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Protective Effect of *Annona Squamosa* Fruit Pulp on Motor Responses Following Intra-Medial Forebrain Bundle Injection of 6-Ohda In Rat Model of Parkinson Disease

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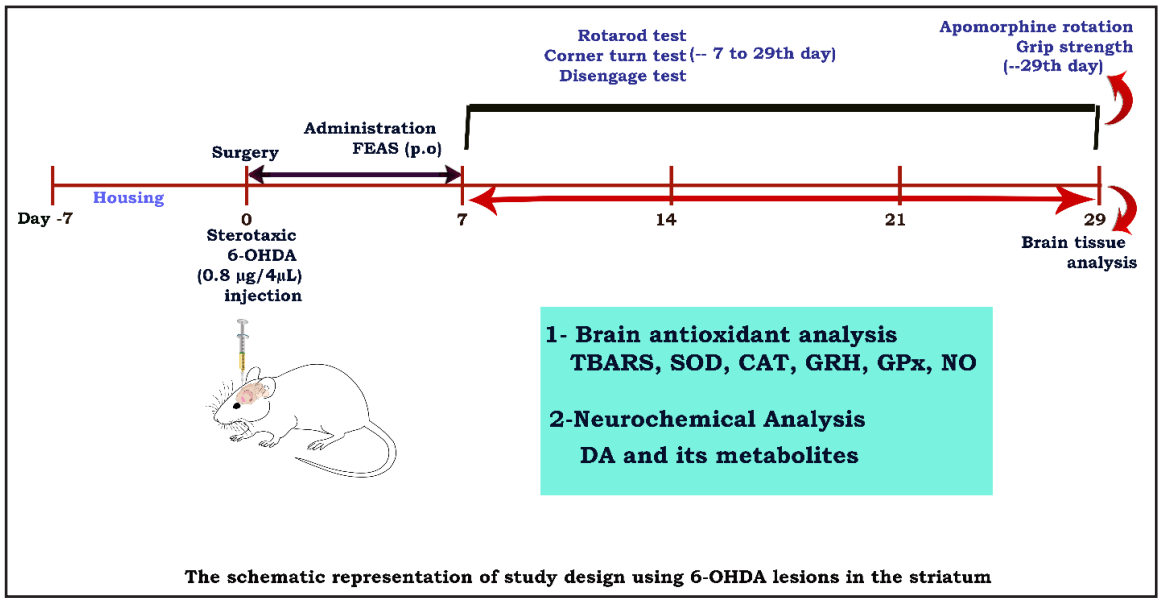
Graphical Abstract



The schematic representation of study design using 6-OHDA lesions in the striatum

Abstract

Objective: The present study investigated the ethanolic extract of fruit pulp of Annona squamosa that ameliorates the 6-OHDAinduced behavioural, biochemical, and neurochemical changes which resemble Parkinson's disease (PD)-like symptoms.

Materials and Methods: Various behavioural and biochemical parameters were carried out to evaluate the activity of ethanolic extract of fruit pulp of Annona squamosa (FEAS) on 6-OHDA treated rats. To determine the therapeutic significance of FEAS on PD, different behavioural tests such as apomorphine rotation, narrow beam maze, rotarod, grip strength, sensorimotor and disengage test and some biochemical tests along with neurochemical findings were

done.**Results:**6-OHDA caused physical and behavioral abnormalities in animals, including abnormal posture, weak grip strength, and motor deficit. Biochemical analysis of brain homogenates in FEAS treated rats showed altered oxidative stress and elevated lipid biomarkers. Neurochemical alterations of the striatum of FEAS treated rats exhibit altered levels of catecholamines. FEAS administered for 07 days significantly enhanced motor function and behaviour tasks and further restored the invitro antioxidant changes in the brain. Furthermore, FEAS-II treatment significantly improved oxidative damage,which is denoted by the alterations in neurochemical changes of rat brain.**Conclusion:**In this research work, FEAS-I & II (200 mg/kg & 400 mg/kg) provided a remarkable neuroprotective impact, which was evidenced by behavioural and biochemical tests. It restored the behavioural and biochemical alterations caused by 6-OHDA and confirmed the strong neuroprotective mechanism of FEAS in 6-OHDA-intoxicated behavior and motor abnormalities.

