Neuroprotective effects of *Boswellia serrata* extract in Huntington's disease: Antioxidant and anti-inflammatory mechanisms in Wistar rats

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KEYWORDS

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ABSTRACT

Using in vitro and in vivo rat models, the present study examined the phytochemical content and neuroprotective potential of *Boswellia serrata* extract in reducing the symptoms of Huntington's disease (HD) through its anti-inflammatory and antioxidant qualities.

For this study, eight Wistar rats per group received oral dosages of *Boswellia serrata* extract at 100, 200, and 400 mg/kg for 14 days. 3-nitropropionic acid (10 mg/kg/day, intraperitoneally) was used for two weeks to develop Huntington's disease. Antioxidant activity was measured using DPPH, ABTS, and reducing power assays. Neuroprotection was evaluated through histopathological analysis, behavioral testing, and assessment of oxidative stress markers.

Phytochemical analysis revealed high levels of acetyl-11-keto-β-boswellic acid (AKBA) in *Boswellia serrata* and its interaction with multiple HD-associated molecular targets. The extract showed strong antioxidant and anti-inflammatory properties by lowering lipid peroxidation, nitrite levels, and pro-inflammatory cytokines. In 3NP-induced HD models, *Boswellia serrata* significantly improved motor function, restored oxidative balance, and preserved neuronal architecture, indicating its efficacy in mitigating HD progression.

The extract from *Boswellia serrata* leaves has strong neuroprotective effects due to its anti-inflammatory and antioxidant properties. These results underline its potential as a therapeutic drug and call for more research into its molecular processes and therapeutic uses.

Introduction

Huntington's disease is a neurological illness that is unstoppable and has a significant impact on both individuals and society. Five to ten cases are thought to occur for every 100,000 persons worldwide [1], HD typically manifests in mid-adulthood, progressively deteriorating motor abilities, cognitive faculties, and psychological well-being. The clinical hallmarks of HD include involuntary choreiform movements, cognitive impairment, and psychiatric disturbances, all of which cumulatively devastate the patient's quality of life and impose a significant strain on caregivers [2]. At the molecular level, HD is brought on by an abnormal growth of CAG repeats in the huntingtin (HTT) gene. A series of neurotoxic processes that preferentially target striatal and cortical neurons and cause their gradual degeneration are triggered by this genetic aberration, which causes a huntingtin protein mutant with an elongated polyglutamine (polyQ) tract to be produced [3].

The pathogenesis of HD is multifaceted, involving mitochondrial dysfunction, oxidative stress, excitotoxicity, and chronic neuroinflammation. These mechanisms culminate in cellular energy deficits, neuronal loss, and compromised neural networks [4]. Researchers frequently utilize animal models replicating the disease's neuropathological features to understand HD better and assess potential therapeutic agents. Among these, 3-nitropropionic acid (3NP)-induced models are widely used. 3-NP, a mitochondrial complex II inhibitor, mimics HD pathology by causing succinate dehydrogenase inhibition, energy failure, and subsequent striatal neurodegeneration [5]. These models recapitulate HD-like behavioral deficits and biochemical changes, serving as valuable tools for studying the efficacy of candidate neuroprotective agents.

Despite advances in understanding HD's genetic and molecular underpinnings, effective disease-modifying therapies remain elusive. This unmet need has driven interest in exploring alternative approaches, particularly those derived from natural products. Plant-based bioactive compounds, with their multitargeted mechanisms and minimal adverse effects, have emerged as a promising area of investigation [6]. Notably, *Boswellia serrata* is a natural tree from India that has attracted a lot of attention due to its high oleo-gum resin acetyl-11-keto-β-boswellic acid (AKBA). AKBA and other boswellic acids have strong anti-inflammatory, antioxidant, and neuroprotective properties that have shown promise in lowering the pathogenic pathways driving neurodegenerative diseases [7].

In light of HD, the current study methodically assesses the neuroprotective potential of *Boswellia serrata* extract. Using both in vitro and in vivo experimental paradigms, it investigates how well the extract reduces oxidative stress, controls inflammatory pathways, and preserves neuronal integrity. By elucidating the phytochemical profile and underlying therapeutic mechanisms of *Boswellia serrata*, this study aims to provide strong scientific evidence of its potential as a novel therapeutic method for the treatment of Huntington's disease.

Materials and Methods

Plant Material

The Botanical Survey of India certified and procured fresh *Boswellia serrata* leaves and bark from confirmed places. The plant material was crushed into a fine powder and stored safely for use in subsequent extraction procedures after being allowed to air dry for 30 days at room temperature.

