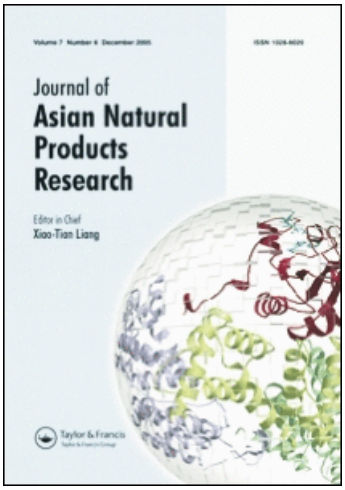

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Sesamol attenuate 3-nitropropionic acid-induced Huntington-like behavioral, biochemical, and cellular alterations in rats

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Sesamol attenuate 3-nitropropionic acid-induced Huntington-like behavioral, biochemical, and cellular alterations in rats

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Sesamol (SML) obtained from sesame seeds (*Sesamum indicum*, Linn, Pedaliaceae) has been used as a traditional health food in India and other countries since a long time. Besides its good antioxidant activity, SML is currently receiving considerable attention in relation to neurological disorders. Therefore, the present study has been designed to explore the protective role of SML in 3-nitropropionic acid (3-NP)-induced neurotoxicity in animals. Male rats were given 3-NP (10 mg/kg) treatment for 14 days. Various behavioral observations (body weight, locomotor activity), oxidative damage (lipid peroxidation, nitrite level, superoxide dismutase, and catalase enzyme), and mitochondrial enzyme complex functions were also assessed in the striatum, cortex, and hippocampal regions of the brain. 3-NP treatment significantly impaired locomotor activity, motor coordination, body weight, oxidative damage, and mitochondrial enzyme complex functions as compared with vehicle-treated groups. SML (5, 10, and 20 mg/kg) pre-treatment significantly improved body weight, locomotor activity, motor coordination, and attenuated oxidative damage in different regions of rat brain. Besides these, SML treatment also significantly improved mitochondrial enzymes in all regions of the brain as compared with the respective control (3-NP) group. The present study suggests that SML could be used as effective agents in the management of Huntington's disease.

Keywords: Huntington's disease; mitochondrial dysfunction; 3-nitropropionic acid; oxidative stress; sesamol

1. Introduction

Huntington's disease (HD) is an autosomal dominant inherited progressive neurodegenerative disorder, affecting people of middle age, characterized by the progressive development of involuntary choreiform movements, cognitive impairment, neuropsychiatric symptoms, and premature death. The pathogenesis of HD is unknown, but increasing evidence suggests the involvement of altered gene transcription, mitochondrial dysfunction, excitotoxicity, and oxidative damage [1]. Despite this heterogeneity, mitochondrial involvement is likely to be an important common key in HD [1]. The mitochondrial enzymes are the seat of a number of important cellular functions, including essential pathways of intermediate metabolism, amino acid biosynthesis, fatty acid oxidation, and apoptosis [2]. The mitochondrial enzyme dysfunction leads to reduced ATP production, impaired calcium buffering, and increased generation of reactive oxygen species (ROS) [3].

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Various lines of evidence demonstrate the involvement of mitochondrial dysfunction in the pathogenesis of HD [3].

3-Nitropropionic acid (3-NP) is a mycotoxin isolated from fungus *Arthinium* and was first described in the 1970s when several children accidentally ingested contaminated sugar cane, which resulted in motor dysfunction and neurodegeneration within the basal ganglia. 3-NP irreversibly binds to succinate dehydrogenase (SDH) enzyme and inhibits its activity by interfering with the ATP synthesis [2]. Long-term exposure to 3-NP leads to a significant loss of medium spiny neurons ($>90\%$) in the basal ganglia, particularly the γ-aminobutyric acid or substance P neurons and preservation of intermediate interneurons [4]. 3-NP induces motor dysfunction, striatal lesions, decreased mitochondrial function, and oxidative stress as seen in HD patients.

Various dietary supplements are currently being used to treat neurological disorders across the world. Sesamol (SML; Figure 1) is a dietary supplement known for its antioxidant activity, currently being used as traditional health food in India as well as in other oriental countries [5]. SML is effective against various diseases, including atherosclerosis, hyperlipidemia, hypertension, anticancer, and anti-aging effects [6]. Studies have reported that SML shows good antioxidant activity [5]. Sesame oil provides protection against increased blood pressure, hyperlipidemia, and lipid peroxidation by increasing enzymatic and non-enzymatic antioxidants [6]. Studies have also demonstrated that SML has strong antitumor properties.

