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Original Article

Ethanolic *Persea americana* (Avocado) Seed Extract Exhibits Antidepressant Effects and Restores BDNF Levels in Reserpine-Induced Depressive Rats

Baby Shalini S N, Jyothi Yachamaneni*, Sahana H S

Department of Pharmacology, Krupanidhi College of Pharmacy, Rajiv Gandhi University of Health Science, Bengaluru, Karnataka, INDIA.

ABSTRACT

Background: *Persea americana* (Avocado) seeds contain flavonoids, phenolic compounds, rutin, and catechin derivatives possessing antioxidant and neuroprotective activities. Previous studies have reported free radical scavenging and anti-inflammatory properties of avocado seed extracts, suggesting their potential relevance in depressive disorders. Therefore, the present study was designed to evaluate the antidepressant-like activity of ethanolic *Persea americana* seed extract in reserpine-induced depressive behaviour in Wistar rats. **Materials and Methods:** Thirty-five female Wistar rats were divided into five groups (n=7). Depression was induced using reserpine (0.2 mg/kg, i.p.) for 14 days. Amitriptyline (25 mg/kg, p.o.) and ethanolic *Persea americana* seed extract (250 and 500 mg/kg, p.o.) were administered orally. Behavioural assessments included Tail Suspension Test (TST), Actophotometer Test, and Elevated Plus Maze (EPM) Test were carried out. Brain-Derived Neurotrophic Factor (BDNF) levels and histopathological alterations in brain tissues were also evaluated. **Results:** Reserpine administration produced significant depressive-like behaviour characterized by increased immobility duration, reduced locomotor activity, decreased open-arm exploration, reduced BDNF levels, and neuronal degeneration in brain tissues. Treatment with ethanolic *Persea americana* seed extract significantly improved behavioural performance, restored BDNF levels, and reduced histopathological alterations compared with the positive control group. The high-dose extract-treated group demonstrated effects comparable to amitriptyline treatment. **Conclusion:** Ethanolic *Persea americana* seed extract exhibited significant antidepressant-like and neuroprotective activity in reserpine-induced depressive behaviour, suggesting its potential therapeutic relevance in depressive disorders.

Keywords: Amitriptyline, Avocado seed extract, BDNF, Depression, Neuroprotection, Reserpine.

Correspondence:
Dr. Jyothi Yachamaneni
Professor and Head, Department of Pharmacology, Krupanidhi College of Pharmacy, Rajiv Gandhi University of Health Science, Bengaluru-560035, Karnataka, INDIA.
Email: jokiran05@gmail.com

INTRODUCTION

Depression is a multifactorial neuropsychiatric disorder characterized by persistent low mood, cognitive impairment, reduced motivation, and disturbances in emotional and behavioural functions.[1] In addition to monoamine depletion, recent evidence indicates that oxidative stress, neuroinflammation, and reduced Brain-Derived Neurotrophic Factor (BDNF) contribute significantly to neuronal dysfunction and impaired neuroplasticity in depression.[2]

Although currently available antidepressant drugs are clinically effective in many patients, their therapeutic use is often limited by delayed onset of action, incomplete response, relapse, and adverse effects such as sedation, weight gain, and cardiovascular complications.[3] These limitations have encouraged increasing interest in plant-derived therapeutic agents possessing multitarget neuroprotective and antioxidant properties. Natural products rich in polyphenols and flavonoids have attracted considerable attention because of their ability to modulate oxidative stress, inflammatory mediators, and neuronal signaling pathways involved in depressive disorders.[4]

Persea americana (Avocado) seeds have recently gained pharmacological importance because they contain several biologically active phytoconstituents, including flavonoids, rutin, catechin derivatives, chlorogenic acid, tannins, and procyanidins.[5] Previous investigations have demonstrated that avocado seed extracts possess potent antioxidant, anti-inflammatory, free radical scavenging, and neuroprotective activities.[6] Polyphenolic compounds present in avocado seeds are reported to reduce oxidative neuronal injury and may contribute to preservation of neuronal architecture under pathological conditions.[7]

Several reports published within the past five years have also shown that Avocado seed extracts exhibit potent free-radical scavenging activity, inhibit neuroinflammatory mediators, and modulate neurotransmitter levels, suggesting their potential relevance in depressive disorders.[8]

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Despite the promising pharmacological properties of *Persea americana* seeds, their antidepressant potential remains insufficiently investigated in validated experimental models. Furthermore, limited information is available regarding their influence on BDNF restoration and histopathological alterations associated with depressive behaviour. Therefore, the present study was designed to evaluate the antidepressant-like and neuroprotective effects of ethanolic *Persea americana* seed extract in reserpine-induced depressive behaviour in Wistar rats through behavioural assessments, estimation of BDNF levels, and histopathological examination of brain tissues.