Preparation of Extracts

The powdered leaves and bark of *Boswellia serrata* were sequentially extracted. Initially, defatting was carried out using petroleum ether, followed by ethanol extraction via Soxhlet apparatus for 60 hours. Subsequently, chloroform extraction was performed, and an aqueous extract was obtained through cold maceration. All extracts were filtered, concentrated in a rotary evaporator, and stored in a controlled condition for additional analysis [8].

Organoleptic and Physicochemical Analysis

The organoleptic properties of *Boswellia serrata* leaves and bark were assessed for color, odor, taste, and texture. Physicochemical analyses included measurements of moisture content, pH, extractive values (using alcohol and water), loss on drying, and ash content (total, water-soluble, acid-insoluble, and sulfated) to evaluate purity and stability, were determined following WHO, 2004 guidelines [9].

Preliminary Phytochemical Screening
Alkaloids, glycosides, steroids, fixed oils, lipids, phenolic compounds, carbohydrates, flavonoids, proteins, saponins, sterols, acids, and terpenoids were among the bioactive substances found by phytochemical screening of all extracts.

Quantitative Estimation
The total phenolic content was calculated and reported in milligrams of gallic acid equivalents per gram of extract using the Folin–Ciocalteu reagent method. The aluminum chloride colorimetric method was used to measure the content of all flavonoids; the results were reported in milligrams of quercetin equivalents per gram of extract.

In-Vitro antioxidant assay:
To evaluate antioxidant activity, extract solutions (1-5 µg/ml) were combined with 0.1 mM DPPH (2,2-diphenyl-1-picrylhydrazyl) in ethanol and incubated in the dark for 30 minutes. The absorbance was measured at 517 nm, using ascorbic acid as the standard reference [10]. To evaluate H_2O_2 (Hydrogen Peroxide) scavenging activity, extracts (10–320 µg/ml) were mixed with 40 mM H_2O_2 and phosphate buffer (pH 7.4) for 10 minutes at room temperature. At 230 nm, absorbance was measured with ascorbic acid serving as the control [11]. To assess reducing power, extracts (10-320 µg/ml) were incubated with potassium ferricyanide and sodium phosphate buffer at 50°C for 20 minutes. After centrifugation, the absorbance was measured at 700 nm. The EC50 value was computed with ascorbic acid as the reference standard [12]. All assays were carried out in triplicate, and statistical analysis was used to establish the significance of the findings. Cytotoxicity was investigated using the MTT assay to evaluate cell viability after 24 hours of exposure to ethanolic and chloroform extracts from *Boswellia serrata* leaves and bark. Cell viability exceeded 75%, indicating good biocompatibility and potential for therapeutic use. Assays were conducted in triplicate to ensure reliability [13].

In Vivo Study
Experimental animals: According to the CPCSEA guidelines and the protocol approved by the Institutional Animal Ethics Committee (IAEC), adult male Wistar rats weighing 250–300 g were housed in a typical laboratory setting with a 12:12 light-dark cycle, 23 ± 2°C, and 55 ± 10% humidity. The rats were given a standard pellet diet and unfettered access to water.

Induction of Huntington's disease-like symptoms: For 14 days in a row, an intraperitoneal injection of 3-nitropropionic acid (3-NP) at a dose of 10 mg/kg/day produced symptoms like those of Huntington's disease (HD). This neurotoxin mimics the pathophysiology of HD in rats by selectively inhibiting succinate dehydrogenase, which results in mitochondrial failure and gradual neuronal degeneration.

Acute Toxicity Study
An acute toxicity study was conducted using Wistar rats (150–200 g) divided into three groups in compliance with OECD guidelines: As a vehicle control, 0.5% carboxymethyl cellulose (CMC) was given to Group I; 300 mg/kg of the separated phytoconstituent was given to Group II in 0.5% CMC; and 2000 mg/kg of the phytoconstituent was given to Group III in 0.5% CMC. The maximum tolerated dose was determined for further experiments after behavioral monitoring was carried out for 14 days in order to detect toxicity.

Experimental Design and Treatment Protocol
Five groups of eight Wistar rats each participated in the study: In order to induce neurological impairment, Group II received an injection of 3-NP (10 mg/kg) for 14 days, while Groups III, IV, and V received oral doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg of ethanol extract of *Boswellia serrata* (EEBS) for the same duration as 3-NP. Group I (vehicle control) received no treatment.

Body weight measurement
Body weight was measured at the start and end of the trial, and the percentage change in body weight was calculated by comparing the final weight with the initial weight.