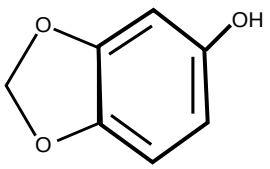

Figure 1. Structure of SML.

The neuroprotective effect of SML is also demonstrated in the focal cerebral ischemia model of Sprague–Dawley rat, where SML attenuated nitrite production, inducible nitric oxide (NO) synthase (iNOS) mRNA, nuclear factor-κB, and p38 mitogen-activated protein kinase activation in lipopolysaccharide-stimulated murine BV-2 microglia [6]. It also significantly reduced the generation of ROS in H_2O_2-induced BV-2 cells and in H_2O_2-cell-free conditions. But no reports are available regarding the use of SML in the animal models of HD.

The present study was undertaken to explore the protective effect of SML against 3-NP-induced behavioral, oxidative stress, and mitochondrial alterations in the discrete areas of rat brain.

2. Results

2.1 Effect of SML on body weight in the 3-NP-treated rats

There was no change in the initial and final body weight of vehicle-treated animals. However, 3-NP (10 mg/kg) treatment caused significant decrease in the body weight on the 15th day as compared with the vehicle-treated group. SML *per se* treatment had no effect on the body weight as compared with the 3-NP-treated (10 mg/kg) group ($P < 0.05$). Furthermore, SML (5, 10, and 20 mg/kg) treatment significantly improved body weight in 3-NP-treated (10 mg/kg) rats ($P < 0.05$; Figure 2). However, the effect was not dose-dependent.

2.2 Effect of SML on locomotor activity in the 3-NP-treated rats

3-NP (10 mg/kg) treatment significantly caused impairment in locomotor activities as compared with vehicle-treated group ($P < 0.05$). Furthermore, chronic treatment with SML (10 and 20 mg/kg) significantly improved locomotor activities as compared with the 3-NP-treated (10 mg/kg) rats (Figure 3). SML *per se*

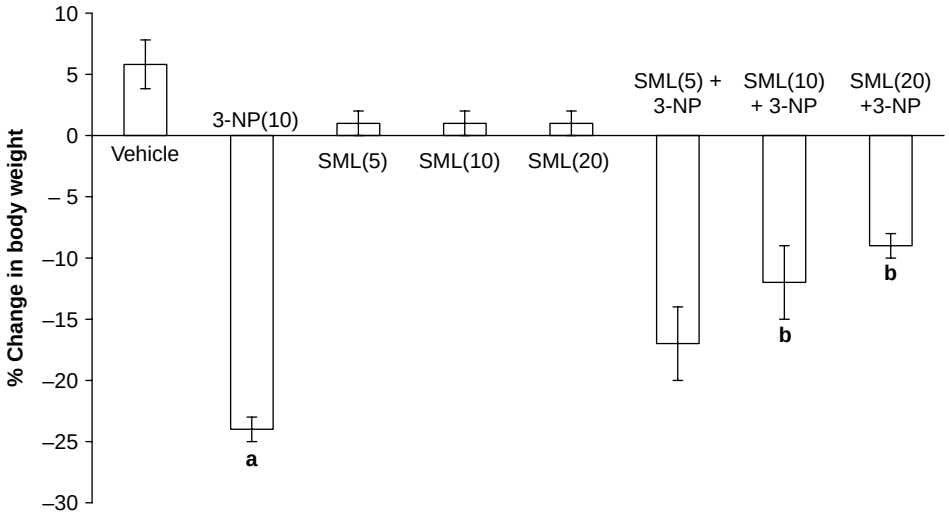

Figure 2. Effect of SML (5, 10, and 20 mg/kg) treatment on the body weight in the 3-NP-treated rats. Values are mean ± SEM. [a]$P < 0.05$ versus naïve and [b]$P < 0.05$ versus 3-NP, treated group (one-way ANOVA followed by Tukey's test). SML, sesamol; 3-NP, 3-nitropropionic acid.

