Neuroprotective Evaluation of Leaf Extract of *Dalbergia sissoo* in 3-Nitropropionic Acid Induced Neurotoxicity in Rats

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ABSTRACT

This research was performed to characterize the neuroprotective effect of ethanolic extract of *Dalbergia sissoo* leaves in 3-Nitropropionic acid induced neurotoxic rats. The ethanolic extract of *Dalbergia sissoo* leaves was administered orally at different doses (300 and 600 mg/kg) to neurotoxic rats. During treatment psychopharmacological parameters were recorded, 24 hours after experiment antioxidant profiles from brain isolate were estimated and histopathology of brain was performed. The ethanolic extract significantly attenuated behavioral alterations, oxidative damage, mitochondrial dysfunction, and striatal/hippocampus damage in 3-Nitropropionic acid treated rats. These results suggest that ethanolic extract of *Dalbergia sissoo* leaves may have potential therapeutic value in the treatment of some neurological disorders, probably by its anti-inflammatory, antioxidant and estrogenic properties.

Keywords: 3-Nitropropionic acid, Huntington’s disease, Excitotoxicity, Oxidative stress, *Dalbergia sissoo*.

INTRODUCTION

3-Nitropropionic acid (3-NP) is a neurotoxin which is produced by various fungal species and is naturally present in leguminous plants which are commonly used to feed animals and thus also responsible for extensive economic losses in the ranching and cattle industries of the western United States. [1] 3-NP is also associated with an epidemic of acute encephalopathy in children who ate moldy sugarcane containg 3-NP, which was reported for the first time in China in 1980. The children exposed to 3-NP developed gastrointestinal disturbance followed by encephalopathy and coma, with dystonia and choreiform movements in survivors. [2] In animals, decreased motor performance with lesions primarily in the striatum, but also in hippocampus and thalamus was reported. [3] 3-NP is an irreversible inhibitor of the electron transport enzyme succinate dehydrogenase, a mitochondrial Complex II enzyme responsible for the oxidation of succinate to fumarate in Krebs cycle. Subsequently it blocks the transport of electrons in oxidative phosphorylation, causing ATP levels in the brain to fall. [4-5] Normally brain requires the highest amount of energy, and this toxin thus affects normal brain electrical activity. Oxidative stress has been suggested to play a role in 3-NP toxicity; however, the process behind the oxidative damage is not fully understood. [6] 3-NP was found to act on NMDA receptors thereby inducing excitotoxicity and leading to generation of free radicals. The inflammation associated with 3-NP, also acts as a contributing factor for neuronal damage and free radical generation. 3-NP is one of the several mitochondrial toxins which were used as model for HD. [7-8] Numerous reports have suggested that mitochondrial energy impairment is related to Huntington disease. [7] In-vivo, intrastriatal injection of mitochondrial toxin in rats produce excitotoxic lesions, while chronic administration of 3-NP produces excitotoxic-like lesions regionally restricted to striatum in both rats and non-human primates. [2, 5] The changes are similar to the changes described in the HD striatum. [9] Pharmaceutical approach for novel prevention and treatment strategies of neurodegeneration involves the use of neuroprotective agents in order to delay or stop neuronal cell death or to strengthen cellular defense system. But effective therapies still remain elusive. [10] A number of natural compounds are being used as brain tonic to help restore debilitated conditions. Since plants produce significant amount of antioxidants, they represent a potential source of new compounds with antioxidant activity. In view of this, *Dalbergia sissoo* Roxb. has been selected based on its use in traditional systems of medicine for augmenting neurological health and was reported to be brain tonic [11], which appear to offer very promising outcomes for neuroprotection. *Dalbergia sissoo* Roxb. (family-fabaceae) also called
‘shisham’ is used time immemorial for treatment of various ailments like burning sensations, dysentery, dyspepsia, leucoderma, and skin ailment, anti-inflammatory, memory enhancer and leaves have significant levels of flavonoids which showed antioxidant activity twice of commonly used antioxidants like vitamin C and selenium. It possesses antioxidant and prevents central nervous system damage however the effectiveness of Dalbergia sissoo in controlling 3-NP induced excitotoxicity is not carried out. The present study aims to evaluate the effect of ethanolic leaf extract of Dalbergia sissoo in chemically induced neurotoxic rats. Antioxidant, anti-inflammatory and estrogenic activities of the plant may be responsible for its neuroprotective effect.