Behavioral assessment
The effect of Behavioral parameters was assessed on the 0^{th}, 7^{th}, and 14^{th} days.
Behavioral and Motor Function Assessments: Motor activity was evaluated using an automated electronic activity meter (Opto-Varimex 4, Columbus Instruments, USA), which tracked horizontal and vertical movements through infrared beams along the x and y axes. Interruptions of these beams were recorded digitally for two minutes per animal, allowing precise quantification of locomotor activity [14]. Evaluating 3-NP-induced motor impairments was the main goal of the movement analysis. Using a scoring system modified from Ludolph et. al., (1991), behavioral deficits such as incoordination, aberrant gait, hindlimb paralysis, and recumbency were assessed. This system categorized motor performance based on the severity of impairments, ranging from normal behavior to complete inability to move [15]. Grip strength was assessed using the string test, where rats were positioned 50 cm above a cushioned surface and made to grasp a 2 mm steel wire. The amount of time the animal could hold onto the wire was noted as an indirect indicator of grip strength [16]. The limb withdrawal test involved placing the rats on a 20 cm high Perspex platform with holes designed for forelimb and hindlimb placement. The retraction time of the hind limbs, assessed after they were placed into the holes, provided a measure of hindlimb function and striatal degeneration [17]. These tests collectively quantified locomotor abilities, motor deficits, grip strength, and hindlimb function in a standardized and reproducible manner.

Assessment of biochemical parameters
On the 15th day after 3-NP injections, biochemical tests were conducted right after behavioral observations.

Tissue Preparation: Rats from all experimental groups were anesthetized using ether, and blood samples were collected for biochemical analysis. Twenty-four hours after the final treatment (day 15), the animals were sacrificed by cervical dislocation. The brain regions, including the striatum, midbrain, and cortex, were carefully dissected and homogenized separately in 0.1 M phosphate buffer solution (pH 7.4) at 4°C for 15 minutes. The resulting homogenates were centrifuged, and the supernatant was used for the estimation of various biochemical markers, including oxidative stress parameters, neuroinflammatory factors, and neurotransmitter levels.

Biochemical Assessments:
Biochemical parameters were evaluated immediately following behavioral tests on day 15 post-3-NP administration. Lipid peroxidation was assessed by measuring malondialdehyde (MDA) levels using the thiobarbituric acid reactive substances method, with absorbance read at 532 nm and results expressed as nmol/g tissue [18]. Reduced glutathione (GSH) was estimated through Ellman's method, where absorbance at 412 nm determined GSH levels in nmol/g tissue [19]. Nitrite levels, as an indicator of nitric oxide production, were quantified using Greiss

reagent, with absorbance measured at 540 nm and results expressed as μmol/mg protein [20]. Catalase activity was analyzed using Sinha's method by measuring hydrogen peroxide decomposition, with absorbance read at 620 nm and expressed as μmol/min/mg protein [21]. Superoxide dismutase (SOD) activity was determined following Misra and Fridovich's method, measuring the inhibition of epinephrine auto-oxidation at 480 nm, expressed as a percentage of control activity [22]. Protein content was determined using the biuret method with bovine serum albumin as the standard. Pro-inflammatory cytokines, including IL-1β and TNF-α, were quantified in tissue homogenates using ELISA kits, with concentrations calculated from standard curves [23]. Standard drugs and chemicals, such as sodium nitroprusside, nicotinamide, and lipid profile kits, were sourced from reliable suppliers like Sigma-Aldrich Ltd. and Erba Diagnostics Pvt. Ltd., and all solutions were freshly prepared for the experiments.

Histopathological Analysis

Brain tissues were collected from sacrificed rats and preserved in 10% neutral buffered formalin for fixation. The samples were then dehydrated through a graded series of ethanol and embedded in paraffin wax. Thin sections, approximately 5 μm in thickness, were cut using a rotary microtome and subsequently stained with hematoxylin and eosin (H&E) for microscopic examination. The stained sections were observed and images were captured under a microscope at 40X magnification to assess histological changes [24].

Statistical analysis

The data were displayed as mean ± S.EM. for continuous variables, and the mean difference was identified using a t-test. Tukey's multiple comparison test was used to process the data for comparisons between more than two groups following one-way analysis of variance (ANOVA). The threshold for statistical significance was set at $P<0.05$. SPSS version 21 was used for the statistical analysis.

Results

Organoleptic and Physicochemical Findings for Boswellia serrata

The organoleptic evaluation of *Boswellia serrata* leaves and bark revealed a brown coloration, a pleasant aroma, a sweet-bitter taste, and a coarse texture. Physicochemical analyses showed a moisture content of 8% for leaves and 7.3% for bark, with pH values ranging from 4.0 to 7.0. Alcohol extraction yielded the highest extractive values, with 70% for leaves and 84.32% for bark. Loss on drying was 0.567% for leaves and 1.056% for bark. Ash content analysis indicated water-soluble ash at 4.247% (leaves) and 2.965% (bark), acid-insoluble ash at 0.920% (leaves) and 0.452% (bark), and sulfated ash at 3.83% (leaves) and 1.03% (bark), respectively.