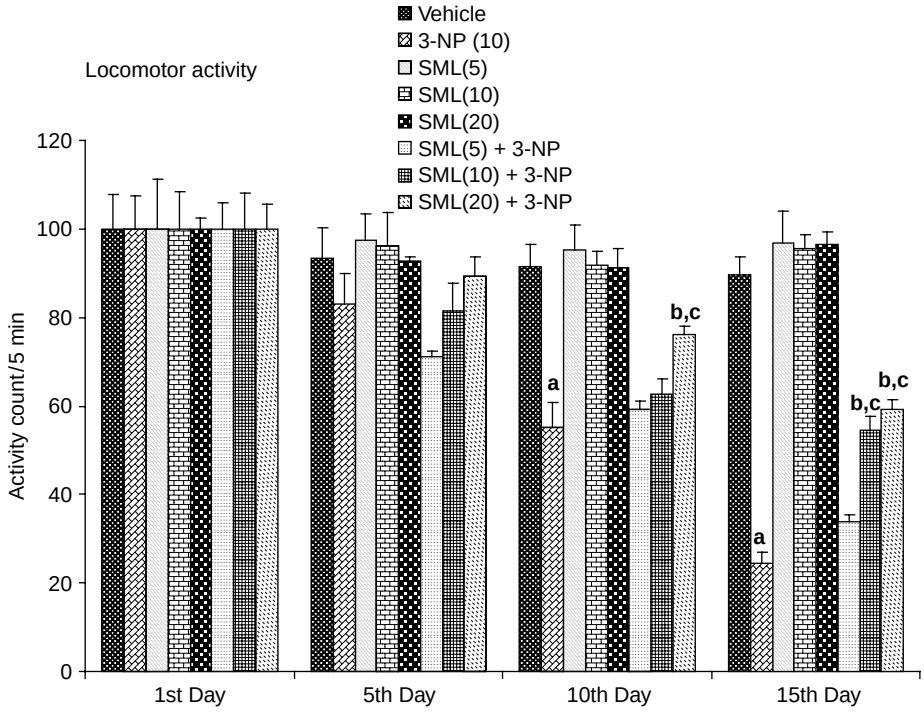

Figure 3. Effect of SML (5, 10, and 20 mg/kg) treatment on locomotor activities in the 3-NP-treated rats. Values are mean ± SEM. [a]$P < 0.05$ versus naïve, [b]$P < 0.05$ versus 3-NP, and [c]$P < 0.05$ versus (3-NP + SML 5 mg/kg), treated group (one-way ANOVA followed by Tukey's test). SML, sesamol; 3-NP, 3-nitropropionic acid.

treated rats did not produce any significant effect at the end of study.

2.3 Effect of SML on rotarod performance in the 3-NP-treated rats

3-NP (10 mg/kg) treatment impaired muscle grip strength as assessed by rotarod test on the 10th and 15th days as compared with the vehicle-treated animals. Furthermore, SML [10, 20 mg/kg, peroral (p.o.)] treatment significantly improved muscle strength on the 15th day in the 3-NP-treated (10 mg/kg) rats ($P < 0.05$; Figure 4). SML *per se* treatment did not produce any significant effect as compared with vehicle-treated animals at the end of study.

2.4 Effect of SML on brain lipid peroxidation, nitrite level, and antioxidant enzyme (SOD activity and catalase) in the 3-NP-treated rats

Systemic administration of 3-NP (10 mg/kg) caused marked increase in lipid peroxidation, nitrite concentration, depleted SOD, and catalase enzyme activity in striatum, hippocampus, and cortex as compared with the vehicle-treated animals. However, SML *per se* treatment did not produce any significant alterations in the malonodialdehyde (MDA), nitrite levels, SOD, and catalase enzyme activity as compared with the 3-NP-treated (10 mg/kg) rats in these regions. However, SML (10 and 20 mg/kg) treatment significantly attenuated MDA, nitrite levels and restored the reduced SOD and catalase activities in the striatum, hippocampus, and cortex of the 3-NP-treated (10 mg/kg) rats ($P < 0.05$; Table 1). However, a lower dose of SML (5 mg/kg) did not produce any significant effect as compared with the control (3-NP) group.

2.5 Effect of SML on mitochondrial complex levels in the 3-NP-treated rats

Systemic 3-NP (10 mg/kg) treatment significantly altered the mitochondrial enzyme complex activities (I, II, and IV) as compared with the vehicle-treated rats. Chronic administration of SML (5, 10, and 20 mg/kg, p.o.) significantly restored the mitochondrial enzyme complex activities as compared with the 3-NP-treated

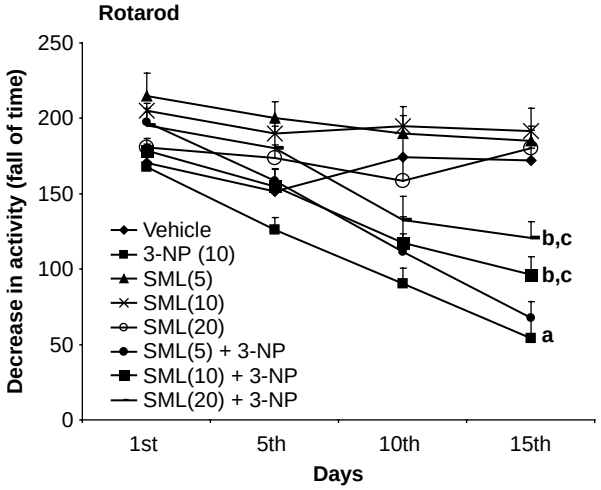

Figure 4. Effect of SML (5, 10, and 20 kg/mg) treatment in the rotarod score in the 3-NP-treated rats. Values are mean ± SEM. [a]$P < 0.05$ versus naïve, [b]$P < 0.05$ versus 3-NP, and [c]$P < 0.05$ versus (3-NP + SML 5 mg/kg), treated group (one-way ANOVA followed by Tukey's test). SML, sesamol; 3-NP, 3-nitropropionic acid.

