Neuroprotective effect of solanesol against 3-nitropropionic acid-induced Huntington’s disease-like behavioral, biochemical, and cellular alterations: Restoration of coenzyme-Q10-mediated mitochondrial dysfunction

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Abstract:

OBJECTIVE: The aim of the present study was to evaluate the solanesol (SNL)-mediated coenzyme-Q10 restoration to ameliorate 3-nitropropionic (3-NP)-induced behavioral, biochemical, and histological changes which resemble Huntington’s disease (HD)-like symptoms in men.

MATERIALS AND METHODS: Various behavioral and biochemical parameters were carried out to evaluate the activity of SNL on 3-NP-treated rats. To determine the therapeutic significance of SNL on HD, different behavioral tests such as memory task, locomotor activity, grip strength, and beam cross and some biochemical test along with histopathological findings were done.

RESULTS: Chronic 3-NP, 10 mg/kg i.p., caused physical and mental abnormalities in animals, including memory impairment, weak grip strength, abnormal posture, and cognitive deficit. Biochemical analysis of brain homogenate in 3-NP-treated rats showed altered mitochondrial complexes, oxidative stress, and elevated lipid biomarkers. Neurohistological alterations of hippocampus, basal ganglia, and cerebral cortex of 3-NP-treated rats exhibit severe neuronal space, irregular damaged cells, and dense pyknotic nuclei-associated marked focal diffused gliosis. SNL administered for 15 days significantly improved motor performance and cognitive behavior task and restored the histopathological changes. Further, SNL treatment significantly improved mitochondrial complexes such as coenzyme-Q10 enzyme activity and attenuated inflammatory and oxidative damage of rat brain.

CONCLUSION: In the present research work, SNL (5, 10, and 15 mg/kg p.o.) provided notable neuroprotective effect, which was confirmed by behavioral paradigms and biochemical test. It restored the behavioral and biochemical alteration caused by 3-NP and confirmed the strong neuroprotective mechanism of SNL in 3-NP-intoxicated memory and cognitive abnormalities.

Keywords: 3-nitropropionic acid, Coenzyme-Q10, Huntington’s disease, mitochondrial dysfunction, solanesol

Introduction

Huntington’s disease (HD) is a hereditary autosomal dominant disorder of central nervous system.¹ The major neuropathological hallmarks are atrophy of the striatal region, caudate and putamen of the striatum of basal ganglia,
cerebral cortex, and hippocampal and subthalamic neurons of brain. It is characterized by chorea, seizures, involuntary movements, dystonia, cognitive decline, intellectual impairment, and emotional disturbances. Mitochondrial dysfunctions are suggested to be involved in HD pathogenesis, which could be linked with direct interaction of mutant Htt with mitochondria (mHtt). Evidence suggests that HD may be associated with impaired energy metabolism and inhibition of mitochondrial complexes (I, II, III, and IV) enzymes resulting in ROS production in striatal regions of HD brain. Mitochondrial dysfunction hypothesis explaining about the pathogenesis of HD was first tested pharmacologically using 3-nitropropionic acid (3-NP), an irreversible inhibitor of succinate dehydrogenase (SDH) which replicates most of the clinical and pathophysiological hallmarks of HD, such as spontaneous choreiform and dystonic movements, cognitive deficits including progressive degeneration of striatal tissue associated with loss of ATP depletion, oxidative–nitrosative stress, and excitotoxicity.

The therapies currently available to HD patients are aimed for symptomatic management, rather than disease cure. However, different laboratory reports suggest the possible involvement of excitotoxicity, neuroinflammatory, oxidative damage, neurochemical disturbance, and mitochondrial dysfunction in its pathogenesis. At present, N-methyl-D-aspartate receptor antagonist, gamma-aminobutyric acid modulators, dopamine blockers, cannabinoid agonists, energy production boosters, and creatine are being tried to treat and manage this disease symptomatically, and a wide variety of therapies has been aimed at downstream events in both preclinical and clinical trials.

Several metabolic modifiers have been tried to ameliorate mitochondrial dysfunctions and oxidative stress in HD, but have been found to be beneficial to a limited extent. Therefore, improving mitochondrial functions has now become a prime focus to combat neurodegeneration in HD. In mitochondrial ETC complexes coenzyme Q\textsubscript{10} also known as ubiquinone (a vitamin-like substance used by the human body to help produce ATP) is a precursor for coenzyme Q\textsubscript{10} that can be obtained externally from Solanaceous plants, especially tobacco (N. tabacum L.) and have been shown to effectively ameliorate various mitochondrial dysfunctions and have potential usefulness in the treatment of numerous human dysfunctions. In addition, SNL participates in cellular energy production and repair of damaged neurons.