Phytochemical Analysis of Boswellia serrata Leaf and Bark Extracts

Ethanolic extracts of *Boswellia serrata* leaves and bark exhibited the highest levels of total phenolics, flavonoids, and tannins in comparison to chloroform and water extracts. With a total phenolic content of 10.4–28.4 mg GAE/100 g extract, tannins of 4.9–12.3 mg GAE/100 g extract, and flavonoids of 13.3–34.56 mg RE/100 g extract, the leaves exhibited a strong antioxidant profile. Ethanol, chloroform, and aqueous extracts of the bark have total phenolic, tannin, and flavonoid concentrations ranging from 6.42 to 18.2 mg GAE/100 g extract, 0 to 9.3 mg GAE/100 g extract, and 12.98 to 22.16 mg RE/100 g extract, respectively.

In-vitro Antioxidant Assay

DPPH Radical-Scavenging Activity:

The DPPH radical-scavenging activity IC50 values for the several *Boswellia serrata* extracts (ELEBS, CLEBS, EBEBS, and CBEBS) in comparison to ascorbic acid. With an IC50 value of 71.22 ± 6.50 µg/mL at 400 µg/mL, ethanolic leaf extracts (ELEBS) had the highest activity, surpassing even ascorbic acid.

Table 1.: DPPH radical-scavenging activity of ELEBS, CLEBS, EBEBS and CBEBS

Samples	Treatment	Dose Concentration	IC_{50} Values
1	Ascorbic Acid	(1-10µg/ml)	42.50 ± 0.74 µg/ml
2	ELEBS	(1-200µg/ml	97.20±4.67µg/ml
3	ELEBS	(1-400µg/ml)	71.22 ± 6.50 µg/ml
4	ELEBS	(1-600µg/ml)	63.50 ± 8.07µg/ml
5	CLEBS	(1-200µg/ml	101.17± 7.09 µg/ml
6	CLEBS	(1-400µg/ml)	88.34 ± 6.34 µg/ml
7	CLEBS	(1-600µg/ml)	73.24 ± 9.16 µg/ml
8	EBEBS	(1-200µg/ml)	100.72 ± 5.59 µg/ml
9	EBEBS	(1-400µg/ml)	86.65 ± 5.22µg/ml
10	EBEBS	(1-600µg/ml)	74.44 ± 5.42 µg/ml
11	CBEBS	(1-200µg/ml	104.07 ± 6.92 µg/ml
12	CBEBS	(1-400µg/ml)	91.14 ± 8.29 µg/ml
13	CBEBS	(1-600µg/ml)	81.56 ± 5.67 µg/ml

Data are represented as mean ± SD of three replicates

"ELEBS (Ethanolic leaf extract of *Boswellia serrata*), CLEBS (Chloroform leaf extract of *Boswellia serrata*), EBEMP (Ethanolic bark extract of *Boswellia serrata*), CBEBS (Chloroform bark extract of *Boswellia serrata*)"

H_2O_2-Scavenging Activity:

The percentage inhibition of H2O2 scavenging by various *Boswellia serrata* extracts at different concentrations. The ethanolic leaf extracts (ELEBS) displayed the highest inhibition, reaching 83.39 ± 5.21% at 160 µg/mL. Chloroform extracts (CLEBS, CBEBS) showed moderate activity, with maximum inhibition at 160 µg/mL being 69.33 ± 2.92% and 60.25 ± 2.19%, respectively.

Table 2.: H_2O_2-scavenging activity of ELEBS, CLEBS, EBEBS, and CBEBS

Samples	Treatment	Dose Concentration	% Inhibition
1	Ascorbic Acid	(100 µg/ml)	93.15 ± 2.18%
2	ELEBS	(1-20µg/ml	45.14 ± 2.95%
3	ELEBS	(1-40µg/ml)	59.87 ± 3.09 %
4	ELEBS	(1-80µg/ml)	78.33 ± 4.05%
5	ELEBS	(1-160 µg/ml)	83.39 ± 5.21%
6	CLEBS	(1-20µg/ml	42.17± 3.39 %
7	CLEBS	(1-40µg/ml)	54.67 ± 3.11%
4	CLEBS	(1-80µg/ml)	64.34 ± 4.16%
5	CLEBS	(1-160 µg/ml)	69.33 ± 2.92%
8	EBEBS	(1-20µg/ml)	40.13 ± 2.15%
9	EBEBS	(1-40µg/ml)	51.67 ± 3.39%
10	EBEBS	(1-80µg/ml)	58.17± 4.32 %
10	EBEBS	(1-160 µg/ml)	62.47± 4.37 %

11	CBEBS	(1-20µg/ml)	36.45 ± 4.27%
11	CBEBS	(1-40µg/ml)	44.79 ± 2.67%
12	CBEBS	(1-80µg/ml)	54.12 ± 3.10%
13	CBEBS	(1-160 µg/ml)	60.25 ± 2.19%

Data are represented as mean ± SD of three replicates.

