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Research Article

Persea americana peel and seed extracts exert neuroprotective effects against 3-nitropropionic acid induced neurotoxicity in male Wistar rats

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Abstract

The relative frequency of occurrence of neurodegenerative diseases has risen dramatically with greater than ten million people suffering from neurodegenerative diseases annually. This present study was designed to determine the phytochemical composition, antioxidant and neuroprotective effects of *P. americana* extracts upon 3-NP induced oxidative stress and neurodegeneration. Adult male Wistar rats were administered with 3-NP (10 mg/kg b.w. i.p.) and co-treated with *P. americana* peel and seed extracts both at two doses (300 and 600 mg/kg b.w. p.o) for 14 days. The qualitative phytochemical screening revealed the presence of phenols, flavonoids tannin, terpenoids, and reducing sugar in the extracts of fruit parts. The flavonoid content (207.18 ± 10.45 - 447.36 ± 5.52 mg/100 mg) and phenolic content (17.45 ± 2.83 - 53.24 ± 3.42 mg/100 mg) correlated with the antioxidant activities of the fruits. Daily administration of *P. americana* peel and seed reversed the effects of 3-NP induced oxidative stress and neurotoxicity in the rats. Therefore, the findings from this study suggest that the neuroprotective effects P. americana peel and seed extracts against 3-NP-induced neurotoxicity might be attributed to their antioxidant potential.

Keywords: *Persea americana*, Neurotoxicity, Oxidative stress, Antioxidants, Neuroprotection

INTRODUCTION

Neurodegenerative diseases are ubiquitous around the globe, posing a critical healthcare issue and financial burden to the countries (Behl et al., 2021). They are a chronic debilitating group of heterogeneous diseases, which include loss of neuronal function and structure, leading to neuronal cell death or progressive degeneration (Behl et al., 2021). Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's diseases, and Amyotrophic lateral sclerosis (ALS) are the major types of neurodegenerative diseases. These diseases are defined by the intensifying loss of specific neuronal cell populations and are related with protein aggregates (Makkar, 2020). Oxidative stress has been implicated in the pathophysiology and progression of neurodegenerative diseases (Singh et al., 2019). The susceptibility of the brain tissue to oxidative stress is due to its high oxygen consumption, the high polyunsaturated fatty acid content in membranes, relatively low antioxidant levels, and regenerative capacity (Ziu et al., 2017).

Persea americana Mill. is commonly known as Avocado (is also known as alligator pear or butter pear). *P. Americana* is consumed globally, but native from Central America and Mexico. It is a dicotyledonous plant from the *Lauraceae* family (Chen, 2011). Avocado fruit (*P. americana*) is a berry that consists of a large central seed and pericarp, which consist of the skin (exocarp), the edible portion (mesocarp), and the inner layer surrounding the seed (endocarp) (Meyer et al., 2011). The antioxidant potential of avocado has been demonstrated in several researches (Antasionasti et al., 2017; Melgar et al., 2018). Agbor et al. (2011) reported that the antioxidant capacity of avocado is due to its high phenolic and flavonoid contents. Several beneficial medicinal properties of compounds present in the avocado

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seed and peel have been reported, which are related to the elevated levels of phenolic compounds (Wang et al., 2010). In addition, the seeds and peels of avocado also contribute 57% and 38% of the antioxidant capacities of the entire fruit, respectively (Wang et al., 2010).

3-Nitropropionic acid (3-NP) is a neurotoxin produced by numerous fungal species and naturally exists in leguminous plants. 3-NP is responsible for the neurodegeneration caused in humans by the ingestion of sugar cane, corn, and peanuts contaminated by Arthrinium fungi (Shivasharan et al., 2013). The systemic administration of 3-nitropropionic acid (3-NP) is a popular method of inducing neurotoxicity similar to what is observed in Huntington's diseases (Tunez & Santamaria, 2009; Tunez et al., 2010). Oxidative stress is one of the major deleterious events in 3-NP-induced neurodegeneration. 3-NP inhibits the succinate dehydrogenase enzyme of mitochondrial respiratory chain complex II. The mechanism by which 3-NP induces neurodegeneration involves mitochondrial membrane depolarization, energy depletion, oxidative stress, and enhanced mitochondrial-dependent apoptosis (Rosenstock et al., 2004; Barnham et al., 2004). The impaired electron transference in mitochondria resulted in an increased generation of reactive oxygen (ROS) and nitrogen (RNS) species, which plays a prominent role in 3-NP pathogenesis (Chen, 2011).