Keywords:6-hydroxydopamine, Annona squamosa Fruit, Parkinson's disease, Dopamine, Neurological disorder,

Introduction

Parkinson disease (PD) is characterised by the progressive degradation of dopamine (DA) neurons in the basal ganglia that innervative substantia nigra pars compacta (SNc)(1)which causes movement abnormalities, cognitive impairment, and sleep disturbances.(2)

Neurological disorders are currently one of the highest global causes of disability.(3)Parkinson's disease is the most prevalent movement disorder, excluding essential tremor, and the second most prevalent neurodegenerative disease after Alzheimer's disease.[4,5]Global PD prevalence estimates for 2019 exceeded 8.5 million individuals (6). PD affects 1-2 people per 1000 at any given moment. PD prevalence increases with the age, and from that 1% of the population older than 60 years is affected (7). PD is pathologically characterized by the loss of nigrostriatal dopaminergic innervation; however, neurodegeneration does not include only nigral dopaminergic neurons but also cells from other parts of the neural network.

The premotor or prodromal phase of PD may begin 12–14 years prior to diagnosis (8). There is now substantial evidence that the disease may begin in the peripheral autonomic nervous system and/or the olfactory bulb, before spreading to the central nervous system and affecting the lower brainstem structures prior to affecting the substantia nigra.(9)

The majority of degenerative PDs belong to one of the two molecular classes -tauopathies or a-synucleinopathies – because ofthe pathological accumulation of the microtubuleassociated tau protein or the presynaptic protein α-synuclein within vulnerable neurons and frequently glial cells as well. α-synuclein folds into -helical structures through its N-terminus upon interaction with negatively charged lipids, like the phospholipids that form cell membranes upon interaction with negatively charged lipids. (10,11) In PD, α-synuclein adopts an amyloid-like β-sheet-rich structure that is prone to aggregate. Indeed, misfolded α-synuclein is found within Lewy Bodies (LBs) as filaments measuring 5–10 nm in length. Serine 129 phosphorylation, ubiquitination, and C-terminal truncation have all been proposed as mechanisms for the conformational changes that lead to abnormal α-synuclein aggregation. (12,13) Different species of α-synuclein, including unfolded monomers, soluble oligomers, protofibrils, and high molecular weight insoluble fibrils, are therefore present in the PD brain.(14)

Mitochondrial dysfunction is considered as a key factor in both idiopathic and familial PD pathogenesis.(15) Both PINK1 and parkin (PARK2 and PARK6, respectively) are essential components of the pathway that controls the removal of dysfunctional mitochondria, a process known as mitophagy.(16) Loss-of-function mutations in either gene cause defective mitochondrial quality control and autosomal recessive PD.(17, 18)

Mitochondrial complex-I deficiency may play a crucial role in DA cell death caused by energy depletion.[15]Neurodegenerative diseases characterized by proteinopathies, which are abnormal protein accumulation, share proteasomal abnormalities as a common characteristic.(19)

In addition to a decrease in activity, the SNpc of PD brains also exhibits a lower expression of various proteasomal components. In particular, the 20S proteasome α-subunit [20] and other molecules involved in the regular function of the Ubiquitin-proteasome system (UPS), such as PA700 and PA28 (protease activators), are diminished.[21] Genetic studies and the discovery that two of the PARK genes linked to monogenic PD encode proteins involved in UPS function, namely parkin (PARK2; E3 ubiquitin ligase) [17,22]and UCH-L1, provide additional evidence (PARK5; Ubiquitin C-terminal hydrolase).[23]

Similar to the UPS system, numerous lysosomal and autophagy-related components are dysfunctional or variably expressed in PD. In nigral neurons of PD brains, the autophagosome marker LC3-II was elevated, indicating an aggregation of autophagic vacuoles. [24,25] At postmortem examination, vital lysosomal membrane proteins (LAMP1 and LAMP2A) and several heat-shock protein family molecular chaperones (such as hsc70 and hsp35) were found to be reduced. [26,27]

Initially believed to be a secondary phenomenon, there is updated evidence that inflammatory responses can contribute to the pathogenesis of the disease on their own. In early studies with rodent models of PD (6-hydroxydopamineand MPTP), inhibition of microglial activation with minocycline pre- and post-neurotoxic insult significantly reduced DA cell death in the SNpc, suggesting that microglia-induced inflammatory processes may be contributing to the degeneration of these cells. [28,29] Survival of dopamine neurons challenged under oxidative stress conditions.

