Neuropharmacological Effect of Aqueous Extract of Bauhinia tomentosa L. Leaves in Experimental Animal Models

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ABSTRACT

Bauhinia tomentosa is a traditional medicine used to treat Diuresis, Impotence, Diabetes, Dysentery, Inflammation and Hyperlipidemia. The present study was carried out to investigate the possible neuropharmacological activities of aqueous extract of Bauhinia tomentosa L. leaves in mice. The effects of the extract on Central Nervous System were evaluated by elevated plus maze, spontaneous locomotor activity, forced swim test, diazepam induced sleeping time, rota rod apparatus and haloperidol induced catalepsy. The extract at doses 200 and 400 mg/Kg revealed the anti-anxiety activity. The Central Nervous System depressant activity was confirmed by spontaneous locomotor activity, forced swim test and diazepam induced sleeping time in mice at the doses of 200 and 400 mg/Kg, p.o. Its nootropic activity was confirmed by increase in inflexion ratio at dose of 400 mg/Kg, p.o. of body weight. The extract has shown significant effect on motor coordination at dose of 400 mg/Kg and potentiated the catalepsy at 90 mins after haloperidol administration at doses, 200 and 400 mg/Kg p.o. The results conclude that the aqueous extract of Bauhinia tomentosa possesses anxiolytic, depressant, nootropic and skeletal muscle relaxant properties along with its anti- psychotic activity.

Keywords: Anxiolytic, Depressant, Nootropic, Muscle relaxant, Anti – Psychotic, Haloperidol.

INTRODUCTION

Medical plants have been utilized in the treatment of various pathological diseases and neurological disorders for many years in folk medicine. The therapeutic plants, in addition, were found to be safe and free from side effects when compared with modern medicines. Several scientific studies demonstrated that the principle bioactive compounds present in the medicinal plants were the main causative agents for their predominant therapeutic effects. Even though 80% of drugs used in developing countries such as India and China are derivatives of phyto extracts, the rational treatment of neurological disorders by plant extract derived drugs is still in its embryonic stage due to the complex chemistry and organization of the central nervous system. Extracts were found to be better than purified compounds since they contain many phytoconstituents which may work synergistically to protect against many disease.

Bauhinia tomentosa (an ornamental plant), is a yellow orchid tree, belonging to the family Caesalpinaceae. It is an erect, branched shrub with 6-12 m height and pale lemon yellow flowers. It is commonly known as ‘Kanchini’ in Tamil and ‘Phalgū’ in Sanskrit. The generic name denotes the bauhin brothers, Jean and Gaspard, the swiss botanist; and the species name ‘tomentosa’ denotes its hairy and velvety pods. It flowers in rainy season and fruits in winter season. It has been used traditionally for its therapeutic properties such as hepatoprotective, astringent, dysentery, antihyperglycemic, antilipidemic, diuretic, tonic, anti-inflammatory, anti-neoplastic, antioxidant, antimicrobial and antioxidant scavenging activities. The presence of four flavonoidal glycosides in leaves like kampferol-7-o-rhamnoside, kampferol-3-o-glucose, quercetin-3-o-glucoside and quercetin-3-o-rutinoside revealed the potential cytotoxic activity. Linoleic, stearic and vernolic acids were reported from the seed oil. Flowers contain glycosides, tannins, alkaloids, phytosterols, flavonoids, saponins, carbohydrates, phenolic compounds and tannins. Bark yields fiber and roots yield carbohydrates, reducing sugars, saponins, tannins, phenolics and flavonoids. According to the literature collected ever time by the author, the present neuropharmacological studies are the first ever studies conducted on the aqueous extract of leaves of Bauhinia tomentosa L.