MATERIALS AND METHODS

Drugs and chemicals
Trichloroacetic acid (TCA), 2 thiobarbituricacid (TBA), 5-5-dithiobis (2-nitrobenzoic acid) (DTNB), Phenazine methosulphate, Nicotinamide adenine dinucleotide (NADH), Nitro blue tetrazolium (NBT), 3-Nitropropionic acid (3-NP) were purchased from Sigma-Aldrich, USA. All other chemicals were of the highest purity commercially available.

Plant material and extraction
The fresh leaves of Dalbergia sissoo Roxb, were collected from Gandhi Krishi Vignan Kendra (GKVK) Karnataka, India in the month of July 2012. The plant was identified and authenticated by Mr. KP Sreenath, taxonomist Department Botany, Bangalore University, Bangalore, India. The collected fresh leaves were shade dried or tray dried for two weeks and then grinded to a fine powder. In the continuous hot extraction method, the plant leaves powder was extracted in ethanol for 3 days at temperature of 78-80°C. The mixture was subsequently filtered and concentrated under reduced pressure at 40°C in rotary flush evaporator. The extract yield was 26% w/w. The extract was stored in desiccator.

Preparation of Dalbergia sissoo leaf extracts suspension
Weighed quantity of ethanolic leaf extract of Dalbergia Sissoo was suspended in distilled water using 0.5% v/v Dimethyl sulphoxide and administered orally to rats. The suspension of extract was prepared freshly every day. The extract was administered at a constant volume of 1 ml for each animal.

Preliminary Phytochemical Investigation
The extracts were used for preliminary phytochemical screening with a battery of chemical tests viz., Molisch’s, Fehling’s, Benedict’s and Barfoed’s test for carbohydrates; Biuret and Millon’s tests for proteins; Ninhydrin’s test for amino acids; Salkowski and Libermann-Burchard’s reactions for steroids; Borntrager’s test for anthraquinone glycosides; Foam test for saponin glycosides; Shinoda and alkaline tests for flavonoids glycosides; Dragendorff’s, Mayer’s, Hager’s and Wagner’s tests for alkaloids; and ferric chloride, Lead acetate tests for tannins and phenols.

Animals
Adult female Wistar rats (180-220 g) bred in animal house of PES College of Pharmacy, Bangalore, were used. The animals were procured from Ragavendra enterprises, Bangalore maintained on a 12 h light: 12 h dark cycle and free excess of food and water. Animals were acclimatized to laboratory conditions before the test. The experimental protocols were approved by the Institutional Animal Ethics Committee (PESCP/IAEC/02/11, Dated 14/12/11) and conducted according to CPCSEA guidelines, Govt. of India.

Acute oral toxicity studies
Acute toxicity studies were carried out on mice according to method proposed by Ghosh, alcoholic leaf extracts at dose of 50, 100, 300, 1000, and 3000 mg/kg body weight were administered to separate groups of the mice (n=6) after overnight fasting. Subsequent to administration of ELDS, the mice observed closely for the first 3 hours for toxic manifestations like increased motor activity, salvation, clonic convulsions, coma and death. The observations were made at regular intervals for 24 hours. The animals were observed for 1 week. The study revealed that ELDS was not toxic up to 3000 mg/kg body weight.

Treatment
3-NP was diluted with saline (adjust pH 7.4) and administered intra-peritoneal to rats for period of 7 days to induce the toxicity. Total 30 rats were randomly divided in to five groups of 6 rats each and treated as follows.

Control group: Receives 1ml of vehicle (0.5% DMSO) alone for 7 days (p.o), from 8th day onward vehicle (0.5% DMSO) followed by 0.5 ml of normal saline (i.p) after 2 hours daily up to 14 days.

3-NP inducing group: Receives 0.5 ml of normal saline alone for 7 days (p.o), from 8th day onward saline followed by 0.5 ml of 3-NP 10 mg/kg (i.p) after 2 hours daily up to 14 days.

ELDS (600 mg/kg) + Normal saline (NS) group:
Pretreatment for 7 days with 1ml of ELDS 600 mg/kg for 7 days (p.o), from 8th day onward ELDS 600 mg/kg followed by 0.5 ml of normal saline (i.p) after 2 hours daily up to 14 days.