On the basis of previous studies, supplementation with SNL can be effective in cognitive and motor performance tests, and this supplementation could ameliorate age-associated mitochondrial functional changes and mitochondria-associated structural damage and oxidative stress in 3-NP-treated animals. Studies aimed at addressing these questions have fallen into two main categories: in vivo behavioral paradigms and in vitro differentiation biochemical and histopathological analyses. Therefore, the aim of the present study was to evaluate the propensity of mitochondrial cofactors and SNL in ameliorating 3-NP-induced behavioral, biochemical, and histological changes.

Materials and Methods

Experimental animals

Male Wistar rats (3 months old, weighing around 220–250 g) were obtained from the animal house of the institute. The animals were housed separately in groups of six per cage (polycarbonate cage size: 29 cm × 22 cm × 14 cm) under standard laboratory conditions with alternate light and dark cycle of 12 h and each. The animals had free access to food and water. The animals were acclimatized to laboratory conditions before behavioral experiments that were carried out between 09:00 and 17:00 h. The experimental protocol was approved by the institutional animal ethics committee, and animal care was taken as per the guidelines of committee for Control and Supervision of Experiments on Animals (CPCSEA), Government of India (888/PO/Re/S/05/CPCSEA).

Drugs and experiment protocol

3-NP acid was purchased from Sigma–Aldrich (USA). SNL was provided as ex gratia sample from BAPEX, Rajasthan. Solutions of the drugs and chemicals were freshly prepared before use. 3-NP was dissolved in 5% dimethyl sulfoxide saline (pH 7.4). SNL was dissolved in water (with 2% ethanol). 3-NP was administered intraperitoneally, and the test drug was administered orally.

Thirty-six animals were randomly divided into six groups: Group 1, normal treated; Group 2, SNL to observe per se effect (15 mg/kg, orally) for 15 days; Group 3, received 3-NP (10 mg/kg, i.p.) for 15 days; and Group 4, 5, and 6, received SNL (5, 10, and 15 mg/kg orally, respectively) 1 h prior to 3-NP administration [Figure 1].

Behavioral tests

Morris water maze

Spatial learning and memory of animals were tested in a Morris water maze (MWM) (Morris, 1984). The animals were given a daily session of three trials per day for 4 days before final trial, i.e., on the 5th and 10th days according to 15-day protocol schedule, each trial having a ceiling time of 120 s and a trial interval of approximately 120 s. If the rat failed to locate the hidden platform within the
maximum time of 120 s, it was gently placed on the platform and allowed to remain there for the same interval of time. The time taken to locate the hidden platform time spent in target quadrant (TSTQ) zone was also measured. Twenty-four hours after the acquisition phase, a probe test (day 15) was conducted by removing the platform. The rats were allowed to swim freely in the pool for 120 s, and the TSTQ, which had previously contained the hidden platform, was recorded. The time spent in the target quadrant indicated the degree of memory consolidation, which had taken place after learning.

**Locomotor activity**

Each animal was observed for 5 min after 15 min for acclimatization in a digital actophotometer (Columbus Inc., USA).

**String test for grip strength**

The length of time the rat was able to hold the wire was recorded on days 1, 5, 10, and 15. This latency to the grip loss is considered as an indirect measure of muscle grip strength.

**Elevated plus maze test**

The elevated plus maze (EPM) served as the behavioral model to evaluate learning and memory in rats. EPM was conducted on the 13th and 14th days of protocol schedule. Each animal was kept at the end of an open arm, facing away from the central platform. Transfer latency (TL) was recorded on the 13th day, i.e., acquisition trial. If the rats did not enter into one of the covered arms within 120 s, it was gently pushed into covered arm and the TL was assigned as 120 s. The rats were allowed to explore the maze for 10 s and then returned to its home cage. Finally, TL was again examined on the 14th day, which is considered as retention latency. TL was taken as the time taken by the animal to move into any one of the covered arms with all its four legs.