Reducing Power Activity:

The reducing power of *Boswellia serrata* extracts at various concentrations, showing the percent inhibition. Ethanolic leaf extracts (ELEBS) exhibited the highest reducing power at 160 µg/mL (72.82 ± 3.07%), significantly outperforming other extracts, including ascorbic acid, which had 87.28 ± 3.48% inhibition at the same concentration.

Table 3.: Reducing power activity of different concentrations of ELEBS, CLEBS, EBEBS, and CBEBS and ascorbic acid.

S. No	Conc.(µg/mL)	ELEBS	CLEBS	EBEBS	CBEBS	Ascorbic Acid
01	20	31.52±0.38	26.14±1.35	23.19±0.79	21.33±0.78	30.14±2.04
02	40	42.54±0.77	38.67±0.87	35.20±1.01	32.14±2.03	56.11±1.88
03	80	57.14±1.34	53.35±2.12	49.12±1.22	44.23±2.34	72.76±2.97
04	160	72.82±3.07	61.20±3.40	59.13±4.12	56.2±2.23	87.28±3.48

Data are represented as mean ± SD of three replicates.

Cytotoxicity Studies

The percentage cell viability of *Boswellia serrata* extracts (ELEBS, CLEBS, EBEBS, CBEBS) at different concentrations. All extracts demonstrated high cell viability, exceeding 75% at concentrations up to 200 µg/mL, indicating that the extracts do not exhibit significant cytotoxic effects and are biocompatible for biological applications.

Table 4.: % Cell viability of ELEBS, CLEBS, EBEBS and CBEBS

Samples	Treatment	Dose Concentration	% Cell Viability
1	Control	-	100%
2	ELEMP	(1-50µg/ml)	89.12±5.82 %
3	ELEMP	(1-100µg/ml)	92.16 ± 6.17 %
4	ELEMP	(1-200µg/ml)	88.46 ± 7.25%
5	CLEMP	(1-50µg/ml)	90.25 ± 8.34%
6	CLEMP	(1-100µg/ml)	87.23± 7.22 %
7	CLEMP	(1-200µg/ml)	90.12 ± 7.34%
8	EBEMP	(1-50µg/ml)	88.32 ± 7.12%
9	EBEMP	(1-100µg/ml)	89.21 ± 5.67%
10	EBEMP	(1-200µg/ml)	91.37± 9.12 %
11	CBEMP	(1-50µg/ml	89.45 ± 7.12%
12	CBEMP	(1-100µg/ml)	90.88 ± 5.43%
13	CBEMP	(1-200µg/ml)	88.78 ± 7.87%

Data are represented as mean ± SD of three replicates.

In-Vivo Testing

Acute Toxicity Evaluation of Ethanolic Extract of *Boswellia serrata:*

In the acute toxicity evaluation of the ethanolic extract of Boswellia serrata, rats administered up to 2000 mg/kg body weight showed no signs of toxicity or mortality during the 14-day observation period.

Effect of *Boswellia serrata* Leaves Extract on body weight in 3-nitro propionic acid-treated rats*:*

The percentage change in body weight over the treatment period was calculated by comparing body weights on day 0 and day 14. Animals treated with 3-NP displayed a marked reduction in body weight by day 14, while those receiving vehicle treatment maintained stable weight. Treatment with ELEBS at oral doses of 100, 200, and 400 mg/kg significantly mitigated weight loss in 3-NP-treated rats, with the 400 mg/kg dose showing the most pronounced effect. The 100 mg/kg dose exhibited minimal impact on weight changes.

Table 5.: Body Weight Analysis in 3-Nitro Propionic Acid-Treated Rats with and without *Boswellia serrata* Extract

Treatment (mg/kg)	Change in body weight (%)
VC	4.88±0.68
3-NP	−26.76±4.35a
ELEBS 100+3-NP	−24.64±4.40
ELEBS 200+3-NP	−12.92±3.34b,c
ELEBS 400+3-NP	−6.77±2.90b,c

Data are presented as means ± S.E.M.