ELDS (300 mg/kg) + 3-NP group:
Pretreatment for 7 days with 1ml ELDS 300 mg/kg (p.o), from 8th day onward ELDS 300 mg/kg followed by 0.5 ml of 3-NP 10 mg/kg (i.p) after 2 hours daily up to 14 days.

ELDS (600 mg/kg) + 3-NP group:
Pretreatment for 7 days with 1 ml of ELDS 600 mg/kg (p.o) from 8th day onward ELDS 600 mg/kg followed by 0.5 ml of 3-NP 10 mg/kg (i.p) after 2 hours daily up to 14 days.

Measurement of body weight change
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ELDS (600 mg/kg) + 3-NP group:
Pretreatment for 7 days with 1 ml of ELDS 600 mg/kg (p.o) from 8th day onward ELDS 600 mg/kg followed by 0.5 ml of 3-NP 10 mg/kg (i.p) after 2 hours daily up to 14 days.

The gap between 3-NP and ELDS administration is 2 h. The dose of 3-NP and ELDS is selected based on previous literature. During the drug treatment rats were observed for the behavioral changes for 50 min daily. On 14th day after 4 h of 3-NP administration rats were evaluated for neurological scoring, ambulatory behavior, elevated plus maze test, Rota rod performance and hanging wire test. On 15th day, elevated plus maze test was performed and rats were sacrificed and brain were isolated for estimation of GSH, CAT, LPO, SOD and total protein, histopathological study.

Parameters monitored

Measurement of body weight change
Animal body weight was noted on the first day and last day of the experimentation. Percentage change in body weight was calculated in comparison to the initial body weight on the first day of the experimentation.

Behavioral parameters

Movement analysis
Neurotoxins are associated with several motor disturbances which prevent normal ambulatory movement of the animal. Severity of the motor abnormalities in these groups was therefore evaluated using a quantitative neurological scale. A neurological score was determined for each animal on 14th day after 4 h of last dose in comparison to control animals.
Preparation of tissue homogenate: assays described below. After the treatment period, experimental and control mice were sacrificed by decapitation under mild anesthesia. Brains were dissected from the intact brain carefully on ice plate (4 ± 2°C). A 10% brain homogenate was prepared with ice-cold phosphate buffered saline (0.1 M, pH 7.4) using Teflon-glass homogenizer. The homogenate was centrifuged at 10,000 rpm at -4°C for 15 min and the pellet discarded. The supernatant obtained was used for the quantification of antioxidant levels like GSH, CAT, LPO, SOD, total protein levels. Swaroop et al. / Neuroprotective Evaluation of Leaf Extract of Dalbergia sissoo in 3-Nitropropionic Acid

Locomotor activity

The spontaneous loco motor activity was monitored using photo actometer (INCO Pvt. Ltd., Ambala, India) equipped with infrared sensitive photocells, the apparatus was placed in darkened, light and sound attenuated and ventilated testing room. Before loco motor task, animals were placed individually in the activity meter for 2 min for habituation. Thereafter, loco motor activity was recorded for a period of 5 min. The loco motor activity was expressed in terms of total photo beam counts/ 5 min.

Elevated plus maze test for special memory

Memory dysfunction is evaluated using elevated plus maze, which consists of two opposite open arms (50 cm × 10 cm), crossed with two closed arms of same dimensions with 40 cm high wall. The arms are connected with Central Square (10 cm × 10 cm). Acquisition of memory was assessed on day 7th after initiating 3-NP treatment. Rat was placed individually at one end of an open arm facing away from the central square. The time taken by animal to move from open arm and enter in to one of the closed arm was recorded as initial transfer latency (ITL). Rat was allowed to explore the maze for 30 s after recording ITL and returned to its home cage. Retention transfer latency (RTL) was noted again on 8th day of first 3-NP dose. The percent retention of memory was calculated by the formula. Similarly the activity was repeated in 14th and 15th day.

% Memory retention= (ITL –RTL)/RTL× 100

Rota-rod performance assessment for motor coordination

The rota rod (rotating rod) test is widely used in rodents to test the ability of an animal to remain on a rotating surface. The rota rod consists of a rotating rod, 75 mm in diameter, which was divided into four parts by compartmentalization to permit the testing of four rats at a time. Briefly, in a training session, the rats were placed on the rota rod that was set to 25 rpm and the performance time that each rat was able to remain on the rota rod was recorded.

