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Original Article

Effect of *Shorea robusta* resin extract in 3-nitropropionic acid-induced Huntington's disease symptoms in Sprague-Dawley rats

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Abstract

Background and purpose: Huntington's disease (HD) is a neurodegenerative disease characterized by neuronal death in the striatum. Asiatic acid is an active component of *Shorea robusta* (Dipterocarpaceae) plants with neuroprotective activity and is considered an acceptable therapeutic candidate for different neurodegenerative diseases. In the present study, the beneficial pharmacological action of *Shorea robusta* resin extract (SRRE) was assessed in 3-nitropropionic acid (3-NP)-induced HD in rats.
Experimental approach: The neuroprotective effect of SRRE (285.7 and 666.7 mg/kg, p.o., 14 days) was studied in 3-NP (10 mg/kg)-induced rats by measuring body weight, behavioral parameters including neurological scoring, motor coordination, spatial memory, and depression-like behavior, neuro-biochemical parameters (gamma-aminobutyric acid and acetylcholinesterase), and oxidative stress parameter in the brain. Histopathology of the rat's brain was also studied.
Findings/Results: SRRE treatment (285.7 mg/kg and 666.7 mg/kg) substantially restored body weight, motor coordination, and mitochondrial enzyme complex I function and improved memory impairment as compared to 3-NP-treated rats. Furthermore, SRRE treatment significantly restored the antioxidant enzyme activity in brain tissue and ameliorated the histopathological changes induced by 3-NP.
Conclusion and implications: The neuroprotective effect of SRRE on 3-NP-induced HD in rats was mediated by a reduction in oxidative stress which may favor the usefulness of *Shorea robusta* in HD.

Keywords: Asiatic acid; Brain tissue; Huntington's disease; Neurodegenerative disease; 3-Nitropropionic acid; *Shorea robusta.*

INTRODUCTION

Huntington's disease (HD) is a hereditary brain disorder resulting in irregular movements together with psychiatric disturbances and progressive cognitive impairment. This is attributed to the progressive neuronal loss in the cerebral cortex, striatum, and hippocampus region (1,2). The pathological changes in the striatum of HD patients are the most severe, which mainly include neuronal degeneration of spiny neurons, those containing γ-amino butyric acid (GABA) and enkephalin.

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Furthermore, neurochemically decreased GABA, enkephalin, and acetylcholine (ACh) levels, as well as enzymes glutamate decarboxylase and choline acetyltransferase are found in the caudate of HD patients. Despite considerable progress in the understanding of the etiopathogenesis of HD, the development of a suitable treatment has been slow (3). Preclinical evaluation of interrupted pathways in HD has triggered the development of functional therapeutic candidates for HD. 3-Nitropropionic acid (3-NP) is a neurotoxin that causes behavioral, histopathological, and neuro-biochemical symptoms of HD both in animals and humans (4). 3-NP inhibits succinate dehydrogenase (SDH) which is involved in both the tricarboxylic acid cycle and the electron transport chain and thus, causes mitochondrial damage. This leads to an increase in electron leakage from the mitochondria, the production of reactive oxygen and nitrogen species, accompanied by depletion of antioxidant defenses. Preventing or postponing neuronal degeneration is a good strategy for the therapy of neurodegenerative diseases (5).

Shorea robusta is a widely known plant in Indonesia, Malaysia, the Philippines, and some areas of northern and eastern India. Various parts of the plant have been proven to exhibit attenuating effects in haemorrhoids, itchiness, leprosy, genital warts, cough, pain, and headache. The resin exuded from the stem has astringent and detergent properties, acting as a cellular oxidative defense mechanism and protecting the mitochondrial functions (6,7). Different monoterpenes and sesquiterpenes, *i.e.* asiatic acid were reported from the oil of resin (8-10). Asiatic acid is one of the triterpenes that reduces H_2O_2-related cell death, decreases intracellular free radical concentration, and activates the cellular oxidative defense pathway (11,12). Furthermore, asiatic acid has neuroprotective activity and serves as an acceptable therapeutic candidate for different neurodegenerative diseases (13,14). Based on this context, we investigated the beneficial pharmacological actions of *S. robusta* resin extract in 3-NP-induced HD in Sprague-Dawley rats.

MATERIALS AND METHODS

Plant material

The dried resin of *S. robusta* (gum) was procured from Inparco India Pvt Ltd, Bengaluru, India. The dried resin was finely powdered using a small electric mill and placed in the refrigerator before the extraction process.

Extract preparation

The weighed powder of *S. robusta* resin was extracted with petroleum ether in the soxhlet apparatus for 36 h and evaporated to dryness. Then, the extract was treated with acetonitrile and an aliquot of the acetonitrile phase was stored at 4 °C for 2 days. The major part of the fat was precipitated in the cold condition. The precipitate was separated by filtration and the filtrate was evaporated and a semisolid, yellowish substance was obtained. The yield of the extract was 12% w/w. Dried powders of the *S. robusta* resin extract were used for Fourier transform infrared (FTIR) analysis and *in vivo* evaluation in rats.