Table 1. Effect of SML treatment on oxidative damage against 3-NP-induced biochemical changes in the striatum, cortex, and hippocampus of rat brain.

Treatment		MDA nmol/mg protein (% of control)	Nitrite level μmol/mg protein (% of control)	SOD unit/mg protein (% of control)	Catalase μmol of H_2O_2 decomposed/min/mg protein (% of control)
Vehicle	Striatum	1.54 ± 0.21 (100)	241 ± 2.3 (100)	7.86 ± 1.2 (100)	3.83 ± 0.7 (100)
	Cortex	1.55 ± 0.22 (100)	263 ± 1.5 (100)	7.86 ± 1 (100)	3.80 ± 0.4 (100)
	Hippocampus	1.45 ± 0.26 (100)	210 ± 1.8 (100)	6.65 ± 2 (100)	3.12 ± 0.8 (100)
3-NP (10)	Striatum	3.98 ± 0.31[a] (258)	461 ± 1.6[a] (191)	3.9 ± 1.6[a] (49)	1.8 ± 1.3[a] (47)
	Cortex	2.98 ± 0.27[a] (192)	370 ± 1.5[a] (141)	5 ± 2.3[a] (64)	2.2 ± 2.0[a] (58)
	Hippocampus	3.3 ± 0.18[a] (228)	385 ± 1.5[a] (183)	3.9 ± 1.5[a] (59)	2.3 ± 1.5[a] (68)
SML (5)	Striatum	1.66 ± 0.05 (106)	249 ± 3.1 (103)	8.1 ± 2.5 (104)	4.3 ± 1.5 (112)
	Cortex	1.63 ± 0.08 (103)	269 ± 0.9 (102)	8.4 ± 1.4 (107)	4.0 ± 0.9 (105)
	Hippocampus	1.55 ± 0.09 (107)	220 ± 1.5 (105)	6.9 ± 1.5 (104)	3.3 ± 1.5 (105)
SML (10)	Striatum	1.64 ± 0.09 (106)	255 ± 1.2 (106)	9.4 ± 0.6 (120)	4.2 ± 2.3 (110)
	Cortex	1.65 ± 0.05 (106)	275 ± 1.5 (105)	7.9 ± 0.5 (102)	3.9 ± 2.5 (105)
	Hippocampus	1.60 ± 0.06 (110)	105 ± 1.5 (220)	7.2 ± 1.5 (108)	3.4 ± 1.5 (109)
SML (20)	Striatum	1.75 ± 0.05 (114)	260 ± 1.2 (108)	8.9 ± 0.8 (114)	4.3 ± 0.5 (113)
	Cortex	1.70 ± 0.04 (110)	279 ± 1.3 (106)	7.8 ± 0.3 (100)	3.9 ± 0.6 (102)
	Hippocampus	1.68 ± 0.08 (117)	230 ± 1.5 (110)	7.4 ± 1.5 (111)	3.45 ± 0.5 (110)
SML (5) + 3-NP (10)	Striatum	3.5 ± 0.10[NS] (227)	367 ± 1.2[NS] (152)	4.5 ± 1.8[NS] (57)	2.0 ± 2.1[NS] (53)
	Cortex	2.4 ± 0.05[NS] (155)	355 ± 1.4[NS] (135)	5.8 ± 1.2[NS] (74)	2.19 ± 0.5[NS] (57)
	Hippocampus	2.99 ± 0.08[NS] (206)	370 ± 1.7[NS] (176)	4.5 ± 1.3[NS] (68)	2.2 ± 1.7[NS] (70)
SML (10) + 3-NP (10)	Striatum	2.8 ± 0.08[b,c] (182)	310 ± 2.5[b,c] (129)	5.0 ± 1.5[b] (65)	2.5 ± 1.8[b,c] (66)
	Cortex	1.98 ± 0.05[b,c] (128)	320 ± 3.5[b,c] (122)	6.4 ± 1.4[b] (82)	3.1 ± 1.9[b,c] (81)
	Hippocampus	2.5 ± 0.07[b,c] (172)	290 ± 2.8[b,c] (138)	5.6 ± 1.6[b,c] (85)	2.5 ± 2.5[b,c] (80)
SML (20) + 3-NP (10)	Striatum	2.3 ± 0.068[b,c,d] (149)	267 ± 3.4[b,c,d] (111)	5.9 ± 2.1[b,c,d] (75)	3.08 ± 0.5[b,c,d] (81)
	Cortex	1.75 ± 0.07[b,c,d] (113)	295 ± 2.9[b,c] (112)	7.1 ± 2.2[b,c] (91)	3.5 ± 1.8[b,c,d] (92)
	Hippocampus	2.1 ± 0.08[b,c,d] (145)	265 ± 2.7[b,c] (126)	5.8 ± 2.3[b] (87)	2.6 ± 1.5[b] (83)