String test for grip strength

The rat was allowed to hold with the forepaws a steel wire (2 mm diameter and 80 cm in length), placed at a height of 50 cm over a cushion support. The length of time the rat was able to hold the wire was recorded. This latency to the grip loss is considered as an indirect measure of grip strength cut off time was taken as 90 s.

Animal autopsy and isolation of brains

After the treatment period, experimental and control mice were sacrificed by decapitation under mild anesthesia. Brains were immediately isolated, rinsed in ice cold saline to remove blood and stored at -20°C immediately until used in assays described below.

Estimation of antioxidant enzyme levels in brain tissue

Preparation of tissue homogenate: The whole brain dissected out, blotted dry and immediately weighed. The brain regions cerebral cortex (Ct), cerebellum (Cb), hippocampus (Hc) and striatum (St) were subsequently dissected from the intact brain carefully on ice plate (4 ± 2°C). A 10% brain homogenate was prepared with ice-cold phosphate buffered saline (0.1 M, pH 7.4) using Teflon-glass homogenizer. The homogenate was centrifuged at 10,000 rpm at -4°C for 15 min and the pellet discarded. The supernatant obtained was used for the quantification of antioxidant levels like GSH, CAT, LPO, SOD, total protein levels.

Catalase (CAT)

In brief, the incubation mixture contained 0.1 ml of diluted homogenate, 1.0 ml of phosphate buffer and 0.4 ml of distilled water to which 0.5 ml of H₂O₂ solution was added to initiate the reaction, while the H₂O₂ solution was left out in control tubes. After incubating for 1 min at 37°C the reaction was stopped by addition of 2 ml of potassium dichromate acetic acid reagent. The samples were kept in boiling water bath for 15 minutes, finally cooled and the absorbance measured at 570 nm against control. The catalase content was calculated by using molar extinction coefficient = 58.03 × 10⁻³ M⁻¹ cm⁻¹ and the values are expressed as nmols/mg protein.

Lipid peroxidation (LPO)

Briefly, the reaction mixture contained 0.1 ml of brain regions homogenate (1 mg protein), 1.5 ml of 20% acetic acid (pH 3.5), 1.5 ml of 0.8% thiobarbituric acid (0.8 % w/v) and 0.2 ml Sodium dodecyl sulphate. Following these additions, tubes were mixed and heated at 95°C for one hour on a water bath and cooled under tap water before mixing 1 ml of distilled water and 5 ml mixture of n-butanol and pyridine (15:1). The mixture was centrifuged at 2200 g for 10 min. The amount of MDA/TBARS formed was measured by the absorbance of upper organic layer at a wave length of 532 nm. The results are expressed as nmol MDA/mg protein. The absorbance of the clear pink color supernatant was measured at 532 nm against appropriate blank. The amount of lipid peroxidation was determined by using molar extinction coefficient 1.56 × 10⁶ M⁻¹ cm⁻¹ and the results were expressed as nmols MDA/g of protein.

Reduced Glutathione (GSH)

The assay is based on the principle of Ellman’s reaction. The sulfhydryl group of glutathione reacts with DTNB (5, 5'-dithiobis-2-nitrobenzoic acid) and produces a yellow colored dithiobis-2-nitrobenzoic acid (DTNB) solution was added to

Super oxide dismutase (SOD)

The assay mixture contained 0.1 ml of sample, 1.2 ml of sodium pyrophosphate buffer (pH 8.3, 0.052M), 0.1 ml of phenazine methosulphate (186μm), 0.3 ml of nitro blue tetrazolium (300μm), 0.2 ml of NADH (750μm). Reaction was started by addition of NADH. After incubation at 300°C for 90 sec, the reaction was stopped by the addition of 1 ml
of glacial acetic acid. The mixture was allowed to stand for 10 min. The color intensity of the chromogen was measured at 560 nm against blank and concentration of SOD was expressed as units/min/mg of protein.\textsuperscript{[28-30]}

**Total protein (TP)**
The total protein of brain tissue was determined by biurett method in ERBA diagnostic kit.\textsuperscript{[30-31]}

Total protein (g/dl) = Absorbance of test/Absorbance of standard*concentration of standard (g/dl)

**Histopathological study of rat brain**
A section of the brain was fixed with 10% formalin and embedded in paraffin wax and cut into sections of 5μm thickness. The sections were stained with haematoxylin and eosin dye for histopathological observations. Depending on the model, either hippocampal or striatal neurons were observed for morphological changes.\textsuperscript{[32]}

**Statistical evaluation**
The data were expressed as Mean ± S.E.M. Statistical comparisons were performed by one way ANOVA followed by Tukey’s post-test using Graph Pad Prism version 5.0. *P<0.5, **P<0.01, ***P<0.001 will be considered as significant compared to toxic control.