MATERIALS AND METHODS

Collection of Plant Material

Fresh leaves of Bauhinia tomentosa L. were collected from the local fields of Tamil Nadu. The plant specimen was identified and authenticated by Botanical Survey of India (BSI), Tamil Nadu Agricultural University Camps, Southern Regional Central, Coimbatore, Tamil Nadu. A Voucher specimen (No.:BSI/SRC/5/23/09-10) is preserved in the herbarium of department of BSI for future references. Leaves were separated from adulterants, shade dried and powered coarsely.
Plant Extraction

The plant extract was prepared by blending and macerating 500g of the fresh leaves of Bauhinia tomentosa with 100ml of distilled water and was kept at 40°C for 24 hours for extraction to take place. The resulting mixture was filtered. The concentration of the extract recovered from the filtrations was calculated using the expression:

\[
\text{Concentration} = \frac{(X - Y)}{Z} \text{ g/ml}
\]

Where \( X \) = Weight of fresh leaves before blending
\( Y_g \) = Weight of leaves after filtration
\( Z_{ml} \) = Volume of water after filtration

Finally the colour consistency of aqueous extract was noted and the fresh preparation was used for each experimental study.

Experimental Animals

Swiss albino mice of either sex, weighing 20-25 gm were used. All animals were obtained from the Animal house, Surya School of Pharmacy, Villupuram, Tamil Nadu. The animals were housed in groups of six in polypropylene cages with soft wood shavings as bedding and housing conditions under 12 hour light-dark cycle. They had free access to tap water and food. A period of at least one week for adaptation to the laboratory facilities was allowed. Each animal was used only once under standard laboratory conditions and all the observations were made at room temperature in a noiseless diffusely illuminated room. All experimental protocols were approved by Institutional Animals Ethics Committee (IAEC) as per provisions of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

Drugs and Chemicals

The drugs used were, Diazepam purchased from NEON Laboratories Ltd, Mumbai, India, Haloperidol procured from SERENACE-RPG Life Sciences Ltd. and normal saline.

Acute Toxicity Study

Acute oral toxicity study of aqueous extract of Bauhinia tomentosa (AEBT) was performed according to the method described by an Organization for Economic Cooperation and Development Guideline (OECD) 423 (Acute Toxic Class Method). Swiss albino mice (20-25 gm, n=6) of either sex were selected by random sampling technique. The animals were kept fasting for 4 hours prior to experiment with free excess of water. The extracts of Bauhinia tomentosa (suspended with 0.5 % W/V, CMC) were administered orally at a dose of 5 mg/Kg body weight to separate group of mice and mortality was observed for 3 days. If mortality was not observed in 4/6 or 6/6 animals, then the dose administered was considered as toxic dose. However, if the mortality was found in single mice out of 6 animals, then the dose was repeated with higher 50, 300, 500, 1000 and 2000 mg/Kg of body weight. Behavioural changes and mortality of experimental mice were observed for 24 hour. After that continued observation were composed to the 14th day.

Anxiolytic Activity

Elevated Plus Maze Model

The elevated plus maze model was used as a standard model to assess anxiolytic activity of drugs. The apparatus consist of two open arms (30×5 cm) and two closed arms (30×5×20 cm) elevated to the height of 50 cm. The floor of each arm is wooden and the walls of the closed arms are also wooden. The edges of the open arms are 0.5 cm in height to keep the mouse from falling down and the edges of the closed arms are 15 cm in height. Four groups of mice (each contained 6 animals) were used for the study. First group, treated with vehicle (10 ml/Kg/p.o.), third and fourth groups with AEBT (200 and 400 mg/Kg/p.o.), respectively. Second group received the reference standard drug Diazepam (1 mg/kg/i.p.) 60 minutes before the commencement of the study.

The mouse was placed at the centre of maze, its head facing one of the closed arm and the readings were noted for 5 minutes. The number of entries into open and closed arms and the time spent in the open and closed arms were recorded during the test period of 5 minutes. Arm entries were counted when the animal placed all of its four paws on it. After each trial, the plus maze was wiped with hydrogen peroxide and dried with sponge, carefully. Experiments were carried out in a sound attenuated room, illuminated only by dim light.

Antidepressant Activity

Locomotor Activity

The central nervous system depressant activity of AEBT was evaluated by Spontaneous Locomotor Activity of mice using an Actophotometer. This photocell activity cage was utilized to determine the degree of depression. The units of the activity counts were based on the beam breaks by movement of mice. The mice were divided into three groups each containing six animals. The animals of each group were placed individually in the activity cage for 10 minutes. The basal activity score of all the animals were monitored and recorded. The mice were re-tested for activity scores 30 min. after Diazepam (1 mg/kg/i.p.) and 60 min. after AEBT (200 and 400 mg/Kg/p.o.) administration, respectively. Each animal served as its own control.