MATERIALS AND METHODS

Plant material and extract preparation

Avocado (*Persea americana*) seeds were procured from Shivay Herbals and Healthcare, Jaipur, India (Batch No. SHAH/1191/2025; Vendor Certificate No. SH/VC-2025/1191). The seeds were thoroughly washed, shade-dried for 7 days, and coarsely ground into a powder. A 500 g portion of the seed powder was subjected to Soxhlet extraction using 70% ethanol for 8 hr (10-12 extraction cycles). The resulting extract was concentrated under reduced pressure using a rotary evaporator and subsequently dried in a vacuum oven at 40°C. The dried extract was stored in airtight amber containers at 4°C until further use.[9]

Preliminary phytochemical studies

Preliminary phytochemical screening of the ethanolic extract of *Persea americana* (Avocado) seed was performed using standard qualitative tests for flavonoids, tannins, saponins, and phenolic compounds.[10]

Drugs and Chemicals

Reserpine (Yucca Enterprises, India) and amitriptyline (Krupanidhi College of Pharmacy, Bengaluru) were used in the study. All reagents and chemicals used in study were of analytical grade.

Dose selection

Amitriptyline was used as the standard antidepressant (25 mg/kg, p.o.).[11] Reserpine (0.2 mg/kg, i.p.) was selected based on literature for inducing depressive-like behaviour.[12] Avocado Seed extract Doses (250 and 500 mg/kg, p.o.) were selected based on acute oral toxicity studies conducted according to OECD guideline 423.[13]

Experimental animals

Thirty-five female Wistar rats (150-200 g) were obtained from Krupanidhi College of Pharmacy Animal Facility (Registration No. KCP/AF/CCSEA/2024; Vendor Certificate No. KCP/AF-VC-2024/07). The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC No. KCP/IAEC/PCOL/161/JULY-2024) on 06 August 2024. Animals were housed in polypropylene cages (42 × 27 × 15 cm) containing corncob bedding, changed twice weekly. Environmental conditions were maintained at 25 ± 4°C, 50-60% humidity, 12-hr light/dark cycle, with free access to standard pellet feed and water *ad libitum*. Animals were acclimatised for 10 days before the commencement of the experiment.

Experimental Design

Thirty-five female Wistar rats were randomly assigned into five groups, with seven animals in each group ($n = 7$). Group 1 (negative control) received normal diet and water. Group 2 (positive control) received reserpine (0.2 mg/kg, i.p.) for 14 consecutive days. Group 3 received reserpine along with amitriptyline (25 mg/kg, p.o.), while Groups 4 and 5 received reserpine along with low-dose (250 mg/kg, p.o.) and high-dose (500 mg/kg, p.o.) ethanolic *Persea americana* seed extract, respectively, for 14 consecutive days. All treatments were administered 60 min after reserpine injection each day. Baseline behavioural assessments were performed on Day 0 prior to treatment initiation, and final behavioural evaluations were conducted on Day 14. At the end of the experimental period, animals were euthanized by overdose of anaesthesia, and brain tissues were collected for histopathological examination and BDNF estimation.[14,15]

EVALUATION OF ANTI-DEPRESSION ACTIVITY

The study evaluated behavioral and tissue-related parameters. Behavioral assessments included the Tail Suspension Test, Actophotometer Test and Elevated Plus Maze Test to assess depressive behavior, locomotor activity, and anxiety-like behavior. Tissue-based evaluation included Histopathological Examination and estimation of Brain-Derived Neurotrophic Factor (BDNF) levels in brain tissues to investigate structural and neurochemical alterations associated with depression and treatment outcomes.

Tail suspension test

The Tail Suspension Test (TST) was performed to evaluate depressive-like behaviour in rats. Each animal was suspended individually by adhesive tape placed approximately 1 cm from the tip of the tail and fixed to a horizontal bar at a height of 58 cm above the floor. The total duration of the test was 5 min, and the immobility time was recorded. Rats were considered immobile when they remained completely motionless except for respiratory movements.[16]

Actophotometer test

Locomotor activity was assessed using an actophotometer to evaluate the effect of treatment on spontaneous motor activity in rats. Each animal was placed individually in the actophotometer chamber, and locomotor activity was recorded for a period of 5 min. Every interruption of the photoelectric beam was considered as one activity count. The total activity score recorded during the test period was used as an index of locomotor activity.[17]

Elevated plus maze test

The Elevated Plus Maze (EPM) test was performed to assess anxiety-like behaviour associated with depressive states. Each rat was placed at the center of the maze facing an open arm and allowed to explore freely for 5 min. The duration spent in the open arms was recorded during the test period. Increased open arm exploration was considered indicative of improvement in depressive-like behaviour.[18]