### Table 1: Observation table of Chemical tests

<table>
<thead>
<tr>
<th>S. No</th>
<th>Tests</th>
<th>Present(+)/Absent(-)</th>
</tr>
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<tbody>
<tr>
<td>1.</td>
<td>Carbohydrates</td>
<td></td>
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<tr>
<td></td>
<td>Molish’s test</td>
<td>+</td>
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<tr>
<td></td>
<td>Benedikt’s test</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Fehling’s test</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Barfoed’s test</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td>Proteins</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Biuret test</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Millon’s test</td>
<td>+</td>
</tr>
<tr>
<td>3.</td>
<td>Amino acids</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ninhydrin’s test</td>
<td>+</td>
</tr>
<tr>
<td>4.</td>
<td>Steroids</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Salkowski reaction</td>
<td>+</td>
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<tr>
<td></td>
<td>Libermann-burchard’s reaction</td>
<td>+</td>
</tr>
<tr>
<td>5.</td>
<td>Flavonoids glycosides</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Shanoda test</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Alkaline test</td>
<td>+</td>
</tr>
<tr>
<td>6.</td>
<td>Anthraquinone glycosides</td>
<td></td>
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<tr>
<td></td>
<td>Borntrager’s test</td>
<td>+</td>
</tr>
<tr>
<td>7.</td>
<td>Saponin glycosides</td>
<td></td>
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<tr>
<td></td>
<td>Fearn test</td>
<td>+</td>
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<tr>
<td>8.</td>
<td>Alkaloids</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dragendorff’s test</td>
<td>-</td>
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<tr>
<td></td>
<td>Mayer’s test</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Hager’s test</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Wagner’s test</td>
<td>-</td>
</tr>
<tr>
<td>9.</td>
<td>Tannins and phenols</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ferric chloride test</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Lead acetate test</td>
<td>+</td>
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</tbody>
</table>

### RESULTS

**Phytochemical analysis**
Phytochemical study Extract subjected for phytochemical study showed the presence of carbohydrates, proteins, amino acids, steroids, phenolic compounds, tannins, glycosides and flavonoids (Table 1).

**Acute toxicity studies**
The ethanolic extract did not show any signs and symptoms of toxicity and mortality up to 3000 mg/kg dose.

**Monitored Parameters**

**Effect of ELDS on Body weight change in 3-NP induced neurotoxicity in rats**
Administration of 3-NP (10 mg/kg i.p. for 7 days) resulted in change in body weight when compared to normal rats. In case of 3-NP induced rats, the body weight was significant (P<0.001) decreased initial body weight. Treatment with ELDS (300 and 600 mg/kg p.o.) markedly prevented the 3-NP induced decrease in body weight. The effect of ELDS at 300 mg/kg was found to be much better (P<0.05) than its higher dose when compared to 3-NP control (Table 2).

**Effect of ELDS on Behavioral characters in 3-NP induced neurotoxicity in rats**

**Neurological scoring**
Intra-peritoneal administration of 3-NP resulted in significant (P<0.001) motor abnormalities, out of six rats three rats showed in-coordination and hind limb paralysis, two rats showed hind limb and forelimb paralysis and one rat showed marked gait abnormalities. They showed increase neurological score when compared to normal control rats. Pretreatment with ELDS (300 and 600 mg/kg) in 3-NP induced rats showed a significant (P<0.001 and P<0.01) improvement in behavioral changes when compared to 3-NP induced rats (Table 2).

**Locomotor activity**
Administration of 3-NP from 8-14 days resulted in significantly (P<0.01) decrease in locomotor activity when compared to normal control animals. Animals pretreated with ELDS (300 and 600 mg/kg) in 3-NP induced rats prevented significantly (P<0.05) decreased locomotor activity compared to control animals (Table 2).