The findings were examined using Tukey's multiple comparison test and one-way ANOVA; the 3-NP control group was compared to aP<0.05, the vehicle control group to bP<0.05, and cP<0.05 as compared to (ELEBS 100+3-NP) group

Neurobehavioral study

Effect of *Boswellia serrata* Leaves Extract on Neurological Function in 3-Nitro Propionic Acid-Treated Rats:

Table 6.: Assessment of Neurological Function Post-Treatment with *Boswellia serrata* Leaves Extract in 3-Nitro Propionic Acid-Treated Rats

Parameter	VC (Vehicle Control)	3-NP (Control)	ELEBS 100 + 3-NP	ELEBS 200 + 3-NP	ELEBS 400 + 3-NP
No. of Days	**Locomotor activity**				
0 Day	388 ± 5.45	367 ± 8.32	395 ± 19.72	377 ± 15.68	389 ± 19.54
7th Day	400 ± 7.21	238 ± 12.66a	289 ± 12.98	302 ± 10.87b,c	355 ± 16.35b,c
14th Day	395 ± 6.68	178 ± 9.45a	232 ± 13.67	316 ± 13.39b,c	352 ± 17.62b,c
	Motor Function score				
0 Day	5.8 ± 0.3	5.9 ± 0.8	5.6 ± 0.7	5.8 ± 0.5	5.4 ± 0.4
7th Day	6.0 ± 0.7	3.6 ± 0.5a	3.6 ± 0.4	4.7 ± 0.5b,c	5.4 ± 0.6b,c
14th Day	6.2 ± 0.6	1.8 ± 0.4a	2.5 ± 0.3	3.9 ± 0.3b,c	5.3 ± 0.5b,c
	Limb Retraction Time				
0 Day	1.0 ± 0.1	1.0 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	1.0 ± 0.1
7th Day	1.0 ± 0.1	52.16 ± 4.16a	46.39 ± 4.12	28.49 ± 4.24b,c	23.28 ± 1.25b,c
14th Day	1.0 ± 0.1	79.66 ± 5.20a	68.45 ± 5.75	24.53 ± 6.14b,c	10.87 ± 1.89b,c
	Grip Strength Latency				
0 Day	50.7 ± 4.15	54.7 ± 3.89	55.32 ± 4.24	50.43 ± 3.77	49.8 ± 4.14

7th Day	53.59 ± 3.19	12.12 ± 50.07a	15.28 ± 2.28b,c	34.19 ± 5.12b,c	42.28 ± 1.25b,c
14th Day	55.21 ± 5.7	5.67 ± 0.70a	11.18 ± 1.12b,c	39.12 ± 3.11b,c	41.18 ± 3.18b,c

Data are presented as mean ± S.E.M.
The findings were examined using Tukey's multiple comparison test and one-way ANOVA; the 3-NP control group was compared to aP<0.05, the vehicle control group to bP<0.05, and cP<0.05 as compared to (ELEBS 100+3-NP) group

The impact of ethanolic leaf extract (ELEBS) from *Boswellia serrata* on locomotor activity, motor function scores, limb retraction times, and grip strength delay in rats treated with 3-nitro propionic acid (3-NP) is shown in Table No. 6. The findings demonstrated that ELEBS treatment at 200 and 400 mg/kg considerably alleviated the motor impairments brought on by 3-NP, with the 400 mg/kg dose exhibiting the greatest improvement. The potential neuroprotective effects of *Boswellia serrata* in this HD model were demonstrated by the notable restoration of locomotor activity, motor function scores, and limb retraction times, as well as the significant improvement in grip strength latency in the ELEBS-treated groups when compared to the 3-NP control group.

Biochemical studies

Effect of *Boswellia serrata* leaves extract on Oxidative stress and Pro-inflammatory Cytokines in 3-Nitro Propionic acid-treated rats

Table 7.: Comparative Analysis of *Boswellia serrata* Leaves Extract on Oxidative and Inflammatory Biomarkers in 3-Nitro Propionic Acid-treated Rats

Treatment	TBARS	GSH	SOD	CAT	Nitrite	IL-1β (pg/mg tissue)	TNF-α (pg/mg tissue)
VC	2.82 ± 0.07	5.92 ± 0.09	4.38 ± 0.06	3.51 ± 0.04	1.18 ± 0.08	170.08 ± 11.68	120.67 ± 14.27
3-NP	8.21 ± 0.80	1.67 ± 0.04a	1.32 ± 0.02a	1.09 ± 0.02a	5.19 ± 0.60a	732.23 ± 14.36a	621.57 ± 42.49a
ELEBS 100 + 3-NP	3.52 ± 0.20	1.99 ± 0.06	1.52 ± 0.03	1.36 ± 0.05	5.06 ± 0.47	700.56 ± 44.52	591.45 ± 39.68
ELEBS 200 + 3-NP	5.88 ± 0.65b,c	3.17 ± 0.07b,c	2.42 ± 0.04b,c	1.89 ± 0.06b,c	3.67 ± 0.59b,c	408.67 ± 33.62b,c	373.88 ± 30.16b,c
ELEBS 400 + 3-NP	7.12 ± 0.56b,c	4.64 ± 0.07b,c	3.68 ± 0.05b,c	2.97 ± 0.04b,c	2.02 ± 0.46b,c	270.65 ± 28.11b,c	243.98 ± 34.56b,c

