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RESEARCH ARTICLE

EVALUATION OF ANTI-DEPRESSANT ACTIVITY OF *CALENDULA OFFICINALIS* L. FLOWER EXTRACT IN EXPERIMENTAL ANIMALS

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ABSTRACT

Objective: Depressive disorder is a prevalent psychiatric disorder, which affects 21% of the world population. The presently using drugs can impose a variety of side-effects. In the traditional system of medicine is useful in treating various ailments. The main objective of the work was to evaluate the antidepressant activity of aqueous extract *Calendula officinalis* L. flowers using forced swim test (FST) and tail suspension test (TST) in mice.

Methods: The aqueous extract (10 to 50 mg/kg) was administered to albino mice for 14 days for evaluating antidepressant activity using forced swim test (FST) and tail suspension test (TST) in mice.

Results: In case of forced swim test the aqueous extract *Calendula officinalis* L.flowers showed significant dose dependent effect on immobility and climbing behavior. As it was evident, oral administration of 50mg/kg of flower extract effectively decreased the time of immobility in both TST and FST compared to control. Potentiality of *Calendula officinalis* L.was almost similar to that of standard drug Imipramine in alleviating depression in both the animal models.

Conclusion: The results indicate that aqueous extract of *Calendula officinalis* L.flowers contained flavonoids, glycosides and saponins which might be active in case of Forced swim test (FST) and Tail Suspension Test (TST) to show anti-depressant activity, which support the ethno medicinal application of the plant as an antidepressant agent.

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INTRODUCTION

Depression is a multifactorial, chronic and life-threatening disease with globally high prevalence. Currently, 29% of the world population suffers from depression, considered to be one of the 10 leading causes of death (Menken, 2000; Grundmann, 2010; Cassani, 2015 and Singer, 2011). According to the World Health Organization, depression will be the second leading disease in the developed countries by 2020 (AbbasiMaleki, 2013 and Reynolds, 2013). The use of plant products for the treatment of human ailments has been a natural approach to health care since the beginning of civilization. In the search for new therapeutic products for the treatment of neurological disorders, medicinal plant research worldwide, has progressed constantly, demonstrating the pharmacological effectiveness of different plant species in a variety of animal models (Zhang, 2004). Many phytoresources have been investigated for their efficacy in controlling neurological disorders like depression (Rajput, 2011; Gautam, 2013). *Calendula officinalis* L. (pot marigold) is one of the commonly used medicinal plants in India, China, Europe and the US (Muley, 2009). *Calendula* was known as "gold" in old English accepted version associated with Virgin Mary and Queen Mary, hence the name marigold (Grieve, 1931 and Kemper, 1999). *Calendula officinalis* L. is globally known for its medicinal importance containing various phyto chemicals including carbohydrates, amino acids, lipids, fatty acids, carotenoids, terpenoids, flavonoids, quinones, coumarins and other constituents (Muley, 2009; Grieve, 1931; Kemper, 1999 and Muley, 2009). The major active constituents of the plant include triterpendiol esters, saponins, and flavonoids including rutin and hyperoside. The orange flower contains a high

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content of carotenoids including astaxanthin and fucoxanthin (Braun and Cohen, 2005; Neukiron, 2004 and Roopashree, 2008). Some important biological activities studiedinclude wound healing, immuno-stimulant, spasmogenic and spasmolytic, hepatoprotective, genotoxic and antigenotoxic, anti-amylase, anti-inflammatory, anti-oedematous, anti-bacterial and anti-fungal, antioxidant, antidiabetic, anti-HIV and anti-cancerous, nephron-protective, prevention of oropharyngeal mucositis, hypoglycemic and gastroprotective activities with no toxic effect (Kirtikar, 1993; Weiner, 1990). The flower extracts of the plant have anti-viral effects on HIV (Kalvatchev, 1997). In the present study effort has been made to evaluate the antidepressant activity of aqueous extract *Calendula officinalis* L. flowers in mice model employing tail suspension test (TST) & forced swim test (FST). Standard antidepressant drug Imipramine has been employed to standardize the animal models of depression.

MATERIALS AND METHODS

Plant material preparation

Calendula officinalis L. flowers were collected from Western Ghat region of India. Flowers were washed and dried under shade. Powdered flower sample was subjected to aqueous extraction by cold maceration method. The extract was filtered and the total volume was reduced to three fourth of its original volume.

Experimental animals

Adult Swiss albino mice with body weight ranging from 25 – 30g were obtained from Bharathi College of Pharmacy, Bharathi Nagar, Mandya, India. Mice were maintained at temperature 21-23^{o}C with a relative humidity of 50-60% under a 12 h light/dark cycle with free access to food and water.

Drug treatment and acute toxicity study

OECD-423, 2001 guidelines (OECD Guideline for Testing of Chemicals, 2002), were followed to determine the acute toxicity of test drug. Mice were fasted for 4 h by feeding with only water. Test drug administration was followed by thorough observation of animal for any behavioral changes periodically for 24 h till next 14 days. The test drug was found to be non-toxic even at the dose of 1500mg/kg. For the antidepressant study, the dose ranging from 10-50mg/kg was used.

Grouping of animal for antidepressant study

Mice were grouped into five batches where each group contained six mice. The animals were administered orally with drug/Vehicle 1 h before the study was carried out.

