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Original Article

Antidepressant like Property of *Hyoscyamus niger* Linn. in Mouse Model of Depression

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Abstract

Aim: *Hyoscyamus niger* (*Solanaceae*) has been used for treatment of mental disorders, epileptic mania, chronic dementia with insomnia. However, it is not yet studied in condition like depression. The present study was planned with an objective to evaluate its antidepressant like property in animal models of depression and to find out the possible mechanism underlying this action in mouse model of depression. **Materials and methods**: Antidepressant activity was studied in forced swim test (FST) and tail suspension test (TST) in mice. Locomotor and anxiolytic activity was also studied. *Hyoscyamus niger* ethanolic extract was administered to mice by oral route at dose of 25, 50, 100, 200 and 400 mg/kg for 14 days. Further an interaction of *Hyoscyamus niger* ethanolic extract with conventional antidepressant drugs were also studied at sub-effective doses. **Results:** The ethanolic extract (50, 100, 200 and 400 mg/kg) significantly reduced immobility duration of mice in FST and TST. The same doses did not change the motor activity in mice. However, high dose of extract has shown anxiolytic activity. Interaction study with conventional antidepressant drugs reduced the duration of immobility count suggests, possible involvement of biogenic amine in antidepressant action. **Conclusion**: These data suggests that *Hyoscyamus niger* possesses antidepressant like action in mouse model of depression.

Keywords: *Hyoscyamus niger,* Forced swim test, Tail suspension test, Depression.

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1. Introduction

Depression is one of the most common psychiatric disorders. According to the World Health Organization, depression is a medical and social problem affecting 340 million people worldwide [1]. Patients with depression have symptoms that reflects changes in brain monoamine neurotransmitters specifically norepinephrine, serotonin and dopamine [2]. Depression is characterized by a combination of symptoms that interfere with a person's ability to work, sleep, study, eat and enjoy once-

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pleasurable activities and prevents a person from functioning normally.

In spite of its prevalence and sever impact, the efficacy of currently available antidepressant is often inconsistent and many of them exert undesirable side effects such as hypotension, arrhythmia, insomnia and sexual dysfunction [3]. With growing number of herbal medicines being introduced to psychiatric practice, many of them have been chosen as alternative therapies for severe depression [4,5]. *Hypericum perforatum* (St. John's wort) [6], *Curcuma longa* [7], *Schinus molle* [8] have been studied in animal models of depression and are commonly used herbal medications for treatment of depression.

Hyoscyamus niger belongs to family *Solanaceae.* In India, it is commonly known as "Khurasani-ajvayan". It is a biennial plant. It has a long, thick, spindle-shaped, corrugated root, which is of a brown colour externally, but whitish internally. The stem sometimes reaches the height of two feet, but often stops at an altitude of six inches. The leaves are large, oblong, acute, alternate, and of a pale, dull green color. Ancient literature reports that *Hyoscyamus* is largely prescribed in mental disorders, epileptic mania, and chronic dementia with insomnia, paralysis, agitans, convulsions, neuralgia, spasmodic cough and asthama [9]. Crude extract of *Hyoscyamus niger* has hypotensive, cardiosupressant and vasodilator activities [10]. It also has spasmolytic, antidiarrhoeal, antisecretory, bronchodilatory activities [11]. Methanolic extract of *Hyoscyamus niger* possesses the anticonvulsant activity against picrotoxin induced seizures in mice [12].

However, until now, there have not been any experimental reports concerning its efficacy for treatment of depression. In the present study, we examined the antidepressant like property of *Hyoscyamus niger* in forced swim test (FST), tail suspension test (TST), an animal models predictive of antidepressant activity. We also tried to explore anxiolytic activity of *Hyoscyamus niger* in hole-board test. Further we observed interaction of *Hyoscyamus niger* with conventional antidepressant drugs to delineate the possible mechanism of action.

2. Material and methods

2.1 Plant material

Fresh leaves of *Hyoscyamus niger* were collected from hilly area near Kannad, Dist. Aurangabad, Maharashtra India. The plant was taxonomically identified and authenticated by Dr. Anil Kshirsagar, lecturer and Head, Department of Botany, Shivaji Arts, Commerce and Science College, Kannad, Dist. Aurangabad with Ref. No. 2008-09/10.