Data are presented as means ± S.E.M. Data were analyzed by using one-way ANOVA followed by Tukey's multiple comparison test; 'aP<0.01 as compared to Vehicle control Group; bP<0.05 as compared to 3-NP control group and cP<0.05 as compared to (ELEBS 100+3-NP) group
Table no. 7. demonstrates how 3-nitro propionic acid (3-NP)-treated rats' oxidative stress and inflammation are affected by *Boswellia serrata* leaf extract (ELEBS). 3-NP resulted in decreased antioxidant activity and enhanced oxidative damage and inflammation, as demonstrated by elevated TBARS, nitrite, and cytokine levels. ELEBS therapy (200 and 400 mg/kg) restored antioxidant enzyme activity (GSH, SOD, CAT) and dramatically reduced TBARS, nitrite, and pro-inflammatory cytokines (IL-1β, TNF-α). These findings demonstrate ELEBS's neuroprotective properties against inflammation and oxidative stress, pointing to a possible therapeutic use for neurodegenerative illnesses.

Effect of *Boswellia serrata* leaves extract on 3-NP-induced histopathological changes in the striatum and cortex regions of rat brain

Histopathological changes in the striatum of control and 3-NP-treated animals are depicted in Figure no.1.

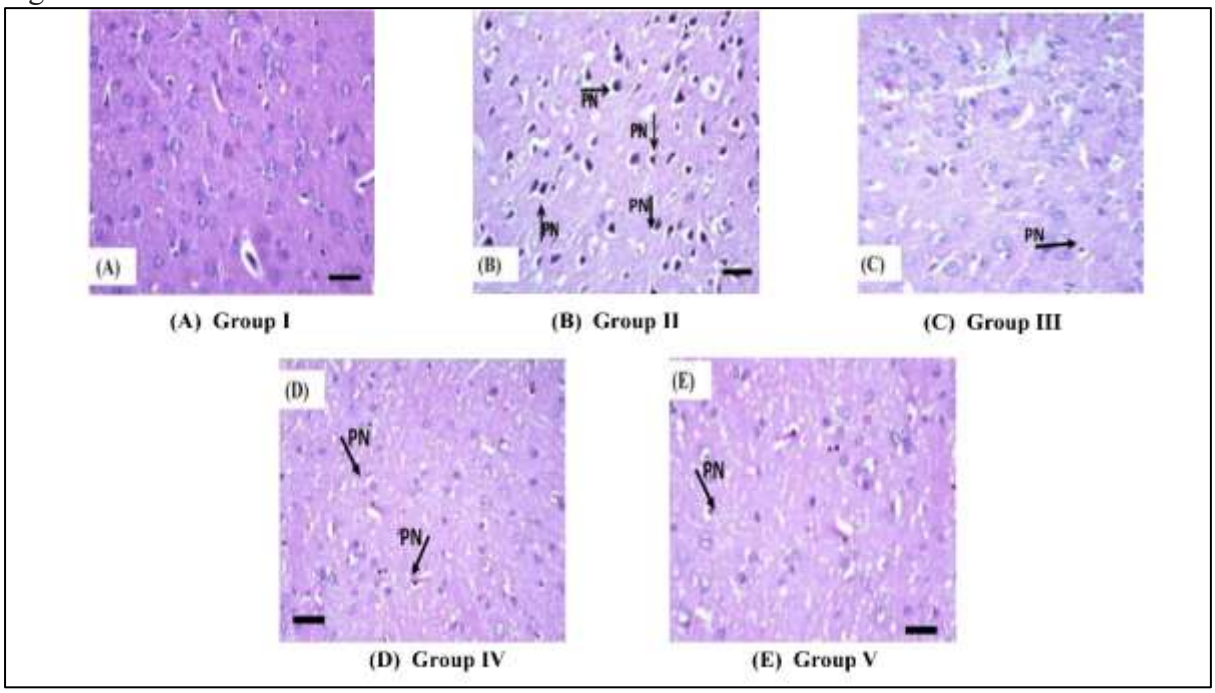

Figure 1.: Impact of *Boswellia serrata* Ethanolic Leaf Extract (ELEBS) on histological changes in the control and 3-NP-treated rats' striatum and cortex, with an emphasis on modifications brought about by the injection of 3-NP to simulate HD. Sections were viewed at a 40x magnification using a light microscope.

