ANTIDEPRESSANT AND NOOTROPIC ACTIVITY OF AQUEOUS EXTRACT OF INDIGOFERA TINCTORIA IN MICE

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ABSTRACT

Objective: The present study was undertaken to evaluate the antidepressant and nootropic potential of aqueous extract of Indigofera tinctoria (ITAE).

Methods: Antidepressant activity of ITAE was evaluated in mice by using the forced swim test (FST), tail suspension test tail suspension test and tetrabenazine induced catalepsy and ptosis models at doses of 250 and 500 mg/kg orally. Nootropic activity was assessed using elevated plus maze (EPM) Morris water maze. Imipramine (25 mg/kg) and Piracetam (300 mg/kg) were used as standard drugs for antidepressant and nootropic activity respectively. Antioxidant assays like DPPH and TBARS was also carried out support the antidepressant and nootropic activity.

Results: Pre-treatment with ITAE showed significant dose dependent reduction in immobility time displayed in both, FST and TST, when compared to that of vehicle. (P<0.05). Also, significant reduction in tetrabenazine induced catalepsy and ptosis was observed. Moreover ITAE showed dose dependent cognitive enhancing activity in MWM and EPM. ITAE exhibited IC50 value of 17.39 μg/ ml in DPPH assay and 398. 71 μgm/ ml in TBARS assay.

Conclusion: ITAE exhibits significant antidepressant and nootropic activities at the dose of 250 and 500 mg/ kg which are comparable to imipramine and piracetam respectively which could be attributed to its effect on neurotransmitters and antioxidant activity respectively.

Keywords: Depression, Nootropic activity, Indigofera tinctoria, Antioxidant.

INTRODUCTION

Depression is a common mental disorder characterized by sadness, loss of interest or pleasure, feelings of low self-worth, disturbed sleep and poor concentration. Worldwide prevalence of depression is consistently high and recent statistics from World Health Organization (WHO) states that approximately 350 million people worldwide are affected by depression. The WHO also predicts that unipolar depression will be the second most prevalent cause of illness-induced disability by 2020[1].

Current therapies of depression like selective serotonin reuptake inhibitors (SSRIs), selective serotonin and noradrenaline reuptake inhibitors (SNRIs) etc mainly act by increasing the catecholamine levels in the brain. However limitations of current therapies include unpleasant side effect profile like sedation, sexual dysfunction and dizziness etc [2]. Furthermore, there is considerable literature available that cognitive function is impaired in depressed patients [3]. Depletion of serotonin (5-HT) in the hippocampus, cortex and thalamus, which are highly innervated with serotonergic and cholinergic neurons, may lead to cognitive impairment. Also, tricyclic antidepressants due to their anti-cholinergic effects may worsen dementia [4]. Thus due to adverse effects of current antidepressants and largely unexplored abundance of natural resources, plants can serve as a promising reservoir of new antidepressant drugs. A number of ayurvedic plants have already been evaluated for their antidepressant potential. Many of these plants have shown positive results in preclinical and clinical studies like Withania somnifera and Celastres paniculatus [5]. Many plants have also been reported to have nootropic activity but very few plants have been reported with both antidepressant and nootropic activity e.g Piperin from Piper longum and Piper nigrum [6]. Hence, finding drugs of plant origin that possess both antidepressant and nootropic activity could be a valuable contribution to the existing assemblage of drugs that possess multiple psychopharmacological effects.

The plant Indigofera tinctoria (IT) belongs to the family Fabaceae and is a branching shrub, which grows throughout India. Traditional systems of medicine reveal its effectiveness in treating asthma, splenomegaly, skin diseases and as a trichogenous agent [7, 8]. The plant shows presence of indicaine, flavonoids such as apigenin, kaempferol, luteolin and quercitin[9] and has been reported to have different pharmacological activities like antianxiety, anti diabetic and antidyslipidaemic activity [8]. There are also reports of the putative neuropharmacological effects of IT, mainly antiepileptic activity. A recent publication has also reported beneficial effects of a compound named SF-6, isolated from IT in Parkinson’s disease [9]. However no reports of the antidepressant or nootropic effects of IT have been reported yet.

Hence the present study was conducted to evaluate the antidepressant and nootropic activity of the aqueous extract of Indigofera tinctoria (ITAE).

MATERIALS AND METHODS

Animals

Swiss albino mice of either sex (20-25 g) were used for the study. The animals were housed into groups of six, in polypolypropylene cages, in a photoperiod of 12 hr/12 hr light and dark cycle at 25 ± 2°C and fed with standard pelleted diet and water ad libitum. All the experiments were carried out between 09:00 and 16:00 hr in the laboratory. The experimental protocols were approved by the Institutional Animal Ethics Committee, (IICT/ PH/IAEC/0506/10).