It is essential to have the relevant disease models in order to comprehend the pathogenesis of PD and develop potential therapies for improved symptom management. The neurotoxin 6-hydroxydopamine (6-OHDA) provides useful animal models of PD by inducing dopaminergic neuronaldegeneration in the substantia nigra pars compacta (SNpc), which leads to a depletion of dopamine level in the striatum.[30-32] To elucidate the underlying neuroprotective mechanism, we studied the effect of Annona squamosa fruit pulp extract (FEAS) on 6-OHDA-induced oxidative stress in rat dopaminergic neurons by examining the post-treatment effects of FEAS therapy on behavioral dysfunction, biochemical alterations, and dopamine level in the rat brain.

Materials and Methods

Extract preparation

The fruits of Annona squamosa Linn. (FEAS) were retrieved from the regionof Namakkal District, Tamil Nadu, India. And obtained authentication from Botanical survey of India, Coimbatore. The fresh fruits were washed, and the pulps were scraped from the seeds using a glovebox under vacuum, lyophilized at -50°C, and ground into a powder. This mixture was then extracted with cold maceration by using 70% ethanol for 48 hours while being continuously stirred, yielding a viscous brownish yellow mass. A small proportion of the extract was subjected to preliminary phytochemical analysis, free radical scavenging activity and the other part was kept for future use in a vacuum desiccator.

Free Radical scavenging activity

The extract FEAS was analysed for free radical scavenging activity such as DPPH radical scavenging, hydroxyl radical scavenging, and superoxide scavenging activities are analysed.[33]

Animals

Twenty-four adults male Wistar rats weighting 230-250 g were housed in a temperature-controlled room (at approximately 25ºC), under 12-h light/dark cycle, with free access to food and water, at 21–22°C and 50–55% humidity. The animal experiments were carried out in ac-

cordance with the approval of Institution animal ethical committee (KMCRET/Ph.D/07/2015-16). According to OECD 423 guidelines, the acute toxicity study was done.

Experimental Design

The experimental rats were divided into the following groups i) Sham operated received saline treatment ii) 6-OHDA lesioned group + saline iii) 6-OHDA lesioned group + Fruit pulp of ethanolic extract of Annona squamosa 200 mg/kg (6-OHDA + FEAS – I) iv) 6-OHDA lesioned group + Fruit pulp of ethanolic extract of Annona squamosa 400 mg/kg (6-OHDA + FEAS – II)

Each group consistedof 4–6 animals. After surgical procedures for the intra-striatal injection of 6-hydroxydopamine (6-OHDA) with saline, the animals were divided into four groups (n = 6 per group). Group I that received saline alone instead of 6-OHDA as a unilateral lesion. Group II received intra-striatal injection of 6-OHDA as a unilateral lesion. Groups III & IV received ethanolic extract of fruit extract of Annona squamosa (FEAS) 200 & 400 mg/kg for 07 days, consecutively after 6-OHDA injection.

0.8 µg 6-OHDA, dissolved in 4 µL physiological saline containing 0.1% ascorbic acid were administrated through Hamilton syringe over 4.50 min; the syringe was left in place for 5 min after injection before being slowly removed. Sham-operated rats injected with saline. The sequence of experiments carried out in this study is graphically outlined in graphical abstract.At the seventh, fifteenth,and twenty-ninth day after surgery, behavioural tests were performed to evaluate the motor abilities of the animals. The animals were sacrificed at 29thday following the beginning of the treatment.

Behavioural assessments:

After receiving a 6-OHDA treatment, the animals' general behaviour was monitored for 4 weeks continuously, including body weight, food and water intake, body temperature, and urine output.[34] All animals underwent baseline measurements of their behavioursin the following behavioural studies (Fig. 1-6).