2.2 Extract Preparation

Fresh leaves of *Hyoscyamus niger* were dried in shadow, powdered and was continuously extracted for 18-20 hours using ethanol (95%) as a solvent. The resulting extracts were concentrated under reduced pressure using rotary vacuum evaporator to get the semisolid mass. This mass was allowed to dry for 2 to 3 hours at room temperature. This *Hyoscyamus niger* leaves ethanolic extract (*HNLEE*) was further used for the evaluation.

2.3 Drugs and treatments

Hyoscyamus niger leaves ethanolic extract (*HNLEE*) was dosed at 25, 50, 100, 200 and 400 mg/kg for 14 days, by oral route to the mice, FST and TST were carried out at 14^{th} day, 1 hr after dosing the animals. Imipramine (20 mg/kg *P.O.*) was used as reference positive standard and was dosed only at day 14. For Locomotor activity and exploratory behavior, *HNLEE* was dosed at 100, 200 and 400 mg/kg for 14 days, by oral route and animals were subjected for test at 14^{th} day, 1 hr after dosing. For interaction of *HNLEE* with conventional antidepressant drugs, *HNLEE* was dosed at sub-effective dose for 14 day and conventional antidepressant were also

administered at sub-effective doses only at 14th day and 1 hr after dosing, animals were subjected for forced swim test.

2.4 Experimental animals

Male Swiss albino mice weighing between 25–30 g bred in animal house facility of the Wockhardt Research Centre Aurangabad, India were used in the present study. The animals were housed under standard laboratory conditions and maintained on 12-hr light and 12 hr dark cycle (lights on at 0800 h), and had free access to food and water. Animals were acclimatized to laboratory conditions for five days before the experiment. Animals were separated randomly into control and experimental groups each containing six mice. Each animal was used only once. All the experiments were carried out between 9:00 a.m. to 3:00 p.m. The experimental protocols were approved by the Wockhardt Animal Ethics Committee (WAEC).

2.5 Experimental protocol

2.5.1 Determination of LD_{50}

Median lethal dose (LD_{50}) of *HNLEE* was estimated in mice as per OECD guideline 423. Briefly, a limit test at 2000 mg/kg of *HNLEE* was performed in mice. A group of three mice was dosed orally with 2000 mg/kg of *HNLEE*. All the animals were observed for 24 hrs for clinical signs and mortality if any.

2.5.2 Forced swim test (FST)

Anti-depressant like property was evaluated by forced swim test (FST) [13, 14, 15] . Briefly, mice were individually forced to swim in a glass jar (height: 25 cm; diameter: 10 cm) containing 15 cm of water maintained at 25 ± 1 º C. After the initial 2-3 minutes (min) of vigorous activity, the animal showed period of immobility by floating with minimum movements. An animal considered to be immobile whenever it remained floating passively in the water in a slightly hunched but upright position, its nose above the water surface (Porsolt et al., 1978). The total duration of immobility was recorded with the help of stop watch during next 4 min of a total 6 min test.

2.5.3 Tail suspension test (TST)

The total duration of immobility induced by tail suspension was measured [16]. Mice both acoustically and visually isolated were suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. The total immobility was recorded for a period of 6 min with the help of stop watch. A mouse was considered immobile when it hangs passively and completely motionless.

2.5.4 Measurement of Locomotor activity

In order to analyze whether changes in immobility were associated with changes in motor activity, a measurement of locomotor activity was performed [17]. Briefly, the locomotor activity (ambulation and stereotypy) of the mice was measured by a computerized LE 8811 IR Motor Activity Monitor (Panlab s.l.). This system was used to measure motor activity in experimentation with animals. It is based on a grid of infrared cells that make it possible to determine the magnitude of motor activity on the basis of analysis of the position and frequency with which the experimental animal break the infrared beams. Mice were placed in the IR frame of 45 X 45 cm containing total of 16 X 16 infrared beams at an interval of 2.5 cm, located on the sides. Mice were placed in IR frame contacting cage, 1 min prior for acclimatization and then locomotion counts were recorded for a period of 10 min. Locomotion was expressed in terms of total number of IR beam counts per 10 min per animal. Chlorpromazine was used as reference positive standard by oral route at dose of 10 mg/kg.