Chemicals

The drugs used in the study were procured from various sources- Imipramine (Depsonil®, Sarabhai Piramal); Tetrabenazine (Revocon®, Sun Pharmaceutical Industries); Scopolamine butyl bromide (Buscopan®, German Remedies Ltd.), Piracetam (Nootropil®- UCB India Pvt Ltd.). 1, 1 Diphenyl, 2-picryl hydrazyl (DPPH) and Thiobarbaturic acid were obtained from Sigma Chemicals and Sisco research lab Ltd. respectively. All other solvents and chemicals used in the study were of analytical grade. Distilled water was used for preparation of aqueous extract.
Collection of plant material

IT leaves were obtained from local market in bulk. IT leaves were washed with distilled water, dried at 50°C in hot air oven, powdered in mixer grinder and stored in a tightly closed container. The identity of the leaves was authenticated by Dr. Ganesh Iyer, Botanist at Ruia College of Arts and Science, Mumbai.

Preparation of extract

Dried powdered leaves were defatted with Petroleum ether (60-80°C) and extracted with water using Soehlet apparatus. The extract so obtained was dried at 50°C in hot air oven and completely dried extract was used for further studies. The aqueous extract for oral administration was prepared in distilled water using 0.2% NaCMC as suspending agent.

Phytochemical screening of IT

Phytochemical screening of ITAE was carried out for the presence of flavonoids, glycosides, saponins and alkaloids [10].

Acute Oral Toxicity study

In accordance with OECD guideline no. 423, acute oral toxicity study of ITAE was performed in mice. The extract was found to be safe till a dose of 2000 mg/kg and hence doses of 250 mg/kg and 500 mg/kg were chosen for in vivo pharmacological evaluations.

Treatment Group

The treatment groups were as follows:

Group I: Mice received 0.2% NaCMC orally, which served as vehicle Control
Group II: Mice were treated with ITAE orally (250 mg/kg)
Group III: Mice were treated with ITAE orally (500 mg/kg)
Group IV: Mice were treated with Imipramine orally (25 mg/kg) which served as Positive Control

The mice were randomly assigned into four groups (n=6) and the mice were pre-treated for 14 days before the experiment.

For nootropic activity, mice were treated with Piracetam orally (300 mg/kg) that served as positive control instead of imipramine.

Antidepressant activity

In the experiments of anti depressant activity, the models of forced swim test, tail suspension test and tetrabenazine induced ptosis and catalepsy was used.

Forced swim test (FST)

The FST described by Porsolt et al [11] was slightly modified for the conduct of the experiment. Each mouse was positioned in a glass cylinder (height 30 cm, diameter 22.5 cm), containing 15 cm of water maintained at 25°C. Animals were pre-screened on the previous day by placing the animals individually in the water filled glass cylinder. After 5-6 min, immobility reaches a plateau where the mice remained immobile for approximately 80% of the time. After 15 min in the water, the mice were removed and allowed to dry before being returned to their home cages. On the day of experiment, 60 min prior to the test, the drugs were administered. The mice were again placed individually in the water filled glass cylinder and the duration of immobility was recorded during the last four min of a six min test. A mouse was considered immobile when floating motionless or making only those movements necessary to keep its head above water surface. The water was changed after each test.

Tail Suspension test (TST)

TST was performed by the method of Steru et al [12] which is a facile method for treating with testing antidepressants. For the test, the mice were suspended on the edge of a shelf 58 cm above a tabletop by adhesive tape placed approximately 1 cm from the tip of the tail. The duration of immobility was recorded for a period of 5 min. Mice was considered to be immobile when they hung passively and completely motionless.

![Fig. 1: Effect of ITAE on Immobility time in FST and TST.](image)

Table 1: Effect of ITAE on Degree of Tetrabenazine induced catalepsy and ptosis in mice (Scores)

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Treatment Group</th>
<th>Scores of catalepsy</th>
<th>Scores of ptosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vehicle control</td>
<td>5 ± 0.04</td>
<td>2.83 ± 0.31</td>
</tr>
<tr>
<td>2</td>
<td>ITAE 250 mg/kg, p.o</td>
<td>4 ± 0.1</td>
<td>1.3 ± 0.47**</td>
</tr>
<tr>
<td>3</td>
<td>ITAE 500 mg/kg, p.o</td>
<td>3 ± 0.25**</td>
<td>1.65 ± 0.15</td>
</tr>
<tr>
<td>4</td>
<td>Positive control (Imipramine 25 mg/kg, p.o)</td>
<td>4.25 ± 0.23**</td>
<td>1 ± 0.21**</td>
</tr>
</tbody>
</table>

N=6, Values Are Mean ± S.E.M. *P< 0.05, **P < 0.01, when compared to vehicle control group by one-way ANOVA followed by DUNNETT’S COMPARISON TEST.
Evaluation of nootropic activity
In the experiments of nootropic activity, the models of elevated plus maze test and Morris water maze test were used.

