Portulaca oleracea Mitigates 3-Nitropropionic Acid-induced Motor Impairment, Mitochondrial Dysfunction and Free Radical Generation in Male Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

The mitochondrial toxin 3-nitropropionic acid (3-NP) effectively induces behavioral changes and selective striatal lesions in rats mimicking clinical Huntington's disease (HD). In the present study, behavioral tests and biochemical analysis were performed to explore the role of ethanolic extract of

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Portulaca oleracea (POEE) in the experimental animals of HD. Male wistar rats received an intraperitoneal injection of 3-NP (10 mg/kg) for 14 days. Simultaneously in treatment groups, the animals received POEE at a dose of 2 and 4 mg/kg along with 3-NP injection for a period of 14 days. The motor behavioral assessments were performed on days 5, 10 and 15. After the behavioral test on 15th day, the biochemical and histopathological parameters were performed. Administration of 3-NP resulted in body weight change, motor and memory impairments. 3-NP administration also causes marked oxidative stress by showing significantly increased levels of lipid peroxidation (LPO), nitric oxide (NO) and reduced activities of endogenous antioxidants. Further, it damages the mitochondrial complex enzyme succinate dehydrogenase (SDH) and elevated the acetylcholine esterase (AChE) activity. Treatment with POEE at 2 and 4 mg/kg bw doses significantly restored the body weight changes and behavioral impairment in 3-NP induced rats. Further, POEE significantly decreased the oxidative damages, restored the antioxidant enzymes activities and mitochondrial dysfunction. Histopathological sections of the striatal region showed the extent of neuronal loss in 3-NP induced rats and was restored upon POEE treatment. From the results, we suggest that POEE might be effective in clinical HD by virtue of its antioxidant and neuroprotective properties.

1. INTRODUCTION

"Huntington's disease (HD) is a progressive neurodegenerative disorder characterized by degeneration of the striatal neurons, which leads to behavioral, motor and cognitive impairments" [1]. "The neurotoxicity induced by 3-nitropropionic acid (3-NP) is a well-known experimental model for the study of the pathogenesis of HD. The mitochondrial toxin 3-NP is a naturally occurring plant and mycotoxin that produces HD-like symptoms in both animals and humans" [2].

"3-NP induced HD model replicates most of the clinical and pathophysiological hallmarks of HD, including spontaneous choreiform and dystonic movements, frontal-type cognitive deficits, and progressive striatal neuronal degeneration. 3-NP administration also results in ATP depletion, which impairs intracellular calcium buffering thereby leading to production of damaging reactive oxygen species (ROS)" [3].

"The primary mechanism of 3-NP-induced neurotoxicity involves the inhibition of complex-II [succinate dehydrogenase (SDH)] enzyme in the mitochondrial electron transport chain (ETC). SDH is an important enzyme located in the inner domain of the mitochondrial membrane, which catalyzes the oxidation of succinate to fumarate. 3-NP irreversibly blocks the SDH enzyme, decreases ATP levels and accelerates neuronal apoptosis" [4].

Currently, no efficacious therapies are available to cure HD pathogenesis. Existing HD medical care targets only the neuro-psychiatric symptoms to enhance the living standard [5]. Thus, the study was framed to develop a therapeutic strategy to treat HD pathogenesis.

Plants that contain flavonoids have received a lot of focus in the scientific community recently as they have been reported to have antioxidant properties against free radicals to treat pathogenesis. *Portulaca oleracea* L. (Portulacaceae) commonly called purslane which is an extensively utilized plant renowned for its high content of α-linolenic acid, β-carotene, glutathione (GSH) and omega-3 fatty acids [6,7]. Numerous pharmacological actions of Portulaca oleracea (PO) have been documented, including wound-healing, analgesic, antibacterial, and anti-inflammatory properties [8,9]. The study from Chen et al. [10] demonstrated the anti-hypoxic property of ethanolic extract of *Portulaca oleracea* (POEE) via increasing the erythropoietin level in the hypoxic mice neural system. Recent research from Chetehouna et al. [11] showed "a beneficial effect of purslane with zinc against Alzhemier's disease (AD) in experimental animals". "Further, the polysaccharides extracted from purslane were shown to improve lead-induced learning and memory behavior in rodents by protecting the hippocampal CA1 and dentate gyrus area of the brain" [12,13-16]. "The anti-parkinsonism effect was exhibited by purslane against rotenone toxin via halting apoptosis by increasing striatal B-cell lymphoma-2 (Bcl-2) and lowering caspase-3 activity. The study also highlighted the anti-inflammatory effect of purslane via inhibiting

inducible nitric oxide synthase (iNOS) and NF-κB expression and oxidative stress through attenuating ROS, nitrite/nitrate, and lactate dehydrogenase, as well as thiobarbituric acid reactive substances in the rat striatum" [17]. "Oleracein E, a tetrahydroisoquinoline present in purslane markedly reduced the Bcl-2-associated X (Bax) amount, caspase-3 activation, and cytochrome C release, ROS, and ERK1/2 phosphorylation in an in-vitro and *In-vivo* models" [18].

Considering all the available evidence, the current study was formulated to assess the neuropharmacological potential of POEE against 3-NP induced neurotoxicity in male albino rats.

2. MATERIALS AND METHODS

2.1 Preparation of Ethanolic Extract of *Portulaca Oleracea* (POEE)

Portulaca oleracea plant was procured from the market at chennai, India and authenticated by Dr. A. Sasikala, Captain Srinivasa murthy Central Ayurveda Research Institute, Chennai, India. The dried aerial part of the plant was roughly ground into powder and then extracted using 90% ethanol in a Soxhlet apparatus. The solvent was subsequently eliminated via evaporation utilizing a Bucchi Rotary Evaporator [19]. The resulting extract yield was calculated to be 49% w/w of the dried plant material.

2.2 Animals and Treatment Schedule

Male Albino rats weighing 250-300 g were obtained from Central Animal House, Dr. ALM Post Graduate Institute for Basic Medical Sciences, University of Madras, Taramani Campus, Chennai, Tamil Nadu, India.