BDNF Estimation

Hippocampal tissues were homogenized in Phosphate-Buffered Saline (PBS) containing protease inhibitors and centrifuged at 10,000 rpm (~10,000 × g) for 15 min at 4°C. The resulting supernatants were collected for estimation of Brain-Derived Neurotrophic Factor (BDNF). BDNF levels were quantified using a commercial Rat BDNF ELISA kit (Elabscience Biotechnology Inc., USA; detection range: 15-10,000 pg/mL) according to the manufacturer's instructions and laboratory standard operating procedures (Lab Code: 4914/BS-KNC; analysis performed on 09 July 2025). All samples were analyzed in triplicate, and BDNF concentrations were expressed as pg/mg protein.[19]

Histopathological examination

Brains were collected 12 hr after the final treatment, rinsed with normal saline, and preserved in 10% formalin for 48 hr. The tissues were embedded in paraffin, sectioned at 5 µm thickness, and stained with Hematoxylin and Eosin (H&E). Three non-consecutive sections from each brain region, including the hippocampus, cerebral cortex, and substantia nigra, were examined under a light microscope. Neuronal morphology and histopathological alterations were evaluated, and representative photomicrographs were captured.[20]

Statistical analysis

Statistical analysis was performed using GraphPad Prism 8.0. Data were expressed as Mean ± SEM and analyzed using one-way ANOVA followed by Dunnett's *post hoc* test. Data normality was assessed using the Shapiro-Wilk test. A value of $p<0.05$ was considered statistically significant.

RESULTS

Plant Extraction Yield

Percentage yield was calculated as:

$$\text{Yield (\% w/w)} = \frac{\text{Weight of dried extract(g)}}{\text{Weight of powdered seed(g)}} \times 100$$

The percentage yield of the ethanolic extract of *Persea americana* seed was found to be 12.4% (w/w).

Phytochemical investigation for various compounds

Preliminary phytochemical examination of the ethanolic extract of *Persea americana* (Avocado) seed confirmed the presence of alkaloids, flavonoids, tannins, saponins, phenols, terpenoids, and carbohydrates (Table 1).

Tail suspension test

No statistically significant differences in immobility duration were observed among the experimental groups during baseline assessment on Day 0. Following 14 days of reserpine administration, the positive control group showed increased immobility duration compared with the negative control group. Simultaneous treatment with ethanolic *Persea americana* seed extract reduced immobility duration compared with the positive control group. Greater reduction in immobility duration was observed in the high-dose extract-treated group, with effects comparable to the standard-treated group receiving amitriptyline (Figure 1).

Actophotometer

At baseline on Day 0, no statistically significant differences in locomotor activity scores were observed among the experimental groups. Following 14 days of reserpine administration, the positive control group showed decreased locomotor activity compared with the negative control group. Simultaneous treatment with ethanolic *Persea americana* seed extract increased locomotor activity scores compared with the positive control group. Greater increase in locomotor activity was observed in the high-dose extract-treated group than in the low-dose group. Increased locomotor activity was also observed in the standard-treated group receiving amitriptyline compared with the positive control group (Figure 2).

Elevated Plus Maze Test

No statistically significant differences in open-arm exploration time were observed among the experimental groups at baseline (Day 0). After 14 days of reserpine administration, the positive control group exhibited reduced open-arm exploration compared with the negative control group. Concurrent treatment with ethanolic *Persea americana* seed extract increased the time spent in the open arms compared with the positive control group, with the high-dose extract-treated group showing greater improvement than the low-dose group. The standard-treated group receiving amitriptyline also demonstrated increased open-arm exploration compared with the positive control group (Figure 3).

Levels of BDNF (Brain-Derived Neurotrophic Factor)

BDNF levels were estimated to assess the neuroprotective effect of Avocado seed extract in reserpine-induced rats. Reserpine treatment significantly reduced BDNF concentration compared with the negative control group.[20] Treatment with Avocado seed