Elevated plus maze test (EPM)
The elevated plus maze apparatus used in the study consisted of two open arms 50 x 10 x 40 cm and two closed arms of the same dimensions with an open roof facing each other. The maze was elevated at a height of 50 cm from the ground.

The mice were placed individually 60 min after oral administration of either vehicle or test drug at the end of either of the open arms and the time taken by the animal to move from open to closed arm (transfer latency) was noted on the first day. The time elapsed between the time that the animal was placed on the open arm and the time at which all four legs were inside the enclosed arms, was noted as the transfer latency. The transfer latency was again recorded 24 hr after the first exposure. TL measured on the 1st and 2nd day served as parameters for acquisition and retrieval, respectively [14].

Table 2: Effect of ITAE on Transfer latency using Elevated plus maze model

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Time to reach the open arm (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
</tr>
<tr>
<td>Vehicle Control</td>
<td>26 ± 3.39</td>
</tr>
<tr>
<td>ITAE 250 mg/kg p.o</td>
<td>31 ± 7.69</td>
</tr>
<tr>
<td>ITAE 500 mg/kg p.o</td>
<td>21 ± 4.24</td>
</tr>
<tr>
<td>Positive control</td>
<td>2.32 ± 5.52</td>
</tr>
<tr>
<td>(Piracetam 300 mg/kg p.o)</td>
<td></td>
</tr>
</tbody>
</table>

N=6, Values Are Mean ± S.E.M, *P< 0.05, **P < 0.01, when compared to control group on each day by one-way ANOVA followed by DUNNETT’S Comparison Test

Morris water maze (MWM) test
The experimental groups used for spatial and working memory testing in MWM were similar to that used in elevated plus maze described earlier. The MWM consisted of a large water tank (48 cm x 28 cm x 18 cm) filled with water, which was made opaque by adding milk. Due to water, homogeneous intramaze environment is generated, thus eliminating any olfactory intrusion. A rectangular escape platform (7x7 cm) was constructed of water resistant material and covered with material that allows the animal to remain on top when it is submerged. The platform was 10 cm in height so that it could be submerged 2 cm below the level of water surface. The water temperature was maintained at 26°C [15]. The spatial and working memory test was done by the method of Annapurna et al.

Table 3: Effect of ITAE on Scopolamine Induced Spatial Memory Impairment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Latency to reach the platform in sec</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
</tr>
<tr>
<td>Control</td>
<td>10.7 ± 0.93</td>
</tr>
<tr>
<td>ITAE 250 mg/kg p.o</td>
<td>8.46 ± 0.37</td>
</tr>
<tr>
<td>ITAE 500 mg/kg p.o</td>
<td>9.59 ± 0.89</td>
</tr>
<tr>
<td>Positive control</td>
<td>8.67 ± 0.67</td>
</tr>
<tr>
<td>(Piracetam 300 mg/kg p.o)</td>
<td></td>
</tr>
</tbody>
</table>

N=6, values are Mean ± S.E.M.*P< 0.05, **P < 0.01, when compared to normal group by one-way ANOVA followed by DUNNETT’S TEST.

Spatial memory test
Drugs were administered orally and after 60 min all the groups were exposed to a training schedule that consisted of the mice swimming in the MWM and finding the submerged hidden platform. This procedure was repeated at 24 hr interval for two more days until each animal acquired minimum time interval to reach the submerged platform in the pool. On fourth day, after complete training, all groups were treated with scopolamine butyl bromide (2 mg/kg, i.p.). 30 min later, they were treated with test extract/standard, and were tested for spatial memory after further 90 min. Mice with memory impairment took more time to reach the platform. The animals were also tested for spatial memory on fifth day to check the ability of test extract to restore scopolamine-induced amnesia [15].