For the current study, a quasi-experimental approach was used, in which animals were not divided into groups at random, but rather based on things they already had in common, such as age and gender. The adult male rats were divided into 5 groups with 6 animals each. Group I - Control rats were given with normal saline intraperitoneally. Group II - Animals received 3-NP (10 mg/kg bw) intraperitoneally for 14 days. Group III - Rats were treated with POEE (2 mg/kg bw) orally 1 h prior to 3-NP i.p. injection for 14 days. Group IV - Rats were treated with POEE (4 mg/kg b.w) orally 1 h prior to 3-NP i.p. injection for 14 days. Group V - Rats were orally treated with POEE (4 mg/kg b.w) alone for 14 days.

2.3 Animal Body Weight Measurement

The body weight of each animal was recorded on the first and last days of the experimental period. The body weight difference of each animal was analyzed using the formula.

[Body weight (15th day -1st day) ×100]/1st day body weight

2.4 Behavioral Observations

All behavioral assessments were conducted in a serene environment at room temperature, devoid of external disturbances. All experiments were carried out within the timeframe of 10:00 am to 6:00 pm.

2.4.1 Rotarod activity

Before the rotarod performance, the rats were subjected to a previous training session. Rats were positioned at a 25 rpm rotational rod speed. The rats were given 180 s to complete the rotarod task, and three trials totaling 5 min were recorded for each rat. The length of time the animal spent on average within the revolving rod was noted [20].

2.4.2 Morris Water Maze test (MWM)

Morris water mazes were implemented to investigate the acquisition and retention of spatial navigation tasks [21]. For the maze acquisition test, the animals were trained to swim in a circular pool with a depth of 40 cm, and a platform was affixed to the center of one of the four quadrants. Starting with the initial day of 3-NP administration, the animals were subjected to four trials over the course of four days. The beginning position was distinct in each trial. The animal's time to locate the platform was recorded for two minutes. The initial procurement latency was recorded as the time it took to reach the platform on the fifth day. The platform was removed on the 15th day, and the animal was randomly released by facing the pool wall. The retention of memory was then tested. The time spent in the target quadrant was also measured on the 15th day.

2.4.3 Open Field test (OFT)

A wooden circle with a diameter of 90 cm served as the open field test apparatus. "The sole source of illumination was a 60W light bulb that was positioned 90-100 cm above the center.

Each animal was dropped in center of the open field, the number of squares crossed, and rearing were measured through direct visual observations for 5 min" [22].

2.5 Tissue Sample Preparation

The animals were sacrificed on the 15th day, and the brain striatum was removed via dissection, followed by a 10% (w/v) tissue homogenate preparation with 0.1M phosphate buffer (pH 7.4). The homogenate was centrifuged at 10,000 × g for 15 minutes. The supernatant was collected and utilized for biochemical analysis.

2.5.1 Biochemical parameters

A technique outlined by Utley et al. [23] was used to determine the level of lipid peroxidation. The Green et al. [24] technique was used to calculate the nitric oxide level. Superoxide dismutase activity was analyzed using the Marklund and Marklund method [25]. The catalase enzyme activity was assayed by the method described by Aebi [26]. Glutathione content was evaluated using the method of Ellman [27]. One indicator of poor mitochondrial metabolism in the brain is succinate dehydrogenase. Kumar et al. [28] approach was followed for the quantitative determination of brain succinate dehydrogenase level. The activity of acetylcholinesterase activity was assessed using the method of Ellmann [29]. The Lowry et al. [30] method was used to estimate the protein content.

2.6 Histopathological Studies

On day 15, animals were sacrificed via cervical decapitation, and the brain was promptly treated with 10% formalin saline for 24 hours before being dehydrated with solvent. The specimens were paraffin waxed and sectioned at 4 μm thickness using a slide microtome. Tissue slices were mounted on glass slides, deparaffinized, stained with hematoxylin and eosin stains, and examined under a light microscope [31].

2.7 Statistical Analysis

Data were analyzed by using analysis of variance (ANOVA) followed by Tukey's Post-hoc test. All the values are expressed as mean ± SD. The $P < 0.05$ was considered statistically significant.

3. RESULTS

3.1 Effect of POEE on 3-NP Induced Changes in Body Weight Mass in Control and Experimental Animals

On day 15, systemic 3-NP (10 mg/kg) injection led to a substantial ($P<0.01$) drop in body weight compared to control animals. POEE (2 mg/kg) and POEE (4 mg/kg) treatments significantly reduced body weight loss ($P<0.05$) compared to the 3-NP-induced group. No significant change was seen in the POEE alone treated group (Fig. 1).

3.2 Effect of POEE on 3-NP Induced Alteration in the Rotarod Activity of Control and Experimental Animals

Systemic 3-NP induction substantially ($P<0.01$) reduced grip strength performance in rotarod test on 10th and 15th days compared to control group, while no significant change in muscle coordination was found on day 5. POEE at 2 mg/kg ($P<0.05$) and 4 mg/kg ($P<0.01$) substantially increased muscular strength (delayed fall of time) compared to the 3-NP induced group on the 10th and 15th day. However, animals administered with POEE alone showed no significant influence on grip strength performance (Fig. 2).

3.3 Effect of POEE on 3-NP Induced Alterations in Morris Water Maze Task in Control and Experimental Animals

In the 3-NP-treated group, the average escape latency of trained rats gradually decreased from day 1 to day 5. However, rats induced with 3-NP revealed ($P<0.01$) delayed mean latency to reach platform on the 10th and 15th day compared to the control group. POEE therapy substantially improved memory performance on the 10th and 15th day ($P<0.05$) in POEE (2 mg/kg) and ($P<0.01$) in POEE (4 mg/kg) compared to the 3-NP group (Fig. 3a). POEE (2 mg/kg and 4 mg/kg) therapy substantially enhanced target quadrant time ($P<0.05$ and $P<0.01$, respectively) on the 15th day compared to the 3-NP induced group. There was no change in the POEE alone treated group (Fig. 3b).