Apomorphine turning behavior

The evaluation of motor function was carried out by apomorphine-induced rotation test on day 29 after 6-OHDA injection (Fig. 7). Apomorphine hydrochloride (0.5 mg/kg, i.p.) causes rotation inrats. After one-minuteadministration of apomorphine, the rotations were recorded; perfect and complete rotations were counted in a cylindrical container (33 cm diameter and 35 cm height) for 1 h in a noiseless separated room. Net numbers of rotations by turning contralateral & ipsilateral to the side of lesion were noted.[35]

Rotarod

Motor performance was also evaluated with a Rotarod equipment, under the protocol which were previously described by MonvilleC.[36] Before administering 6-OHDA, the first three testing days provided as a training period. The animals were put through a four-trial test using an accelerating protocol that lasted 4 to 40 rpm in 5 minutes and given at least 20 minutesrest between trials. On the 7th, 15th, 21st, and 29thday, using the similar protocol, the latency to fall was then recorded.

Narrow beam maze

Animals were positioned on a narrow beam that was 1 m long and 80 cm above the ground. To ensure that the animal wouldn't be hurt if it fell, a 12 cm thick foam was placed exactly beneath the beam. The animal was set at one end of the beam, while the animal's residence cage was set at the other. A stopwatch was used to measure how long it took the animals to travel from their initial starting positions to the other end. The test was recorded as "timed out" at 2 minutes if animals refused to finish a beam run. The latency to start and total time to cross the beam were both recorded throughout this test.[37]

Grip strength

Forelimb strengths of rats were measured using a grip strength meter.[38] To produce a reliable assessment of holding capability, the animals were pretrained for six training sessions on 1st-day, and the grip strength test was carried out on 29thday. Rat forelimbs were placed on the tension bar as they were positioned facing the grip strength meter's T bar. The rat was gently and steadily dragged away from the T bar by the root of the tail when it grabbed the bar. The grip strength meter was automatically calculated and recorded the force that each animal could produce in grams. Each animal measures were computed. Between measurements, the rats were given 30s to rest.

Sensorimotor test

The corner test assesses the direction pattern of sensorimotor dysfunction. It was initially applied on rats. It has been an established technique for evaluating sensory-motor function which is a valid way to spot and measure sensory and postural asymmetry. [39,40] It offers a quick approach for identifying ipsilateral and contralateral steering deviation.

Disengage test

A tactile stimulus was placed towards the vibrissae, and a stopwatch was used to measure how long it took the rat to turn around and touch the probe with a paw. Paw extension was thus measured for the disengage test.[40]

Biochemical Studies

After behavioral assessment (i.e., 29 days after surgery), the animals were deeply anesthetized. The brains were quickly removed and placed on an ice-cold Petridish. Rat brains will be fixed using a Microwave Fixation System. Striata from both sides are dissected from sham-operated, 6-OHDA-treated, extracts treated group of animals.[41] To eliminate debris, the striatal tissue is homogenized in 250 µl acetonitrile before being centrifuged at 13,000 g for 30 min. A stream of nitrogen is used to evaporate the acetonitrile after the supernatant has been collected and cleaned with heptane. Re-suspending the sample in 75 µl of mobile phase (37.5 mM phosphoric acid, pH 8.5) for the estimation of total protein and lipid peroxidation. The supernatant was again centrifuged at 15000 rpm for 1 h at 4°C. The supernatant obtained was used for further estimation of superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), and lipid peroxidation (TBARS).

TBARS activity

Thiobarbituric acid reactive substances (TBARS) in the homogenate were estimated by the method of Ohkawa*et al.,*[42] The amount of lipid peroxidation was determined by using ε = 1.56 × 105$M^{-1}cm^{-1}$ and expressed as TBARS nmoles/mg of protein.

Superoxide dismutaseactivity

Superoxide dismutase activity was determined based on SOD's potential to stop adrenaline's auto-oxidation to adrenochrome in an alkaline pH. The SOD activity (U/mg of protein) was calculated using the usual plot.[43]

Catalase activity

The Caliborne method was used to analyze the catalase activity.[44] Changes in absorbance were noticed at 240 nm. nM H_2O_2 consumed/min/mg protein was used to measure catalase activity.