2.5.5 Hole-board test

Assessment of anxiety behaviour of mice was studied by using hole board apparatus (UGO BASILE) [18]. The board is elevated so that the mouse dipping its head into the hole does not see the bottom. Head dipping was thought to indicate curiosity and was measured by electronic devices. One hour after dosing mouse were placed on hole board and number of head dipping were recorded for 5 min. An increase in the head dipping response reveals a positive anxiolytic like effect [19]. Diazepam was used as reference positive standard at dose of 1 mg/kg by oral route.

2.5.6 Interaction with conventional antidepressant drugs

We investigated interaction of *HNLEE* with conventional antidepressant drugs such as imipramine (dual reuptake inhibitor), fluoxetine (selective serotonin reuptake inhibitor –SSRI), venlafaxine (dual reuptake inhibitors of serotonin and nor epinephrine) and clorgyline (selective monoamine oxidase- A inhibitor). An attempt was made to observe synergistic and or additive effect if any, of *HNLEE* with conventional antidepressant drugs. For this purpose *HNLEE* was administered at sub-effective dose (25 mg/kg) for 14 days and all other antidepressants were administered only on 14th day at their sub-effective doses. Doses studied were imipramine (2 mg/kg), fluoxetine (5 mg/kg), venlafaxine (2 mg/kg) and Clorgyline (125 µg/kg). The doses of the drugs used were selected according to previous studies conducted in our laboratory and as reported in the literature [20].

2.6 Statistical analysis

Results expressed as mean (seconds) ± S.E.M and the data were analyzed by One-way analysis of variance (ANOVA) followed by Newman-Keuls's post-hoc test wherever appropriate. Differences with $P<0.05$ was considered statistically significant. Stastical tests were applied by using computerized GraphPad Prism software (V.4.0)

3 Results

3.1 Determination of LD_{50}

Mice were treated with *HNLEE* at dose of 2000 mg/kg by oral route, exhibited dullness, lethargy upto 4 hrs post dosing. After that all the mice were normal. This lethargy and dullness could be because of *HNLEE* dosing at such a high dose.

Table 1: Effect of *HNLEE* and Chlorpromazine in spontaneous locomotor activity of mice

Group	Ambulatory movement	Stereotypic movement
Vehicle control	1601.33 ± 46.48	1291.67 ± 36.10
HNLEE 100 mg/kg	1547.50 ± 59.84^{NS}	1250.0 ± 47.41 ^{NS}
HNLEE 200 mg/kg	1570.83 ± 46.78 ^{NS}	1271.67 ± 52.64 ^{NS}
HNLEE 400 mg/kg	1558.33 ± 34.57 ^{NS}	1254.17 ± 58.16 ^{NS}
Chlorpromazine 10 mg/kg	737.50 ± 57.60*	649.17 ± 40.39*

Values are expressed as Mean ± S.E.M. of total number of IR beam counts per 10 min per animal. Comparisons were made by using one way ANOVA. NSP>0.05, * P<0.001 when compared with vehicle control.

None of the mice showed mortality. Hence as per OECD 423 guideline, LD_{50} of *HNLEE* could not be determined and it was considered to be above 2000 mg/kg by oral route.

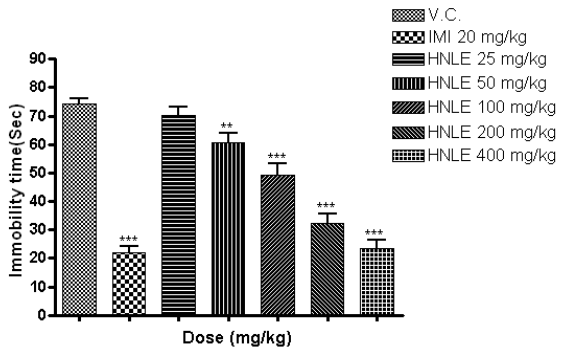


Fig. 1 The effect of *HNLEE* (25, 50, 100, 200 and 400 mg/kg, *P.O.*) and Imipramine (20 mg/kg, *P.O.*) on FST in mice. Data represents Mean ± S.E.M. of immobility time in sec. Comparisons were made by using One way ANOVA followed by Newman-Keul's post hoc test, , **P<0.01, ***P<0.001 compared to control.