3.4 Effect of POEE on 3-NP Induced Changes in Open Field Test in Control and Experimental Animals

On day 15, rats induced with 3-NP had significantly lower ($P<0.01$) locomotory activity

(Fig. 4a) and exploratory activity (Fig. 4b) compared to the control group. POEE therapy reversed locomotor deficit, increasing ambulation counts and rearing behavior considerably ($P<0.05$) in POEE (2 mg/kg) and ($P<0.01$) in POEE (4 mg/kg), compared to the 3-NP induced group. The POEE-treated mice showed no experimental alterations.
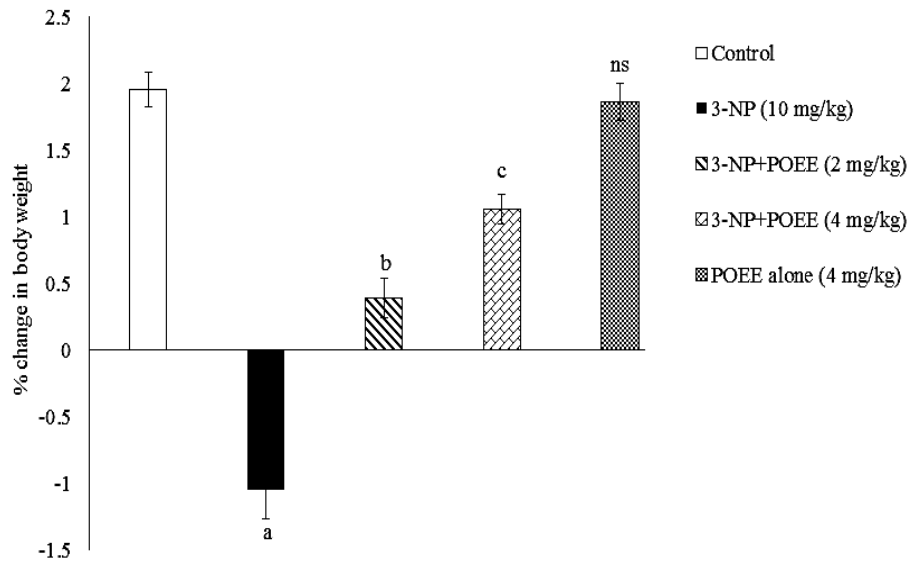

Fig. 1. Effect of POEE on 3-NP induced changes in the body weight mass of control and experimental animals

Data represents mean ± SD (n=6). Unit was expressed as % of change in body weight. aP<0.01 (p value = 0.00021) compared to vehicle-treated control group; bP<0.05 (p value = 0.03756) and cP<0.01 (p value = 0.00077) compared to 3-NP induced group; ns-non significant (p value = 0.11786) compared to control group by one way ANOVA with Tukey's post-hoc test.

Fig. 2. Effect of POEE on 3-NP induced alteration in rotarod activity in control and experimental animals

Data represents mean ± SD (n=6). ᵃP<0.01 (p value = 0.00016) compared to vehicle-treated control group; ᵇP<0.05 (p value = 0.02495) and ᶜP<0.01 (p value = 0.00051) compared to 3-NP induced group; ns-non significant (p value = 0.09215) compared to control group by one way ANOVA with Tukey's post-hoc test.
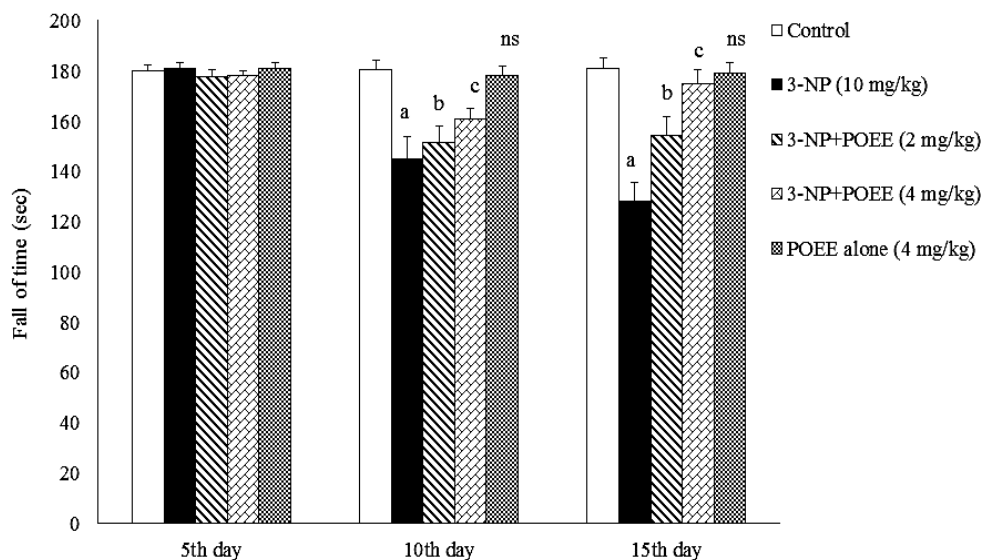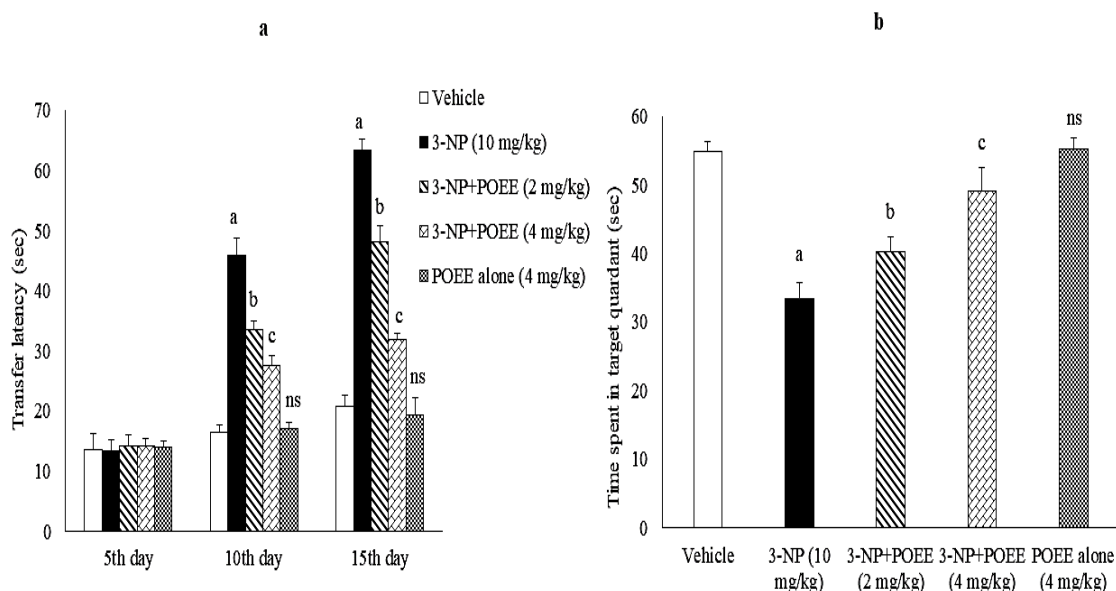
Fig. 3. Effect of POEE on 3-NP induced changes in acquisition and retention memory in control and experimental animals