GRH activity

Glutathione reductase (GRH) activitywas determined spectrophotometrically as described previously byCarlberg I.[45] The amount of nmol NADPH•min•mg^{-1}protein used to express the enzyme activity.

GPx activity

The dismutation of H_2O_2 at 340 nm was used to measure GPx activity spectrophotometrically using the approach previously described by Wendel A.[46]The enzymatic activity was denoted as nmol NADPH•min•mg^{-1} protein.

Total protein content

Total thiols were assayed on the principle of formation of relatively stable yellow color by sulfhydryl groups of DTNB. Protein content in the samples was determined by the method of Lowry et al.,[47]

Nitric oxide estimation

The level of nitrite in the rat striatal tissue is an indicator of the production of nitric oxide (NO), which was determined according to the method of Kumar et al. (2012).By measuring nitrite, nitrate, and total nitrite indirectly in rat brain extract supernatants that were produced after centrifugation, nitric oxide was detected. The absorbance was then noted at 620 nm and 550 nm, respectively.[48]

Neurochemical estimation

The method described by Patel was used to estimate the levels of brain catecholamines with a little modification.[49]By employing an electrochemical detector and a C18 reverse phase column, HPLC was used to determine the concentrations of catecholamines (DA) and their metabolites (DOPAC, HVA) in the striatum. Based on the standard curve created by employing standards with concentrations of 10-100 ng/ml that were homogenized in a solution containing 0.2 M perchloric acid, the quantities of the neurotransmitter and its metabolites were determined.

Statistical Analysis

The results were expressed as mean ± standard error mean (SEM). Behavior parameters were analyzed by ordinary and repeated measures of one-way ANOVA (analysis of variance), followed by Dunnett's post hoc test, $p < 0.05$ was considered significant throughout the analysis.

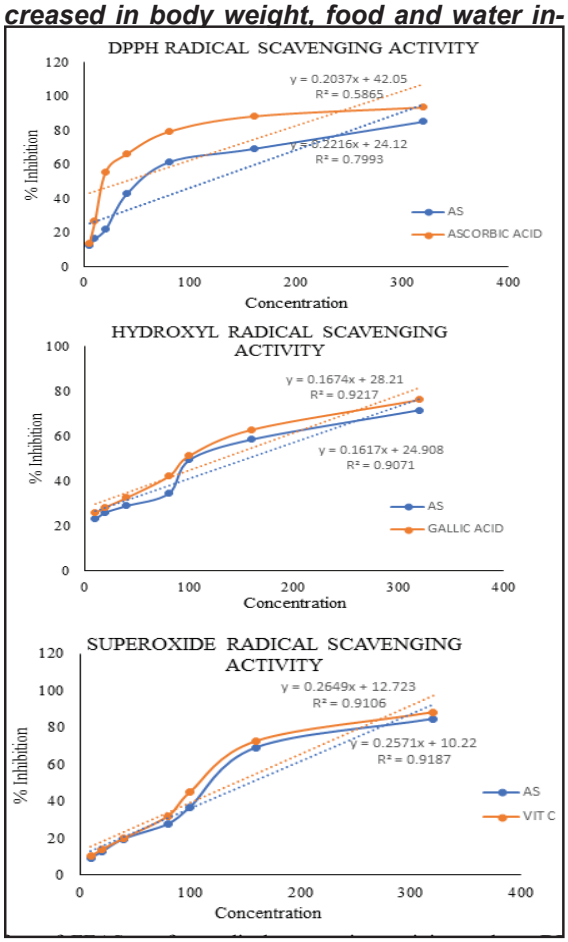
Results and Discussion

Effect of FEAS onpreliminary phytochemical analysis

The extract was subjected to preliminary phytochemical screening. Tests for alkaloids, proteins, steroids, phenols, tannins, flavonoids, gums and mucilage, glycosides and saponins were tested positive using Trease GE and Evans WC methods. And from the radical scavenging activity the IC50 value was found to be 116.78, 155.17, and 140.72 respectively, which shows that the inhibitory concentration of FEAS was higher than that of standards. (Fig.1)

Fig 1. Effect of FEAS on free radical scavenging activity such as DPPH, and superoxide Scavenging activities.