**Beam-crossing task**

It is used to measure motor coordination ability of animals. The beam consisted of two platforms (8 cm in diameter) connected by a wooden beam (50 cm × 2.0 cm × 0.5 mm). The beam was elevated 50 cm above ground. To adapt to the elevated beam, a rat was allowed to explore it for 5 min before training on day 15. A training trial was started by placing the rat on the platform at one end. When a rat walked across the beam from the one end to the other end, slipping of its feet occurred and number of slip in each trial was recorded. Motor performance of the rats was scored on a scale ranging from 0 to 4. A score of 0 was assigned to animal that could readily transverse the beam. Scores 1, 2, and 3 were given to animals demonstrated mild, moderate, and severe impairment, respectively. Score 4 was assigned to the animals completely unable to walk on the beam.

**Biochemical estimations**

**Preparation of homogenate**

After behavioral test, the animals were sacrificed by decapitation; the brain was isolated and washed with ice-cold isotonic saline solution. Individual brain tissue samples (Cortex, Striatum and Hippocampus) were then homogenized with ice-cold 0.1 M phosphate buffer (7.4). The homogenate was centrifuged at 10,000 × g for 15 min using refrigerated centrifuge, and the supernatant was used for biochemical estimation.

**Estimation of complex-V activity (ATP)**

Aliquot of homogenate was sonicated immediately in ice-cold perchloric acid (0.1 N) to inactivate ATPases. After centrifugation (14000 × g, 4°C, 5 min), supernatants containing ATP were neutralized with 1N NaOH and stored at −80°C until analysis. ATP level in supernatants were quantified using a reverse-phase HPLC (Perkin Elmer). The mobile phase contains 100 mM KH2PO4 buffer solution (pH 6.0) whose flow rate was maintained at 1.2 mL/min. The column was maintained at the temperature 25°C, and the detection wavelength was set 254 nm. A reference solution of ATP was prepared according to dissolving standard (Sigma, St. Louis. MO, USA).

**Protein estimation**

The protein content was measured using Agappe protein estimation kit (Biuret method).

**Estimation of complex-II activity (succinate dehydrogenase)**

SDH is a marker of impaired mitochondrial metabolism in the brain. The quantitative measurement levels in the brain were performed according to the method as described in previous reports.
**Lactate dehydrogenase assay**
A diagnostic kit (Trans Asia, India) was used to measure lactate dehydrogenase (LDH) activity in rat brain homogenate and expressed as IU/L.[21]

**Estimation of acetylcholinesterase levels**
The quantitative measurement of acetylcholinesterase (AchE) activity in the brain was performed according to the method described by Ellman et al. The assay mixture contained 0.05 ml of supernatant, 3 ml of 0.01M sodium phosphate buffer (pH 8), 0.10 ml of acetylthiocholine iodide, and 0.10 ml of DTNB (Ellman’s reagent). The change in absorbance was measured immediately at 412 nm spectrophotometrically. The enzymatic activity in the supernatant was expressed as µM/mg protein.[22]

**Estimation of glutathione levels**
Reduced glutathione (GSH) in the brain was estimated in accordance with a previously described method by Ellman. The concentration of GSH in the supernatant was expressed as µM/mg protein using a spectrophotometer at 412 nm.[23]

**Estimation of malondialdehyde levels**
The quantitative measurement of malondialdehyde (MDA) – end product of lipid peroxidation – in brain homogenate was performed according to the method of Wills (1996). The amount of MDA was measured, after its reaction with thiobarbituric acid, at 532 nm using a spectrophotometer. The concentration of MDA was expressed as nM/mg protein.[24]

**Estimation of catalase activity**
Catalase activity was measured by Aebi method (80). 0.1 ml of supernatant was added to the cuvette containing 1.9 ml of 50 mM phosphate buffer (pH 7.0). The reaction was started by the addition of 1.0 ml of freshly prepared 30 mM H₂O₂. The rate of decomposition of H₂O₂ was measured spectrophotometrically at 240 nm. The activity of catalase was expressed as µM/H₂O₂ decomposed/ min activity.[25]

**Estimation of superoxide dismutase activity**
Superoxide dismutase (SOD) activity was assayed according to the method of Misra and Fridovich (1972). The supernatant (0.2 ml) of the brain homogenate was mixed with 0.8 ml 50 mM glycine buffer (pH 10.4), and the reaction was started by the addition of 0.02 ml of epinephrine. After 5 min, the absorbance was measured spectrophotometrically at 480 nm. The activity of SOD was expressed as %control.[26]