Data represents mean ± SD (n=6). [a]P<0.01 (p value = 0.00037) compared to vehicle-treated control group; [b]P<0.05 (p value = 0.03398) and [c]P<0.01 (p value = 0.00028) compared to 3-NP induced group; ns-non significant (p value = 0.10842) compared to control group by one way ANOVA with Tukey's post-hoc test. a-Acquisition memory; b-Retention memory.
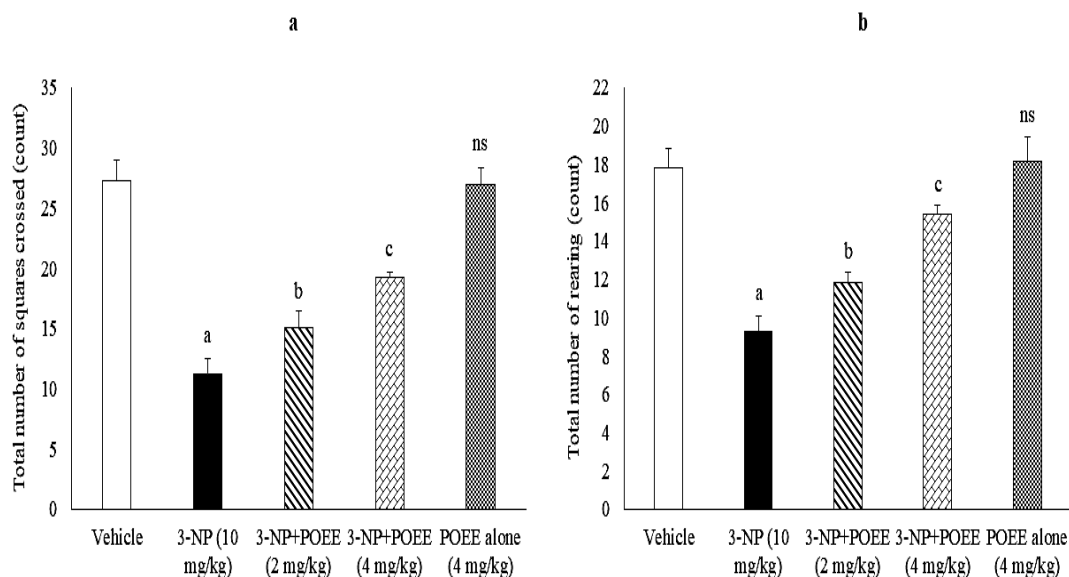
Fig. 4. Effect of POEE on 3-NP induced changes in open field test in control and experimental animals

Data represents mean ± SD (n=6). [a]P<0.01 (p value = 0.00093) compared to vehicle-treated control group; [b]P<0.05 (p value = 0.02719) and [c]P<0.01 (p value = 0.00041) compared to 3-NP induced group; ns-non significant (p value = 0.07329) compared to control group by one way ANOVA with Tukey's post-hoc test. a-Locomotor activity; b-Exploratory activity.

3.5 Effect of POEE on 3-NP Induced Alteration in the Levels of LPO and NO in Control and Experimental Animals

3-NP-induced rats had considerably higher levels of LPO and NO compared to control animals ($P<0.01$). POEE (2 mg/kg) and POEE (4 mg/kg) significantly reduced LPO and NO levels ($P<0.05$ and $P<0.01$, respectively). Furthermore, no significant differences were seen in rats treated with POEE alone (Table 1).

3.6 Effect of POEE on 3-NP Induced Abnormalities in the Activities of Antioxidants Status in Control and Experimental Animals

Intraperitoneal injection of 3-NP decreases SOD, CAT, and GSH activities significantly ($P<0.01$) compared to control rats. POEE (2 mg/kg) and POEE (4 mg/kg) significantly restored SOD, CAT, and GSH activities to near-normal levels ($P<0.05$ and $P<0.01$, respectively), compared to the 3-NP administered group. There were no significant differences seen in rats treated with POEE alone (Table 2).

3.7 Effect of POEE on 3-NP Induced Changes in the Activities of SDH and AChE Enzymes in Control and Experimental Animals

Chronic 3-NP induction substantially ($P<0.01$) decreases SDH enzyme activity in comparison to control animals. Further, 3-NP administration significantly ($P<0.01$) elevated the activity of AChE compared to control animals. POEE (2 mg/kg) and (4 mg/kg) significantly recovered SDH and AChE activities compared to 3-NP-induced rats ($P<0.05$ and $P<0.01$, respectively). There was no significant difference in the POEE alone treated group (Table 3).