Forced Swim Test

Forced swim test model was employed for the determination of antidepressant activity of all major classes of antidepressant drugs. Animals were placed individually in a glass cylinder (30 cm in height, 22.5 cm in diameter) filled with water upto a height of 15 cm, at room temperature. Each animal was trained to swim for 15 min., constituted the pre-test session. After 24 hours, the animals were treated with AEBT (200 and 400 mg/Kg/p.o.) or vehicle (control group) and was again forced to swim in similar environment for a period of 6...
min., in a test session. Duration of immobility for each mouse was recorded. The mouse was assessed to be immobile when it remains floating motionless in water and struggling to keep its head above water. Four groups of animals (n=6) were chosen by means of completely randomized schedule and were used for the experiment\(^\text{14}\).

**Diazepam-Induced Sleeping Time**

Three groups of animals (each contained 6 mice) were used to determine the effect of AEBT on duration of diazepam-induced sleep test. Mice were subjected to pretreatment by treating the control group with normal saline and the test groups with AEBT (200 and 400 mg/Kg/p.o.), respectively. The pretreatment was carried out 30 min. prior to the treatment. In treatment, Diazepam (25 mg/Kg, i.p.) was given to all groups. Each animal was observed for the duration of sleep and the readings were recorded as the time interval between the loss and regaining of righting reflex\(^\text{15,16}\).

**Nootropic Activity**

**Elevated Plus Maze Model**

The elevated plus maze served as the exteroceptive behavioural model, wherein the stimulus existed outside the body was used to evaluate the retention of learning and memory. The apparatus used was same as described earlier. On the first day, Mice were placed individually at the end of an open arm facing away from the central platform and the time taken by the mouse to move from the end of open arm to either of the closed arm was recorded as transfer latency, TL. Animals were divided into three groups of six mice each, randomly and were allowed to explore the plus maze for 3 min. after the measurement of TL. On the second day, mice received vehicle, or extract (200 and 400 mg/Kg, p.o.) 1 hour before their placement on the elevated plus maze and TL was noted again for 90 seconds. The TL was expressed as retention after 24 h calculating the “inflexion ratio (IR)” using the formula,

$$IR = \frac{(L_0-L_1)}{L_1}$$

Where, \(L_0=\) initial transfer latency in seconds and \(L_1=\) transfer latency after 24 h\(^\text{17,18}\).

**Motor Coordination Activity**

**Rota rod test**

The effect of extracts on the motor coordination was studied by using a rota-rod apparatus. The apparatus consisted of a base platform and an iron rod of 3 cm diameter and 30 cm length, with a non slippery surface. The rod was separated into four equal sections by three disks, enabling four mice to walk on the rod at the same time. The instrument was set at a rate of 25 rpm. Three groups of mice (n=6) were used for the study. Animals of each group were placed individually on the rod and the fall off time of each animal was recorded for 5 min. Mouse that remained on the rod for 3 min were selected for the study. Then, the first group (served as reference) received diazepam at the dose of 4 mg/Kg body weight, i.p. and the other two groups received AEBT at the doses of 200 and 400 mg/Kg body weight, p.o., respectively. The animals were again tested for fall off time 60 min. before and after administration of the drug and the extracts. Each animal served as its own control\(^\text{19}\).

**Catalepsy Activity**

**Haloperidol (Hp)-Induced Catalepsy**

Catalepsy, defined as a reduced ability to initiate movement and a failure to correct posture, was measured by means of the bar test. Standard bar test was used to measure the cataleptic activity. Three groups of mice (containing six animals each) were used for the study. Catalepsy was evaluated by placing both forepaws of the mouse over a horizontal bar (diameter, 1 cm), elevated 4 cm from floor. The length of the time the mice maintained this position was recorded by stopwatch for 180 seconds. The mice maintained the position for 30 seconds were said to be cataleptic. The end point of catalepsy was measured when the animal moved its head in an exploratory manner or when both the front paws were removed from the bar. The control group was treated with haloperidol (2 mg/Kg/i.p.) 30 min. and 90 min. prior to the experiment. The test groups were administered with AEBT (200 and 400 mg/kg, p.o.) 30 min. before haloperidol treatment and the experiment was repeated\(^\text{20,21}\).