MATERIALS AND METHOD

Chemicals and Reagents

The 3-Nitropropionic acid was purchased from Sigma Chemical Co., USA. All other reagents used for the study were of analytical grades.

Plant Samples

Avocado (*Persea americana*) fruits were purchased from Ketu Jakande market in Kosofe Local Government, Lagos, Nigeria. The fruits were identified and authenticated by Dr. Kadiri, A., of the Department of Botany, University of Lagos, Nigeria, and the Voucher specimen number (LUH: 8049) was allotted to the *P. americana* fruit.

Preparation of *P. americana* Pulp, Seeds, and Peels

The fruits were washed and sliced into two halves. The peels and the seeds were separated from the pulp. Chopped peels and the seeds were dried in the open air and later oven-dried at 50°C for 30 min and milled into a rough powder. The milled seeds and peels were extracted by the maceration process. The pulp, milled seeds, and peels were soaked in 80% ethanol for 7 days with occasional stirring. The ethanol extracts of the fruit parts were concentrated and allowed to evaporate to dryness at 50°C using a rotary evaporator.

Qualitative Phytochemical Screening of *P. americana*

P. americana pulp, peel, and seed extracts were tested for the presence of the main families of phytochemicals as described by the methods of Harborne, 1973; Trease & Evans, 1989; and Sofowora, 1993. The presence of flavonoids was determined by lead acetate test. A Ferric chloride test was used to evaluate the presence of phenolic compounds and tannins. Wagner's test was used to confirm the presence of alkaloids in the extract. Keller-Kiliani test was used to determine the existence of glycosides in the extracts. Salkowski test was used for sterols and triterpenoids. Froth test was used to detect the presence of saponins. The presence of anthraquinones was determined by Borntrager's test.

Quantitative Analysis of the Phytochemicals

Quantitative analysis of phenolics (Singleton et al., 1999), total flavonoid (Zou et al., 2014), Cardiac Glycoside; Alkaloids (Harborne, 1973, Hinneburg et al., 2006; Obdoni & Ochuko, 2001), Saponin Content (Obdoni & Ochuko, 2001), Tannins (Van-Burden & Robinson, 1981), Terpenoids, Reducing Sugar (Miller, 1972).

Determination of the Antioxidant Activity of *P. americana*

The antioxidant potentials of the *P. americana* extracts were determined using 2, 2-Diphenyl-1-picryl-hydrazyl (DPPH) assay (Bondet et al., 1997), Nitric oxide scavenging activity assay reducing power assay (Green et al., 1982).

Animals and Treatment Schedule

Induction of Neurotoxicity using 3-Nitropropionic Acid:

3-Nitropropionic acid (3-NP) was used to induce neurotoxicity in the rats according to the method of Thangarajan et al., 2016.

Experimental Design

Thirty-six adult male Wistar rats were obtained from the laboratory animal center, College of Medicine, University of Lagos, Lagos, Nigeria. The rats were kept in a group of six in clean and spacious and transparent plastic cages under standard laboratory conditions including a well-aerated room, good lighting, with suitable temperature (30 ± 20C) in a neat environment and at a 12-h light/dark cycle. The treatment of the rats was followed standard environmental and ethical conditions in conformity of National Research Council Guide for care and use of laboratory animals (NRC, 2011). The animals were randomly divided into six (6) groups (n = 6 per group) and acclimatized for 2 weeks, where they had access to standard rat chow and water ad *libitum*.

Grouping

The animals were divided into six groups (n = 6 per group). The grouping was based on the treatment received by the rats according to the following.

I. Group A (control group): rats will receive 0.9% saline i.p. for 14 days.

II. Group B: Animals received 3-NP (10 mg/kg body weight dissolved in 0.9% saline) intraperitoneally for 14 days.

III. Group C: rats were treated with *P. americana* peel extract (300 mg/kg body weight dissolved in water) orally along with 3-NP (10 mg/kg body weight i.p.) for 14 days.

IV. Group D: rats were treated with *P. americana* peel extract (600 mg/kg body weight dissolved in water) orally along with 3-NP (10 mg/kg body weight i.p.) for 14 days.