3.8 Effect of POEE on 3-NP Induced Histological Changes in Rat Striatum

Histological changes in the striatum of control and experimental groups of animals were depicted in Fig. 5. The control group exhibited the normal histology of striatum (Fig. 5a). Sections of the 3-NP induced group exhibited irregularly damaged granular cells displayed shrunken and pyknotic nuclei (Fig. 5b). Animals treated with POEE (2 mg/kg) exhibited a moderate number of damaged granular cells with pyknotic nuclei and few normal cells (Fig. 5c). Animals treated with POEE (4 mg/kg) exhibited an increased number of normal granular cells with few damaged cells (Fig. 5d). Drug alone administered rats resembling that of control histoarchitecture (Fig. 5e).

Sections were visualized under light microscope at a magnification of 400x. (a) Control section showing normal striatal histology. (b) 3-NP induced section showing increased number of irregularly damaged cells with pyknotic nuclei (denoted by arrow). (c) POEE (2 mg/kg) treated group showing a presence of moderate number of degenerated cells with pyknotic nuclei and few normal neurons (denoted by arrow). (d) POEE (4 mg/kg) treated group showing increased number of normal granular cells and few damaged cells (denoted by arrow). (e) POEE alone administered rats resembling that of control histo-architecture.

Table 1. Effect of POEE on 3-NP induced changes in the level of oxidative stress markers in the striatum of control and experimental animals

Parameters	LPO		NO	
	(nmol of MDA released/mg protein)	p value	(nmol/mg protein)	p value
Control	2.18 ± 1.09	-	0.85 ± 0.11	-
3-NP (10 mg/kg)	5.68 ± 1.19	0.00063	3.19 ± 0.17	0.00021
3-NP+POEE (2 mg/kg)	4.02 ± 2.21	0.03567	2.42 ± 0.19	0.04162
3-NP+POEE (4 mg/kg)	2.65 ± 1.31	0.00076	1.76 ± 0.11	0.00045
POEE alone (4 mg/kg)	2.08 ± 2.12	0.10456	0.98 ± 0.10	0.08646

Data represents mean ± SD (n=6). 3-NP induced group compared to vehicle-treated control group; POEE treated groups compared to 3-NP induced group by one way ANOVA with Tukey's post-hoc test. LPO- Lipid Peroxidation; NO – Nitric Oxide

Table 2. Effect of POEE on 3-NP induced alteration in the activities/levels of antioxidants in the striatum of control and experimental animals

Parameters	SOD		CAT		GSH	
	(Units/mg protein)	p value	(nmol of H_2O_2 consumed /min/mg protein)	p value	(µmol of glutathione/ min/mg protein)	p value
Control	12.14 ± 0.71	-	3.19 ± 0.79	-	48.38 ± 1.93	-
3-NP (10 mg/kg)	5.41 ± 1.01	0.00046	1.38 ± 0.62	0.00038	29.33 ± 2.42	0.00015
3-NP+POEE (2 mg/kg)	6.97 ± 0.65	0.04025	2.01 ± 0.97	0.02547	35.26 ± 1.51	0.03459
3-NP+POEE (4 mg/kg)	9.84 ± 0.45	0.00101	2.65 ± 0.84	0.00056	42.65 ± 1.75	0.00029
POEE alone (4 mg/kg)	11.65 ± 0.86	0.06238	3.32 ± 0.77	0.09274	47.71 ± 1.32	0.17162

Data represents mean ± SD (n=6). 3-NP induced group compared to vehicle-treated control group; POEE treated groups compared to 3-NP induced group by one way ANOVA with Tukey's post-hoc test. SOD- Superoxide Dismutase; CAT- Catalase; GSH- Reduced Glutathione.

Table 3. Effect of POEE on 3-NP induced abnormalities in the activity of succinate dehydrogenase in the striatum of control and experimental animals

Parameters	SDH		AChE	
	(nmol of succinate oxidized/min/mg protein)	p value	(µmol/L/min/mg protein)	p value
Control	63.27 ± 2.53	-	2.31 ± 0.14	-
3-NP (10 mg/kg)	36.85 ± 3.19	0.00018	5.78 ± 0.47	0.00033
3-NP+POEE (2 mg/kg)	45.22 ± 1.61	0.02756	4.22 ± 0.34	0.02415
3-NP+POEE (4 mg/kg)	52.46 ± 2.84	0.00038	3.09 ± 0.71	0.00064
POEE alone (4 mg/kg)	61.28 ± 3.19	0.12186	2.18 ± 0.26	0.07217

Data represents mean ± SD (n=6). 3-NP induced group compared to vehicle-treated control group; POEE treated groups compared to 3-NP induced group by one way ANOVA with Tukey's post-hoc test. SDH- Succinate dehydrogenase; AChE- Acetylcholine esterase.
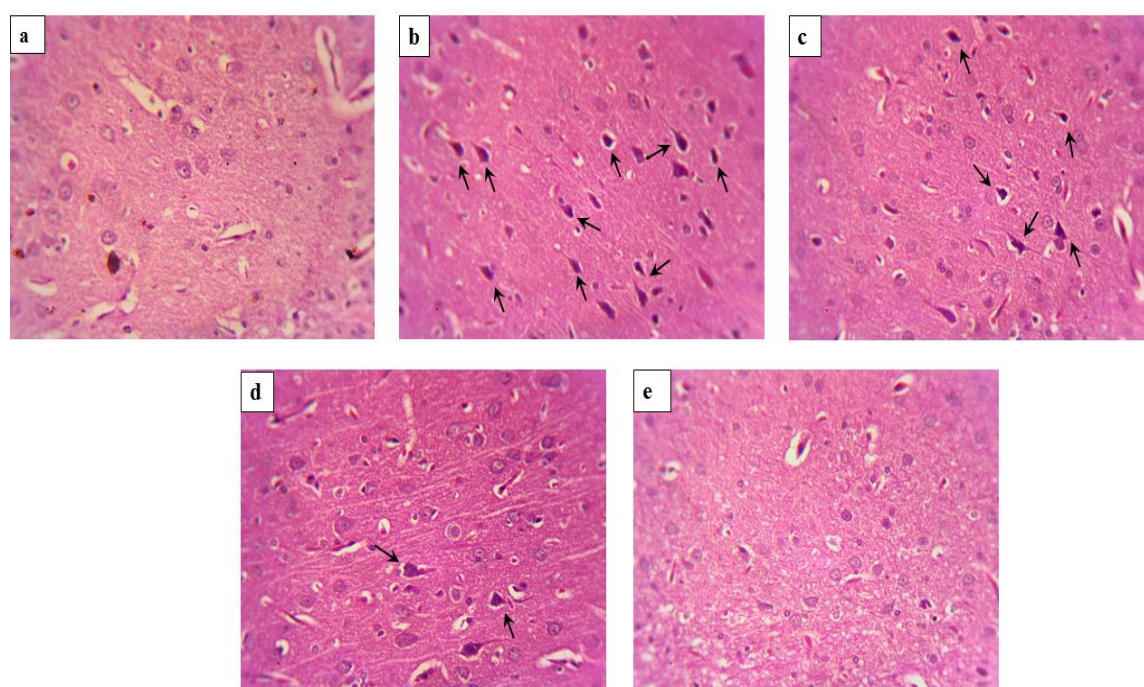