Values expressed as percentage of vehicle-treated group. NS, not significant.
[a] $P < 0.05$ versus control.
[b] $P < 0.05$ versus 3-NP.
[c] $P < 0.05$ versus [3-NP + SML (5)].

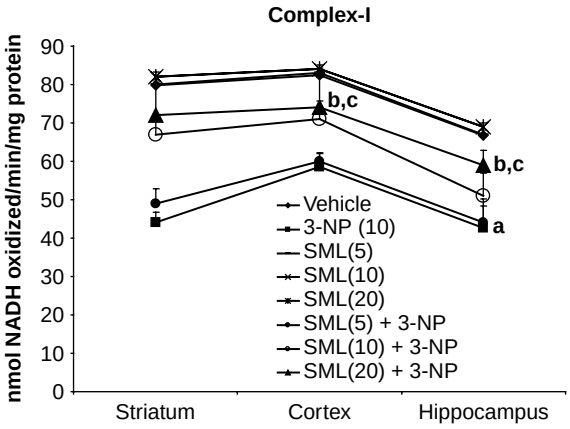

Figure 5. Effect of SML (5, 10, and 20 mg/kg) treatment in mitochondrial complex-I in the 3-NP-treated rats. Values are mean ± SEM. [a]$P < 0.05$ versus naïve, [b]$P < 0.05$ versus 3-NP, and [c]$P < 0.05$ versus (3-NP + SML 5 mg/kg), treated group (one-way ANOVA followed by Tukey's test). SML, sesamol; 3-NP, 3-nitropropionic acid.

group ($P < 0.05$; Figures 5–7). A low dose of SML (5 mg/kg) did not influence significantly these mitochondrial enzyme complex activities (I, II, and IV; $P < 0.05$). SML (5, 10, and 20 mg/kg, p.o.) *per se* treatment did not cause any significant change in the mitochondrial enzyme complexes (I, II, and IV) as compared with the 3-NP-treated group ($P < 0.05$).

2.6 Effect of SML on 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-H-tetrazolium bromide (MTT) ability in the 3-NP-treated rats

Systemic 3-NP (10 mg/kg) treatment significantly declined the number of viable cell as compared with the vehicle-treated rats, as measured in the MTT assay. Chronic administration of SML (10 and 20 mg/kg, p.o.) significantly increased the count of viable cells as measured in the MTT ability assay as compared with the 3-NP-treated group ($P < 0.05$; Figure 8). A low dose of SML (5 mg/kg) and *per se* (5, 10, and 20 mg/kg, p.o.) treatment did not cause any significant change in the viable cell count as compared with the 3-NP-treated group ($P < 0.05$).

3. Discussion

3-NP (fungal toxin) irreversibly inhibits the mitochondrial enzyme SDH [2] and invariably causes cell death in the caudate, putamen resulting in severe dystonia in humans and animals [3]. 3-NP produces HD-like symptoms in animals. The 3-NP model is a reliable animal model for studying HD pathogenesis, because it mimics a downstream process of cell death similar to what is seen in the HD brain, namely mitochondrial impairment. Impaired glucose metabolism in the brain cells as a result of enzyme deficiencies causes decreased production of ATP. Several enzymes are involved in the tricarboxylic acid cycle (TCA) and the electron transport chains are downregulated in the brain of HD patients [7]. 3-NP treatment causes several behavioral changes, as examined in HD patients, possibly due to the loss of ATP. In the present study, we examined basic indices of animal health, including body weight, behavioral signs of intoxication, oxidative stress, antioxidant enzymes levels, and mitochondrial complexes. 3-NP inhibits the respiratory chain reaction and inhibits ATP generation, which is one of the