Characterization of the extract by FTIR spectra acquisition

FTIR spectra of SRRE were obtained from the FTIR spectrometer (Perkin Elmer, Spectrum BX FT-IR). The uniform KBr pellets were prepared by taking 10 mg of dried extract powder. The sample of extract specimens was loaded in an FTIR spectrometer. FTIR spectra were collected at frequency regions of 4000-450 cm^{-1} with a resolution of 4 cm^{-1}.

Animals

Twenty-four male rats (190-230 g) were acclimatized for 7 days and housed under standard laboratory conditions, temperature (25 ± 2 °C) and humidity (55-65%) with 12/12-h light/dark cycle, with food and water *ad libitum* in photobiological light exposure chambers. All studies were approved by the Institutional Animal Ethics Committee as per the committee for control and supervision of experiments on animals (CPCSEA) guidelines (Ethical code: SSR/IAEC/2019/04).

Acute toxicity study

The median lethal dose (LD_{50}) of SRRE was determined as per the organization for economic cooperation and development (OECD)-425 (2008) guidelines (15). For the acute toxicity study of the extracts, one rat received SRRE (175 mg/kg) as a single oral dose. After dose administration, the animal was kept in close observation for the initial 30 min, and then periodic observations were taken after 4 h and after every 24 h for 14 days. The dose of the following animals was increased or decreased by 3.2 times higher than the initial dose on the basis of survival and death of animals. The weight of the animals, clinical observations based on their behavioral, neuronal, and other abnormalities were noted for each animal for 14 days.

Experimental design of 3-NP-induced HD

One day before the initiation of treatment (day 0: last day of acclimatization), all animals were randomly grouped into four groups (n = 6). Group I (normal saline) was administered normal saline (1 mL/kg, *i.p.*) for 14 days; group II (3-NP) was treated for 14 days with 3-NP (10 mg/kg/day, *i.p.*); group III (3-NP + LD SRRE) was treated with a low dose of SRRE (285.7 mg/kg/day, per *p.o.*, 14 days) and co-administered 3-NP (10 mg/kg/day, *i.p.*) for 14 days; group IV (3-NP + HD SRRE) was treated with a high dose of SRRE (666.7 mg/kg/day, *p.o.*, for 14 days) and co-administered 3-NP (10 mg/kg/day, *i.p.*) for 14 days (Fig. 1). SRRE was suspended in distilled water and given 1 h before treatment with 3-NP. Based on the acute oral toxicity analysis and previous work (7), doses of SRRE were selected. On the 14th day, after 2 h of 3-NP administration, rats were tested for behavioral functions in the sequence of neurological scoring followed by a T-maze test and rotarod test with 1-h intervals. Rats were also subjected to a forced swim test (after 3 h of rotarod test). On the 15th day, rats were sacrificed and brain samples were collected and used for the determination of biochemical and oxidative stress parameters and histopathological studies.

Body weight

The body weight was determined on the 1st and 14th days of the experimental study. The percentage of alteration in body weight on the 14th day was calculated concerning body weight on the 1st day of the study.

Behavioral parameters

Neurological scoring

The motor disruption created by 3-NP was calculated by neurological scoring (16). The scoring was done as per our earlier work: score 0 for normal, score 1 for general slowness of displacement due to mild hind limb impairment, score 2 for coordination loss and significant abnormalities in gait, score 3 for hind limb paralysis, score 4 for inability to move due to impairment in both forelimbs and hind limbs, and score 5 for recumbency.

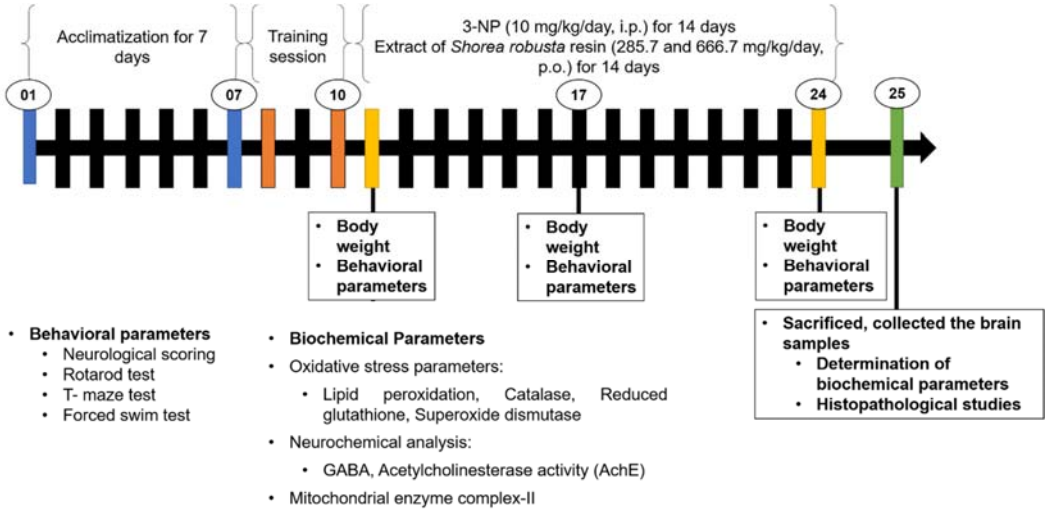

Fig. 1. Experimental design of 3-NP-induced Huntington's disease rats. NP, 3-Nitropropionic acid.