Effect of FEAS on 6-OHDA induced decreased in body weight, food and water intake, body temperature, and urinary output



It was observed that administration of 6-OHDA significantly reduced the body weight, food and water intake, urinary output ($p=0.2080$;

p=0.0223; p=0.0337; p=0.1272) but increased body temperature (p<0.0001) when compared to sham operated groupsrespectively. However, treatment with FEAS-I & II (200mg/kg& 400 mg/kg) started to restore the body weight, food and water intake, and volume of urine output compared to the 6-OHDA treated group. Moreover, treatment with FEAS-II (400 mg/kg/day) significantly and dose-dependently ameliorated the loss in body weight compared to FEAS-I (200 mg/kg/day) treated group (Figs. 2, 3, 4, 5, 6).

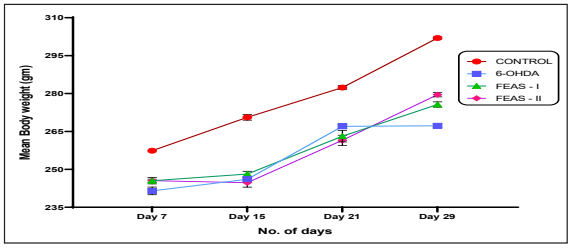


Fig 2. Effect of 6-OHDA and FEAS post-treatment on the body weights (Mean ± SEM) of each group of rats were recorded. Data are shown as Mean ± SEM; n = 06 rats for each group. *p ≤ 0.05, **p ≤ 0.01, **p ≤ 0.001, ***p ≤ 0.0001, as compared with sham groups. Statistical analysis was done by one-way analysis of variance (ANOVA) and Dunnett's post hoc comparison test.

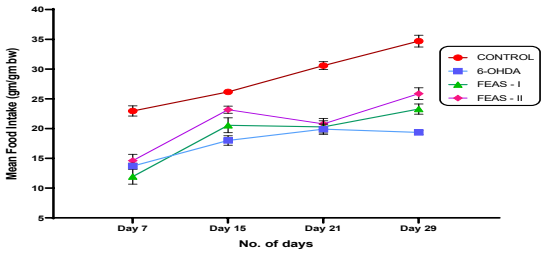


Fig 3. Effect of 6-OHDA and FEAS post-treatment on the food intake (Mean ± SEM) of each group of rats were recorded. Data are shown as Mean ± SEM; n = 06 rats for each group. *p ≤ 0.05, **p ≤ 0.01, **p ≤ 0.001, ***p ≤ 0.0001, as compared with sham groups. Statistical analysis was done by one-way ANOVA and Dunnett's post hoc comparison test.

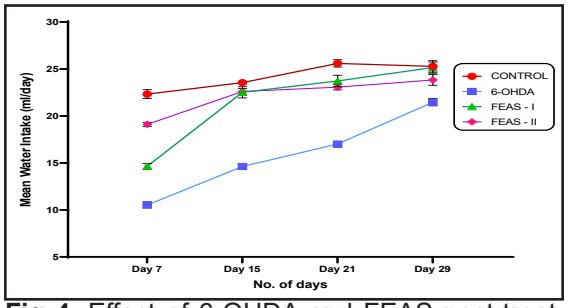


Fig 4. Effect of 6-OHDA and FEAS post-treatment on the water intake (Mean ± SEM) of each group of rats were recorded. Data are shown as Mean ± SEM; n = 06 rats for each group. *p ≤ 0.05, **p ≤ 0.01, **p ≤ 0.001, ***p ≤ 0.0001, as compared with sham groups. Statistical analysis was done by one-way ANOVA and Dunnett's post hoc comparison test.

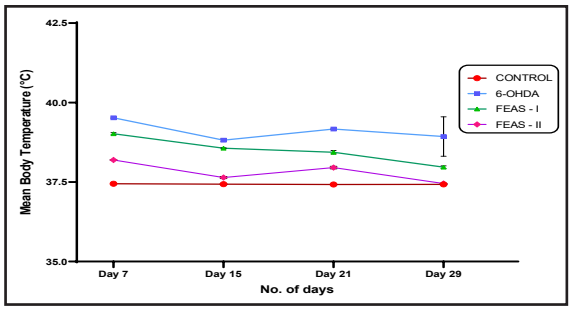


Fig 5. Effect of 6-OHDA and FEAS post-treatment on the body temperature (Mean ± SEM) of each group of rats were recorded. Data are shown as Mean ± SEM; n = 06 rats for each group. *p ≤ 0.05, **p ≤ 0.01, **p ≤ 0.001, ***p ≤ 0.0001, as compared with sham groups. Statistical analysis was done by one-way ANOVA and Dunnett's post hoc comparison test.