**Estimation of nitrite levels**
Nitrite, an indicator of the production of nitric oxide, was estimated using Griess reagent as described by Green et al. A measure of 500 µl of Griess reagent (0.1% N-(1-naphthyl) ethylenediamine dihydrochloride, 1% sulfanilamide, and 2.5% phosphoric acid) was added to 100 µl of supernatant, and the absorbance was determined at 546 nm spectrophotometrically. The concentration of nitrite in the supernatant is determined from sodium nitrite standard curve and expressed as µM/mg protein.[27]

**Histopathological studies**
The individual brain samples (cortex, striatum, and hippocampus) were fixed in 10% formalin solution before embedding in paraffin wax. The tissue was then processed and sectioned (10 µm 290 thick) using a rotary microtome. The sections were hematoxylin 291 and eosin stained.[28] The slides were then mounted with DPX 292 (mixture of 10 g of distyrene 80, 5 ml of dibutyl phthalate and 35 ml of 293 xylene) and viewed under the light microscope (higher-power views, 294 x 400) and photographed using a digital camera (Nikon, Japan).

**Statistical analysis**
All the results were expressed as mean and standard deviation. The data were analyzed using two-way ANOVA followed by Bonferroni post hoc test and one-way ANOVA followed by post hoc Tukey’s multiple comparison test. P < 0.05 was considered statistically significant.

**Results**

**Effect of solanesol on Morris water maze**
Escape latency (ELT) period was increased in 3-NP-treated rats when compared to normal and SNL per se rats (P < 0.05) (46.43 ± 3.42 s [day 5] and 59.06 ± 7.42 s [day 10]). SNL per se administration did not show any significant changes when compared to normal rats. Coadministration of SNL (5, 10, and 15 mg/kg p.o.) significantly decreased ELT period when compared to 3-NP-only-treated rats, indicating remarkable improvement in learning [Figure 2].

3-NP-treated rats showed remarkable decrease in TSTQ when compared to normal and SNL per se treated rats (P < 0.05) (8.04 ± 2.29 s). Per se group did not show changes during TSTQ. SNL 5, 10, and 15 mg/kg p.o. administration showed a remarkable increase in TSTQ when compared to 3-NP-treated rats, which clearly define enhancement of cognitive behavior [Figure 3].

**Effect of solanesol on locomotor activity**
There was no significant difference found between all treatment groups on day 1. There was a significant decrease in ambulatory movements in chronic 3-NP-administered rats as compared to normal and SLN per se rats (P < 0.05) (310.0 ± 50.13, 149.34 ± 10.83, 117.0 ± 11.34, and 55.05 ± 13.45). SNL...
Effect of solanesol on grip strength

Initially, on day 1, there was no significant difference found in groups in grip strength task. Chronic administration of 3-NP produced a significant loss in grip strength, measured by the reduction in time to hold the metal wire as compared to normal rats. Coadministration of SNL (5, 10, and 15 mg/kg p.o.) showed significantly dose-dependent changes in locomotor activity of rats when compared to 3-NP-treated rats [Figure 4].

Effect of solanesol on elevated plus maze

Initial TL was significantly increased in chronically treated 3-NP rats as compared to normal and SNL per se rats (P < 0.05) (13.35 ± 3.35 s). Statistically, SNL per se administration did not reveal any change when compared to normal rats in TL. The administration of SNL (5, 10, and 15 mg/kg p.o.) exhibited a notable decrease in TL when compared to 3-NP-treated rats [Figure 6].

Effect of solanesol on balance beam crossing

As shown in Figure 7, there was a significant increase in the number of slips in 3-NP treatment groups, indicating impairment in beam-walking performance as compared to vehicle-treated group (P < 0.05) (13.34 ± 1.34). In addition, neurological score of 3-NP-treated rats also found high when compared to both control and SLN per se groups (P < 0.05) (3.65 ± 0.28) [Figure 8]. There was no significant difference found between normal and SNL per se treated rats. Pretreatment with SNL (5, 10, and 15 mg/kg p.o.) significantly and dose dependently decreases the number of slips as well as improves balance beam-walking performance when compared to the 3-NP-treated group [Figures 7 and 8].