Rotarod test

The Rotarod device was used to evaluate drug effects on motor coordination, balance, strength of grip, and motor learning in rats (17). Before the main test was executed, the animals were trained to walk on a rotarod. On the rotating rod, rats were placed and allowed to walk on the rod at a constant speed of 12 rpm and 300 s of cut-off time was maintained. Rats were observed and there was a record of the average fall time.

T-maze test

T-maze was used to assess working spatial memory (18). For rats, the maze was made up of a T-shaped platform (870 × 200 mm stem, 400 × 100 mm arms, and 50-mm walls at the perimeter of the platform). The food cup (10 mm wide and 15 mm deep) is a reward located 10 mm from the distal end of one arm and punishment at the other arm of the maze. Each animal was placed at the starting platform and allowed to move without restriction through both arms of the maze. Memory improvement was recorded in terms of transfer latency on the first, seventh, and fourteenth days of the experimental study. Transfer latency is the time taken by rats to move from the starting point to the food cup containing the distal end of the arm. Before performing the main test, the animals were well-trained.

Forced swim test

The forced swim test was performed to study depressive-like behavior in rodents (19). Rats were placed individually in a cylinder-like container containing water up to 250 mm (height: 400 mm and diameter: 180 mm). After 5 min, rats were taken out of the cylinder and the total duration of immobility was measured (rats remained passively floating in the water).

Brain homogenate

Mitochondria were isolated from brain homogenates as mentioned by Kola et al. (20). The brains were washed in isotonic saline (ice-cold) and homogenized with extraction buffer (0.44 M sucrose in 0.01 M tris-HCl, pH 7.4, 0.01 M ethylenediaminetetraacetic acid (EDTA), and 0.1% bovine serum albumin (BSA)). Then, the supernatant was obtained from the homogenates by centrifuging at 3000 g for 45 min at 4 °C. For the estimation of acetylcholinesterase (AChE) and oxidative stress, a part of the supernatant was used. The remaining supernatant was centrifuged at 3000 g for 45 min at 4 °C. After re-centrifugation, the initial mitochondrial pellets were washed with extraction buffer and centrifuged at 17000 g for 30 min at 4 °C. The final mitochondrial pellet was resuspended in buffer (0.44 M sucrose in 0.01 M Tris-HCl, pH 7.4). Suspensions of the mitochondrial pellet were used for estimating mitochondrial enzymes.

Oxidative stress parameters

Lipid peroxidation

The lipid peroxidation was assessed as mentioned by Shahgond *et al.* with minor changes (21). Brain homogenate (0.5 mL), 10% trichloroacetic acid (1 mL), and distilled water (0.5 mL) were combined in a test tube, centrifuged for 12 min at 2000 *g* 4000 rpm, and the supernatant was collected. Then, 0.1 mL of TBAR solution was transferred to the supernatant and the mixture was stored for 45 min at 80 °C. Later, the absorbance was recorded at 532 nm (extinction coefficient, 1.56×10^5 $M^{-1} \cdot cm^{-1}$).

Catalase

The estimation of catalase was performed as described by Akhtar *et al.* (22). Phosphate buffer (1.9 mL, 0.05 M, pH 7), H_2O_2 (1 mL, 30 mM), and homogenate (0.1 mL) were added to the test tube. The absorption of the blend was spectrophotometrically recorded at 240 nm with intervals of 60 s (extinction coefficient, 43.6 $M^{-1} \cdot cm^{-1}$).

Reduced glutathione

Reduced glutathione was measured as described by Patel et al (23). In a test tube, an equal volume of brain homogenate and tricarboxylic acid (10%) and centrifuge the above mixture for 20 min to separate the supernatants. Then, the supernatant (1 mL) was mixed with phosphate buffer (3 mL, 0.2 M, pH 8) and 5,5'-dithiobis 2-nitrobenzoic (DTNB) reagent (0.5 mL). The absorption of the above blend was then spectrophotometrically recorded at 412 nm (extinction coefficient, 1.36×10^3 $M^{-1} \cdot cm^{-1}$).

Superoxide dismutase

The estimation of superoxide dismutase activity was performed as described by Marklund *et al*. with minor changes (24). EDTA (0.1 mM), sodium carbonate (50 mM), and nitro blue tetrazolium (96 mM) were added to a test tube. Then, 2 mL of the above mixture, 0.05 mL of hydroxylamine, and 0.05 mL of supernatant brain homogenate were added to the cuvette. The absorbance of the above reaction mixture was taken spectrophotometrically at 560 nm for 120 s at 30-s intervals.

Neurochemical analysis

Estimation of GABA

The brain sample was homogenized repeatedly with ice-cold ethanol (80% *v/v*). Ethanol was evaporated from the above extract and distilled water was added to the residue. The test sample and standard solution of GABA (2 mM) were spotted by paper chromatography with the mobile phase, butanol: acetic acid: water (12:3:5). Then, the chromatography paper was dried and ninhydrin reagent was used as a spraying reagent. After spraying, dry the paper in the oven at 105 °C for 5 min. The spotted region was sliced, eluting the substance into 75% ethanol with 0.005% $CuSO_4$. The absorption of the above elution at 515 nm was taken spectrophotometrically (25).