V. Group E: rats were treated with *P. americana* seed extract (300 mg/kg body weight dissolved in water) orally along with 3-NP (10 mg/kg body weight i.p.) for 14 days.

VI. Group F: rats were treated with *P. americana* seed extract (600 mg/kg body weight dissolved in water) orally along with 3-NP (10 mg/kg body weight i.p.) for 14 days.

Open field test:

The open field test (OFT) apparatus was a circle made of wood, 90 cm in diameter. A 60 W light bulb was fixed at 90–100 cm above the center and provided the only source of illumination in the resting room. Each rat was allowed to occupy the center of the open field, the number of squares crossed (locomotor behavior) and rearing numbers and assisted rearing (exploratory behavior) were measured through direct visual observations for 5 min on day 14.

Elevated Plus Maze:

The maze was prepared by using a customized plus-shaped maze which is elevated 50cm above the ground and consists of two opposite closed arms, two opposite opened arms, and a central square of 5cm sides. The open and closed arm measures 30 × 5 cm with 15 cm high walls that enclosed the closed arms. The rats were placed at the center square of the maze facing one side of the open arms opposite the experimenter. The rats were monitored for 5 min before being returned to their home cages. The maze was cleaned with 70% ethanol to remove all dirt and stain before and after each rat was being tested.

Antioxidant Parameters Assay

Sample Homogenization:

Due to the small sizes of all the test samples, each group was homogenized distinctively with 5 ml 0.1 M phosphate buffer (pH 7.2). The homogenates were placed in a mortar; laboratory sand was added to it (acid-washed sand) and was blended in the mortar with pestle. The resulting homogenate was centrifuged at 2500 rpm for 15 min. The supernatant was decanted and stored at -20°C until further analysis.

Reduced Glutathione (GSH)

The reduced glutathione (GSH) content of the test samples was estimated according to the method described by Sedlak and Lindsay (1968). To the homogenate, 10% TCA was added and centrifuged. 1.0 ml of supernatant was treated with 0.5 ml of Ellman's reagent (19.8 mg of 5, 5-dithiobisnitro benzoic acid (DTNB) in 100 ml of 0.1% sodium nitrate) and 3.0 ml of phosphate buffer (0.2M, pH 8.0). The absorbance was read at 412 nm.

Superoxide Dismutase:

Superoxide Dismutase (SOD) activity was determined by its ability to inhibit the auto-oxidation of epinephrine determined by the increase in absorbance at 480 nm as described by Sun and Zigma (1978). The reaction mixture (3 ml) contained 2.95 ml 0.05 M sodium carbonate buffer (pH 10.2), 0.02 ml of each sample homogenate and 0.03 ml of epinephrine in 0.005 M HCl was used to initiate the reaction. The reference cuvette contained 2.95 ml buffer, 0.03 ml of the substrate (epinephrine), and 0.02 ml of distilled water. Enzyme activity was calculated by measuring the change in absorbance at 480 nm for 5 min.

Determination of Catalase (CAT) Activity:

Catalase activity was determined according to Sinha et al., (1972). It was assayed colorimetrically at 620nm and expressed as μmoles of H_2O_2 consumed/min/mg protein at 25°C. The reaction mixture (1.5 ml) contained 1.0 ml 0.01M phosphate buffer (pH 7.0), 0.1 ml of tissue homogenate and 0.4 ml of 2M H_2O_2. The reaction was stopped by the addition of 2.0 ml of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in a 1:3 ratio).

Lipid peroxidation using Malondialdehyde (MDA):

Malondialdehyde, an index of lipid peroxidation, was determined using the method of Buege and Aust (1978). 1.0 ml of the supernatant was added to 2 ml TCA-TBA-HCl reagent (thiobarbituric acid 0.37%, 0.24N HCl, and 15% TCA) tricarboxylic acid-thiobarbituric acid-hydrochloric acid in the ratio (1:1:1) and the reagent boiled at 100°C for 15 min and allowed to cool. Flocculent materials were removed by centrifuging at 3000 rpm for 10 min. The supernatant was removed and the absorbance read at 532 nm against a blank.

Estimation of nitric oxide concentration in the striatum:

The level of nitric oxide (NO) in the striatum homogenate was measured by assaying total nitrate/nitrite, the stable

products of NO oxidation, as described by Green *et al*. (1982). The nitrite concentration was measured spectrophotometrically using the Griess reagent (1% sulfanilamide in 5% phosphoric acid (sulfanilamide solution and 0.1% N-1-naphthylethylenediamine dihydrochloride in double-distilled water). A standard curve was plotted. The nitrite concentrations in the samples were expressed as nmol/mg protein.