**Hanging wire test**
Animals treated with 3-NP from 8-14 days resulted in significantly (P<0.001) decrease in grip strength when compared to normal control animals. Pretreatment with ELDS (300 and 600 mg/kg) in 3-NP induced rats improved significantly (P<0.001 and P<0.05) the grip strength compared to 3-NP alone treated rats (Table 2).

**Elevated plus maze paradigm**
In the present experiment, mean initial transfer latency (ITL) on day 14th day was relatively stable in all the animals within the group. 3-NP alone administered rats for 7 days showed significant (P<0.001) increase in mean retention transfer latency (RTL) compared to normal control animals. Normal control animals entered closed arm quickly and mean RTL was shorter when compared to its own ITL (initial transfer latency). In contrast, 3-NP treated rats performed poorly and showed an increased mean RTL compared to its own ITL. This indicates there is cognitive dysfunction in 3-NP treatment. Chronic pretreatment with ELDS (300 and 600 mg/kg p.o.) to 3-NP treated rats showed significant (P<0.01) improvement in memory performance when compared to 3-NP alone treated rats (Table 2).

**Rota rod test**
Administration of 3-NP from 8-14 days significantly (P<0.001) decreased motor coordination and body balance when compared to normal control rats. Pretreatment with ELDS (300 and 600 mg/kg) in 3-NP induced rats significantly (P<0.001 and P<0.01) improved the motor coordination and body balance and showed increase in latency to balance on the beam (Table 2).

**Antioxidant Parameters**
Effect of ELDS on levels of CAT, LPO, GSH, SOD, and TP: Results clearly revealed increase in the levels of MDA and hydroperoxides in 3-NP induced toxic group compared to control group. Treatment with extracts significantly prevented this raise in levels.CAT, GSH, SOD and TP content have significantly increased in extract treated groups whereas toxic group has shown significant decrease in levels.
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<table>
<thead>
<tr>
<th>Table 2: Effect of ELDS on Body weight change and Behavioral characters in 3-NP induced neurotoxicity in rats</th>
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<tbody>
<tr>
<td>Treatment</td>
</tr>
<tr>
<td>Normal control group</td>
</tr>
<tr>
<td>Inducing group (3-NP)</td>
</tr>
<tr>
<td>High dose (D.S 600 mg/kg)+NS</td>
</tr>
<tr>
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</tr>
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Each value are expressed as mean ± SEM (n = 6), *P < 0.001, *P < 0.01, *P < 0.05 when compared to 3-NP alone treated rats. *P < 0.001, *P < 0.01, *P < 0.05 when compared to control rats. One-way ANOVA followed by Tukey’s post test.

<table>
<thead>
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<th>Table 3: Effect of ELDS on antioxidant levels in 3-NP induced neurotoxicity in rats</th>
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<tr>
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compare to control group. Ethanolic extract (300mg/kg) has shown maximum protection (Table 3).

**Histopathologic observations of hippocampal region in 3-NP induced neurotoxicity in rats**

**A. Control group:** The CA3 region shows intact pyramidal cells in tight clusters [Fig. A(I), Arrow]. The interconnected neuropil fibers in CA3 region appear intact. The CA1 region shows intact pyramidal cells [Fig. A(II), Long Arrow] along with intact neuropil fibres [Fig. A(II), Short Arrow].

**B. 3-NP inducing group:** The CA3 region shows intact pyramidal cells in clusters [Fig. B(I), Arrow]. The interconnected neuropil fibers in CA3 region appear intact. The CA1 region shows decreased number of intact pyramidal cells and most of the pyramidal cells show degenerative changes [Fig. B(II), Long Arrow] along with decreased in neuropil fibres [Fig. B(II), Short Arrow].

**C. Low dose ELDS (300 mg/kg) +3-NP:** The CA3 region shows few intact pyramidal cells [Fig. C(I), Short Arrow] and damaged pyramidal cells [Fig. C(I), Long Arrow] with dense inflammatory cells. The interconnected neuropil fibers in CA3 region appear reduced. The CA1 region shows few intact pyramidal cells [Fig. C(II), Short Arrow] and pyramidal cells show degenerative changes [Fig. C(II), Long Arrow] along with dense inflammatory cells and reduced neuropil fibres.