Fig. 5. Effect of POEE on 3-NP induced histological changes in control and experimental animals

4. DISCUSSION

Among HD toxin models, 3-NP is noteworthy because it may be utilized to simulate the striatal lesions and motor deficits that are indicative of the pathophysiology of HD [32]. The systemic intraperitoneal infusion of 3-NP (10 mg/kg) for 14 days in this experiment causes weight loss in the rats. This might be attributed to several factors, including ATP depletion, free radical production, metabolic impairment, and striatal lesions that directly reduce the rat appetite and food intake [33]. Our results are in good agreement with the Mahdy et al. research [34]. Furthermore, the body weight of the animal was greatly increased with POEE administration. The body weight gain

of the animal might be achieved by limiting the free radical generation and neuroinflammation are well aligned with the antioxidant status of the present investigation.

The dorsal striatum's medium spiny and cholinergic neurons are extremely susceptible to 3-NP neurotoxic, which impairs motor function in the test animals [35]. In support of this, 3-NP induction showed a markedly elevated mean fall-off time in the rotarod test, illustrating the stiffness and movement issue. In the Morris Water Maze, additional 3-NP induction decreased the amount of time spent in the target quadrant and postponed the transfer latency. Our findings from the OFT of rats given 3-NP

revealed reduced exploratory feeling and hypolocomotion. POEE treatment dramatically reduced the motor and memory impairments in mice inebriated with 3-NP, indicating that it has antidepressant, anti-anxiety, and muscle-relaxing qualities [36,37]. Further, POEE stimulates the antioxidant and anticholinesterase effects to boost motor and memory behavior in the rodents.

The primary cause of HD pathogenesis is oxidative stress, which is brought on by the Htt mutation [5]. 3-NP induction results in oxidative stress via a secondary excitotoxic cell death pathway [38]. The current investigation demonstrated that systemic 3-NP injection decreased the levels of endogenous antioxidant enzyme activities and elevated LPO and NO levels, indicating the formation of ROS and RNS as well as oxidative damage to the striatum. It has been observed that lipid peroxidation plays a significant role in the loss of cell function in neurodegenerative illnesses caused by oxidative stress [39]. In 3-NP-induced rats, co-treatment with POEE dramatically reduced LPO and NO levels; this may be because of POEE antioxidant properties [40].

Antioxidant enzymes such as catalase and superoxide dismutase often serve as the first line of defense against the production of reactive oxygen species (ROS). GSH, a non-enzymatic antioxidant, prevents the production of hydroxyl radicals and supports the health of brain cells. Reduced GSH levels have been connected to both neurodegenerative illnesses and typical aging. An earlier study found that the antioxidant enzymes were significantly reduced after 3-NP therapy, indicating a potential role for oxidative stress in the neurodegenerative process [41]. The current investigation revealed a decrease in antioxidant status in the 3-NP-induced group, which may have been caused by an increased use of these antioxidants to combat oxidative stress. Treatment with POEE, however, reversed the alterations, indicating that it had an antioxidant-like action [40].

Acetylcholinesterase (AChE), a cholinergic enzyme, cleaves the neurotransmitter acetylcholine to disrupt cholinergic neurotransmission [42]. A disrupted cholinergic neurotransmission in basal ganglia might negatively impact other neuronal populations, subsequently resulting in behavioral alterations [43]. Similar impairment of cholinergic neurotransmission following induction of 3-NP was observed in the present study. Treatment

with POEE inhibited the enhanced activity of AChE, which accelerated the turnover of acetylcholine at the synaptic junction and permitted the signal transmission. This data is consistent with the behavioral analysis of the current investigation, which demonstrated enhanced memory and learning behavior after POEE therapy.

According to several investigations, 3-NP can affect the function of the mitochondrial enzyme complex by blocking the SDH of the TCA cycle and ETC [44]. As a result, a decreased SDH activity in the 3-NP induced state was noted in the current investigation. The treatment with POEE considerably reduced the toxicity of 3-NP and the inhibition of mitochondrial complex-II.

The light microscopy characteristics of 3-NP-induced rat showed irregularly damaged cells that were condensed and vacuolized in the striatum, mimicking the histological changes that are comparable to those seen in clinical HD cases [45]. The observed histological alterations may be ascribed to heightened oxidative stress induced by 3-NP. Because of its characteristics, POEE therapy lessened such histopathological changes in 3-NP-induced rats. Our findings corroborate a previous work by Hussein et al. [46], which found that purslane reduced the histopathological damage to the brain in rodents via regulating the pathway involving proinflammatory cytokine TNF-α and nuclear factor kappa B (NF-κB).