HPLC Analysis (Liquid-Liquid Extraction)

Sample preparation:

One ml of the homogenized sample was mixed with 1 ml of cooled methanol. The mixture was vortex mixed for 1 min, sonicated for 10min using ultrasonic birth then centrifuged for 10 min at 3000 rpm. The supernatant was collected using a Pasteur pipette into another plain sample bottle. The supernatant was filled using an acrodysc syringe filter 0.45 µm (micrometer). The filtrate was concentrated at 50°C using a water bath, the concentrate was reconstituted using 1 ml of 100% ethanol with was ready for HPLC analysis. Rat brain was carefully excised after cervical dislocation and the striatum anatomically removed for determination of catecholamines (dopamine and serotonin).

Statistical Analysis:

The results were expressed as means ± standard deviation (SD) and the statistical difference between treatments tested using one-way analysis of variance (ANOVA) followed by Dennett's multiple comparison tests using Graph Pad prism 8. Differences in p values below 0.05 were considered statistically significant (Tables 1-4).

Table 1. Phytochemical composition of *P. americana* pulp, seed and peel extracts.

Phytochemical	*P. americana* pulp (hexane)	*P. americana* pulp	*P. americana* seed	*P. americana* peel
Saponins	-	+	+	-
Tannins	+	+	+	+
Flavonoids	+	+	+	+
Phenol	+	+	+	+
Terpenoids	+	+	+	+
Cardiac glycoside	+	-	+	+
Steroids	-	-	-	-
Anthraquinone	-	-	-	+
Alkaloids	-	-	+	+
Reducing sugar	+	+	+	+

Table 2. Phytochemical Composition of Avocado (*P. americana)* pulp, seed and peel extracts.

	Alkaloids (%)	Saponins (%)	Tannin (mg/100g)	Cardiac glycoside (%)	Terpenoids (mg/100g)	Phenol (mg/100g)	Total Flavonoid (mg/100g)
P. Americana Pulp extract (hexane)	ND	ND	63.51 ± 2.81	3.60 ± 0.43	84.86 ± 4.95	20.58 ± 1.82	355.79 ±13.22
P. Americana Pulp extract	ND	6.51± 0.22	64.05 ± 2.63	ND	154.75± 1.63	17.45 ± 2.83	207.18 ±10.45
P. Americana Seed extract	3.05±0.23	4.20 ±0.14	54.03 ± 3.68	3.53 ± 0.70	160.99 ± 3.63	53.24 ± 3.42	316.81± 21.32
P. Americana Peel extract	9.2 ± 0.07	ND	61.84 ± 2.15	2.50 ± 0.70	163.28 ± 1.32	29.79 ± 3.12	447.36 ± 5.52

Table 3. Antioxidant activities of *P. americana* extracts.

	DPPH IC50 (mg/mL)	Nitric oxide scavenging IC50 (mg/mL)	Reducing power IC50 (mg/mL)
P. americana pulp extract (hexane)	2.16 ± 0.10	2.93 ± 0.23	9.24 ± 1.35
P. americana pulp extract	2.57 ± 0.07	2.36 ± 0.06	17.39 ± 3.92
P. americana seed extract	2.05 ± 0.11	2.08 ± 0.03	10.88 ± 1.49
P. americana peel extract	1.83 ± 0.02	2.00 ± 0.11	12.95 ± 2.51

Table 4. Correlation coefficients between antioxidants activities and the total phenolic and flavonoid contents of avocado.

Phytochemical Contents	DPPH	Nitric oxide scavenging	Reducing power
Phenolics	0.72	0.70	0.40
Flavonoids	0.95	0.19	0.58

RESULTS

Effect of *P. americana* peel and seed extracts on 3-NP induced changes in body weight mass of control and experimental rats.

Systemic administration 3-NP (10 mg/kg) induction significantly (P<0.05) decreased the bodyweight of the animals as compared to the control group. Further, the loss in body weight was attenuated significantly (P<0.05) upon simultaneous treatment with the extracts of the *P. americana* as compared to 3-NP induced group. No significant difference between body weight changes was observed in the control and the group administered 600mg/kg b.w. seed extract (**Figure 1**).