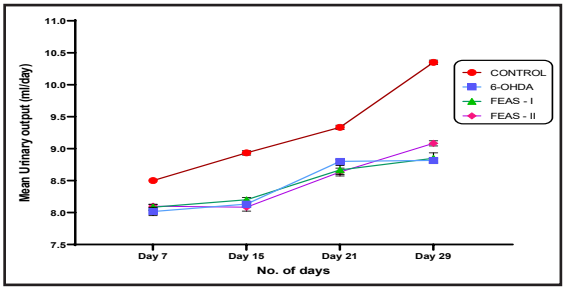


Fig 6. Effect of 6-OHDA and FEAS post-treat-

ment on the urinary output (Mean ± SEM) of each group of rats were recorded. Data are shown as Mean ± SEM; n = 06 rats for each group. *p ≤ 0.05, **p ≤ 0.01, **p ≤ 0.001, ***p ≤ 0.0001, as compared with sham groups. Statistical analysis was done by one-way ANOVA and Dunnett's post hoc comparison test.

Effect of FEAS on 6-OHDA induced changes in apomorphine activity, rotarod and grip strength, and narrow beam walk performance of rats

Apomorphine activityand narrow beam maze (NBM) walk performance was significantly increased on 6-OHDA administration (on the 29thday) as compared to sham operated group ($p<0.001$). Treatment with FEAS-I & II (200 and 400 mg/kg) significantly attenuated the total turns per hour in apomorphine rotation and latency to start and transfer latency in NBM as compared to 6-OHDA group ($p<0.001$). Moreover, concomitant administration of FEAS-II (400 mg/kg/day) significantly reduced the turns start latency and transfer latency as compared to FEAS-I (200 mg/kg/day) treated group (Figs. 7, 8).

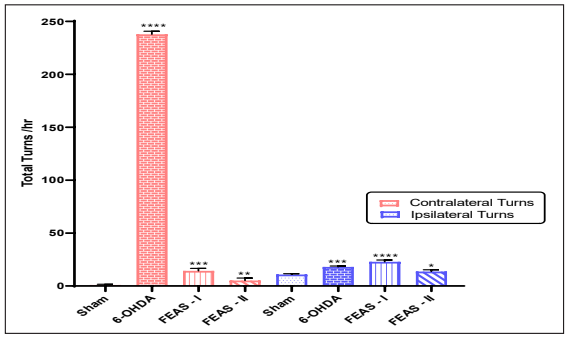


Fig 7. Effect of 6-OHDA and FEAS post-treatment on the apomorphine rotation (Mean ± SEM) of each group of rats were recorded. Data are shown as Mean ± SEM; n = 06 rats for each group. *p ≤ 0.05, **p ≤ 0.01, **p ≤ 0.001, ***p ≤ 0.0001, as compared with sham groups. Statistical analysis was done by one-way ANOVA and Dunnett's post hoc comparison test.

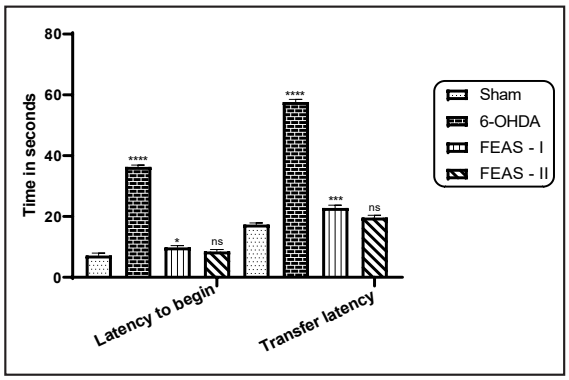


Fig 8. Effect of 6-OHDA and FEAS post-treatment on the activity on narrow beam maze (Mean ± SEM) of each group of rats were recorded. Data are shown as Mean ± SEM; n = 06 rats for each group. *p ≤ 0.05, **p ≤ 0.01, **p ≤ 0.001, ***p ≤ 0.0001, as compared with sham groups. Statistical analysis was done by one-way ANOVA and Dunnett's post hoc comparison test.