Effect of solanesol on ATP, succinate dehydrogenase, lactate dehydrogenase, and acetylcholinesterase levels in brain homogenates of rats

As shown in Table 1, 3-NP treatment significantly decreased the level of ATP and SDH in brain homogenate (P < 0.05) as compared to normal and SNL per se group. In SNL (5, 10, and 15 mg/kg p.o.)-treated brain homogenate (striatum, cortex, and hippocampus), we found a remarkable increase in the level of ATP and SDH when compared to 3-NP-treated group. Chronic 3-NP treatment resulted in a significant increase in LDH and AChE enzymatic activity in brain homogenate samples (striatum, cortex, and hippocampus) when compared to normal and SNL per se group. Chronic SNL significantly reversed the effect of 3-NP on above-mentioned biochemical changes that occurred in the experimental animals, providing some degree of protection [Table 1].

Effect of solanesol treatment against 3-nitropropionic-induced biochemical changes (malondialdehyde, glutathione, nitrite, superoxide dismutase, and catalase levels) in the striatum, cortex, and hippocampus of rat brain homogenate

Reduced GSH levels and enzymatic activities of SOD and catalase significantly decreased in the brain of 3-NP-treated rats as compared to normal rats.
Furthermore, it was found that chronic 3-NP treatment induced lipid peroxidation as indicated by increased level of MDA on rat brain homogenates as compared to normal and SNL per se rats. In the treatment group, SNL 5 mg/kg, p.o. and 10 mg/kg, p.o. administration there was remarkable increase in GSH, SOD, and catalase activity when compared to 3-NP-treated rats, while the SNL 15 mg/kg, p.o. administration was found more effective to modulates biochemical activities when compared with 3-NP-treated rats. The test sample also significantly reduced the oxidative marker (MDA and nitrite) [Table 2].

**Effect of solanesol on 3-nitropropionic-induced histopathological changes in the striatum, cortex, and hippocampus of rat brain**

Histological individual section of striatum, cortex, and hippocampus of the normal [Figure 9] and SNL per se [Figure 10] treated rat brain showed optimally sized, undamaged pyramidal shape neuronal cells with a clearly observable cell’s nucleus and continue cell membrane. The 3-NP treated [Figure 11] brain section (striatum, cortex and hippocampus) showed several neuronal space and irregular damaged cells and dense pyknotic nuclei accompanied with marked focal diffuse gliosis as compared to normal and SNL per se treated rat brains [Figure 12].

**Discussion**

The present study was planned to evaluate the consequences of three factors, namely mitochondrial complexes activation, brain energy restoration, and modulation of brain anti-oxidative defense system by employing specific phytochemicals, i.e., SNL. In addition, the present study aimed to explore the possible mechanism involved in 3-NP-induced neurodegeneration which could lead to pathogenesis of HD. In defiance of the present therapeutic alternatives, HD is not fully controlled. However, on the basis of the current challenges, there are phytochemicals and herbal treatments...
extracts are in process to ameliorate the symptomatic and therapeutic prevention of HD. These natural therapies decrease the oxidative neuronal dysfunctions and perform ameliorative treatment strategies in various brain disorders.

3-NP, a mycotoxin, has been well known as a mitochondrial complex-II enzyme (SDH) inhibitor, causing dysfunction of mitochondrial electron transport chain system and energy failure and found to cause various behavioral and biochemical abnormalities related to memory and motor functioning. In our experiment, behavioral memory was evaluated using MWM, wherein 3-NP-intoxicated rats showed remarkable acquisition and retention reduction when compared with normal and SNL per se-ingested animals. Furthermore, chronic 3-NP-treated animals showed marked motor and behavioral dysfunctions such as bradykinesia, muscle weakness, and rigidity. The present findings reveal the

Table 1: Effect of solanesol treatment against 3-nitropropionic-induced biochemical changes (adenosine triphosphate, succinate dehydrogenase, lactate dehydrogenase, and acetylcholinesterase levels) in the striatum, cortex, and hippocampus of rat brain homogenate