**Statistical Analysis**

Results were expressed as Mean ± SEM. Data were analyzed using one-way analysis of variance (ANOVA) followed by Dunnet’s test and paired t-test. The results were regarded as significant at \(p<0.05\), very significant at \(p<0.01\) and highly significant at \(p<0.001\), respectively.

**RESULTS**

**Acute Toxicity Study**

The aqueous extract of leaves of Bauhinia tomentosa L. was evaluated for its acute toxicity in mice. No mortality was observed to a dose as high as 2000 mg/Kg, p.o. of AEBT leaves and the results showed that the extract can be used safely in animals upto a dose of 2000 mg/Kg, p.o.

**Anxiolytic Activity**

**Elevated Plus Maze Model**

The result in table 1 showed the effect of oral administration of AEBT (200 and 400 mg/Kg) in Elevated plus maze model. The extract (200 and 400 mg/Kg, p.o.) and diazepam (1 mg/Kg, i.p.) significantly increased the mean number of entries and mean time spent in open arms and decreased the preference to the closed arms, when compared with control.
Table: 1 Effect of aqueous extract of leaves of *Bauhinia tomentosa* L. on Elevated plus maze model for Anxiolytic activity

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean No of Entries (sec)</th>
<th>Mean time spent (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Open arm</td>
<td>Closed arm</td>
</tr>
<tr>
<td>Control (Vehicle)</td>
<td>3.246±0.3628</td>
<td>11.426±0.5438</td>
</tr>
<tr>
<td>Diazepam (1 mg/Kg, i.p.)</td>
<td>11.244±0.7246**</td>
<td>5.324±0.7824**</td>
</tr>
<tr>
<td>AEBT (200 mg/Kg, p.o.)</td>
<td>5.854±0.4224**</td>
<td>9.236±0.2484**</td>
</tr>
<tr>
<td>AEBT (400 mg/Kg, p.o.)</td>
<td>8.122±0.3566**</td>
<td>7.794±0.5126**</td>
</tr>
</tbody>
</table>

**Antidepressant Activity**

**Locomotor Activity**

The effect of AEBT (200 and 400 mg/Kg, p.o.) and diazepam (1 mg/Kg, i.p.) on spontaneous locomotor activity was summarized in table 2. The photocell activity count in the extract treated groups was significantly reduced when compared to basal value. The same effect was revealed by the standard treated group as well.

**Diazepam-induced sleeping time**

The extract potentiated diazepam-induced sleeping time in mice (Figure 1). The average sleeping time due to dopamine (25 mg/Kg, i.p.) alone was found to be 105.0±1.77 in minutes. AEBT (200 mg/Kg, p.o.) significantly potentiated the sleeping time, whereas the extract (400 mg/Kg, p.o.) did not show any significant change in sleeping time induced by diazepam.

**Nootropic Activity**

**Elevated Plus Maze Model**

In the elevated plus maze paradigm, the *Bauhinia tomentosa* extract showed a decrease in transfer latency (TL) on day-2, when compared with control. The extract at dose 400 mg/Kg, p.o. revealed significant increase in inflexion ratio (IR), whereas at dose 200 mg/Kg, p.o. the IR of the extract was found to be non-significant. The results are shown in table 3.

**Motor Coordination Activity**

**Rota rod test**

The AEBT extract at dose 400 mg/Kg, p.o. and diazepam (1 mg/Kg, i.p.) shown a significant decrease in fall off time when compared with basal value. Whereas the extract at dose 200 mg/Kg, p.o. was found to be non-significant. The results are given in table 5.
Table: 4 Effect of aqueous extract of leaves of Bauhinia tomentosa L. on Elevated plus maze for Nootropic activity