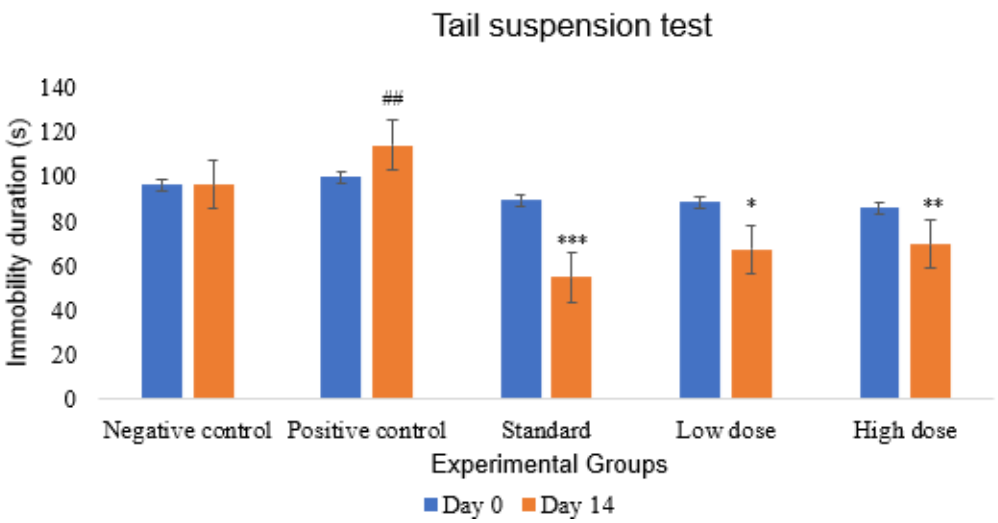

Figure 1: Immobility duration in the Tail Suspension Test. Data are expressed as Mean±SEM (*n*=7). ## *p*<0.001 compared with the negative control group; *p<0.05, **p<0.01, ***p<0.001 compared with the positive control group using one-way ANOVA followed by Dunnett's *post hoc* test.

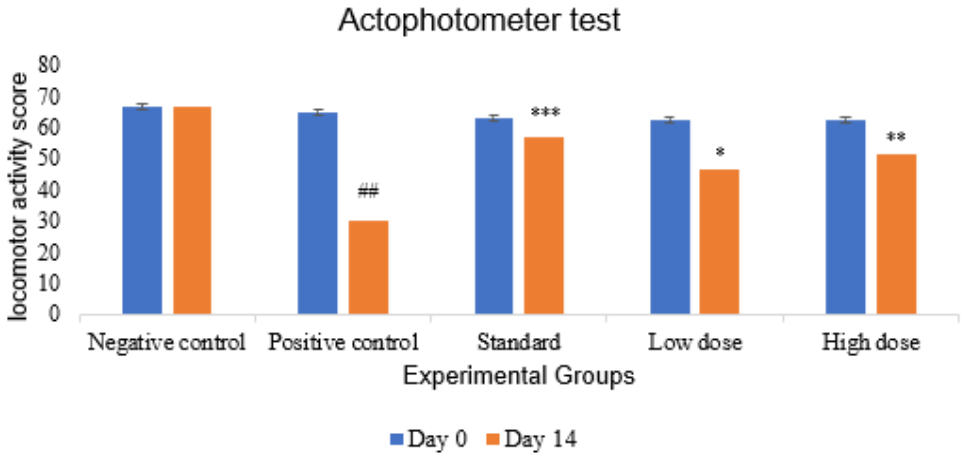

Figure 2: Locomotor activity score in the Actophotometer Test. Data are expressed as Mean±SEM (*n*=7). ## *p*<0.001 compared with the negative control group; *p<0.05, **p<0.01, ***p<0.001 compared with the positive control group using one-way ANOVA followed by Dunnett's *post hoc* test.

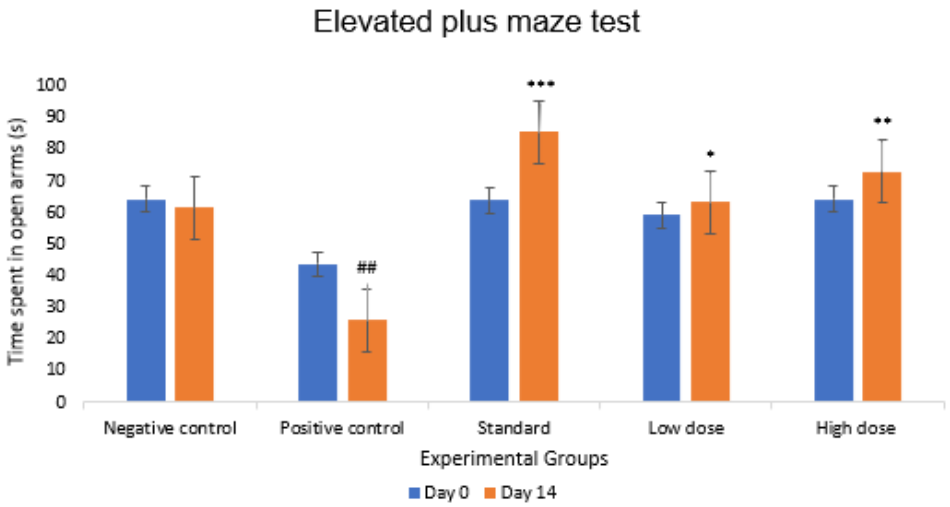

Figure 3: Open-arm exploration in the Elevated Plus Maze Test. Data are expressed as Mean ± SEM (*n*=7). ## *p*<0.001 compared with the negative control group; *p< 0.05, **p<0.01, *** p<0.001 compared with the positive control group using one-way ANOVA followed by Dunnett's *post hoc* test.

extract increased BDNF levels at both dose levels, with greater improvement observed in the high-dose group. Amitriptyline treatment also restored BDNF levels toward normal values (Figures 4-7).