| Treatment          | Individual brain sample | ATP (m/g tissue) | SDH (unit/mg protein) | LDH (IU/L) | AChE (m/mg protein) |
|--------------------|-------------------------|------------------|-----------------------|------------|---------------------|
| Normal             | Striatum                | 650.4±5.72       | 5.21±0.61             | 404.3±32.11| 10.16±0.54          |
|                    | Cortex                  | 580.2±8.02       | 5.09±0.43             | 324.4±21.55| 12.24±0.46          |
|                    | Hippo                   | 557.3±12.04      | 3.77±0.15             | 326.4±9.12 | 13.92±0.54          |
| SNL per se         | Striatum                | 643.3±6.55       | 3.94±0.25             | 365.5±16.6 | 13.84±0.54          |
|                    | Cortex                  | 590.3±7.34       | 5.38±0.32             | 330.5±16.23| 12.47±1.14          |
|                    | Hippo                   | 562.4±15.25      | 3.24±0.36             | 316±15.84  | 14.95±0.55          |
| 3-NP control       | Striatum                | 188.4±6.34*      | 0.62±0.52*            | 1098±29.92*| 45.74±1.65*         |
|                    | Cortex                  | 220.3±12.41*     | 0.91±0.21*            | 998±29.25* | 40.74±1.05*         |
|                    | Hippo                   | 249.3±9.83*      | 0.65±0.15*            | 965±30.73* | 48.65±1.36*         |
| SNL 5+3-NP         | Striatum                | 235.3±8.53*      | 1.26±0.25*            | 854±41.84* | 31.65±1.25*         |
|                    | Cortex                  | 285.2±15.13*     | 1.99±0.24*            | 767.4±39.26*| 33.25±1.06*         |
|                    | Hippo                   | 320.1±12.63*     | 0.84±0.16*            | 804.4±45.24*| 42.84±3.05*         |
| SNL 10+3-NP        | Striatum                | 311.6±14.43*     | 2.67±0.43*            | 698.4±51.35*| 28.35±2.44*         |
|                    | Cortex                  | 344.3±32.62*     | 3.10±0.43*            | 629±30.95* | 26.62±1.02*         |
|                    | Hippo                   | 409.10±19.43*    | 2.73±0.74*            | 634±36.44* | 32.05±1.86*         |
| SNL 15+3-NP        | Striatum                | 420.2±29.53*     | 3.79±0.43*            | 536.7±58.97*| 22.57±1.03*         |
|                    | Cortex                  | 492.4±32.82*     | 4.44±0.54*            | 454.3±52.38*| 23.22±2.07*         |
|                    | Hippo                   | 476.3±21.90*     | 3.04±0.14*            | 493.6±55.35*| 24.74±2.16*         |

Values are means±SD (n=6). *Signifies P<0.05 as compared to normal and SNL per se, #P<0.05 versus 3-NP, **P<0.05 as compared to SNL (5+3-NP) and SNL (10+3-NP). ATP=Adenosine triphosphate, SDH=Succinate dehydrogenase, LDH=Lactate dehydrogenase, AChE=Acetylcholinesterase levels, SNL=Solanesol, 3-NP=3-Nitropropionic, SD=Standard deviation

Figure 8: Effect of solanesol on balance beam-walking performance in 3-nitropropionic-treated rats (neurological score). Values are mean ± standard deviation (n = 6), *signifies P < 0.05 as compared to normal and solanesol per se, #P < 0.05 versus 3-nitropropionic, **P < 0.05 as compared to solanesol (10 + 3-nitropropionic) and solanesol (15 + 3-nitropropionic)

Figure 9: Panel A1 (basal ganglia), panel A2 (cortex) and panel A3 (hippocampus): photomicrographs of H and E-stained brain sections of normal rats
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behavioral and biochemical alterations associated with 3-NP-intoxicated rats.\textsuperscript{[31]} It has been explained in various studies that body weight get reduced in case of HD patients.\textsuperscript{[32]} Moreover, slow motor movement symptoms are mainly present in the end stage of the HD patients. These bodyweight reduction and motor disturbances mimic the same dysfunction associated after 3-NP injected intoxicated rats. During EPM, transfer latency is the symbol to observe the memory function.\textsuperscript{[33]} During EPM task, chronic administration of 3-NP remarkably increased the TL as compared to normal and SNL per

Table 2: Effect of solanesol treatment against 3-nitropropionic-induced biochemical changes (malondialdehyde, glutathione, nitrite, superoxide dismutase, and catalase levels) in the striatum, cortex, and hippocampus of rat brain homogenate