Effect of *P. americana* peel and seed extracts on 3-NP induced changes in locomotor and exploratory activities on an open field test in control and experimental rats.

The 3-NP induction resulted in a significant (P<0.05) reduction in the number of squares crossed, rearing, and assisted rearing on day 14 in the 3-NP induced group when compared with the control. Simultaneous treatment with the peel and seed extracts significantly (P<0.05) increased these parameters (**Figure 2**).

Effect of *P. americana* peel and seed extracts on the percentage of time spent in the open and closed arm in the elevated plus-maze test

3-NP alone treated rats spent a reduced period in the open arm which was found to be significant ($p < 0.05$) when compared with the control. The treatment with the *P. Americana* peel and seed at 600mg/kg b.w significantly reversed this effect toward the normal (**Figure 3**).

Effect of *P. americana* peel and seed extracts on lipid peroxidation, nitric oxide, and glutathione levels in control and experimental rats.

3-NP induction (10 mg/kg/day; i.p) significantly (p<0.05) increased lipid peroxidation, nitric oxide concentration and depleted glutathione concentration in the brain striatum as compared to control group (**Figures 4-6**). The treatment with *P. americana* peel extract at 300mg/kg and seed extracts (300mg and 600mg/kg) significantly (p<0.05) attenuated lipid peroxidation, normalized nitric oxide concentration when compared with control, and restored levels of antioxidant glutathione as compared to 3-NP alone treated group.

Effect of *P. americana* peel and seed extracts on 3-NP induced abnormalities in antioxidant enzymes in the brain striatum of control and experimental rats.

Treatment with 3-NP caused a significant (p<0.05) decrease in the activities of the antioxidant enzymes superoxide dismutase (SOD), and catalase (CAT) when compared to control animals. Treatment with *P. americana peel and* seed extracts at 600mg/kg b.w. significantly (p<0.05) restored the activities of these antioxidant enzymes when compared with the control animals (**Figures 7-9**).

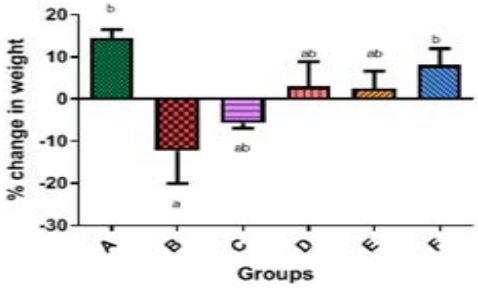

Figure 1. Percentage change in body weight of control and experimental rats. Values are expressed as mean ± standard deviation. [a]P<0.05 when compared with the control group; [b]P<0.05 when compared with 3-NP group (one-way ANOVA followed by Dunnett's multiple comparison test).

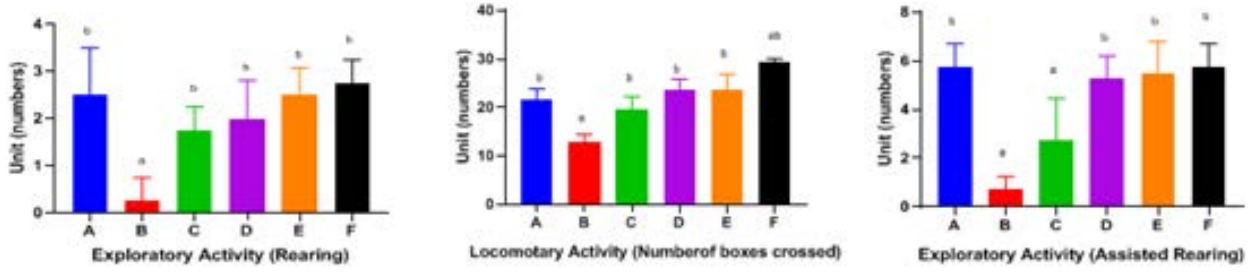

Figure 2. Locomotor (2a) and exploratory activities (2b and 2c) on open field test in control and experimental rats induced with 3-NP (treated and untreated). Values are expressed as mean ± standard deviation. [a]P<0.05 when compared with the control group; [b]P<0.05 when compared with 3-NP group (one-way ANOVA followed by Dunnett's multiple comparison test).