AchE activity

The AchE activity was assessed by the method of Ellman et al (26). Brain homogenate (0.05 mL), phosphate buffer (3 mL, 0.2 M, pH 8), iodide acetylthiocholine (0.1 mL), and DTNB (100 µL) were added to the test tube. The absorbance of the above reaction mixture was measured spectrophotometrically at 412 nm for 120 s at 30-s intervals.

Mitochondrial enzyme complex II

SDH activity was measured as described by King (27). In the test tube, phosphate buffer (1.5 mL, 0.2 M, pH 7.8) and succinic acid (0.2 mL, 0.6 M, pH 7.8) were added. Then, bovine serum albumin (0.3 mL, 1% *w/v*) and potassium ferricyanide (0.1 mL, 0.03 M) were added and thoroughly mixed. 0.05 mL of mitochondrial suspension was mixed in the above reaction mixture. The absorbance of the above reaction was measured spectrophotometrically at 420 nm for 180 s at 30-s intervals.

Histopathology of rats' brain

The individual brain samples for the histopathology study were fixed in formalin solution, embedded in paraffin wax, and sectioned using a rotary microtome (28). The sections were stained by hematoxylin and eosin (H&E) and viewed under the microscope.

Statistical analysis

The findings were subjected to a one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparisons test except for neurological scoring. Statistical analysis of neurological scores was carried out using the Chi-square ($\chi 2$) test. All columns were compared with the control column. The GraphPad Prism 5 software was used to perform statistical analysis. The values are expressed as mean ± SD and $P < 0.05$ was considered significant.

RESULTS

Characterization of the extract by FTIR spectra acquisition

The FTIR spectra extract is displayed in Fig. 2. FTIR spectrum of the extract was characterized by peaks at 1694.76 cm^{-1} (C=O stretching), 2936.16 cm^{-1} (C-H aliphatic asymmetric), 3422.81 cm^{-1}(O-H), 2869.53 cm^{-1} (C-H aliphatic symmetric), 1460.35 cm^{-1}(C=C), 1386.03, 1189.59, 1153.31 cm^{-1}(C-O of carboxylic acid). Similar peaks were also obtained from an IR spectrum of asiatic acid (29). These data depicted the presence of asiatic acid in the petroleum ether extract of *S. robusta* resin (Fig. 3).

Acute toxicity study

No treatment-related mortality or behavioral changes or toxicity were observed throughout the 14 days in all five animals (data presented in Table S1-S3). In clinical observation, fur, skin, eyes, mucous membrane, gait, posture, and respiration appeared normal. No lacrimation, clonic or tonic movement, salivation, piloerection, diarrhea, stereotype, or bizarre behaviors were observed. This shows that the LD_{50} value of SRRE was assumed to be > 2000 mg/kg. Hence, 285.7 and 666.7 mg/kg doses of the extract were selected for further study.

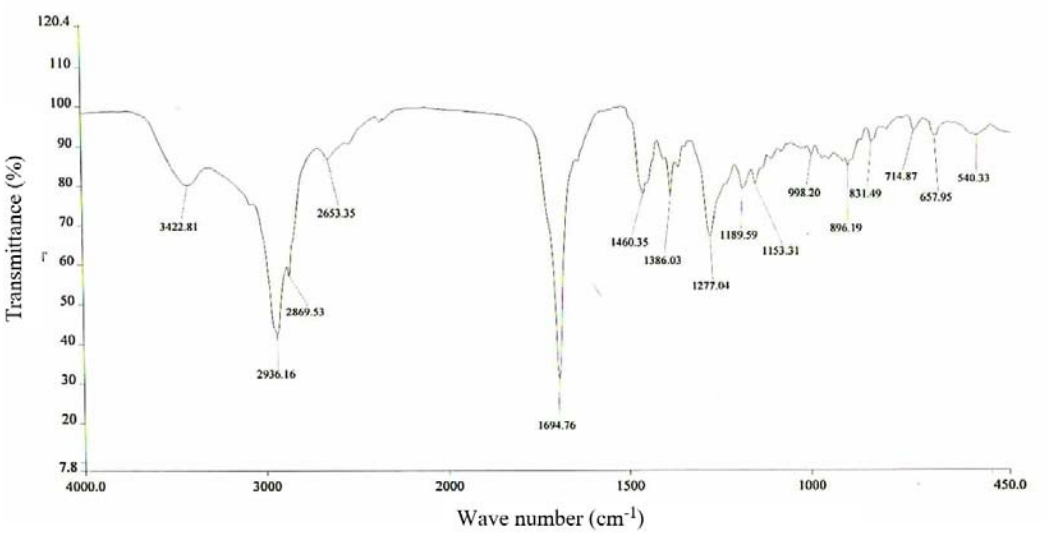

Fig. 2. The FTIR spectra of extract.

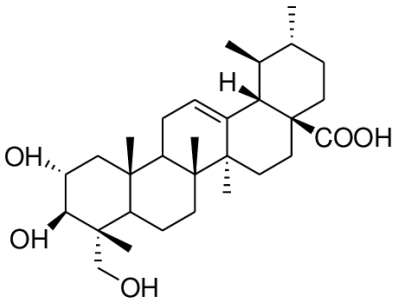

Fig. 3. Structure of asiatic acid.