DISCUSSION

The goal of the study demonstrated that ethanolic *Persea americana* (Avocado) seed extract exhibited significant antidepressant-like and neuroprotective effects in reserpine-induced depressive behaviour in Wistar rats. Administration of the extract improved behavioural performance, restored BDNF levels, and attenuated histopathological damage in brain tissues, suggesting its beneficial role against reserpine-induced neuronal alterations.

Reserpine administration significantly increased immobility duration in the Tail Suspension Test, indicating the development of depressive-like behaviour in experimental rats. Treatment with ethanolic *Persea americana* seed extract reduced

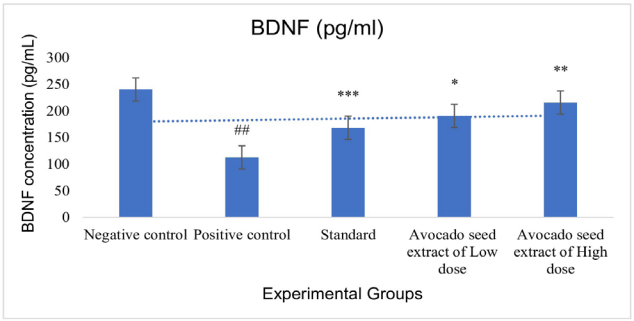

Figure 4: BDNF levels in reserpine-induced rats. Data are expressed as Mean ± SEM (n=7). ##p<0.001 compared with the negative control group; * p<0.05, ** p<0.01, *** p<0.001 compared with the positive control group using one-way ANOVA followed by Dunnett's *post hoc* test.

