EVALUATION OF ANTIOXIDANT POTENTIAL OF SMILAX ZEYLANICA LINN. IN REVERSING HALOPERIDOL INDUCED CATALEPSY IN RATS

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ABSTRACT

Ethanolic root extract from Smilax zeylanica was investigated for its antioxidant and anti cataleptic effects in the haloperidol-induced catalepsy rat model of the disease by measuring behavioral and biochemical parameters. Catalepsy was induced by administration of haloperidol (1 mg/kg, i.p) in male albino rats. A significant (P < 0.01) reduction in the cataleptic scores were observed in all the drug-treated groups as compared to the haloperidol-treated group; with maximum reduction observed in the Smilax zeylanica (500 mg/kg body weight) administered group. To estimate biochemical parameters: the generation of thiobarbituric acid reactive substances (TBARS); reduced glutathione (GSH) content and glutathione-dependent enzymes; catalase; and superoxide dismutase (SOD) in the brain were assessed. Haloperidol administration increased generation of TBARS and significantly reduced GSH, which were restored to near normal level with the Smilax zeylanica treatment. Catalase and SOD levels were also increased to normal levels, having been reduced significantly by haloperidol administration. Our findings of behavioral studies and biochemical estimations shows that Smilax zeylanica reversed the haloperidol-induced catalepsy in rats. We conclude that the antioxidant potential has contributed to the reduction in the oxidative stress and catalepsy induced by haloperidol administration.

Keywords: Smilax zeylanica, Antioxidant, Haloperidol, Land Catalepsy.

INTRODUCTION

Haloperidol is an antipsychotic drug which is used in the treatment of schizophrenia and other affective disorders. It blocks dopaminergic action in the nigrostral pathway leading to a high frequency of extra pyramidal motor side effects. In animal models, haloperidol induces a behavioral state known as catalepsy in which the animals are unable to correct externally imposed postures. There is pharmacological evidence for stimulation of dopaminergic neurons by noradrenergic neurons in the brain. Locomotor activity and alertness appear to be regulated both by central noradrenergic and dopaminergic neurons. The use of haloperidol has been associated with an increased level of oxidative stress in the brain. The brain is made up of 70% lipid and any kind of stress is usually manifested by lipid peroxidative damage. The extent of this damage can be used to evaluate the degree of cellular harm. Stress-induced lipid peroxidative damage in the brain can be quantified by either determining the amount of peroxidative products or the rates of enzyme-catalyzed reactions neutralizing free radical intermediates such as superoxide dismutase (SOD). SOD is a primary, natural, and free radical scavenging and antioxidant enzyme in the body. The estimation of the activity of such antioxidant enzymes such as SOD, catalase, or glutathione peroxidase, can be used to assess the therapeutic effects of different antioxidant agents.

Smilax zeylanica Linn. is an evergreen woody climber endemic to Western Ghats of Southern India. It is a slow growing riparian species distributed up to 1200 m altitude. The roots are used to treat syphils, gonorrhea, swellings, abscesses and boils. In the folkloric system of medicine, the plant was used in veneral diseases, to promote healing of wounds, swellings, abscesses, in rheumatism and pain in lower extremities, skin diseases, leucorrhoea, colic, dysentery, dysuria and fever. Chophachine is an important drug used in ayurveda for the treatment of several diseases like diseases of the nervous system, epilepsy, psychosis, urinary disorders, polyuria, hemiplegia, Parkinson’s disease, congenital diseases, leprosy, rejuvenator; blood purifier, while S. zeylanica may be a potential alternate source of Chophachine. The antiepileptic activity in S. zeylanica, which is one of the properties of the drug Chophachine, was established. The roots of S. zeylanica have a steroidal saponin glycoside diosgenin. However no scientific study on anti cataleptic activity of the plant has been reported. The present investigation was undertaken to study the anti cataleptic activity of Smilax zeylanica roots on haloperidol induced catalepsy in rats.