<table>
<thead>
<tr>
<th>Groups</th>
<th>Transfer latency in seconds</th>
<th>Inflexion ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day-1</td>
<td>Day-2</td>
</tr>
<tr>
<td>Control (Vehicle)</td>
<td>36.899±1.846</td>
<td>17.212±1.346</td>
</tr>
<tr>
<td>AEBT (200 mg/Kg, p.o.)</td>
<td>46.542±1.724**</td>
<td>16.213±0.8456**</td>
</tr>
<tr>
<td>AEBT (400 mg/Kg, p.o.)</td>
<td>56.168±2.635**</td>
<td>12.064±0.833**</td>
</tr>
</tbody>
</table>

Table: 5 Effect of aqueous extract of leaves of Bauhinia tomentosa L. on Rota rod test

<table>
<thead>
<tr>
<th>Groups</th>
<th>Fall off time (Seconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before administration</td>
</tr>
<tr>
<td>Diazepam (1 mg/kg, i.p.)</td>
<td>274.5±4.18</td>
</tr>
<tr>
<td>AEBT (200 mg/Kg, p.o.)</td>
<td>222.5±3.13</td>
</tr>
<tr>
<td>AEBT (400 mg/Kg, p.o.)</td>
<td>254.0±2.23</td>
</tr>
</tbody>
</table>

Catalepsy Activity

Haloperidol (Hp)-Induced Catalepsy

Haloperidol (1 mg/Kg, i.p.) exhibited a significant cataleptic behaviour in the treated animal (Figure 2). Pre-treatment with AEBT extract at dose 200 and 400 mg/Kg, p.o. significantly potentiated the cataleptic effect of Haloperidol after 90 min time interval. However, the extract (200 and 400 mg/Kg, p.o.) produced less significant cataleptic behaviour after 30 min time interval.

DISCUSSION

This study investigated the acute toxicity profile of AEBT orally. The extract was evaluated for anxiolytic (Elevated plus maze model), Antidepressant (Spontaneous locomotor activity, Forced swim test, Diazepam-induced sleeping time), Nootropic (Elevated plus maze model), Motor-coordination (Rota rod test), Antipsychotic property (Haloperidol-induced Catalepsy).

Acute toxicity was done according to OECD-423 guideline. No mortality was observed to a dose as high as 2000 mg/Kg, p.o. of AEBT leaves and the result showed that the extract can be used safely in the animals upto the dose of 2000 mg/Kg. Dose levels for the present study were chosen as 200 mg/Kg and 400 mg/Kg, respectively.

Anxiety results from disturbances in coordination of various neurotransmitters such as gamma amino-butyric acid (GABA), serotonin, dopamine, nor adrenaline, opioid peptides endocannabinoids, corticotrophin-releasing hormone, neuropeptide Y and oxytocin in number of neurological pathways of brain. GABA, the major inhibitory neurotransmitter in the brain, by activation of its receptor (GABA_A receptor), increases chloride conductance across the cell membrane and causes neuronal failure to generate an action potential, thereby leading to inhibition. The elevated plus maze was found to be an etiologically valid animal model of anxiety since it uses natural stimuli, such as, the fear of a new, brightly lit open space and the fear of balancing on a relatively narrow raised platform. Moreover, it is well understood that anxiolytic agents increases the number of entries and the time spent in open arms of the elevated plus maze.

The AEBT (200 and 400 mg/Kg, p.o.) and diazepam (1 mg/Kg, i.p.) significantly increased the mean number of entries and mean time spent in the open arms and decreased the preferences to the closed arms when compared with control. The result that was obtained from
anxiety-related behavior model in mice revealed that the AEBT leaves possess an anxiolytic-like effect. Flavonoids were reported as an anxiolytic agents through the γ-amino butyric acid type A receptors in the central nervous system (CNS). Hence the reported anxiolytic activity of the extract might be due to the presence of flavonoids as their phytoconstituents.