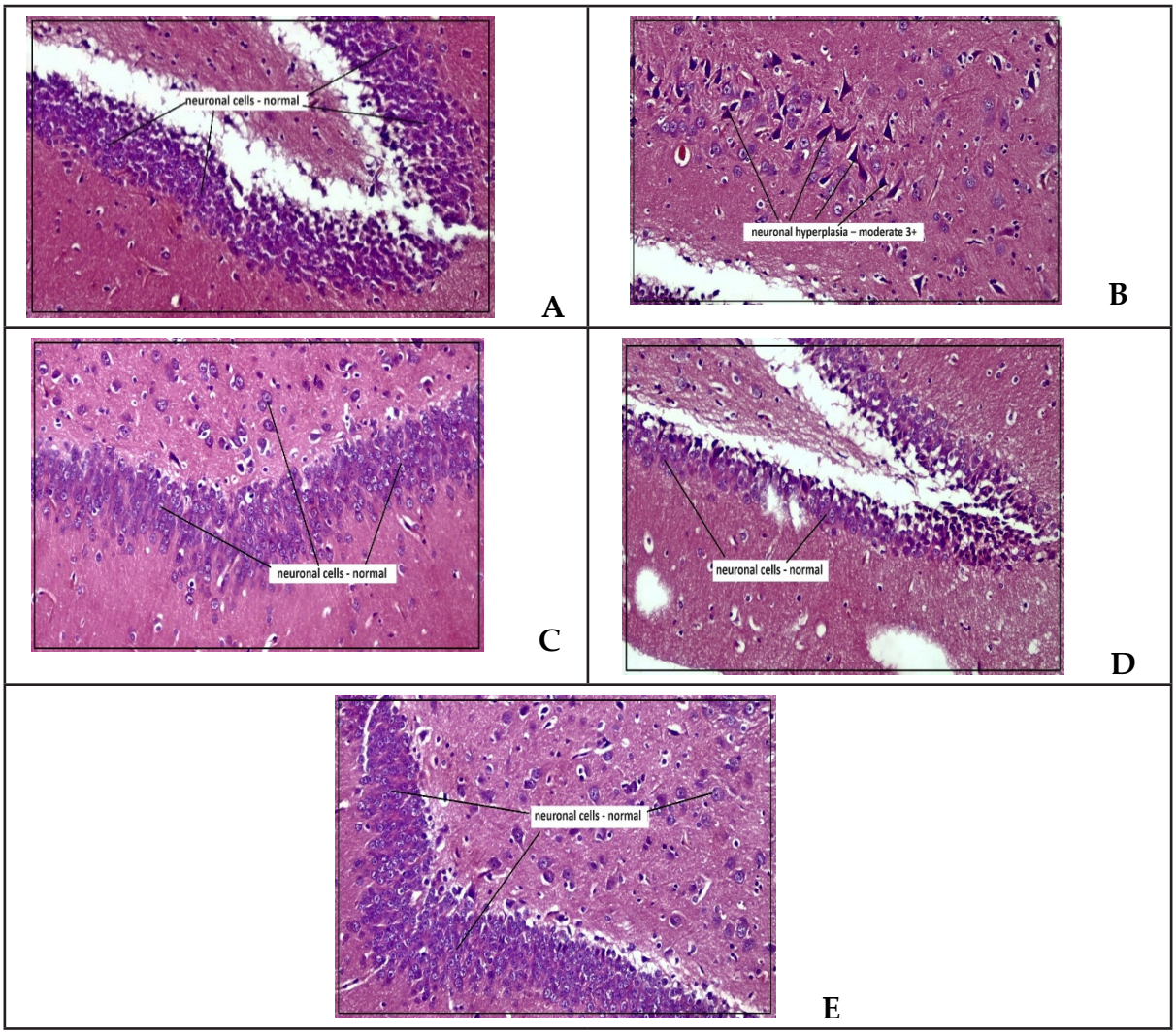

Figure 5: Histopathological evaluation of the hippocampus (H and E stain, ×100). (A) Negative control group showed normal neuronal architecture, while (B) positive control group showed neuronal degeneration and histopathological alterations. (C) Standard-treated group showed preserved neuronal morphology, whereas (D) low-dose and (E) high-dose extract-treated groups showed reduced histopathological alterations compared with the positive control group.

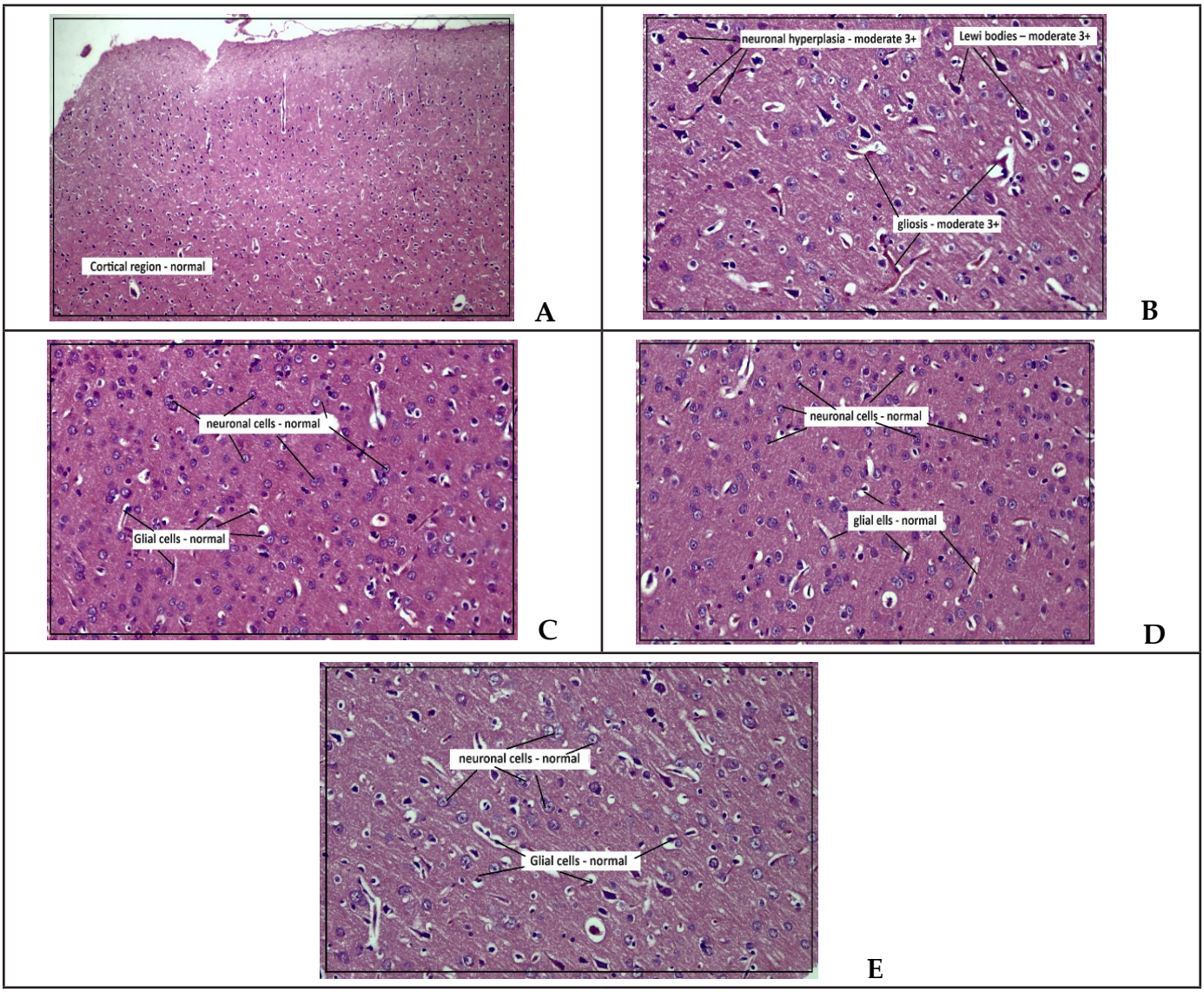

Figure 6: Histopathological evaluation of the cerebral cortex (H and E stain, ×100). (A) Negative control group showed normal cortical neuronal morphology, while (B) positive control group showed gliosis, apoptotic changes, and neuronal degeneration. (C) Standard-treated group showed preserved cortical architecture, whereas (D) low-dose and (E) high-dose extract-treated groups showed improved neuronal morphology with reduced histopathological alterations compared with the positive control group.

immobility duration in a dose-dependent manner, suggesting antidepressant-like activity comparable to the standard-treated group.