**D. High dose ELDS (600 mg/kg) +3-NP:** The CA3 region shows some intact pyramidal cells in clusters [Fig. D(I), Short Arrow] and some damaged pyramidal cells [Fig. D(I), Long Arrow]. The interconnected neuropil fibers in CA3 region appear intact. The CA1 region shows moderate number of intact pyramidal cells and some of the pyramidal cells show degenerative changes [Fig. D(II), Long Arrow] along with intact neuropil fibres [Fig. D(II), Short Arrow].
E. High dose ELDS (600 mg/kg) + NS: The CA3 region shows intact pyramidal cells [Fig. E(I), Short Arrow] and some damaged pyramidal cells [Fig. E(I), Long Arrow]. The interconnected neuropil fibers in CA3 region appear reduced. The CA1 region shows pyramidal cells and few of the pyramidal cells show degenerative changes [Fig. E(II), Arrow] along with decreased neuropil fibers.

DISCUSSION

3-NP toxic model is one of the important models offering inferential information on those toxic events occurring in Huntington’s disease (HD). The treatment of rats with 3-NP was produced significant motor and behavioral abnormalities including bradykinesia, muscles weaknesses and rigidity. These findings are in agreement with earlier report who also observed a variety of neurobehavioral abnormalities and motor deficit in rats following 3-NP administration. [33] The symptoms developed by sub chronic administration of 3-NP are akin to juvenile onset and late hypokinetic stages of HD. [34] In the present study, sub-chronic administration of 3-NP for 7 days produced significant alterations in body weight change, motor and cognition related behaviors. The weight reduction was not shown by the animals which were treated with ELDS. This reduction in weight could be due to metabolic impairment caused by 3-NP, i.e. impairment in energy metabolism, mobilization of energy stores and lipid peroxidation which constitute peripheral effects. [35,36] 

However, striatal lesions and bradykinesia must be acting as central contributing factors for weight loss. [18,35]

3-NP administration can cause both hypoactivity and hyperactivity depends on frequency and time of dosing. [18,36] Animals which received 3-NP for 7 days exhibited significant hypoactivity along with marked neuronal loss in the dorsolateral striatum depicting that rigidity and movement disorders are related to basal ganglia lesions. [34] In the present study, ELDS pretreatment improved the hypoactivity, as evident by several behavioral investigations such as movement analysis and locomotor activity. It was also observed that there was significant increase in transfer latency of animals in elevated plus maze test in 3-NP administered rats. 3-NP administration in early reports also showed cognitive deficit, motor deficit and hypokinetic activity which resembles clinical symptom related to HD. [34-35]

Pretreatment and treatment with both the doses of ELDS significantly protected the 3-NP induced increase in transfer latency on elevated plus maze and behavior and motor deficit.

The involvement of oxidative damage and mitochondrial dysfunction are implicated in 3-NP toxicity along with possible involvement of excitotoxicity and generation of free radicals due to glutamate release. [33] This increased glutamate causes influx of Ca\(^{2+}\) and may lead to superoxide production and activation of calcium-dependent nitric oxide synthase, causing an increase in oxidative and nitrosative stress activation. [34-35] In the present study, administration of 3-NP for 7 days, produced oxidative stress (increased levels of LPO (as evident by increased MDA levels) and depleted levels of endogenous antioxidant enzyme (catalase, reduced glutathione, SOD levels). These results supports the oxidative stress based theory of neurotoxicity caused by 3-NP. [33] The mitochondrial dysfunction due 3-NP leads to decreased level of succinic dehydrogenase (SDH), which is believed to be major mechanism of 3-NP toxicity. In the present study, we could not able measure the levels of SDH, but however, several articles supports hypothesis of depletion. [17] It is well documented that polyphenol and flavonoid content of stem bark extracts is significantly more and having antioxidant activity and leaf extract possess anti-inflammatory activity which is due to bioactive flavonoids found in the leaf extract. On the other hand treatment with ELDS has significantly reversed the 3-NP induced changes.

Hence it can be concluded that ELDS protected 3-NP induced neurodegeneration attributed to its antioxidant and behavioral properties. The study also suggests that the protection of antioxidant enzymes activity along with the direct antagonism of 3-NP may be beneficial. This activity of Dalbergia sissoo can be further explored in stroke, epilepsy and other degenerative conditions in which the role of glutamate and inflammatory mediators known to play vital role in the pathogenesis.

REFERENCE

Swaroop et al. / Neuroprotective Evaluation of Leaf Extract of Dalbergia sissoo in 3-Nitropropionic Acid………