Table 4: Effect of ITAE on Scopolamine Induced Working Memory Impairment

<table>
<thead>
<tr>
<th>Quadrant</th>
<th>Treatment</th>
<th>Latency to reach the platform in sec</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 0</td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>10.7 ± 0.93</td>
</tr>
<tr>
<td></td>
<td>ITAE 250 mg/kg p.o</td>
<td>8.46 ± 0.37</td>
</tr>
<tr>
<td></td>
<td>ITAE 500 mg/kg p.o</td>
<td>9.59 ± 0.89</td>
</tr>
<tr>
<td></td>
<td>Positive control</td>
<td>8.67 ± 0.67</td>
</tr>
<tr>
<td></td>
<td>(Piracetam 300 mg/kg p.o)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Control</td>
<td>7.2 ± 0.22</td>
</tr>
<tr>
<td></td>
<td>ITAE 250 mg/kg p.o</td>
<td>5.56 ± 1.17</td>
</tr>
<tr>
<td></td>
<td>ITAE 500 mg/kg p.o</td>
<td>5.12 ± 0.63</td>
</tr>
<tr>
<td></td>
<td>Positive control</td>
<td>5.6 ± 0.54</td>
</tr>
<tr>
<td></td>
<td>(Piracetam 300 mg/kg p.o)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Control</td>
<td>8.24 ± 0.83</td>
</tr>
<tr>
<td></td>
<td>ITAE 250 mg/kg p.o</td>
<td>5.49 ± 0.74</td>
</tr>
<tr>
<td></td>
<td>ITAE 500 mg/kg p.o</td>
<td>5.87 ± 1.06</td>
</tr>
<tr>
<td></td>
<td>Positive control</td>
<td>6.55 ± 0.86</td>
</tr>
<tr>
<td></td>
<td>(Piracetam 300 mg/kg p.o)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Control</td>
<td>8.93 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>ITAE 250 mg/kg p.o</td>
<td>7.1 ± 0.63</td>
</tr>
<tr>
<td></td>
<td>ITAE 500 mg/kg p.o</td>
<td>6.54 ± 0.43</td>
</tr>
<tr>
<td></td>
<td>Positive control</td>
<td>6.88 ± 0.24</td>
</tr>
</tbody>
</table>

N=6, Values Are Mean±S.E.M. *P< 0.05, **P < 0.01, when compared to control group on each day by one-way ANOVA followed by DUNNETT’S TEST.
Working memory test
This test was applied after the acquisition phase of testing was completed. It is important that the mice demonstrate that they know the location of hidden platform before beginning the test. This method has been referred as the reversal test. Again here the time taken by the mice for finding the submerged platform was recorded but the platform’s location was changed sequentially to all four quadrants i.e. North, South, East and West in the MWM and average time to reach the submerged platform in each quadrant was recorded.

Evaluation of spatial memory and working memory tests
Latency to reach the platform in sec (mean values) was recorded on day 0, day 1, day 2, day 4 and day 5. Day 1 is the day from which animals were treated with the test extract/standard. Day 4 is the day on which animals were treated with scopolamine butyl bromide 2mg/kg, i.p [15].

Evaluation of antioxidant activity
DPPH radical scavenging assay
DPPH radical scavenging assay was performed spectrophotometrically by the method of Deshmukh et al. The DPPH scavenging activity was measured spectrophotometrically by monitoring the decrease in absorption at 517 nm [16]. The assay was performed in duplicates.

Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the formula
\[
\text{% inhibition} = \left(\frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{test}}}{\text{Abs}_{\text{control}}}\right) \times 100
\]

TBARS Assay
The lipid peroxidation level is measured as the thiobarbituric acid reactive substance (TBARS), as per the method of Okhawa et al. Mouse brain homogenate was used for induction of lipid peroxidation, mediated by FeSO4 as pro-oxidant. The absorbance was read at 532 nm in a UV spectrophotometer[17]. The assay was performed in duplicates.

The percent inhibition of lipid peroxidation was calculated by comparing the results of the tests with those of controls, not treated with the extracts, as per the following formula:
Inhibition (%) = \left(\frac{\text{Control} - \text{test}}{\text{Control}}\right) \times 100

DISCUSSION
Depression is associated with a significant negative impact on quality of life, morbidity/mortality, and cognition. Many factors are proposed for causing depression, such as changes in brain monoaminergic transmission (e.g., 5-Hydroxy Tryptamine, Norepinephrine, Dopamine), abnormalities in function of neurotransmitter receptors (e.g., Adenylyl Cyclase-cAMP pathway), dysregulation of hypothalamic pituitary adrenal axis (cortisol), increased proinflammatory cytokines (e.g., Interleukin-6, Tumour necrosis factor-α), increased nitric oxide (NO) and increased oxidative stress (e.g., lipid and DNA damage)[18, 19].