In the Actophotometer Test, reserpine-treated animals showed reduced locomotor activity, reflecting behavioural suppression and reduced exploratory behaviour. Administration of avocado seed extract significantly improved locomotor activity compared with the positive control group, indicating improvement in behavioural performance.

In the Elevated Plus Maze Test, reserpine administration decreased open-arm exploration, suggesting anxiety-like behavioural alterations associated with depressive conditions. Treatment with avocado seed extract increased the time spent in the open arms, particularly at the higher dose, indicating improvement in anxiety-related behavioural changes. Overall, the behavioural improvements observed following treatment with ethanolic *Persea americana* seed extract suggest its beneficial effect against reserpine-induced behavioural alterations in rats.

The observed behavioural effects may be attributed to the phytoconstituents identified during preliminary phytochemical screening, including flavonoids, phenolic compounds, tannins, and saponins. Among these, rutin and catechin derivatives are widely recognized for their antioxidant and neuroprotective properties. These compounds may help reduce oxidative neuronal injury and improve neuronal function under depressive conditions.

A significant finding of the present study was the restoration of BDNF levels following treatment with avocado seed extract.

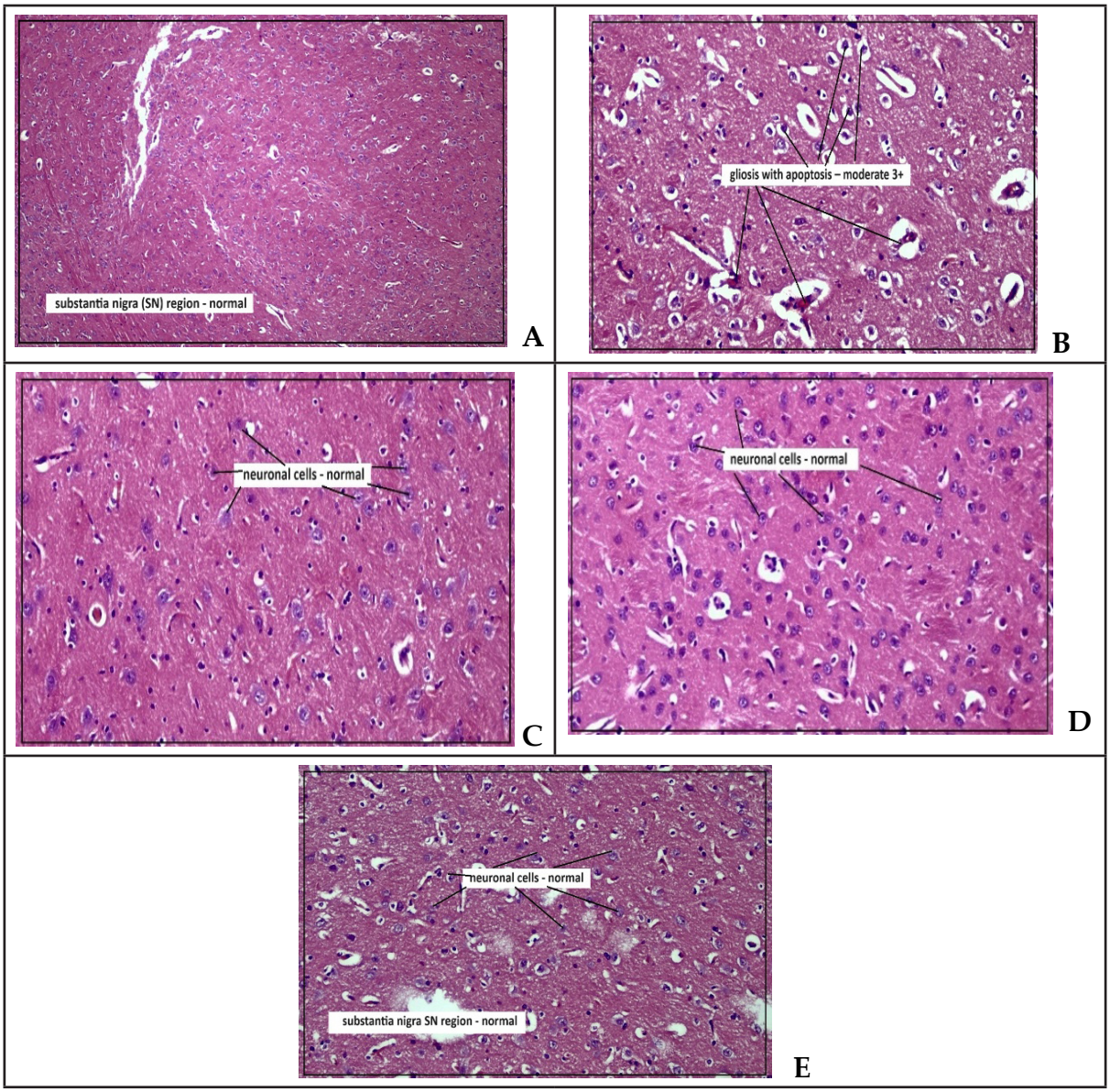

Figure 7: Histopathological evaluation of the substantia nigra (H&E stain, ×100). (A) Negative control group showed normal neuronal morphology, while (B) Positive control group showed neuronal degeneration, gliosis, and apoptotic changes. (C) Standard-treated group showed preserved neuronal architecture, whereas (D) Low-dose and (E) high-dose extract-treated groups showed improved neuronal morphology with reduced histopathological alterations compared with the positive control group.

Table 1: Result of Phytochemical investigation.

Compound Type	Test	Results
Flavonoids	Alkaline reagent, Ferric chloride, Conc. H_2SO_4, Ammonia	+
Phenols	Iodine, Ferric chloride, Lead acetate	+
Tannins	Gelatin, Braymer's, NaOH	+
Saponins	Foam test	+
Alkaloids	Dragendroff, Wagner's, Mayer	+
Terpenoids	Salkowski, Liebermann-Burchard, Sulphur powder	+
Carbohydrates	Molisch, Fehling, Benedict	+

Reduced BDNF expression is closely associated with impaired neuroplasticity and neuronal dysfunction in depressive disorders. The increase BDNF levels observed in the treated groups suggests improvement in neuronal survival and functional recovery. The observed effects may be associated with the presence of polyphenolic compounds known for their neuroprotective potential.

Histopathological examination further supported the protective effect of the extract. Reserpine-treated animals exhibited neuronal degeneration, gliosis, and disruption of normal cellular architecture in the hippocampus, cerebral cortex, and substantia nigra. In contrast, treatment with avocado seed extract preserved neuronal morphology and reduced tissue damage, particularly at the higher dose. These findings correlate with the behavioural and biochemical improvements observed in the study and indicate attenuation of neuronal injury.