MATERIALS AND METHODS

Plant materials

Dried roots of Smilax zeylanica were purchased from an herbal Market (Hyderabad, Andhra Pradesh, India.) and authenticated by Dr.K.Madhava Chetty, Assistant Professor, Department of Botany, SV University, Tirupati. A specimen voucher was deposited at the Department of Pharmacology, Nizam Institute of Pharmacy, India.

Preparation of ethanol extract of Smilax zeylanica

The dried roots were coarsely powdered and weighed quantity of powder was subjected to continuous hot percolation in sox hlet apparatus with ethanol at 65-70°C. The extract was evaporated under reduced pressure using Rota flash evaporator until all the solvent had been removed. The yield of the extract was 10% w/w. when compared to the dried starting material. The extract obtained was suspended in 1% v/v Tween 80 for oral administration.

Experimental animals

Inbred adult Wistar rats of either sex, weighing 150–200 gm were obtained from the animal house of the Nizam Institute of Pharmacy, Deshmukhi, Ramoji Film City, Hyderabad. Before and during the experiment, the animals were maintained in a well-ventilated room with a 12-hour light/dark cycle in standard polypropylene cages under controlled temperature (26 ± 1°C) and humidity (30%–40%). They were fed with a standard pellet diet obtained from Gold Moher, Lipton India Ltd, Hyderabad and water ad libitum throughout the experimental period. All animal experiments were carried out in accordance with the guidelines of CPCSEA and study was approved by the IAEC (Institutional animal ethical committee) with registration number. (13/30/AC/10/CPCSEA).

Acute toxicity

Rats selected by a random sampling technique were used in the study. Acute oral toxicity was performed as per Organization for Economic Co-operation and Development (OECD)-423 guidelines. Three male Wistar rats weighing between 150–200g were used for...
each dose. The dose levels of 5mg, 50mg, 500mg, 1000mg, 2000mg, and 5000 mg/kg/body weight, per oral dose were selected. The lethal dose (LD)-50 value of the extract was determined. The drug was administered orally to rats, which were fasted overnight with water ad libitum before the administration of the drug. The body weight of the rats was noted before and after treatment. The animals were observed for toxic symptoms such as behavioral changes, locomotion, convulsions and mortality for 72 hours.

**Experimental Design**

Adult male Wistar rats (150-200gm) were divided into six groups each containing six animals. Group I received the vehicle 1% Tween 80 solution and served as the control, group II received haloperidol alone and served as the negative control without any drug treatment, group III received combination of L-dopa and carbidopa (100 mg ± 25 mg/kg by intraperitoneal administration) and served as positive control and Groups IV, V, VI received Smilax zeylanica at doses of 100, 250, 500 mg/kg body weight, respectively for 15 days. Catalepsy was induced by the intraperitoneal administration of haloperidol at a dose of 1mg/kg body weight in normal saline. All the behavioral studies were performed at room temperature in a calm room without any external interference. After the 15 days, animals were sacrificed by cervical dislocation and the whole brain was immediately dissected out and washed in ice-cold saline to remove all traces of blood. The brains were weighed and a 10% tissue homogenate was prepared in 0.025 M Tris–HCl buffer at pH 7.5 and used to measure the activities of thiorubarbitric acid reactive substances (TBARS). Enzyme activity was assayed in 10% brain homogenates prepared in 0.2 M phosphate buffer, pH 8.0.

**Behavioral studies**

**Measurement of catalepsy by block method**

This scoring method followed is in three steps. **Step I:** The rat was taken out of the home cage and placed on a table. If the rat failed to move when touched or pushed gently on the back, a score of 0.5 was assigned. **Step II:** The front paws of the rats were placed alternately on a 3-cm high block. If the rat failed to correct the posture within 15 seconds, a score of 0.5 for each paw was added to the score of step I. **Step III:** The front paws of the rats were placed alternately on a 9-cm high block, if the rat failed to correct the posture within 15 seconds a score of 1 for each paw was added to the scores of steps I and II. Thus, the highest score for any animal was 3.5 (cutoff score) and that reflects total catalepsy.