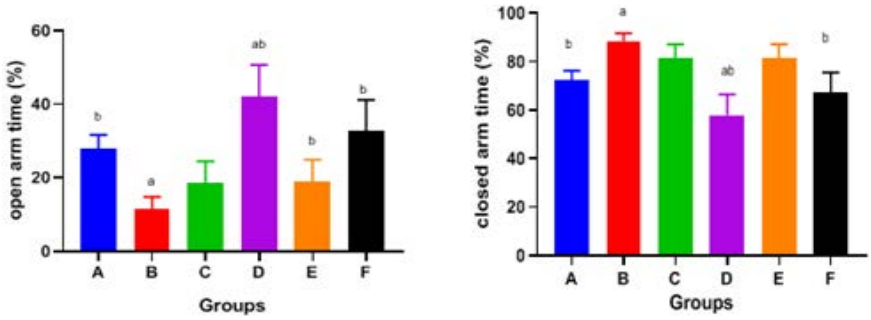

Figure 3. Open arm time and closed arm time in control and experimental rats induced with 3-NP (treated and untreated). Values are expressed as mean ± standard deviation. [a]P<0.05 when compared with the control group; [b]P<0.05 when compared with 3-NP group (one-way ANOVA followed by Dunnett's multiple comparison test).

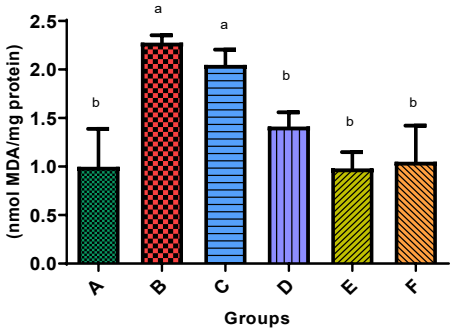

Figure 4. Lipid peroxidation in the brain of control and experimental rats induced with 3-NP (treated and untreated). Values are expressed as mean ± standard deviation. [a]P<0.05 when compared with the control group; [b]P<0.05 when compared with 3-NP group (one-way ANOVA followed by Dunnett's multiple comparison test).

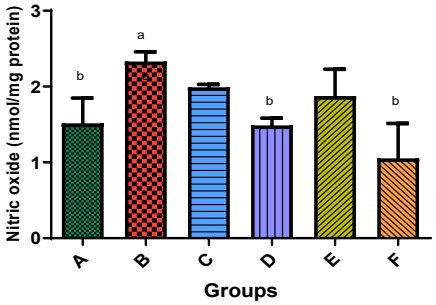

Figure 5. NO level in the brain of control and experimental rats induced with 3-NP (treated and untreated). Values are expressed as mean ± standard deviation. [a]P<0.05 when compared with the control group; [b]P<0.05 when compared with 3-NP group (one-way ANOVA followed by Dunnett's multiple comparison test).

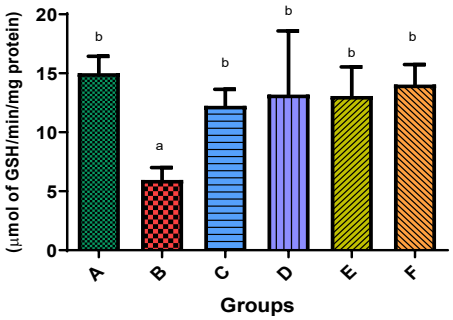

Figure 6. GSH level in the brain of control and experimental rats induced with 3-NP (treated and untreated). Values are expressed as mean ± standard deviation. [a]P<0.05 when compared with the control group; [b]P<0.05 when compared with 3-NP group (one-way ANOVA followed by Dunnett's multiple comparison test).

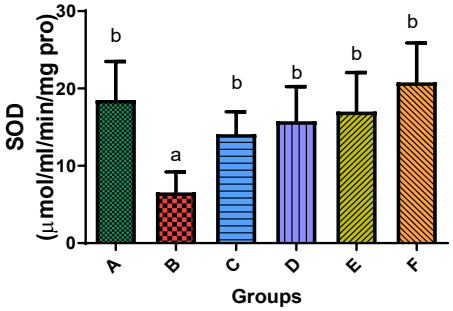

Figure 7. SOD activity in the brain of control and experimental rats induced with 3-NP (treated and untreated). Values are expressed as mean ± standard deviation. [a]P<0.05 when compared with the control group; [b]P<0.05 when compared with 3-NP group (one-way ANOVA followed by Dunnett's multiple comparison test).