| Treatment       | Individual brain sample | MDA (nM/mg protein) | GSH (µM/mg protein) | Nitrite (µM/mg protein) | SOD (% control) | Catalase (% control) |
|-----------------|-------------------------|---------------------|---------------------|-------------------------|-----------------|---------------------|
| Normal          | Striatum                | 1.09±0.27           | 12.93±0.46          | 0.92±0.45               | 100.0±0.0       | 100.0±0.0           |
|                 | Cortex                  | 0.86±0.16           | 11.36±0.35          | 9.35±0.43               | 100.0±0.0       | 100.0±0.0           |
|                 | Hippo                   | 0.86±0.37           | 10.92±0.74          | 0.96±0.47               | 100.0±0.0       | 100.0±0.0           |
| SNL perse       | Striatum                | 1.35±0.82           | 11.47±0.48          | 1.16±0.53               | 96.42±1.73      | 94.41±3.04          |
|                 | Cortex                  | 0.95±0.26           | 10.25±0.64          | 1.25±0.56               | 95.61±2.01      | 93.46±1.72          |
|                 | Hippo                   | 0.99±0.21           | 9.92±0.35           | 1.25±0.54               | 94.39±1.28      | 95.06±1.43          |
| 3-NP control    | Striatum                | 11.64±0.53*         | 1.28±0.37*          | 6.57±0.38*              | 7.01±0.27*      | 33.27±4.50*         |
|                 | Cortex                  | 9.04±0.95*          | 1.64±0.37*          | 7.63±0.21*              | 6.25±0.45*      | 34.46±4.94*         |
|                 | Hippo                   | 9.34±0.52*          | 1.54±0.28*          | 7.45±0.72*              | 7.10±0.49*      | 35.06±4.71*         |
| SNL 5+3-NP      | Striatum                | 8.42±0.54*          | 3.94±0.63*          | 6.23±0.45*              | 48.37±4.27*     | 58.46±5.33*         |
|                 | Cortex                  | 7.32±0.35*          | 4.96±0.48*          | 5.82±0.39*              | 49.52±4.31*     | 56.26±3.42*         |
|                 | Hippo                   | 6.94±0.42*          | 4.65±0.37*          | 5.57±0.58*              | 45.46±4.15*     | 55.73±4.75*         |
| SNL 10+3-NP     | Striatum                | 6.47±0.48*          | 5.11±0.37*          | 4.36±0.58*              | 59.93±5.43*     | 69.43±3.46*         |
|                 | Cortex                  | 6.35±0.29*          | 4.92±0.45*          | 4.02±0.84*              | 60.05±3.36*     | 71.73±3.54*         |
|                 | Hippo                   | 5.94±0.73*          | 4.60±0.32*          | 4.79±0.29*              | 57.40±3.73*     | 74.73±3.58*         |
| SNL 15+3-NP     | Striatum                | 4.03±1.25*          | 7.94±0.94*          | 3.49±0.84*              | 79.52±4.37*     | 80.34±3.47*         |
|                 | Cortex                  | 3.93±0.46*          | 7.96±0.58*          | 3.06±0.40*              | 76.65±4.30*     | 79.35±5.17*         |
|                 | Hippo                   | 3.63±0.57*          | 8.51±0.57*          | 2.99±0.53*              | 75.32±4.21*     | 82.74±3.93*         |

Values are mean±SD (n=6), *Signifies P<0.05 as compared to normal and solanesol perse, †P<0.05 versus 3-NP, ‡P<0.05 as compared to SNL (5+3-NP) and SNL (10+3-NP). SNL=Solanesol, 3-NP=3-Nitropropionic, SD=Standard deviation, MDA=Malondialdehyde, GSH=Glutathione, SOD=Superoxide dismutase
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set treated group, indicating a marked reduction in memory performance. Whereas, SNL at the dose of 20, 40, and 60 mg/kg p.o. exceptionally reduced increased TL duration as compared to 3-NP-treated group. The observed results revealed the ameliorative effect of SNL on 3-NP-intoxicated memory dysfunctions due to involvement of brain cholinergic system.

