

ANTIDEPRESSANT ACTIVITY OF ETHANOLIC EXTRACT OF *EUPHORBIA THYMIFOLIA* LINN. IN THE FORCED SWIMMING TEST IN RATS

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Summary

In continuation of our work on *E. thymifolia*, the preliminary antidepressant activity of ethanolic extract of the plant was performed by behavioural despair (forced swimming test) in rats. The ethanolic extract (50 & 100 mg/kg, i.p.) produced a significant ($p < 0.01$) reduction in the time of immobility (in sec.) from 255 ± 8.4 to 165 ± 4.33 and 112 ± 3.87 respectively, which was comparable to imipramine (107 ± 4.74), used as reference standard. The phytochemical screening of the ethanolic extract showed the presence of phenolics, terpenes and flavonoids.

Keywords: Antidepressant activity, *Euphorbia thymifolia*, Euphorbiaceae, Forced swimming test.

Introduction

Depression is a major neurological disorder affecting nearly 13-20% of the population. In spite of the introduction of the tricyclic antidepressants (TCAs), selective reversible inhibitors of monoamine oxidase A (MAO-A), selective serotonin reuptake inhibitors (SSRIs) and specific serotonin-noradrenaline reuptake inhibitors (SNRIs), depression continues to be a major health problem. In search for new therapeutic products for the treatment of neurological disorders, medicinal plant research has progressed constantly worldwide, demonstrating the promising pharmacological effectiveness of various plant species in animal models [1]. The plants of genus *Euphorbia* contain diversified classes of compounds in both free and conjugated form viz. tannins, phenolics [2], flavonoid glycosides [3], terpenes [4] and steroids [5]. The genus *Euphorbia* is reported for antidepressant [6], cytotoxic activity [4], antinociceptive activity [7], and antioxidant activities [8]. Plants of genus *Euphorbia* are also reported to be used as purgative, anthelmintic, antianaphylactic, anti-arthritic, antidiarrhoeal, anti-inflammatory, in warts treatment, antiasthmatic, astringent, narcotic, diuretic and immunosuppressive properties. *Euphorbia thymifolia* Linn. (syn. *Chamaesyce thymifolia* L.; Euphorbiaceae), which showed various biological activities including antibacterial, anti-fungal, antioxidant, antiviral, as stimulant, in worm infection, pain and jaundice. Recently, we have reported anti-inflammatory and hepatoprotective activities of ethanolic extract of the plant [9]. The presence of polyphenolics [10], flavonoids [11] and alkaloids [12] has been reported from *Euphorbia thymifolia* Linn. In the present study, the phenolics rich ethanolic extract of *E. thymifolia* was used to evaluate its antidepressant activity, which was not reported earlier.

Materials and methods

Plant material, extraction and isolation

The whole plants of *Euphorbia thymifolia* were collected from Tamil Nadu and were authenticated by Prof. K.N. Dubey (Department of Botany, Banaras Hindu University, Varanasi, India). A voucher specimen (specimen No. PCRL 36) has been deposited in the Pharmaceutical Chemistry Research Laboratory, Department of Pharmaceutics, Institute of Technology, Banaras Hindu University, Varanasi, India, for future reference. The shade-dried powder of whole plant was passed through sieve No. 40 & 600 g was extracted (soxhlation) with petroleum ether (60-80°C) for 24 h and subsequently with ethanol (30 h). The extractives were concentrated *in vacuo*. Qualitative determination of ethanolic extract was done for the presence of tannins, flavonoids, sterols, terpenes and alkaloids using standard methods [13].

Animals

Male albino rats (Charles foster strain) weighing 120-180 g were obtained from M/S Asian Fauna Store, Varanasi. Animals were randomly housed in groups of five in polypropylene cages at an ambient temperature with a 12 h light: 12 h dark cycle. The animals were allowed free access to laboratory diet (M/s Hindustan Lever Ltd., Mumbai, India) and water *ad libitum*. The animals were fasted overnight before the experiment. Experiments were performed in accordance with the current guidelines for the care of laboratory animals and the ethical guidelines for the investigation of experimental pain in conscious animals [14].

Reagents and Chemicals

All the chemicals and solvents used for this work were obtained from Merck (Germany) and S.D. Fine (Mumbai). The chemicals purchased were of analytical reagent grade or were purified by standard methods prior to use [15].

Acute toxicity studies

The acute toxicity for all test compounds was carried out in albino rats weighing 120-180g which were fasted overnight. The dosage was varied from 50-1000 mg/kg body weight. The animals were observed for 24 h for any signs of acute toxicity such as increased/decreased motor activity, tremors, convulsion, sedation, lacrimation etc. No mortality of the animals was observed even after 24 h. Hence, the LD₅₀ cut off value of the test compounds was fixed as 1000 mg/kg and 1/10th of cut off value (i.e. 100 mg/kg) was taken as maximum screening dose for the evaluation of antidepressant activity.

Antidepressant Activity

Behavioural despair (forced swimming test) in rats

This test was performed according to the method described by Porsolt et al. [16] with slight modifications [17]. The rats are individually forced to swim inside a vertical transparent glass vessel (40 cm height × 18 cm diameter) containing 23 cm of water maintained at 25 ± 2 °C for 15 min. The total duration of immobility (in sec.) was measured during the last 4 min of the 6 min test session. Groups of five rats were treated with single dose of vehicle, standard drug, imipramine (25 mg/kg, i.p.) and ethanolic extract of *E.thymifolia* (50, 100 mg/kg, i.p.) for 7 consecutive days. The last dose was given 1 h before the test. Rats were exposed to pre-test for 15 min., 24 h prior to the 6 min. swim test. The period of immobility (remained floating in water without struggling and making only those movements necessary to keep its head above water) during the last 6 min. test period was noted. After the test the rats were removed from the water, dried and placed in cages. The results of antidepressant activity are given in the Table 1.

Table 1. Effect of ethanolic extract of *E.thymifolia* and imipramine on behavioral despair model of depression in rats (n = 5, mean \pm S.E.M.).

Group	Dose (mg/kg, i.p.)	Immobility period (Seconds)
Control	-	255 \pm 8.4
Std.(Imipramine)	25	107 \pm 4.74*
Ethanolic extract	50	165 \pm 4.33*
	100	112 \pm 3.87*

*p < 0.01 compared to control.

Results and Conclusions

Immobility is a state of lowered mood or hopelessness, which the rats experience when they are allowed to swim in a restricted space from which they cannot escape. This is thought to reflect either a failure to persist in escape directed behavior after persistent stress or the development of passive behavior that disengages the animal from active forms of coping with stressful stimuli [17]. This behavioral immobility reflecting a state of despair is reduced by a broad spectrum of antidepressant drugs [16]. The ethanolic extract showed significant antidepressant activity by decreasing the duration of immobility (sec.), when compared with the control and with the standard drug, Imipramine. The dose of 50 & 100 mg/kg, i.p. produced a reduction in the time of immobility (sec.) from 255 \pm 5.4 to 165 \pm 4.33 and 112 \pm 3.87 respectively and it is comparable to that of the standard drug, imipramine (107 \pm 4.74) (Table 1). Thus, the present observations indicate the antidepressant activity of ethanolic extract of *E. thymifolia*. Further, neurochemical studies would unravel the possible mechanisms involved in the antidepressant activity of ethanolic extract.

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