Group I: Control, administered with phosphate buffered saline 5mL/kg.
Group II: Positive control, administered with standard drug Imipramine 15mg/kg.
Group III: Received *Calendula officinalis* L. flowers extract 10mg/kg.
Group IV: Received *Calendula officinalis* L. flowers extract 30mg/kg.
Group V: Received *Calendula officinalis* L. flowers extract 50mg/kg.

Forced Swim Test (FST)

The test was performed according to the method described by Porsolt *et al*. (1978). FST is a behavioral despair model employed to test antidepressant activity. Mice were allowed to swim individually for 6 minutes in a beaker of 20cm height, 10cm diameter, filled with water to the depth of 10cm at 25 ± 2^{o}C the duration of immobility was measured for 4 min from the point when the mice start floating by making only movements which are necessary to keep its head above the water. Fresh water was used for every swimming test. The decrease in immobility time is the indication of antidepressant activity.

Tail Suspension Test (TST)

On the 14th day immediately after administration of the last dose, each mouse was individually suspended on the edge of the table 50 cm above the floor by adhesive tape placed 01 cm from the tip of the tail for the period of 05 minutes using a stop watch and Immobility duration was recorded. Mice are considered immobile when they hanged passively and completely motionless. The principle of this test is that suspending mice suspended upside down leads to characteristic behavior of immobility which resembles human depression. The decrease in immobility duration is considered as behavioral profiles that indicates an antidepressant-like action (Yamaguchi, 2000).

Statistical Analysis

The result obtained from the study was expressed as mean ± S.E.M. The data was analyzed by one way ANOVA followed by Dunnet's multiple comparison tests while $p<0.05$ was considered statistically significant in all cases.

RESULTS AND DISCUSSION

Aqueous extract *Calendula officinalis* L. flowers did not produce any sign of acute toxicity till the oral dose of 1500 mg/kg hence the extracts were used in the range of 10–50 mg/kg orally assuming that LD50dose is 1500 mg/kg. From both FST and TST it was observed that the aqueous extract of *Calendula officinalis* L. showed dose dependent effect on immobility and climbing behavior. At the dose of 50mg/kg significantly decreased the immobility time in both animal model FST and TST than control and it was comparable to the effect of standard drug Imipramine used at the dose of 15mg/kg. The observations are given in Table 1, FST and TST models of depression are widely used to screen new anti-depressant drugs (Porsolt, 1978).

Table 1. Effect of aqueous extract of *Calendula officinalis* L. flowers on immobility in tail suspension test (TST) and forced swim test (FST) in mice model

Groups	Treatment	Duration of Immobility	
		TST	FST
I	Control	188±3.98	178±2.54
II	Imipramine (15mg/kg)	130±4.16	112±1.86
III	*C. officinalis* extract (10mg/kg)	155±5.48	141±4.14
IV	*C. officinalis* extract (30mg/kg)	146±4.72	133±2.34
V	*C. officinalis* extract (50mg/kg)	136±4.28	120±2.51

The tests are quite sensitive and relatively specific to all major classes of anti-depressant drugs including TCAs, SSRIs,

MAOI, atypical antidepressants. The FST is the most widely used tool for assessing antidepressant activity pre□clinically. TST is less stressful than FST and has a greater pharmacological sensitivity (Vikasgupta, 2010). Depression is a common, debilitating, life threatening illness with a high incidence. Numerous anti-depressant compounds are now available, which presumably act via different mechanisms involving the serotonergic, noradrenergic and/or dopaminergic systems. Heterogeneity of clinical response to antidepressant and mood-stabilizing drugs and susceptibility to adverse effects are major clinical problems (Detke, 1995). Many classes of antidepressants such as TCAs, SSRIs, SNRIs, MOIs are currently being used to treat Depression, those elicit unwanted effects on patients which limits the further use of these drugs (25). Since the arisen of civilization, plants have been used for prevention and cure of diseases. Recently herbal products are gaining more attention as they are natural, safe, affordable and accessible for everyone (Eddouks, 2012). *Calendula officinalis* L. possess great medicinal/ pharmacological properties are evident by a number of good studies (Kirtikar, 1993; Weiner, 1990; Kalvatchev, 1997). The present study was taken up to demonstrate the antidepressant activity of aqueous extract of *Calendula officinalis* L. fruit extract in mice behavioral despair model FST and TST. Antidepressant activity of the aqueous extract of the flower was dose dependent where the maximum concentration used for this study i.e. 50mg/kg was very effective in reducing the immobility duration ($p<0.01$) compared to control. The floating or immobile posture in mice reflects a state of behavioral despair or entrapment. *Calendula officinalis* L. was proved to be as capable as standard drug Imipramine in reducing depression ($p<0.01$).

Test drugs were administered by oral route 1h prior to the test. Each value represents the mean ± SEM (n=6), $p<0.05$, $p<0.01$ vs. control.

Conclusion

The present results show that aqueous extract of *Calendula officinalis* L. flowers produced an anti-depressant-like effect as it decreases the immobility time during depression in animal model (FST &TST).Thus aqueous extract of *Calendula officinalis* L. flowers may have potential therapeutic value for the management of depressive disorders. The phytochemical flavonoids, glycosides, triterpendiol esters and saponins which might be active in case of forced swim test (FST) and tail suspension test (TST) to show anti-depressant activity can serve as better phytotherapeutic agent in treating the mental disorder like depression.

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