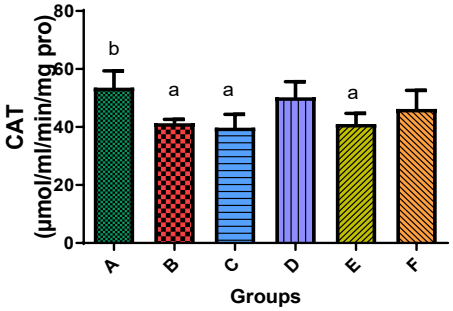

Figure 8. Catalase activity in the striatum of control and experimental rats induced with 3-NP (treated and untreated). Values are expressed as mean ± standard deviation. [a]P<0.05 when compared with control group; [b]P<0.05 when compared with 3-NP group (one-way ANOVA followed by Dunnett's multiple comparison test).

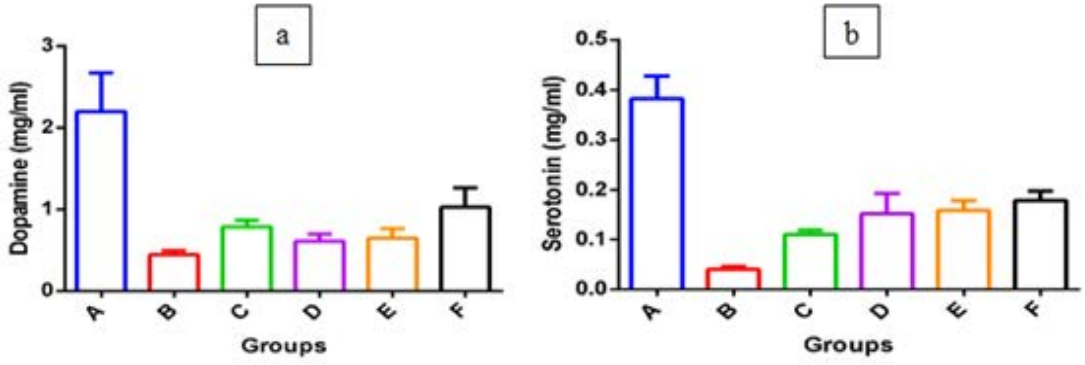

Figure 9. Catecholamine levels (9a: dopamine; 9b: serotonin) in the striatum of control and experimental rats induced with 3-NP (treated and untreated). Values are expressed as mean ± standard deviation. [a]P<0.05 when compared with the control group; [b]P<0.05 when compared with 3-NP group (one-way ANOVA followed by Dennett's multiple comparison test).

Effect of *P. americana* peel and seed extracts on brain striatum catecholamine

Systemic 3-NP (10 mg/kg i.p) treatment caused a significant ($p<0.05$) decrease in levels of catecholamine dopamine (DA) and serotonin (5-HT) in the brain striatum when compared to the control group. Treatment with *P. americana* peel and seed showed a reversal in the decreased level of the catecholamines.

DISCUSSION

The phytochemicals contents such as phenolics, flavonoids, tannins, and terpenoids found in *P. americana* fruit parts were shown to be abundant in this study. This is in line with other researches, which found that the peel, kernel, and pulp of *P. americana* are high in phenolics, which contribute to its antioxidant capacity. *P. americana* has a

high phenolic content and antioxidant activity, according to Wang et al. (2010). There was a link between the fruit's antioxidant capacity and its phenolic and flavonoid contents. Several researchers have found that the antioxidant activity of fruits is related to their phenolic and flavonoid contents (Siriamornpun et al., 2015; Zhang et al., 2010; Zhou & Yu, 2006).

The health-promoting properties of plant-based foods have largely been attributed to their wide range of phytochemicals (Duke, 2013). The presence of flavonoids, alkaloids, terpenoids, tannins, and saponin in the fruits is an indication of the potential health benefit domiciled in *P. americana* fruit. Phytochemicals with antioxidant activity remove free radicals and inhibit oxidative reactions by being oxidized themselves (Yashin et al., 2017). The presence of antioxidant phytochemicals such as phenol, flavonoids, terpenoids and tannins in *P. americana* is a strong indication of the antioxidant potential of these fruits. Natural antioxidants are promising strategies to counteract the undesirable effects of oxidative stress. Oxidative stress has been identified as a major causative factor in the development and progression of several diseases, including cardiovascular and cancer, as well as neurodegenerative diseases (Liguori et al., 2018).