3.2 Forced swim test (FST)

The effects of *HNLEE* and Imipramine in FST are shown in Fig. 1. *HNLEE* given at 25 mg/kg dose did not produce significant reduction in immobility count of the mice (P>0.05). *HNLEE* following oral administration at doses 50, 100, 200 and 400 mg/kg produced dose dependent decrease in the immobility duration. This reduction in immobility time was significant when compared with vehicle control (P<0.001). Imipramine was used as reference positive standard, significantly reduced the immobility time in FST (P<0.001).

Percent decrease in immobility time with *HNLEE* at 25, 50, 100, 200 and 400 mg/kg and Imipramine at 20 mg/kg dose was 5.60, 18.61, 33.85, 56.72, 68.38 and 70.63 % respectively. ED_{50} of *HNLEE* in FST determined by Finneys probit analysis method [21] was found to be 179.15 mg/kg with fiducial limit of 78.97- 406.41

3.3 Tail suspension test (TST)

The effect of *HNLEE* and Imipramine in TST is shown in Fig. 2. *HNLEE* exhibited dose dependent decrease in immobility duration in mice. Reduction in immobility time with 25 mg/kg dose of extract was comparable with that of vehicle control (P>0.05), however, other doses of *HNLEE* 50 (P<0.05), 100, 200 and 400 mg/kg produced significant reduction in immobility time in mice (P<0.001). Imipramine, a reference positive standard given at 20 mg/kg dose produced significant reduction in immobility time (P<0.001). The percent decrease in immobility time with extract at dose of 25, 50, 100, 200 and 400 mg/kg and Imipramine at 20 mg/kg dose is 2.48, 12.38, 28.57, 50.29, 69.52 and 69.14 % respectively. ED_{50} of *HNLEE* determined by TST method was found to be 205.84 mg/kg with fiducial limit of 93.44 – 453.44.

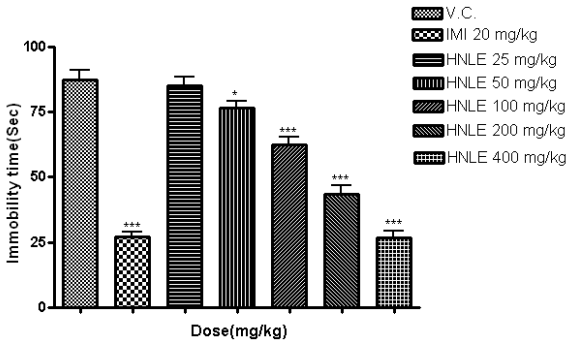


Fig. 2 The effect of *HNLEE* (25, 50, 100, 200 and 400 mg/kg, *P.O.*) and Imipramine (20 mg/kg, *P.O.*) on TST in mice. Data represents Mean ± S.E.M. of immobility time in sec. Comparisons were made by using One way ANOVA followed by Newman-Keul's post hoc test, *P<0.05, **P<0.01, ***P<0.001 compared to control.

3.4 Locomotor activity

The effect of HNLEE and chlorpromazine on spontaneous locomotor activity of mice is shown in Table 1. *HNLEE* at doses 100, 200 and 400 mg/kg by oral route did not produce significant difference in ambulatory and stereotypic activity in mice when compared with vehicle treated animals (P>0.05). Chlorpromazine a positive control at dose of 10 mg/kg produced significant reduction in ambulatory and stereotypic movements of the mice.

3.5 Hole-board test

Effects of *HNLEE* on anxiety levels of mice were studied with hole board apparatus and are presented in Fig. 3. *HNLEE* at dose of 400 mg/kg produced mild to moderate anxiolytic effect in hole board test when compared with vehicle treated mice (P<0.05). Effects at other doses of *HNLEE* were comparable with that of vehicle treated mice. At the same time, diazepam (DPZ) following oral administration at 1 mg/kg enhanced the anxiolytic effect (P< 0.01).