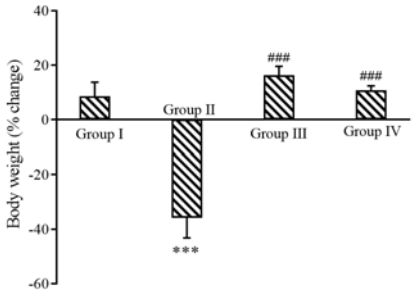

Fig. 4. The effect of *Shorea robusta* resin extract on body weight changes in 3-nitropropionic acid-induced rats. The data are expressed as mean ± SD, n = 6. ***$P \leq 0.001$ Indicates significant differences compared to group I and ###$P \leq 0.001$versus group II.

Body weight

The body weight of rats administered with only 3-NP was found to be significantly reduced compared with normal rats (Fig. 4). At doses of 285.7 mg/kg and 666.7 mg/kg, respectively, the SRRE treatment groups, group III and group IV, significantly restored body-weight loss induced by 3-NP.

Behavioral assessment

Neurological scoring

In contrast with the control rats (group I), 3-NP administration triggered a motor disturbance. The SRRE treatment groups, groups III and IV at the dose of 285.7 mg/kg and 666.7 mg/kg, respectively, diminished the 3-NP-induced motor disturbance as compared with the group II rats (Table S4).

Rotarod test

3-NP administered rats (group II) showed significantly reduced fall time compared with group I (the normal saline group of rats). SRRE treatment at doses of 285.7 mg/kg and 666.7 mg/kg showed a significant increase in motor control/muscle grip strength compared to group II (3-NP-treated only) rats as shown in Fig. 5 on the 14th day of the treatment regimen.

T-maze test

The transfer latency period was remarkably increased in 3-NP-treated rats when compared to vehicle-treated normal rats, indicating memory impairment (Fig. 6). SRRE (285.7 mg/kg and 666.7 mg/kg) significantly decreased the transfer latency period when compared to 3-NP-treated rats (group II), indicating restored memory.

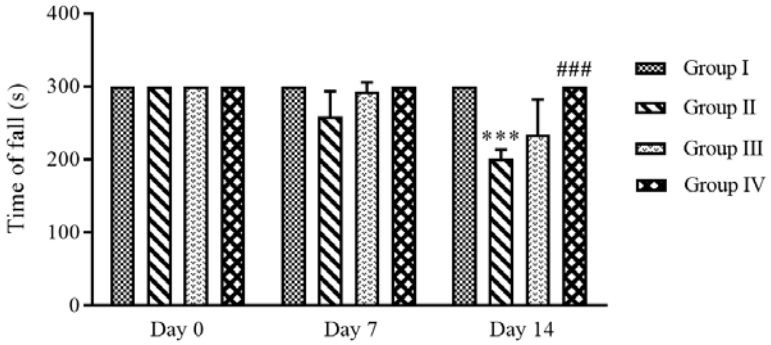

Fig. 5. The effect of *Shorea robusta* resin extract on motor coordination in 3-nitropropionic acid-induced rats. The data are expressed as mean ± SD, n = 6. ***$P \leq 0.001$ Indicates significant differences compared to group I and $^{###}P \leq 0.001$versus group II.

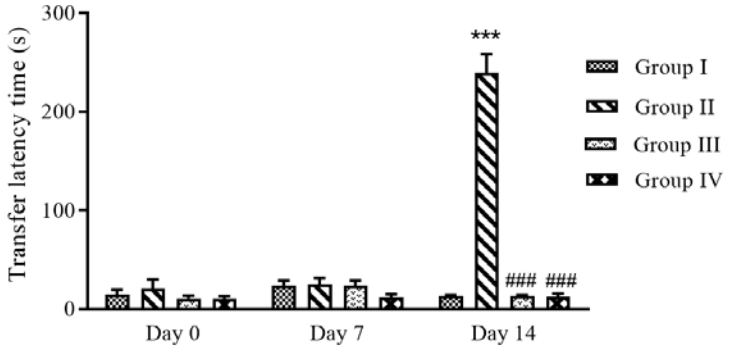

Fig. 6. The effect of *Shorea robusta* resin extract on memory impairment in 3-nitropropionic acid-induced rats. The data are expressed as mean ± SD, n = 6. ***$P \leq 0.001$ Indicates significant differences compared to group I and $^{###}P \leq 0.001$versus group II.

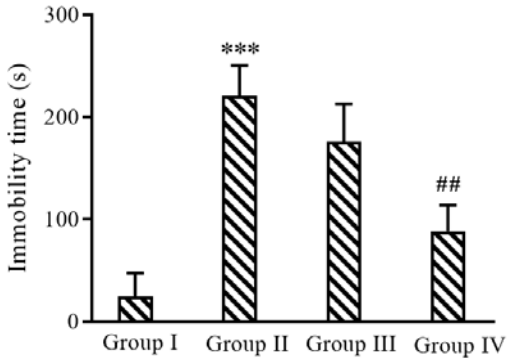

Fig. 7. The effect of *Shorea robusta* resin extract on forced swim test in 3-nitropropionic acid-induced rats. The data are expressed as mean ± SD, n = 6. ***$P \leq 0.001$ Indicates significant differences compared to group I and $^{##}P \leq 0.01$versus group II.