**Biochemical studies**

**Estimation of lipid peroxidation products**

Lipid peroxidation was estimated colorimetrically in brain tissue by quantifying TBARS according to the method of Niehaus and Smilax zeylanica. In brief, for the estimation of TBARS the superoxided of the tissue homogenate was treated with trichloroacetic acid (TCA) reagent and mixed thoroughly. The mixture was kept in boiling water bath for 15 minutes. After cooling, the tubes were centrifuged for 10 minutes and the supernatant was used for measurement. The developed color was read at 535 nm using a UV spectrophotometer (Hitachi 912) against a reagent blank and expressed as mM per 100g tissue.

**Estimation of antioxidants**

Catalase (CAT) was assayed colorimetrically at 620 nm and was expressed as micromoles of H2O2 consumed per minute per mg of protein; using the method described by Sinha20. The reaction mixture (1.5 mL, volume) contained 1.0 mL of 0.01 M pH 7 phosphate buffer, 0.1 mL of tissue homogenate and 0.4 mL of 2 M H2O2. The reaction was stopped by the addition of 2 mL of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid mixed in the ratio of 1:3). The assay for SOD was based on SOD mediated inhibition of the reduction of nitro blue tetrazolium to blue formazan by superoxide anions as described by Beauchamp and Fridovich21. The total protein present in the homogenate was estimated following the method described by Lowry22. Units of SOD activity determined were expressed in terms of milligrams of total protein (TP). Reduced glutathione (GSH) was determined by the method of Ellman23. One mL of supernatant was treated with 0.5 mL of Ellman’s reagent and 3 mL of phosphate buffer (0.2 M, pH 8.0). The absorbance was read at 412 nm. The activity of GSH was expressed as nm GSH formed/g tissue.

**Statistical analysis**

Each group of rats assigned to a specific drug treatment each group consisted of 6 animals. All the values are expressed as mean ± standard error of mean (SEM). The data were analyzed by analysis of variance (ANOVA) followed by Dunnett’s multiple comparison test.

**RESULTS**

The phytochemical analysis of ethanol extract of Smilax zeylanica revealed the presence of carbohydrates, proteins, steroids, phenols, flavonoids, saponins, gums and mucilage. The acute oral toxicity was undertaken according to the OECD guidelines 423 (acute toxicity method). There was no considerable change in body weight either before or after experimental treatment and no signs of toxicity were observed. The LD50 test of the ethanol extract was found to be greater than 5000 mg/kg body weight after oral administration.

The anti cataleptic scores, of the present study are given in Table-1 and Fig-1, assessed by block method. Haloperidol induced catalepsy significantly (P < 0.01) at a dose of 1 mg/kg (intraperitoneal administration). Significant reversal in haloperidol-induced catalepsy was observed with the administration of Smilax zeylanica ethanol extract and combination of L-dopa and carbidopa. The maximal decrease (P < 0.01) in catalepsy was observed in the group receiving ethanol extract of Smilax zeylanica at a dose of 500 mg/kg,

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug treatment</th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
<th>150 min</th>
<th>180 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vehicle control</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>2</td>
<td>Haloperidol (1mg/kg)</td>
<td>2.8 ± 0.0***</td>
<td>3.5 ± 0.53**</td>
<td>3.5 ± 0.5</td>
<td>3.7 ± 0.5</td>
<td>3.2 ± 0.5</td>
<td>3.0 ± 0.5</td>
</tr>
<tr>
<td>3</td>
<td>L-dopa + carbidopa (100mg +25mg/kg) + haloperidol (1mg/kg)</td>
<td>0.583 ± 0.0</td>
<td>1.65 ± 0.5</td>
<td>1.25 ± 0.5</td>
<td>1.02 ± 0.5</td>
<td>0.50 ± 0.5</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>4</td>
<td>Smilax zeylanica (100mg/kg) + haloperidol (1mg/kg)</td>
<td>0.325 ± 0.0**</td>
<td>0.486 ± 0.0**</td>
<td>0.546 ± 0.0**</td>
<td>0.056 ± 0.0**</td>
<td>0.316 ± 0.0**</td>
<td>0.247 ± 0.0**</td>
</tr>
<tr>
<td>5</td>
<td>Smilax zeylanica (250mg/kg) + haloperidol (1mg/kg)</td>
<td>1.02 ± 0.0**</td>
<td>2.03 ± 0.0**</td>
<td>1.42 ± 0.0**</td>
<td>1.29 ± 0.0**</td>
<td>1.29 ± 0.0**</td>
<td>1.00 ± 0.0**</td>
</tr>
<tr>
<td>6</td>
<td>Smilax zeylanica (500mg/kg) + haloperidol (1mg/kg)</td>
<td>0.65 ± 0.0</td>
<td>1.12 ± 0.0</td>
<td>1.00 ± 0.0</td>
<td>0.09 ± 0.0</td>
<td>0.60 ± 0.0</td>
<td>0.00 ± 0.0**</td>
</tr>
</tbody>
</table>