It has been observed that chronic treatment with 3-NP significantly reduced the motor coordination. It reduced the grip strength in animal and produced imbalance in beam-waking performance and impaired locomotor function. On the other hand, SNL was found significant to ameliorate 3-NP-intoxicated memory and motor abnormalities. Mitochondrial dysfunction is well documented and explored in HD, and optimal modulation of mitochondrial signaling pathway has been found beneficial to restore cyclic nucleotide pathways for preventing the cases of HD in preclinical experimental research. Furthermore, restoration of mitochondrial functioning is sufficient to ameliorate the memory and motor dysfunctions in rats. Effectiveness of restoration of mitochondrial dysfunction may be due to selective and dominant effect of SNL in the striatal, cortical, and hippocampal region. Moreover, reduction in the ATP levels in the present study was found to be due to 3-NP-induced neurotoxicity. In the present findings, SNL efficiently increases the ATP levels, which could be the sign of proper modulations and restoration of mitochondrial complexes in the individual samples of brain homogenates, i.e., striatum, cortex, and hippocampus. Acetylcholine has been studied frequently as a major neurotransmitter for the formation and storage of memory. Different regions of brain participating in memory and cognitive formation are hippocampus, amygdala, and cerebral cortex, which are highly vulnerable to be damaged by free radical attack and associated with cognitive dysfunctions. HD is mainly associated with imbalance between various neurotransmitters in cholinergic nervous system causing memory and cognitive disturbances. Loss of cholinergic innervations, as demonstrated by elevated AChE enzyme levels, is associated with a large number of neurodegenerative abnormalities. Increased AChE depletes the level of acetylcholine whereas inhibition of AChE represents the prolongation of availability of Ach, which is responsible to enhance cholinergic function. In this study, 3-NP administration significantly increased the AChE levels in hippocampus, which is well reported in various research studies. In the present study, the test drug SNL surprisingly reduced the AChE level as compared to 3-NP-treated group.

Energy depletion is associated with excessive release of free radicals, which initiate the oxidative cascades, which in turns activates the reversible secondary cascade, i.e., excitotoxicity and vice versa. From previous research studies, it has been clearly observed that energy impairment could generate huge amount of free radicals in the brain of HD, especially in hippocampus, basal ganglia, and cortex. In HD, energy impairment is mainly due to abnormalities in mitochondrial functioning and free radical generation, where free radical-mediated cell membrane damage leads to lipid peroxidation and produce its end metabolite, namely MDA. Reduced GSH is one of the major antioxidants present in the brain, which is responsible to scavenge the oxidative stress by neutralizing free radicals. Reduced level of such antioxidant in the brain might be the critical factor for the various neurodegenerative abnormalities, which could finally lead to learning and memory deficits. On the other hand, SOD and catalase are the most powerful antioxidant enzymes that perform a protective role in neutralizing the free radical generation. During 3-NP injection, a remarkable reduction in antioxidant levels of SOD and catalase was found. In the present study, SNL surprisingly restored the enzymatic levels of SOD and catalase which was depleted by 3-NP treatment. Supporting the previous results, 3-NP has also been found to cause a significant decrease in mitochondrial SDH enzyme and GSH levels, and increase M in DA and nitrite levels. LDH was estimated in brain homogenates, where 3-NP-intoxicated rats exhibit severe neuronal cell damage as confirmed by increasing LDH enzyme levels. Whereas, SNL treatment in specific 3-NP-intoxicated

Figure 12: Panel D1 (basal ganglia), panel D2 (cortex) and panel D3 (hippocampus): photomicrographs of H and E-stained brain sections of solanesol 60 mg/kg p.o. treated rats.
rats has remarkably reversed the levels of MDA, nitrite, and LDH and potentiated the SDH and GSH levels too, showing antioxidant and anti-inflammatory activity. Furthermore, in the present study, SNL significantly improved mitochondrial function via elevating ATP levels.

The observed data on the basis of the current experiment schedule confirmed that chronic administration of SNL in per se treated rats did not exhibit any surprisingly neuroprotective effect on learning and memory appearance when compared with normal rats. On the other hand, SNL (20, 40, and 60 mg/kg p.o.) provided notable neuroprotective effect, which was confirmed by behavioral paradigms and biochemical test. It restored the behavioral and biochemical alteration caused by 3‑NP and confirmed the strong neuroprotective mechanism of SNL in 3‑NP‑intoxicated memory and cognitive abnormalities. Moreover, systemic administration of 3‑NP drastically impaired memory retention, resembling Huntington’s type neurodegenerative disorders.\(^{[9]}\)

**Conclusion**

The observed data in this research work also confirmed that SNL increases brain antioxidant activity by neutralizing the free radical generation and improves the major histopathological structural alterations in different regions of rat brain. Therefore, the data from the current study clearly indicate the prophylactic neuroprotective efficacy of SNL in the 3‑NP model. The protective property of SNL is attributed to efficacy to modulate in vivo behavioral paradigms, improve mitochondrial function, and restore cholinergic functions in brain regions culminating in the improved motor/stress behavior. Based on the observed data in the current study, it is proposed that SNL may be utilized as a therapeutic agent/adjuvant in protecting the CNS against or to manage oxidative stress‑mediated neurodegeneration condition.

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**Conflicts of interest**

There are no conflicts of interest.

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