The beneficial effects observed in the present study may possibly be associated with the bioactive polyphenolic constituents present in the extract, potentially through antioxidant and neuroprotective mechanisms. Reduction of oxidative stress-mediated neuronal damage could contribute to the improvement in behavioural and histological parameters observed in the present study.

Overall, the findings of this study suggest that ethanolic *Persea americana* seed extract possesses considerable antidepressant-like and neuroprotective potential in reserpine-induced depressive behaviour. However, further investigations involving neurotransmitter analysis, oxidative stress markers, inflammatory mediators, and molecular studies are necessary to elucidate the underlying mechanisms involved for these effects.

CONCLUSION

The present investigation demonstrated that ethanolic *Persea americana* seed extract produced beneficial effects against reserpine-induced depressive alterations in Wistar rats. Administration of the extract improved behavioural responses, increased BDNF levels, and minimized neuronal damage observed in brain tissues. These findings suggest that avocado seed extract possesses promising neuroprotective and antidepressant-like potential. Nevertheless, further experimental studies are necessary to characterize the active phytoconstituents, evaluate long-term safety, and clarify the underlying biological pathways involved in its therapeutic effects.

ABBREVIATIONS

BDNF: Brain-Derived Neurotrophic Factor; **EPM:** Elevated Plus Maze; **H&E:** Hematoxylin and Eosin; **IAEC:** Institutional Animal Ethics Committee; **i.p.:** Intraperitoneal; **OECD:** Organisation for Economic Co-operation and Development; **p.o.:** Per os (oral administration); **PBS:** Phosphate-buffered saline; **SEM:** Standard Error of the Mean; **TST:** Tail Suspension Test.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

This study investigated the antidepressant-like and neuroprotective potential of ethanolic *Persea americana* (Avocado) seed extract against reserpine-induced depressive behaviour in female Wistar rats. Reserpine administration produced significant depressive-like symptoms characterized by increased immobility, reduced locomotor activity, decreased open-arm exploration, reduced BDNF levels, and neuronal degeneration in brain tissues. Treatment with ethanolic avocado seed extract effectively improved behavioural performance, restored BDNF levels, preserved neuronal architecture, and reduced histopathological alterations in the hippocampus, cerebral cortex, and substantia nigra. The protective effects were more pronounced in the high-dose extract-treated group and were comparable to the standard antidepressant amitriptyline. The study demonstrates the antidepressant-like and neuroprotective potential of *Persea americana* seed extract, possibly mediated through flavonoid- and phenolic-mediated antioxidant, neuroprotective, and BDNF-restorative mechanisms.

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