Values were mean ± SEM (n=6). Statistical analysis by One-way ANOVA, followed by Dunnett’s multiple comparison tests. *P<0.05. **P<0.01.
Fig. 1: Effect of EESZ on haloperidol induced catalepsy by block method

Table 2: Effect of ethanol extract of *Smilax zeylanica* on TBARS, SOD, CAT and GSH levels in Haloperidol administered rat brain.

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug treatment</th>
<th>TBARS (mM/100g tissue)</th>
<th>SOD (UA)</th>
<th>Catalase (UB)</th>
<th>GSH (mg/ 100g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vehicle control</td>
<td>1.13 ± 0.08</td>
<td>7.83 ± 0.70</td>
<td>2.99 ± 0.27</td>
<td>11.53 ± 0.50</td>
</tr>
<tr>
<td>2</td>
<td>Haloperidol (1mg/kg)</td>
<td>1.99±0.12***</td>
<td>4.58±</td>
<td>0.75±</td>
<td>5.82± 0.33***</td>
</tr>
<tr>
<td>3</td>
<td>L-dopa+carbidopa (100mg+25mg/kg)+haloperidol (1mg/kg)</td>
<td>1.22±0.05***</td>
<td>6.42±</td>
<td>2.19±</td>
<td>9.15± 0.28***</td>
</tr>
<tr>
<td>4</td>
<td><em>Smilax zeylanica</em> (100 mg/kg) + haloperidol (1mg/kg)</td>
<td>1.27± 0.82*</td>
<td>6.2± 0.56**</td>
<td>2.00± 0.24*</td>
<td>8.52± 0.56*</td>
</tr>
<tr>
<td>5</td>
<td><em>Smilax zeylanica</em> (250 mg/kg ) + haloperidol(1mg/kg)</td>
<td>1.15±0.15***</td>
<td>6.95±0.62**</td>
<td>2.52± 0.28**</td>
<td>10.00±0.76***</td>
</tr>
<tr>
<td>6</td>
<td><em>Smilax zeylanica</em> (500 mg/kg)+ haloperidol (1mg/kg)</td>
<td>1.14±0.32***</td>
<td>7.43±0.46***</td>
<td>2.78± 0.56**</td>
<td>10.32±0.36***</td>
</tr>
</tbody>
</table>

Values were mean ± SEM of six samples of six observations. Statistical significant test for comparison was done by ANOVA, followed Dunnett’s test.

A. Amount of enzyme required to inhibit 50% of NBT reduction per mg protein.
B. Micromoles of H$_2$O$_2$ consumed per min per mg protein.