5. CONCLUSION

The results of this study conclude that the administration of 3-NP triggers free radical generation followed by the mitochondrial impairment and neuronal death of the striatum. Depletion of striatal neurons altered the behavioral patterns in experimental animals. The POEE treatment considerably corrected the motor deficits and mitochondrial impairment via rejuvenating the antioxidant status to promote neuronal survival. Therefore, with the present data we could conclude that the Portulaca oleracea may be advantageous and might develop an adjuvant treatment for the management of Huntington's disease.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image

generators have been used during writing or editing of manuscripts.

ETHICAL APPROVAL

All experiments and protocols described in the present study were approved by the Institutional Animal Ethics Committee (IEAC) (IEAC No: 02/05/2018) of Dr. ALM Post Graduate Institute for Basic Medical Sciences, University of Madras, Taramani Campus, Chennai.

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COMPETING INTERESTS

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

1. Tong H, Yang T, Xu S, Li X, Liu L, Zhou G, Yang S, Yin S, Li XJ, Li S. Huntington's disease: complex pathogenesis and therapeutic strategies. Int J Mol Sci. 2024; 25(7):3845.
2. Manish S, Sunil K, Nidhi S. Animal models for Huntington's disease: A review. International research journal of pharmacy. 2012;3:20-26.
3. Yan S, Li XJ, Li S. Huntington's disease: From large animal models to HD gene therapy. In: Huntington's Disease, Academic Press. 2024;455-472.
4. Aguilar K, Jakubek P, Zorzano A, Wieckowski MR. Primary mitochondrial diseases: The intertwined pathophysiology of bioenergetic dysregulation, oxidative stress and neuroinflammation. European Journal of Clinical Investigation. 2024;54 (7):e14217.
5. Labbadia J, Morimoto RI. Huntington's disease: underlying molecular mechanisms and emerging concepts. Trends Biochem Sci. 2013;38:378-385.
6. Kumar A, Sreedharan S, Kashyap AK, Singh P, Ramchiary N. A review on bioactive phytochemicals and ethnopharmacological potential of purslane (*Portulaca oleracea* L.). Heliyon. 2021;8: e08669.
7. Melilli MG, Pagliaro A, Scandurra S, Gentile C, Di Stefano V. Omega-3 rich foods: Durum wheat spaghetti fortified with *Portulaca oleracea*. Food Biosci. 2020;37: 100730.
8. Ghorani V, Saadat S, Khazdair MR, Gholamnezhad Z, El-Seedi H, Boskabady MH. Phytochemical characteristics and anti-inflammatory, Immunoregulatory, and antioxidant effects of *Portulaca oleracea* L.: a comprehensive review. Evidence-Based Complementary and Alternative Medicine. 2023;2023(1): 2075444.
9. Fatima N, Syed S, Ahmad M, Jabeen M, Jahan N. A comparison of analgesic and anti-inflammatory activities of *Portulaca oleraceae* leaf and seeds. RADS Journal of Pharmacy and Pharmaceutical Sciences. 2018;6(3):194-9.
10. Chen CJ, Wang WY, Wang XL, Dong LW, Yue YT, Xin HL, et al. Anti-hypoxic activity of the ethanol extract from *Portulaca oleracea* in mice. J Ethnopharmacol. 2009; 124:246–250.
11. Chetehouna S, Derouiche S, Atoussi O. Identification of new alkaloids from Algerian Purslane by HPLC-QTOF-MS and beneficial effect of Purslane enriched with Zinc on experimental Alzheimer disease in rats. Current Trends in Biotechnology and Pharmacy. 2024;18(1):1595-607.
12. Tao H, Ye DL, Wu YL, Han MM, Xue JS, Liu ZH, Chen XT, Wang HL. The protective effect of polysaccharide extracted from Portulaca oleracea L. against Pb-induced learning and memory impairments in rats. Int J Biol Macromol. 2018;119:617-623.
13. Uno UU, PB. Ekpo HO. Ogbe CM. Okolo, UB. Ekaluo. Effect of Soursop (*Annona Muricata* L.) Leaf Extract on Oxidative Stress Caused by Caffeine in Albino Rat Models. Asian Journal of Biology. 2017; 1(2):1-7. Available:https://doi.org/10.9734/AJOB/201 6/31586.
14. Amadi, Chikadibia Fyneface, Benjamin Nnamdi Okolonkwo, Isobo Jack, and Kingsley Kalawari Odiabara. Treatment Options for Erectile Dysfunction in Diabetics. Asian Journal of Biochemistry, Genetics and Molecular Biology. 2023;15 (4):15-25.
15. Netto MB, de Oliveira Junior AN, Goldim M, Mathias K, Fileti ME, da Rosa N,

Laurentino AO, de Farias BX, Costa AB, Rezin GT, Fortunato JJ. Oxidative stress and mitochondrial dysfunction contributes to postoperative cognitive dysfunction in elderly rats. Brain, behavior, and immunity. 2018;73:661-9.

16. Vázquez-Memije ME, Capin R, Tolosa A, El-Hafidi M. Analysis of age-associated changes in mitochondrial free radical generation by rat testis. Molecular and cellular biochemistry. 2008;307:23-30.

17. Moneim AEA, Dkhil MA, Al-Quraishy S. The potential role of *Portulaca oleracea* as a neuroprotective agent in rotenone-induced neurotoxicity and apoptosis in the brain of rats. Pestic Biochem Physiol. 2013;105:203-212.

18. Sun H, He X, Liu C, Li L, Zhou R, Jin T, Yue S, Feng D, Gong J, Sun J, Ji J. Effect of Oleracein E, a neuroprotective tetrahydroisoquinoline, on rotenone-induced Parkinson's disease cell and animal models. ACS chemical neuroscience. 2017;8(1):155-64.

19. Radhakrishnan R, Zakaria MNM, Islam MW, Chen HB, Kamil M, Chan K, et al. Neuropharmacological actions of *Portulaca oleracea* L. v.sativa (Hawk). J Ethnopharmacol. 2001;76:171–176.

20. Kumar P, Kumar A. Possible role of sertraline against 3-nitropropionic acid induced behavioral, oxidative stress and mitochondrial dysfunctions in rat brain. Prog Neuro-Psychoph. 2009;33:100–108.