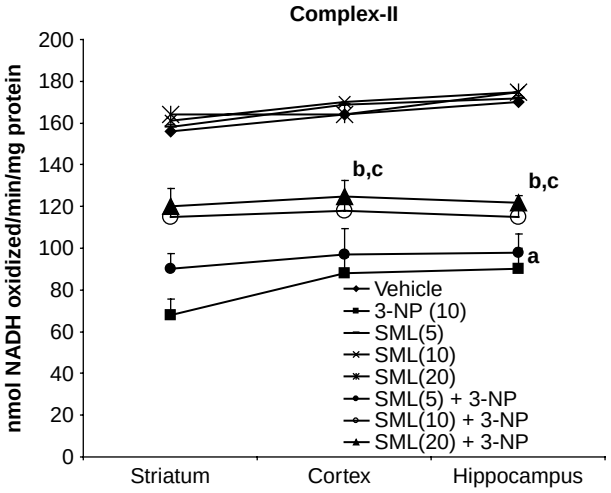

Figure 6. Effect of SML (5, 10, and 20 mg/kg) treatment in mitochondrial complex-II in the 3-NP-treated rats. Values are mean ± SEM. $^{a}P < 0.05$ versus naïve, $^{b}P < 0.05$ versus 3-NP, and $^{c}P < 0.05$ versus (3-NP + SML 5 mg/kg), treated group (one-way ANOVA followed by Tukey's test). SML, sesamol; 3-NP, 3-nitropropionic acid.

reasons for the loss in body weight in animals and humans. The chronic SML treatment for 15 days significantly improves body weight as well as motor coordination suggesting its therapeutic potential. The present findings are further supported by other investigators who reported similar effects of SML in other disease conditions [8,9]. The 3-NP model is capable of mimicking both the hyperkinetic and hypokinetic symptoms against acute and chronic treatments, respectively, in animals [2]. In the present study, chronic 3-NP treatments significantly caused hypoactivity in animals. Weak muscle activities are the major symptoms

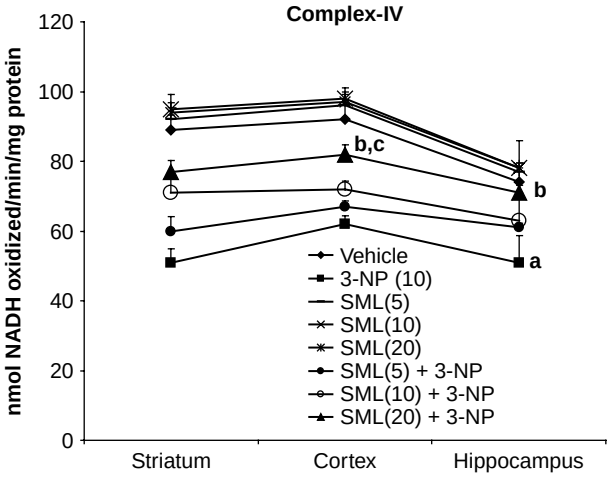

Figure 7. Effect of SML (5 and 10 kg) treatment in mitochondrial complex-IV in the 3-NP-treated rats. Values are mean ± SEM. $^{a}P < 0.05$ versus naïve, $^{b}P < 0.05$ versus 3-NP, and $^{c}P < 0.05$ versus (3-NP + SML 5 mg/kg), treated group (one-way ANOVA followed by Tukey's test). SML, sesamol; 3-NP, 3-nitropropionic acid.

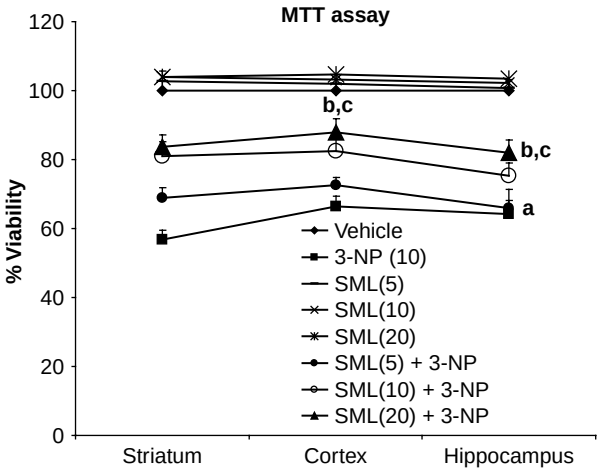

Figure 8. Effect of SML (5, 10, and 20 mg/kg) treatment on the MTT assay in the 3-NP-treated rats. Values are mean ± SEM. [a]$P < 0.05$ versus naïve, [b]$P < 0.05$ versus 3-NP, and [c]$P < 0.05$ versus (3-NP + SML 5 mg/kg), treated group (one-way ANOVA followed by Tukey's test). SML, sesamol; 3-NP, 3-nitropropionic acid.

of HD patient. 3-NP treatment also decreased motor incoordination and balance on the Rotarod apparatus which was significantly reversed by the higher doses of the SML.