Forced swim test

The immobility duration of 3-NP-administered rats (group II) was significantly increased compared with group I. The immobility time of 3-NP-induced rats was dose-dependent and significantly improved by SRRE treatment (Fig. 7).

Oxidative stress parameters

Lipid peroxidation

As shown in Table 1, 3-NP treatment significantly increased lipid peroxidation (81.9%) in the rats' brains compared with group I. The treatment with SRRE (285.7 mg/kg and 666.7 mg/kg) significantly attenuated lipid peroxidation levels towards normal compared to the rats of group II.

Catalase

As shown in Table 1, 3-NP-induced rats displayed a substantial decrease in the brain homogenate level of catalase (52%) relative to normal rats. SRRE (285.7 mg/kg and 666.7 mg/kg) treatment in 3-NP-induced rats showed a substantial increase (18%, and 70.8%, respectively) in catalase levels relative to group II rats.

Table 1. The effect of *Shorea robusta* resin extract on neurochemical and oxidative stress parameters in the brain homogenate of the rat.

Groups	Lipid peroxidation (nmol/g wet tissue)	Reduced glutathione (µmol/mg wet tissue)	Catalase (µmol/ min/g wet tissue)	Super oxide dismutase (Units/g wet tissue)	Succinate dehydrogenase (mol/min/g wet tissue)	Acetylcholine-sterase (µmol/min/g wet tissue)
I	400.51 ± 6.55	36.98 ± 1.34	1.50 ± 0.04	7.92 ± 0.03	8.03 ± 0.20	1.23 ± 0.01
II	728.80 ± 3.77***	15.86 ± 0.23***	0.72 ± 0.01***	4.90 ± 0.02***	3.97 ± 0.44***	4.33 ± 0.02***
III	586.71 ± 2.59$^{###}$	31.82 ± 0.82$^{###}$	0.85 ± 0.02$^{###}$	6.8 ± 0.04$^{###}$	6.53 ± 0.23$^{###}$	2.29 ± 0.01$^{###}$
IV	485.1 ± 5.15$^{###}$	33.91 ± 0.20$^{###}$	1.23 ± 0.02$^{###}$	7.87 ± 0.1$^{###}$	7.64 ± 0.23$^{###}$	2.26 ± 0.02$^{###}$

The data are expressed as mean ± SD, n = 6. $^{***}P \leq 0.001$ Indicates significant differences compared to group I and $^{###}P \leq 0.001$versus group II.

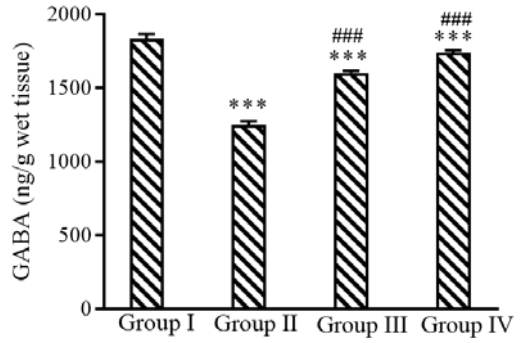

Fig. 8. The effect of *Shorea robusta* resin extract on GABA in 3-nitropropionic acid-induced rats. The data are expressed as mean ± SD, n = 6. $^{***}P \leq 0.001$ Indicates significant differences compared to group I and $^{###}P \leq 0.001$versus group II. GABA, Gamma amino butyric acid.

Reduced glutathione

Administration of 3-NP resulted in a significant decrease in the reduced-glutathione level of brain homogenates in comparison with group I animals (57.1%). SRRE treatment (285.7 mg/kg and 666.7 mg/kg) showed a substantial improvement in reduced glutathione levels relative to group II animals (Table 1).

Superoxide dismutase

The results demonstrated in Table 1 revealed that 3-NP-induced rats (group II) significantly decreased the level of brain superoxide dismutase compared to group I control rats (38%). SRRE treatment (285.7 mg/kg and 666.7 mg/kg) demonstrated a substantial improvement in the amount of superoxide dismutase (40.6% and 60.6%, respectively) relative to animals in group II.

Neurochemical analysis

Estimation of GABA

As shown in Fig. 8, 3-NP-induced rats showed a significant decrease in striatum GABA content as compared to group I rats. SRRE treatment (285.7 mg/kg and 666.7 mg/kg) substantially increased the content of GABA relative to group II rats.

AChE activity

Exposure of rats to 3-NP showed a significant increase in AChE activity compared to the control group I rats. The SRRE-treated groups, groups III and IV at doses of 285.7 mg/kg and 666.7 mg/kg, respectively, demonstrated a substantial decrease in AchE activity compared to group II (Table 1).

Mitochondrial enzyme complex II (SDH) activity

Rat exposure to 3-NP suggested a considerable decrease in the degree of SDH activity relative to controls (Table 1). The treatment with SRRE (285.7 mg/kg and 666.7 mg/kg) in 3-NP-treated rats caused a significant increase in SDH activity compared with those treated with 3-NP only.