21. Nowak G, Szewczyk B, Wieronska JM, Branski P, Palucha A, Pilc A, et al. Antidepressant like effects of acute and chronic treatment with zinc in forced swim test and olfactory bulbectomy model in rats. Brain Res Bull. 2003;61:159–164.

22. Frautschy SA, Hu W, Kim P, Miller SA, Harris MEW, Chu T, Cole GM. Phenolic anti-inflammatory antioxidant reversal of Aβ-induced cognitive deficits and neuropathology. Neurobiol Aging. 2001; 22:993–1005.

23. Utley HC, Bernheim F, Hochslein P. Effect of sulfhydryl reagent on peroxidation in microsome. Arch Biochem Biophys. 1967; 260:521–531.

24. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannebaum SR. Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. Anal Biochem. 1982; 126:131–138.

25. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem. 1974;47:469-474.

26. Aebi H. Catalase in-vitro. Method Enzymol. 1984;105:121–126.

27. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys. 1959;82:70–77.

28. Kumar P, Padi SSV, Naidu PS, Kumar A. Possible neuroprotective mechanisms of curcumin in attenuating 3-nitropropionic acid-induced neurotoxicity. Method Find Exp Clin. 2007;29:1–7.

29. Ellmann GE, Courtney KD, Andersen V, Featherstone RM. A new and rapid colorimetric determination of acetyl cholinesterase activity, Biochem Pharmacol. 1961;7:88–95.

30. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem. 1951; 193:265–275.

31. Hartz JS. Simultaneous histologic fixation and gross determination of calcification. Am J Clin Pathol. 1974;17:750–753.

32. Albekairi TH, Kamra A, Bhardwaj S, Mehan S, Giri A, Suri M, Alshammari A, Alharbi M, Alasmari AF, Narula AS, Kalfin R. Beta-boswellic acid reverses 3-nitropropionic acid-induced molecular, mitochondrial, and histopathological defects in experimental rat model of Huntington's disease. Biomedicines. 2022; 10(11):2866.

33. Upadhayay S, Yedke NG, Rahi V, Singh S, Kumar S, Arora A, Chandolia P, Kaur P, Kumar M, Koshal P, Jamwal S. An overview of the pathophysiological mechanisms of 3-nitropropionic acid (3-NPA) as a neurotoxin in a huntington's disease model and its relevance to drug discovery and development. Neurochem Res. 2023;48(6):1631-1647.

34. Mahdy HM, Mohamed MR, Emam MA, Karim AM, Abdel-Naim AB, Khalifa AE. Puerarin ameliorates 3-nitropropionic acid-induced neurotoxicity in rats: Possible neuromodulation and antioxidant mechanism. Neurochem Res. 2014;39: 321-332.

35. Jellinger KA. Pathomechanisms of behavioral abnormalities in Huntington disease: an update. J Neural Transm. 2024;1-4.

36. Jalali J, Ghasemzadeh Rahbardar M. Ameliorative effects of *Portulaca oleracea* L. (purslane) and its active constituents on

nervous system disorders: A review. Iran J Basic Med Sci. 2023;26(1):2-12.

37. Reddy R, Rajesham VV, Kumar SK, Kumari JP, Ramesh M, Suba V. Neuropharmacological profile of *Portulaca oleracea* L. sativa on animal models. Int J Exp Pharmacol. 2011;1:33-36.

38. Haddadi R, Eyvari-Brooshghalan S, Makhdoomi S, Fadaiie A, Komaki A, Daneshvar A. Neuroprotective effects of silymarin in 3-nitropropionic acid-induced neurotoxicity in male mice: Improving behavioral deficits by attenuating oxidative stress and neuroinflammation. Naunyn-Schmiedeberg's Arch Pharmacol. 2024; 397(4):2447-63.

39. Petrovic S, Arsic A, Ristic-Medic D, Cvetkovic Z, Vucic V. Lipid peroxidation and antioxidant supplementation in neurodegenerative diseases: a review of human studies. Antioxidants. 2020;9(11): 1128.

40. Dkhil MA, Moneim AEA, Al-Quraishy S, Saleh RA. Antioxidant effect of purslane (*Portulaca oleracea*) and its mechanism of action. J Med Plants Res. 2011;5:1589–1593.

41. Kumar P, Kalonia H, Kumar A, Goyal A. 3-Nitropropionic acid-induced neurotoxicity as an experimental model of Huntington's disease: Possible behavioral, biochemical and cellular Alterations. J Pharm Educ Res. 2010;1:26-36.

42. Walczak-Nowicka ŁJ, Herbet M. Acetylcholinesterase inhibitors in the treatment of neurodegenerative diseases and the role of acetylcholinesterase in their pathogenesis. Int J Mol Sci. 2021;22(17): 9290.

43. Jamwal S, Kumar P. Insight into the emerging role of striatal neurotransmitters in the pathophysiology of Parkinson's disease and Huntington's disease: a review. Curr. Neuropharmacol. 2019;17(2): 165-75.

44. Huang LS, Sun G, Cobessi D, Wang AC, Shen JT, Tung EY, Anderson VE, Berry EA. 3-nitropropionic acid is a suicide inhibitor of mitochondrial respiration that, upon oxidation by complex II, forms a covalent adduct with a catalytic base arginine in the active site of the enzyme. Journal of Biological Chemistry. 2006;281 (9):5965-72.

45. Suganya SN, Sumathi T. Effect of rutin against a mitochondrial toxin, 3-nitropropionicacid induced biochemical, behavioral and histological alterations-a pilot study on Huntington's disease model in rats. Metab Brain Dis. 2017;32: 471-81.

46. Hussein RM, Youssef AM, Magharbeh MK, Al-Dalaen SM, Al-Jawabri NA, Al-Nawaiseh TN, et al. Protective effect of *Portulaca oleracea* extract against lipopolysaccharide-induced neuroinflammation, memory decline, and oxidative stress in mice: Potential role of miR-146a and miR-let 7. J Med Food. 2022;25:807-817.

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