The test models of depression (spontaneous locomotor activity, forced swim test and diazepam-induced sleeping time) were done on AEBT leaves. The locomotor activity count in the extract treated groups was significantly reduced when compared to basal value. The standard treated group also revealed the same effect. Central nervous system depressant agents act via reduction of locomotion and rearing. Reduction of locomotor activity may be a sign of CNS depression and it was evidenced by Ray et al. Forced swim test was based on the observations that rats or mice when forced to swim in a restricted space from which there is no possibility of an escape may cease to struggle, surrounding themselves (despair or helplessness) to the experimental conditions and this state is called as the state of depression. The AEBT at doses (200 and 400 mg/Kg, p.o.) shown significant increase in immobility period in a dose dependant manner, compared to control. Diazepam-induced sleeping time has been used to elucidate CNS active properties of drugs in animals. The aqueous extract of Bauhinia tomentosa leaf potentiated the diazepam-induced sleeping time in mice. The average sleeping time due to diazepam (25 mg/Kg, i.p.) alone was found to be 105 in minutes. AEBT (200 mg/Kg, p.o.) significantly potentiated the sleeping time, whereas the extract (400 mg/Kg, p.o.) was found to produce non-significant change in sleeping time induced by diazepam (25 mg/Kg, i.p.). The potentiation of benzodiazepine induced sleep further suggested the sleep inducing property of the plant extract.

All the above anti-depressant screening models suggested the CNS depressant activity of AEBT leaves in mice. The CNS depressant activity is due to the increase in GABA concentration in brain. The present study indicated that the leaf extract significantly increased brain GABA content in mice, and these findings are in agreement with the above anti-anxiety studies that were shown that the anxiolytic like effect of AEBT is mainly due to the presence of flavonoids since they act as a ligand for GABA receptor and found to increase GABA content in brain.

The nootropic drugs belong to the category of psychotropic agents selectively facilitates the effect on intellectual performance, such as learning and memory. The elevated plus maze is again used as a model to study nootropic activity. The increase in IR by the aqueous extract (400 mg/Kg) proved that Bauhinia tomentosa possessed nootropic activity. Thus the aqueous extract was found to improve memory in absence of cognitive deficit. The result supported the view that Gamma amino butyric acid is involved in the nootropic and anxiolytic activity and thus the anxiolytics can be used to improve learning and memory.

Skeletal muscle relaxants are the drugs that reduce muscle tone or cause paralysis by acting centrally in the cerebrospinal axis or peripherally at neuromuscular junction. The muscle relaxant property of AEBT leaves on mice was evaluated using rota rod apparatus. The fall-off time was compared before and after administration of test drug. Diazepam was used as a positive control. Mice ingesting higher dose of test drug (400 mg/Kg, p.o.) fell from the revolving rod significantly earlier than the dose of 200 mg/Kg, p.o. The result proven the effect of AEBT as skeletal muscle relaxant.

Catalepsy is a behavioural immobility that is associated with varying degrees of enhancement in muscular rigidity and decrease in response to stimuli. Typical neuroleptic agents such as chlorpromazine, haloperidol and reserpine induce a cataleptic state in rodents which is widely used as a model to test the extrapyramidal side effects of antipsychotic agents. Neuroleptic-induced catalepsy has been linked to a blockade of postsynaptic striatal dopamine D1 and D2 receptors. Extract also found to produce significant extrapyramidal side effects identical to those induced by neuroleptics. The free radical generation and lipid peroxidation in excess due to the increase in turnover of monoamine may cause haloperidol-induced extrapyramidal disorder. Many preclinical and clinical studies have also proposed reactive oxygen species as causes of haloperidol-induced toxicity. The present work showed that the extract potentiated the haloperidol-induced catalepsy after 30 and 90 minutes of haloperidol administration (1 mg/Kg, i.p.). The extract at doses 200 and 400 mg/Kg, p.o. shown only less significant potentiation of catalepsy after 30 minutes whereas the potentiation of catalepsy was significant after 90 minutes. The results suggested that the extract act as an antipsychotic against schizophrenia. Further studies are required to explore the exact mechanism of action of the extract.

CONCLUSION

In conclusion, the present study evidenced that the aqueous extract of leaves of Bauhinia tomentosa L. demonstrated potential neuropharmacological activities. These findings are consistent with the hypothesis that Bauhinia tomentosa treatment triggers anxiolytic, depressant, nootropic, skeletal muscle relaxant and antipsychotic effects. Further studies will confirm the mechanism of action of Bauhinia tomentosa for central nervous system drug development and its use in the treatment of neurological disorders.

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