Histopathology of rat's brain

Histology was studied at 200× magnification to examine the abnormalities. Marked histopathological differences were observed in the 3-NP treatment group, *i.e*., cell shrinkage and neurofibrillary degeneration in the striatum, in contrast to normal rats as shown in Fig. 9. However, treatment with SSRE countered the histopathological changes induced by 3-NP.

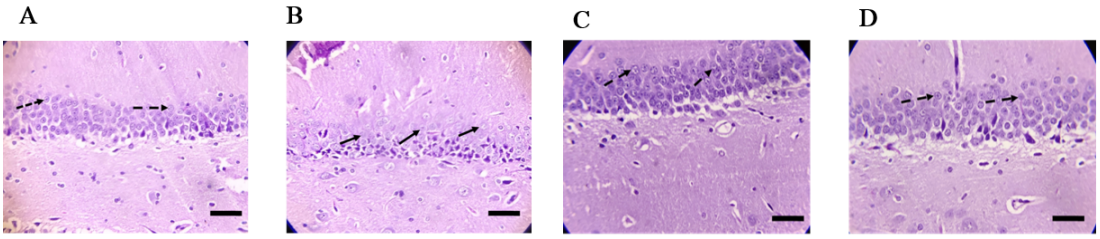

Fig. 9. The effect of SRRE on 3-NP-induced histological alteration (200 ×) in the brain. (A) Group I: normal saline, representing normal nuclear staining and the perikarya (dash type arrow) of striatal cells (mainly projection neurons); (B) group II: 3-NP: cell shrinkage and neurofibrillary degeneration in the striatum (solid arrow represents the pyknotic cells); (C) group III: 3-NP + SRRE (285.7 mg/kg): representing normal nuclear staining and the perikarya (dash type arrow) of striatal cells (mainly projection neurons); (D) group IV: 3-NP + SRRE (666.7 mg/kg): representing normal nuclear staining and the perikarya (dashed arrow) of striatal cells (mainly projection neurons). Scale bar = 50 µm. SRRE, *Shorea robusta* resin extract; 3-NP, 3-nitropropionic acid.

DISCUSSION

HD is a neurological disorder, characterized by behavioral and psychiatric distress with unwanted choreatic moves, motor dysfunction, dementia, and cognitive decline (30). The neuropathological changes linked to HD physical symptoms include advanced neuronal cell death in different regions of the brain, *i.e.* the striatum, cerebral cortex, and hippocampal region (31). Biochemical reports show that the pathophysiology of HD includes oxidative stress and changes in energy metabolism (32).

3-NP, a striatal mitochondrial toxin, successfully induces HD-like manifestation in experimental animals. 3-NP induces cellular energy deficit by irreversible inhibition of succinate dehydrogenase enzyme and excitotoxicity by N-methyl-D-aspartate (NMDA) receptor activation that sources oxidative stress-related neurotoxicity in the striatum leading to neurochemical imbalance (33,34).

Studies have reported that antioxidants like lycopene and epigallocatechin-3-gallate (35), *Withania somnifera* root extract (31), flavonoid kaempferol (36), hesperidin and naringenin (37), sertraline (38), S-allyl cysteine (39), L-carnitine (40), taurine (41), and resveratrol (42) have a beneficial effect on 3-NP-induced HD-like conditions in laboratory animals (26). Based on these reports, the present study was designed to explore the potential of *S. robusta* in the animal models of HD.

Body weight loss may be a sign of 3-NP neurotoxicity due to impaired energy metabolism. Supporting the preceding result, 3-NP-treated rats displayed a decline in body weight with considerably reduced feed intakes throughout our investigation. This was dramatically improved by 14 days of treatment with SRRE (285.7 mg/kg and 666.7 mg/kg) indicating its therapeutic potential. In addition, exposure of rats to 3-NP produced significant disability in the motor performance test (*i.e.* rotarod test). A significant cognitive dysfunction was observed in T-maze by increasing the latency period due to specific effects on the striatum, which controls body movement. The present findings are consistent with the previous results following 3-NP administration (43-46). Treatment with SRRE (285.7 mg/kg and 666.7 mg/kg) substantially improved motor control, muscle grip, memory impairment, and restored mitochondrial enzyme complex I function in 3-NP-induced animals.

Oxidative stress is a major cause of 3-NP-induced neurotoxicity (32,37) and in this study 3-NP significantly increased lipid peroxidation and decreased reduced glutathione, superoxide dismutase, and catalase activity in the brain tissue. The administration of SRRE restored the antioxidant system in the brain to have a neuroprotective impact. The beneficial effect of SRRE is also attributed to a decrease in the excitotoxicity of 3-NP in the brain due to a rise in GABA levels and a decrease in AchE activity in the striatum. Thus, these observations recommend that SRRE has a valuable effect on HD-like symptoms that were induced by 3-NP in rats. Studies have shown that *S. robusta* resin, a rich source of asiatic acid, possesses neuroprotection by preserving the integrity of

the blood-brain barrier, serving as a cellular oxidative defense mechanism, and protecting mitochondrial functions. FTIR analysis also proved that *S. robusta* resin extract contains asiatic acid.