The immobility displayed by rodents when subjected to an unavoidable and inescapable stress in FST and TST has been postulated to mirror depressive disorders in humans. In this study, we provide conclusive evidence that ITAE 250 mg/kg and 500 mg/kg administered by oral route produces antidepressant like effects in FST and TST after two weeks treatment, comparable to the standard drug, Imipramine (P values < 0.01). However, a false positive or negative result can be observed due to enhanced or decreased locomotor activity because of psycho-stimulating effects of the antidepressants in a behavioural despair test [20]. Hence, to eliminate the possibility that the decrease in the immobility time elicited by a drug is due to an increased locomotor activity, the activity cage test was performed. In our current study, no significant changes in locomotor activity in mice were observed at the doses of ITAE 250 mg/kg and 500 mg/kg administered by oral route produces antidepressant like effects in FST and TST after two weeks treatment, comparable to the standard drug, Imipramine (P values < 0.01). However, a false positive or negative result can be observed due to enhanced or decreased locomotor activity because of psycho-stimulating effects of the antidepressants in a behavioural despair test [20]. Hence, to eliminate the possibility that the decrease in the immobility time elicited by a drug is due to an increased locomotor activity, the activity cage test was performed. In our current study, no significant changes in locomotor activity in mice were observed at the doses of ITAE 250 mg/kg and 500 mg/kg. Thus, the antidepressant-like effect of ITAE was not due to increased locomotor activity. Furthermore, significant reversal of tetrabenazine induced ptosis and catalepsy in mice by ITAE suggests that its antidepressant potential may be due to increase in catecholamine levels as tetrabenazine causes significant depletion of catecholamine levels.

The phytoconstituents present in ITAE showed majorly the presence of flavonoids, tannins and saponins. Flavonoids such as apigenin, quercetin and kaempferol which are present in IT are reported to have good antioxidant potential and are also reported to have antidepressant activity [21]. Literature sources reveal that IT contains appreciable amounts of conjugated indoxyl, the precursor of isatin, an indole derivative [22]. Isatin is an endogenous compound which has the ability to inhibit MAO [23]. Isatin’s MAO inhibitory activity could lead to increased amount of neurotransmitters, the levels of which are depleted in depression. Thus, presence of flavonoids and isatin precursors could be evaluated.
hypothesized to be the one of the reasons for the antidepressant potential of ITAE. The results of antioxidant activity displayed that ITAE scavenged DPPH radical in a dose dependent manner. To evaluate the antioxidant potential of the extract, the extract was allowed to react with a stable radical 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) in a methanol solution. In its radical form, DPPH absorbs at 515 nm, but upon reduction by an antioxidant, the absorption disappears. The bleaching of DPPH absorption is representative of the capacity of ITAE to scavenge free radicals independently from any enzymatic activity. Further, ITAE was able to inhibit the lipid peroxidation in the brain homogenate induced by Fe²⁺-ADP-acorbate system. The inhibition could be caused by the scavenging of OH radical or by reducing the rate of conversion of ferrous to ferric. As ITAE showed good DPPH scavenging potential and was able to reverse the lipid peroxidation in mice brain, it may have neuroprotective effects.

There is growing evidence that one of the major causes for depression is oxidative stress [19, 24] and antidepressants may exert its therapeutic action by virtue of its antioxidant activity [24]. Further, oxidative stress has shown to lead to memory impairment and administration of antioxidants reverses this phenomenon [25]. In the present study, the treatment with ITAE at 250 and 500 mg/kg caused reduction in transfer latency in the EPM and also mitigated the memory impairment caused by scopolamine in both spatial and working memory as measured in the MWM test. The results of ITAE 250 mg/kg and 500mg/kg were comparable to that of the standard drug, Piracetam (P < 0.01) in the MWM test.

From the above results, it could be postulated that the cognitive enhancing activities of ITAE could be due to its antioxidant activity. Also, the antidepressant effect of ITAE could be attributed partly to the increase in neurotransmitter levels and partly to its antioxidant potential.

CONCLUSION
In conclusion, the administration of ITAE and ITAE exhibits antidepressant and nootropic activities at the dose of 250 and 500 mg/kg in pretreated mice which can have potential benefits to treat cognitive impairment associated with depression. Further studies are needed to identify the phytoconstituents responsible for these activities and to conduct biochemical estimations of neurotransmitters involved in the antidepressant and nootropic activities of ITAE to elicit the exact mechanism of action.

CONFLICT OF INTERESTS
Declared None

ACKNOWLEDGEMENT
This work was supported by grants from University Grants Commission (UGC), India. The authors gratefully acknowledge the financial assistance and fellowship from University Grants Commission, India.

REFERENCES