Fig. 2: Effect of EESZ on TBARS, SOD, CAT and GSH levels in haloperidol administered rat brain.
DISCUSSION
The present study demonstrates the anti-cataleptic and antioxidant effects of ethanolic extract of Smilax zeylanica in haloperidol-induced catalepsy and oxidative stress in rats. The central nervous system is especially vulnerable to free radical damage because of the brain's high oxygen consumption, its abundant lipid content, and the relative paucity of antioxidant enzymes as compared with other tissues25. Free radicals generated in the brain are also reported to induce gene expression, thus enhancing the synthesis of antioxidant enzymes and repair oxidative cellular damage. The brain is known to synthesize molecules like glutathione and NADPH. Glutathione functions as a major antioxidant in tissue defense against free radicals in the brain. However, the concentration of glutathione is, relatively, in lesser quantities in the brain as compared to the other organs of the body25. The natural antioxidant system present in brain can be in form of enzymes like catalase, peroxidase, superoxide dismutase and low molecular weight antioxidants (ascorbic and lipoic acids, carotenoids or indirectly acting chelating agents)24. Free radical scavengers or antioxidants function as biological bodyguards for essential molecules by either neutralizing reactive species before they mutlate a molecule or they repair damage that has been inflicted. The induction of free radicals in mammals by haloperidol is well established. It is also well established that the administration of haloperidol leads to an increase in the oxidative stress in the brain tissue25. The increase in SOD observed in the present study supports this concept. Superoxide formation is a major factor in oxygen toxicity and the superoxide dismutase enzyme constitutes an essential defense against it. Under normal conditions, decreased activity of antioxidant enzymes, such as SOD, glutathione peroxidase and catalase, in the brain leads to the accumulation of oxidative free radicals resulting in degenerative effects25. An increase in these enzymes under normal conditions would represent increased antioxidant activity and a protective mechanism in neuronal tissue, thus, constituting the first line of defense against oxidative stress in our body. However, in the presence of a free radical-quenching agent, the induction of the antioxidant enzymes is minimized. So, any overall decrease in catalytic scores and SOD activity in the drug treated groups indicates the ability of the drug extract to combat oxidative stress in brain tissue and reduce the severity of haloperidol-induced catalepsy. The altered balance of the antioxidant enzymes caused by the decrease in CAT, SOD, GSH activities may be responsible for the inadequacy of the antioxidant defenses in combating ROS mediated damage. The decreased activities of CAT and SOD may be a response to increased production of H2O2 and O2 by the antioxidation26. It has been suggested that these enzymes play an important role in maintaining physiological levels of oxygen and hydrogen peroxide by hastening the dismutation of oxygen radicals and eliminating organic peroxides and hydroperoxides. Previous studies have shown that dopamine receptors in the striatum are involved in Neuroleptic-induced catalepsy2. It has been demonstrated that the cataleptic effects of haloperidol are apparently mediated by dopamine receptors localized post synaptically on strial neurons2. The degeneration of dopaminergic neurons leads to an increase in population of dopamine receptors27. The haloperidol-induced catalepsy in rats has been proposed to be a direct consequence of antagonism of dopamine D2 receptors28. Neuroleptics like haloperidol exerts multiple effects on dopaminergic signaling and produce DA related behavioral changes and catalepsy29. Neuroleptics like haloperidol exerts multiple effects on dopaminergic signaling and produce DA related behavioral changes and catalepsy29.

From the above results, it was concluded that, treatment with Smilax zeylanica root extract increased the activity of enzymes by quenching the free radicals and restored to normal levels in cataleptic rats. Significantly lower levels of lipid peroxides in the brains of the drug-treated group and increased activities of enzymatic and nonenzymatic antioxidants in the brain suggest that the extract reduces oxidative stress. Such evidence supports our study and indicates that the extract of Smilax zeylanica inhibits the symptoms of haloperidol-induced catalepsy in rats. The action by which the amelioration takes place may be attributed to one (or) more pharmacological/biochemical mechanisms. To conclude, the brain exhibits numerous morphological and functional alterations during oxidative stress, a factor implicated in the pathogenesis of many CNS disorders. Treatment of such neuronal disorders with Smilax zeylanica root extract significantly decreases lipid peroxidation and significantly increases the antioxidants in the brain. The findings of this study suggest the possible antioxidant role of Smilax zeylanica in overcoming behavioral and neurological changes during oxidative stress. Since the possibility of pharmacological interactions between haloperidol and Smilax zeylanica extract should be further investigated in clinical studies.

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