dependent manner, as EFR significantly decreases the immobility time in stressed rats analogous to reference drug.

Various antidepressant drugs are expected to modify cate-cholaminergic or serotonergic neurotransmission in the brain (Blier, 2013; Gonzalez and Aston-Jones, 2008). Further, the role of serotonin in the pathogenesis of depression is well documented in both preclinical and clinical research (Singh et al., 2009; Maes and Meltzer, 1995; Meltzer and Lowy, 1987). 5-HTP induced stereotypical behaviour (head twitch response) in rodents is

an indicative of central serotonergic activity, and involves increase in extracellular serotonin and subsequent activation of 5-HT2A receptors (Schreiber et al., 1995). Additionally, published report on the extract of *F. religiosa* fruits viz., anticonvulsant activity (Goel and Singh, 2013; Singh and Goel, 2009) and anti-amnesic activity (Kaur et al., 2010) were also hypothesize the augmentation of serotonergic pathway. Interestingly, the EFR significantly increases the frequency of 5-HTP induced head twitches, suggesting enhanced serotonergic function in rodent brain.

CONCLUSION

Although the outcome of present findings establishes the potential antidepressant activity of hydro-alcoholic extract of *Ficus religiosa* L. fruits (EFR) in rodent models of depression, favourably by modulating the serotonergic neurotransmission. But the mechanism of adapted serotonin level in brain by the extract can only be confirmed after *ex-vivo* or *in-vivo* experiments involving 5-HT depletion studies and/or direct 5-HT measurements.

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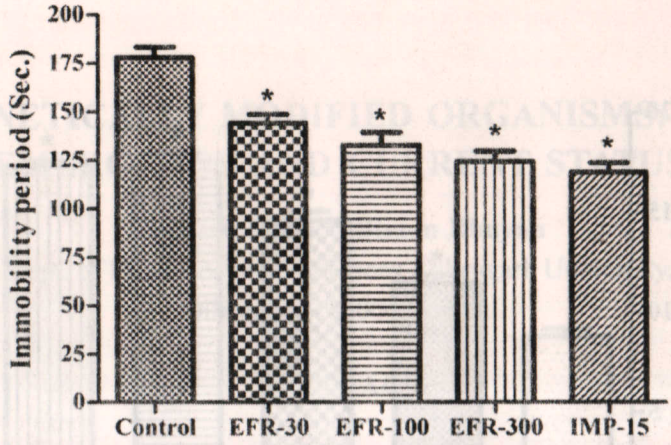

Figure 1: Effect of EFR (30, 100 and 300 mg/kg) and IMP (15 mg/kg) on immobility in FST activity on rats. Each column represents mean ± SEM of immobility period in sec. **P*<0.05 compared to vehicle treated control. EFR = hydro-alcoholic extract of *F. religiosa* fruits, IMP = imipramine, FST = force swim test.

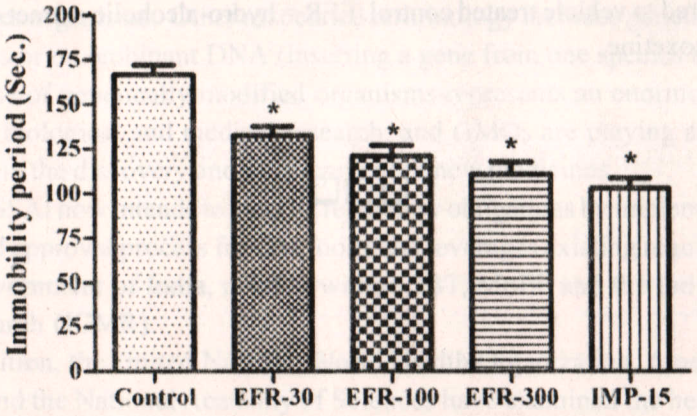

Figure 2: Effect of EFR (30, 100 and 300 mg/kg) and IMP (15 mg/kg) on immobility in TST activity on rats. Each column represents mean ± SEM of immobility period in sec. One **P*<0.05 compared to vehicle treated control. EFR = hydro-alcoholic extract of *F. religiosa* fruits, IMP = imipramine, TST = Tail suspension test.

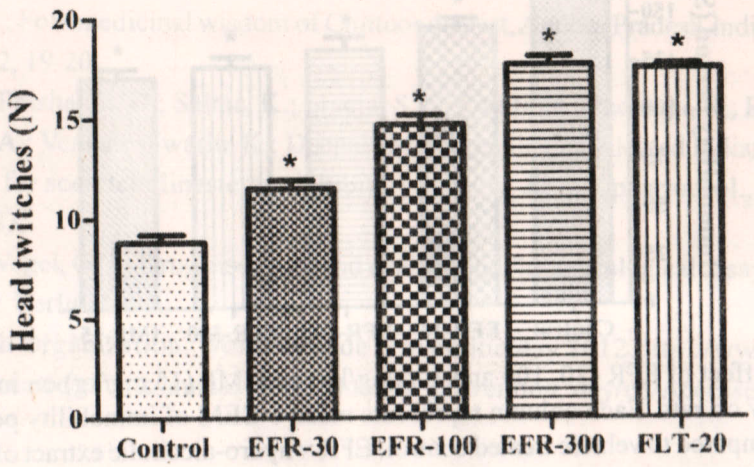

Figure 3: Effect of EFR (30, 100 and 300 mg/kg) and FLT (20 mg/kg) on 5-HTP induced head twitching in mice. Each column represents mean±SEM of No. of head twitches. *$P<0.05$ compared to vehicle treated control. EFR = hydro-alcoholic extract of *F. religiosa* fruits, FLT = fluoxetine.

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