3.6 Interaction with conventional antidepressant drugs

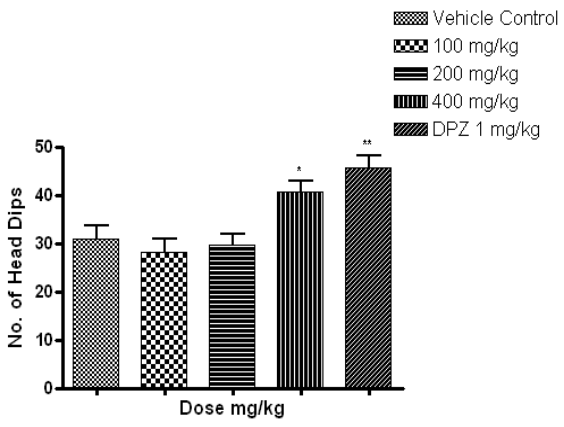
HNLEE and conventional antidepressant drugs were administered simultaneously on 14th day to the mice at their sub-effective doses by oral route. Results are presented in Fig. 4. Reduction in immobility time in FST with sub-effective doses of *HNLEE* and conventional antidepressant drugs was comparable with that of vehicle treated mice (P>0.05). However, when sub-effective dose of *HNLEE* was co-administered with sub-effective doses of Imipramine, fluoxetine, venlafaxine and chlorgylline produced significant reduction in immobility duration when compared with vehicle control group (P<0.001).

4 Discussions

The present study demonstrated the antidepressant like activity of *HNLEE* in FST and TST, a valid animal model for screening antidepressant drugs [16, 22].

The test model of depression (forced swim test and tail suspension test) are based on the observation that rats or mice when forced to swim or suspended in a restricted space from which there is no possibility of an escape, eventually cease to struggle, surrendering themselves (despair or helplessness) to the experimental conditions. This suggested that this helplessness or despair behavior reflected a state of lowered mood in laboratory animals and could serve as a valuable test for screening antidepressant drugs [14, 16,23]

Fig. 3. The effect of *HNLEE* (100, 200 and 400 mg/kg, *P.O.*) and Diazepam (1 mg/kg, *P.O.*) on hole-board test in mice. Values are expressed as Mean ± S.E.M. of total number of head dipping per 5 min per animal. Comparisons were made by using one way ANOVA. ^{NS}P>0.05, * P<0.05, **P<0.01, when compared with vehicle control.

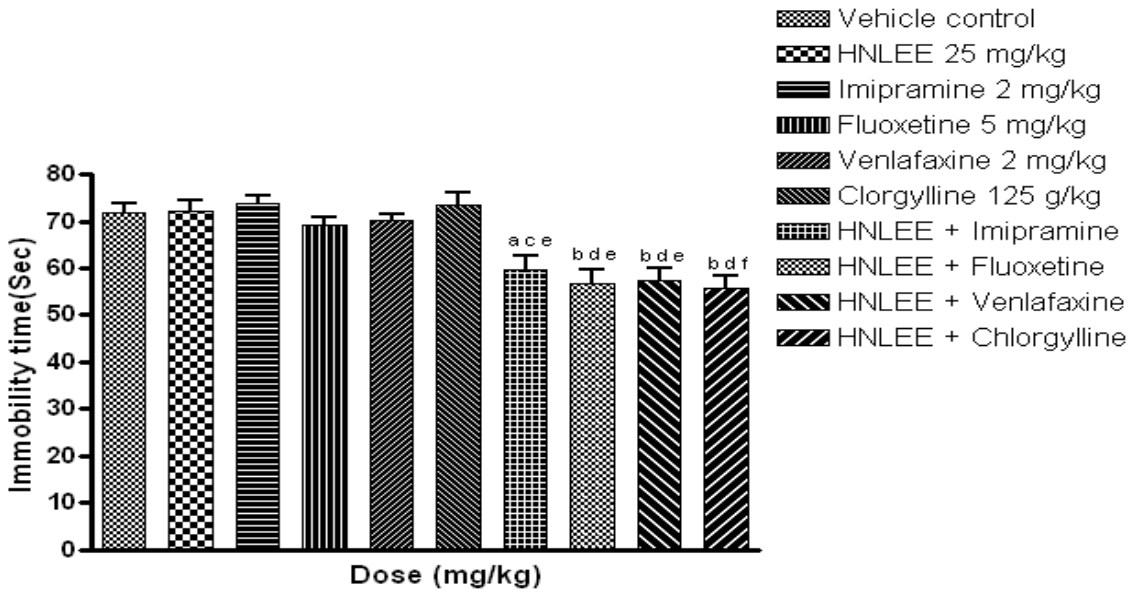


The forced swimming-induced state of immobility in animals claimed to represent a condition similar to human depression [24]. These models are widely accepted to screen antidepressants including tricyclics, selective serotonin re-uptake inhibitors, monoamine oxidase inhibitors [23, 25].