CONCLUSION

In this study, SRRE could shield behavioral modifications and diminish the neurochemical and biochemical changes in the brain induced by 3-NP. This could be attributed to the antioxidant shielding mechanism and the elevated GABA level, which in turn reduced neuronal injury in the brain. Hence, SRRE could be further explored as a favorable candidate for the management of HD-like symptoms.

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Conflict of interest statement

The authors declared no conflict of interest in this study.

Authors' contribution

C. Patel contributed to the conceptualization, design of the study, analysis, and interpretation of the data, drafting of the article, final editing; K. Thakur and L. Shagond contributed to the design of the study, methodology, acquisition of the data, and analysis of the data; S. Acharya contributed to supervision, conceptualization, revising the article critically for important intellectual content; S.H.S. Boddu contributed to drafting, resources, data analysis, and final editing; K. Ranch contributed to supervision, formal analysis, drafting, and revising of the article. The final version of the article was approved by all authors.

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SUPPLEMENTARY MATERIAL

Table S1. Body weight in single dose acute oral toxicity.

Dose (mg/kg)	Body weight (g)		
	Day 0	**Day 7**	**Day 14**
175 (1st animal)	243	247	240
550 (2nd animal)	240	244	246
2000 (3rd animal)	252	256	259
2000 (4th animal)	250	253	257
2000 (5th animal)	253	256	268
Control	245	247	249

Table S2. Mortality record for each dose level.

Time interval	175 mg/kg (1st animal)	550 mg/kg (2nd animal)	2000 mg/kg (3rd animal)	2000 mg/kg (4th animal)	2000 mg/kg (5th animal)
0.5 h	Nil	Nil	Nil	Nil	Nil
1 h	Nil	Nil	Nil	Nil	Nil
2 h	Nil	Nil	Nil	Nil	Nil
3 h	Nil	Nil	Nil	Nil	Nil
4 h	Nil	Nil	Nil	Nil	Nil
Day 1	Nil	Nil	Nil	Nil	Nil
Day 2	Nil	Nil	Nil	Nil	Nil
Day 3	Nil	Nil	Nil	Nil	Nil
Day 4	Nil	Nil	Nil	Nil	Nil
Day 5	Nil	Nil	Nil	Nil	Nil
Day 6	Nil	Nil	Nil	Nil	Nil
Day 7	Nil	Nil	Nil	Nil	Nil
Day 8	Nil	Nil	Nil	Nil	Nil
Day 9	Nil	Nil	Nil	Nil	Nil
Day 10	Nil	Nil	Nil	Nil	Nil
Day 11	Nil	Nil	Nil	Nil	Nil
Day 12	Nil	Nil	Nil	Nil	Nil
Day 13	Nil	Nil	Nil	Nil	Nil
Day 14	Nil	Nil	Nil	Nil	Nil
Mortality	0/1	0/1	0/1	0/1	0/1

Table S3. Clinical signs observed in animals.

Parameters	Cage side observation				
	175 mg/kg (1st animal)	**550 mg/kg (2nd animal)**	**2000 mg/kg (3rd animal)**	**2000 mg/kg (4th animal)**	**2000 mg/kg (5th animal)**
Lacrimation	Normal	Normal	Normal	Normal	Normal
Pupil size	Normal	Normal	Normal	Normal	Normal
Unusual respiration	Nil	Nil	Nil	Nil	Nil
Change in gait	Normal	Normal	Normal	Normal	Normal
Posture	Normal	Normal	Normal	Normal	Normal
Presence of clonic or tonic movements	Nil	Nil	Nil	Nil	Nil
Stereotypes like excessive grooming	Nil	Nil	Nil	Nil	Nil
Repetitive cycling	Nil	Nil	Nil	Nil	Nil
Walking backward	Nil	Nil	Nil	Nil	Nil
Skin and fur	Normal	Normal	Normal	Normal	Normal
Eyes	Normal	Normal	Normal	Normal	Normal
Mucus membrane	Normal	Normal	Normal	Normal	Normal
Any occurrence of secretion and excretion	Nil	Nil	Nil	Nil	Nil

Table S4A. Neurological scoring of rats of group I.

Sr. No.	Neurological scoring		
	Day 0	Day 7	Day 14
1	0	0	0
2	0	0	0
3	0	0	0
4	0	0	0
5	0	0	0
6	0	0	0

Table S4B. Neurological scoring of rats group II.

Sr. No.	Neurological scoring		
	Day 0	Day 7	Day 14
1	0	0	1
2	0	0	1
3	0	0	1
4	0	0	1
5	0	0	1
6	0	0	1

Table S4C. Neurological scoring of rats of group III.

Sr. No.	Neurological scoring		
	Day 0	Day 7	Day 14
1	0	0	1
2	0	0	0
3	0	0	0
4	0	0	1
5	0	0	0
6	0	0	1

Table S4D. Neurological scoring of rats of group IV.

Sr. No.	Neurological scoring		
	Day 0	Day 7	Day 14
1	0	0	0
2	0	0	0
3	0	0	0
4	0	0	0
5	0	0	0
6	0	0	0