Furthermore, 3-NP treatment significantly caused oxidative damage and alterations in mitochondrial enzyme complex (I–IV) activities. Further, 3-NP treatment significantly impaired energy metabolism by altering mitochondrial enzyme complex (II), increased oxidative damage possible by the formation of reactive oxygen and nitrogen species. 3-NP treatment has also been reported to increase various markers of oxidative stress, such as 3-nitrotyrosine, 8-hydroxy-2-deoxyguanosine, and MDA [10]. Moreover, 3-NP induces protein oxidation in synaptosomes from the striatum and cortex [10]. The 3-NP-induced striatal lesion could be attenuated by overexpressing Cu, Zn-SOD or Bcl-2 in mice [11]. Oxidative stress is considered to be one of the major determinants of 3-NP-induced neurotoxicity [2]. In the present study, 3-NP significantly caused oxidative damage as indicated by raised lipid peroxidation, nitrite concentration, and depletion of antioxidant enzyme levels (SOD and catalase) in the striatum, cortex, and hippocampal regions of rat brain. SML treatment significantly attenuated oxidative damage (as indicated by reduced lipid peroxidation, nitrite concentration, and restoration of SOD and catalase enzyme activity) and restoration of the mitochondrial enzyme activities in the striatum, cortex, and hippocampal regions of rat brain. It seems that antioxidant effect of SML could be involved in its protection against 3-NP-induced neurotoxicity. SML has been proven to be a strong antioxidant in a number of animal models [8,12]. Sesame lignans suppress lipid peroxidation of erythrocytes, sesamin and SML in preventing hypoxic or H_2O_2-stressed death of neuronal PC_{12} cells [13]. SML inhibits nitrite production and iNOS expression in the liver of septic rats [14]. SML significantly decreased nitrite levels because of its potential to inhibit expression of iNOS and NO scavenging effects [6]. Recently, it has been reported that SML attenuated oxidative stress by reducing xanthine oxidase [14]. SML pretreatment gives significant protection to cultured human lymphocytes against

γ-radiation-induced cellular damage, inhibits lipid peroxidation, hydroxyl radical-induced deoxyribose degradation, and DNA cleavage [15]. SML is reported to enhance hepatic detoxification, reduce occurrence of chemically induced tumor, and protect against oxidative stress [16].

Mitochondrial oxidative stress has been implicated as a common feature in the etiology of HD [17]. Disruption of mitochondrial enzyme activity has also been associated with the abnormal formation of ROS. Inhibition of enzymes in the electron transport chain can lead to an increase in electron leakage from the mitochondria and production of ROS like the superoxide radical ($O_2^{-\cdot}$), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($OH^{-\cdot}$). ROS cause damage to cellular membranes and genetic material as demonstrated by an increase in the DNA damage marker 8-hydroxydeoxyguanosine [18]. Supporting to several research evidences, 3-NP treatment in the present study causes significant mitochondrial damage by inhibiting SDH, an enzyme involved in the TCA and the electron transport chain [18]. Biochemical studies have also demonstrated multiple defects in the caudate and decreased complex II and III activities of HD patients [20]. In the present study, 3-NP significantly inhibits mitochondrial enzyme complex activities in striatum, cortex, and hippocampal regions. 3-NP specifically inhibits complex-II in striatum as compared with other complexes. SML treatments significantly restored the enzyme activities in the mitochondria, suggesting that SML could influence the respiratory chain or electron transport chain in the cells. Besides, antioxidant mechanism of SML could be involved in reversing the mitochondrial enzyme status in different regions of the brain.

In summary, the present study suggests antioxidant potentials of SML against 3-NP-induced neurotoxicity. However, more investigations are required to elucidate the cellular mechanisms in the protective effect of SML.

4. Experimental

4.1 Animals and treatment schedule

Male Wistar rats bred in the Central Animal House Facility of the Panjab University, Chandigarh and weighing between 250 and 300 g were used. The protocol was approved by the Institutional Animal Ethics Committee and was carried out in accordance with the Indian National Science Academy Guidelines for the use and care of animals.