Neurons require a high energy source to maintain ion gradients across the plasma membrane that is critical for the generation of action potentials (James et al., 2005).

3-Nitropropionic acid (3-NP) is a mitochondrial toxin that irreversibly inhibits succinate dehydrogenase, a respiratory chain complex II enzyme, thereby inhibiting the Krebs cycle energy metabolism (Kumar, 2006). The administration of 3-NP induces symptoms like bodyweight loss, cognitive dysfunction, muscular weakness, rigidity and increased brain oxidative stress, the same as observed in Huntington Disease patients.

The treatment of rats with 3-NP produced considerable weight loss, motor, and behavioural abnormalities. These findings are in agreement with earlier reports that observed a variety of neurobehavioral and motor abnormalities in rats after 3-NP administration. The treatment with *Persea americana* peel and seed extracts to 3-NP treated rats prevented or restored the body weights towards the normal indicating protective potential of these extracts against 3-NP induced mitochondrial toxicity.

The administration of 3-NP to the animals is associated with distorted movement and hypoactivity. Treatment of *P. americana* peel and seed extracts to 3-NP treated animals significantly reversed these movement disorders induced by 3-NP, as evidenced by movement analysis and increased locomotor counts.

The impairment of mitochondrial electron transport is a major contributor to the synthesis of reactive oxygen species (ROS) that creates oxidative damages to the phospholipid bilayer (Thangarajan et al., 2016). In addition, it has been reported that a fall of ATPs in 3-NP induced conditions enhance the nitric oxide synthase (NOS) activity (Brouillet et al., 2005), that renders an elevated nitric oxide state and amplifies the respiratory block in mitochondria. The levels of MDA and nitric oxide in the present study were found to be increased in 3-NP exposed animals. These were reversed significantly by the extracts especially in the group administered 600 mg/kg body weigh seed extract.

The cellular antioxidant mechanism protects against oxidative damages by reactive oxygen species during neural pathogenesis (Singh *et al*., 2019). Superoxide dismutase (SOD) and catalase (CAT) and Glutathione (GSH) are major components of the cellular antioxidant defense mechanism. 3-NP administration affects the activities of SOD, CAT, and level of GSH. There were significantly decreased levels of antioxidants (SOD, CAT, and GSH) in the 3-NP treated rat brains. Treatment with extracts from *P. americana* peel and seed to 3-NP treated rats significantly restored antioxidant status and decreased oxidative stress.

It has been reported that the administration of 3-NP resulted in neurochemical imbalance. The 3-NP caused a significant decrease in the levels of catecholamines (dopamine and serotonin) when compared to the control group (Kumar et al., 2010). The decreased level of dopamine in this study is consistent with the previous reports on 3-NP. Previous findings have suggested that biphasic changes in dopamine neurotransmission occur (Cepeda et al., 2013). It stated that in the early stages, the neurotransmission of dopamine increases and leads to hyperkinesia which can be controlled by depletion of dopamine stores.

But on the other hand, neurotransmission of dopamine decreases and leads to hypokinesia in later stages of the disease, which can be controlled by increasing dopamine function (Cepeda et al., 2013). The decreased level of dopamine observed in this study might be a result of the decreased level of dopamine in the 3-NP treated group. The levels of serotonin (5-HT) and its metabolite (5-HIAA) are also found to be decreased in Huntington's diseases (Caraceni et al., 1979). The findings from this study are consistent with the previous reports as observed in the decreased level of serotonin in the 3-NP induced Huntington's diseases.

The striatum contains neuronal activity related to movements, cognition, rewards and the conjunction of both movement and reward. Striatal neurons show activity related to the preparation, initiation and execution of movements (Baez-Mendoza and Schultz, 2013). Hence, striatum was used in this study.

CONCLUSION

The presence of phytochemicals with antioxidant activities in *Persea americana* seed and peel is an indication that the fruit can prevent oxidative stress a major contributor to the pathogenesis of neurodegenerative diseases. Data of the present study indicate that peel and seed extracts of *P. americana* exhibit neuroprotective potential against 3-nitropropionic acid-induced neurotoxicity in male Wistar rats.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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