(A) The striatum and cortical areas of control rats showed normal histology. (B) Condensed pyknotic nuclei were seen in both striatal and cortical regions of rats treated with 3-NP alone, indicating significant damage. (C) The striatum showed fewer pyknotic nuclei in the ELEBS + 3-NP (100 mg/kg, p.o.) group, suggesting partial protection. (D) A more noticeable reduction in pyknotic nuclei was seen in the ELEBS + 3-NP (200 mg/kg, p.o.) group, indicating enhanced neuroprotective effects. (E) The ELEBS + 3-NP (400 mg/kg, p.o.) group showed the greatest reduction in pyknotic nuclei and significantly better histological preservation in the striatum at higher doses (scale bar—100 μm).

Discussion

The present study highlights the neuroprotective potential of *Boswellia serrata* in reducing the consequences of HD, which is modeled by the neurotoxicity that 3-NP causes in rats. One well-known technique for producing HD-like symptoms is the 3-NP model, which replicates important elements of HD such as neuroinflammation, oxidative stress, and motor dysfunction [25,26]. The present study demonstrates the various ways that BS, and more especially its Boswellic acids, defends against these pathogenic processes. The neuroprotective benefits noted here are in line with earlier research indicating that *Boswellia* has strong anti-inflammatory and antioxidant qualities, both of which are essential for preventing neurodegenerative illnesses [27].

Additionally, the phytochemical study of BS showed a high concentration of bioactive substances, including phenolics and flavonoids, which support the plant's potent antioxidant action. The results of the DPPH and hydrogen peroxide scavenging assays demonstrate that BS effectively neutralizes reactive oxygen species (ROS), a significant cause of the oxidative damage observed in HD. Our findings are supported by other studies that demonstrate the critical role antioxidants play in lowering oxidative stress and preventing cellular damage in neurodegenerative diseases [28].

The in-vivo studies on 3-NP-induced HD rats revealed significant improvements in motor coordination, body weight, and biochemical markers of oxidative stress. Treatment with BS mitigated the weight loss typically observed in HD models, suggesting a protective effect on metabolic functions. Additionally, the behavioral assays, including locomotor activity and grip strength tests, demonstrated that BS enhances motor function, reducing the neuromuscular impairments commonly seen in HD. These findings are in line with prior research that has demonstrated *Boswellia*'s neuroprotective properties, including the restoration of motor function and the enhancement of cognitive outcomes in models of neurodegenerative diseases [27].

The biochemical analyses further supported the antioxidant effects of BS, with significant reductions in lipid peroxidation and elevated levels of catalase and glutathione—key antioxidant enzymes that are often depleted in neurodegenerative conditions. The modulation of these markers indicates that BS restores the brain's endogenous antioxidant defenses, providing critical protection against oxidative damage [29].

Boswellia serrata has been shown to reduce pro-inflammatory cytokine levels, including TNF-α and IL-1β, which can cause HD neuronal damage. Because these cytokines are suppressed, it is possible that BS inhibits neuroinflammation, which would stop microglial activation and additional neuronal damage. This is consistent with previous research that highlights the part inflammation plays in the development of HD and the ability of *Boswellia* to alter immune responses.

The histopathological evaluation further corroborated the neuroprotective potential of BS, with reduced damage to the striatum and cortex observed in treated animals. The presence of pyknotic nuclei, a hallmark of neuronal apoptosis, was significantly reduced in the BS-treated groups, especially at higher doses. These findings suggest that BS not only protects against neuroinflammation and oxidative stress but also contributes to the preservation of neuronal structure and function [30].

The combined effects of *Boswellia serrata* on biochemical markers, cytokine levels, and histopathological changes underscore its potential as a broad-spectrum neuroprotective agent in HD. There is strong evidence to support the use of BS as an adjuvant treatment for HD due to its capacity to lower oxidative stress, regulate inflammation, and stabilize dopaminergic pathways.

Conclusion

This study highlights the potent neuroprotective properties of *Boswellia serrata* in combating Huntington's disease-related neurodegeneration. By effectively reducing oxidative damage and regulating inflammatory responses, *Boswellia serrata* demonstrates its ability to mitigate key pathological features of HD, including neuronal damage and metabolic dysfunction. Its role in stabilizing dopamine pathways further emphasizes its therapeutic potential.

Overall, the findings underscore the promise of *Boswellia serrata* as a plant-based intervention for managing Huntington's disease symptoms. Its antioxidant and anti-inflammatory mechanisms present a compelling case for further research into its clinical applications and potential as an adjuvant treatment for neurodegenerative disorders.

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