Animals were randomly divided into eight groups with 10 animals in each group. Group-1 vehicle-treated group received vehicle for SML (p.o.) and also normal saline [intraperitoneally (i.p.)]; group-2 received 3-NP (10 mg/kg, i.p.) for 14 days; groups 3–5 received sesame oil (5, 10, and 20 mg/kg) *per se* and groups 6–8 received SML (5, 10, and 20 mg/kg) and 3-NP (10 mg/kg, i.p.) for 14 days. In groups 6–8, sesame oil was given 1 h prior to 3-NP administration. 3-NP (Sigma Chemicals, St Louis, MO, USA) was diluted with saline (adjust pH 7.4) and administered i.p. to the animals. SML (Sigma Chemicals, 99% purity) was suspended in 0.5% sodium carboxymethyl cellulose solution and administered by p.o. route in a constant volume of 0.5 ml per 100 g of the body weight.

4.2 Measurement of the body weight

The body weight of animals was recorded on the first and last day of the experimentation. The percentage change in body weight was calculated as

$$\frac{\text{Body weight (1st day} - \text{15th day)}}{\text{1st day body weight}} \times 100.$$

4.3 Behavioral parameters

4.3.1 Assessment of gross behavioral activity (locomotor activity)

The locomotor activity was monitored using actophotometer (IMCORP, Ambala, India). The horizontal motor activity was detected by two perpendicular arrays of 15 infrared beams located 2.5 cm above the floor of the testing area. Each interruption of a beam on the *x*- or *y*-axis generated an electric impulse, which was presented on a digital counter. Each animal was observed over a period of 5 min and the values were expressed as counts per 5 min [19].

4.3.2 Motor activity observation

All animals were evaluated for motor ability and grip strength using the rotarod. Each rat was given a prior training session before initialization of therapy to acclimate them to Rotarod apparatus (Techno, Lucknow, India). Rats were placed on the rotating rod with a diameter of 7 cm (speed 25 rpm). The length of the time on the rod was taken as the measure of competency. Three separate trials were given to each rat at 5-min intervals and 180 was the cut-off time. The average results were recorded as fall of time [20].

4.4 Dissection

On day 15, after behavioral assessments, the animals were randomized into two groups; one group was used for the biochemical assays and the other group was used for the assessment of mitochondrial dysfunction. For the biochemical analysis, animals were killed by decapitation immediately after behavioral assessment. The brains were removed, forebrain was dissected out, and cerebellum was discarded. Brains were put on ice and the striatum, cortex, and hippocampus were separated and weighed. A 10% (w/v) tissue homogenate was prepared in 0.1 M phosphate buffer (pH 7.4). The homogenate was centrifuged at 10,000*g* for 15 min and aliquots of the supernatant were separated and used for biochemical estimations.

4.5 Measurement of lipid peroxidation

The quantitative measurement of lipid peroxidation in the brain was performed according to the method of Wills [21].

4.6 Estimation of nitrite levels

The accumulation of nitrite in the supernatant, an indicator of the production of NO, was determined with a colorimetric assay with Greiss reagent (0.1% *N*-(1-naphthyl)ethylenediamine dihydrochloride, 1% sulfanilamide, and 2.5% phosphoric acid) as described by Green and Tannebaum [22].

4.7 Catalase estimation

Catalase activity was assayed by the method of Luck [23], wherein the breakdown of hydrogen peroxides (H_2O_2) is measured at 240 nm.

4.8 SOD activity

SOD activity was assayed according to the method of Kono [24]. The assay system consisted of 0.1 mM EDTA, 50 mM sodium carbonate, and 96 mM nitroblue tetrazolium. In the cuvette, 2 ml of the above mixture was taken and to it 0.05 ml of post-mitochondrial supernatant and 0.05 ml of hydroxylamine hydrochloride (adjusted to pH 6.0 with NaOH) were added. The auto-oxidation of hydroxylamine was observed by measuring the change in optical density at 560 nm for 2 min at 30/60-s intervals.

4.9 Protein estimation

Protein estimation was done by biuret method using bovine serum albumin as a standard [25].

4.10 Isolation of rat brain mitochondria and mitochondrial enzyme complex estimation

Rat brain mitochondria were isolated by the method of Berman and Hastings [26]. Complex-I activity was measured spectrophotometrically by the method of King and Howard [27]. Complex-II activity was measured spectrophotometrically according to King [28]. Cytochrome oxidase activity was assayed in the brain mitochondria according to the method of Sottocasa *et al.* [29].

4.10.1 MTT ability

The MTT assay is based on the reduction MTT by hydrogenase activity in functionally intact mitochondria. The MTT reduction rate was used to assess the activity of the mitochondrial respiratory chain in isolated mitochondria by the method of Liu *et al.* [30].

4.11 Statistical test

One specific group of rats was assigned to one specific drug treatment condition and each group comprised 10 rats ($n = 10$). All the values were expressed as mean ± SEM. The data were analyzed using one-way ANOVA followed by Tukey's test. In all tests, the criterion for statistical significance was $P < 0.05$.

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