In present study, *HNLEE* was administered by oral route the mice at different dose levels. Lower dose of *HNLEE* was found to be non-effective in altering the immobility duration of mice. However, other doses significantly reduced the immobility time of mice in FST and TST. This reduction in immobility time was found to be dose dependent in both the models. The minimal effective dose of extract was 50 mg/kg in FST and TST model. ED_{50} determined in both the models of depression is comparable with each other. This suggests that *HNLEE* has reproducible antidepressant like activity irrespective of the model. Plant sources can also be the effective alternative remedy to treat the depression is once again proved by our findings. *Tagetes lucida* Cav [26], *Salvia elegans* [27], *Curcuma longa* [7], *Marsilea minuta* [28] are some of the examples to support our findings. Compounds altering motor activity may give false positive/negative effects [17], to ensu re reduction in immobility time by *HNLEE* is not resultant from the changes that occur in motor activity different doses of *HNLEE* were administered to mice by oral route. *HNLEE* did not show any alternations in amubulatory as well as stereotypic activity of mice.

However, reference standard chlorpromazine significantly reduced both ambulatory and

Fig. 4: Effect of HNLEE, Imipramine, Venlafaxine, Fluoxetine and Clorgyline in the FST. Mice were simultaneously treated with HNLEE and conventional antidepressants. After 1 hr of dosing test was carried out. The values are expressed as Mean ± S.E.M. Data were analyzed by one way Analysis of Variance (ANOVA) followed by Newman-Keuls test. aP<0.01, bP<0.001 when compared with vehicle treated animal, cP <0.01, dP < 0.001 when compared to HNLEE alone treated animals, eP< 0.01, fP<0.001 when compared with its respective conventional antidepressant treated animals.



stereotypic activity in mice.

Exploratory behavior or anxiolytic activity of *HNLEE* was studied with the help of hole board apparatus. Generally, exploration is gradually inhibited by anxiety, thereby representing an indirect measurement of anxiety [29]. The inhibition of exploration behavior can be reversed by anxiolytic compounds [30] indicated, in the case of hole board test, by increase in number of head dips [19,31]. Only high dose of *HNLEE* showed increase in number of head dipping compared with vehicle treated group. This suggests that *HNLEE* has anxiolytic activity at high dose.

We also examined the interaction of *HNLEE* with conventional antidepressant drugs at sub-effective doses in FST. Our objective was to observe synergistic or additive effect if any, which will help to delineate the possible mechanism of action underlying the antidepressant like activity of *HNLEE*. Conventional antidepressants, such as tricyclic antidepressants (TCA), selective serotonin reuptake inhibitors (SSRI), serotonin and noradrenaline reuptake inhibitors (SNRI) and monoamine oxidase inhibitors (MAOI), increased the concentration of noradrenaline, serotonin and dopamine by either inhibiting neurotransmitter reuptake or its degradation. Fluoxetine is a potent inhibitor of serotonin reuptake with least or no effect on norepinephrine reuptake [32]. Unlike these agents, imipramine or venlafaxine are dual reuptake inhibitor of serotonin and norepinephrine [32,33]. Low dose of venlafaxine inhibited serotonin reuptake while at high dose it inhibited both serotonin and norepinephrine reuptake and has an action somewhat similar to imipramine [34]. In the present study we observed that *HNLEE*, when administered with standard antidepressant drugs such as imipramine, venlafaxine, fluxetine and chlorgylline at sub effective dose produced synergistic action in forced swim test. This suggests the possible involvement of more than one or all the biogenic amine systems in its antidepressant activity of *Hyoscyamus niger.* The exact involvement of one or all biogenic amine system can further be explored in further study.

Conclusions

The data of our study suggests that *Hyoscyamus niger* extract has antidepressant like activity in FST and TST in mice, a valid animal model to screen antidepressant drugs. This antidepressant activity may involve one or all the biogenic amine system. Apart from this *Hyoscyamus niger* also exhibited anxiolytic activity.

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