Rotarod activityandgrip strength performancewere decreased with 6-OHDA administration (on the 29thday) compared to sham-operated group ($p = 0.004$; $p<0.0001$). Treatment with FEAS-I & II (200 and 400 mg/kg) significantly improved the latency to fall,impaired grip strength, and locomotor activity as compared to 6-OHDA group. Furthermore,continuous administration of FEAS-II (400 mg/kg) extremely attenuated the duration of fall, latency andimpaired grip strength compared to FEAS-I(200 mg/kg)treated group. (Figs. 9, 10).

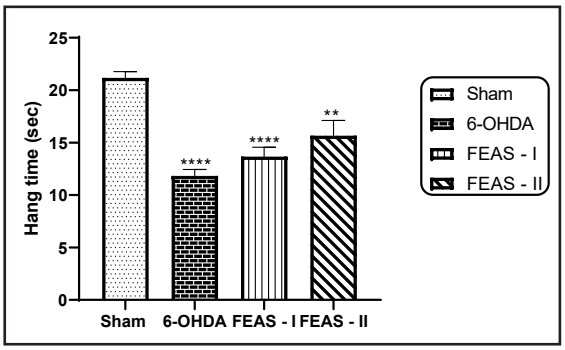


Fig 9. Effect of 6-OHDA and FEAS post-treatment on the rotarod activity (Mean ± SEM) of

each group of rats were recorded. Data are shown as Mean ± SEM; n = 06 rats for each group. *p ≤ 0.05, **p ≤ 0.01, **p ≤ 0.001, ***p ≤ 0.0001, as compared with sham groups. Statistical analysis was done by one-way ANOVA and Dunnett's post hoc comparison test.

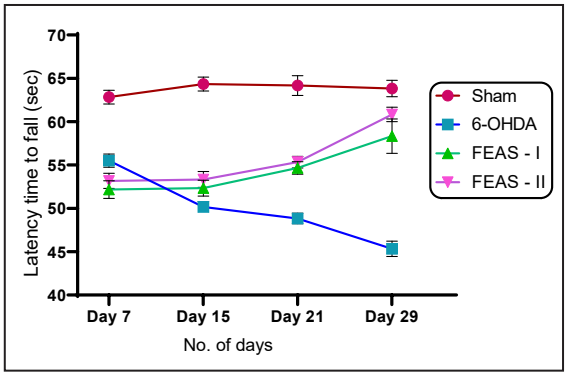


Fig 10. Effect of 6-OHDA and FEAS post-treatment on the grip strength activity (Mean ± SEM) of each group of rats were recorded. Data are shown as Mean ± SEM; n = 06 rats for each group. *p ≤ 0.05, **p ≤ 0.01, **p ≤ 0.001, ***p ≤ 0.0001, as compared with sham groups. Statistical analysis was done by one-way ANOVA and Dunnett's post hoc comparison test.

Effect of FEAS on 6-OHDA induced changes in sensorimotor and disengage activity of rats

In sham-operated rats, the turns on both sides were equal. The total count was calculated by applying the formula, which denotesthe spontaneous turning latency. Thus, the turning latency noted in rats lesioned with 6-OHDA developed a preference for spontaneous turning towards the contralateral side and the latency(0 to 29th day) was significantly increased on 6-OHDA administration as compared to sham operated group (p = 0.0005) as the lesioned rats turns more on contralateral side. Treatment with FEAS-I & II (200 and 400 mg/kg) significantly attenuated the turn latency on the single side.

Duration of forelimb paw extension was increased on 6-OHDA administration (on the 29thday) due to disengage activity compared to sham-operated group (p = 0.0196). Treatment with FEAS-I & II (200 and 400 mg/kg) significantly attenuated the latency of paw extension as compared to 6-OHDA group. (Figs. 11, 12).

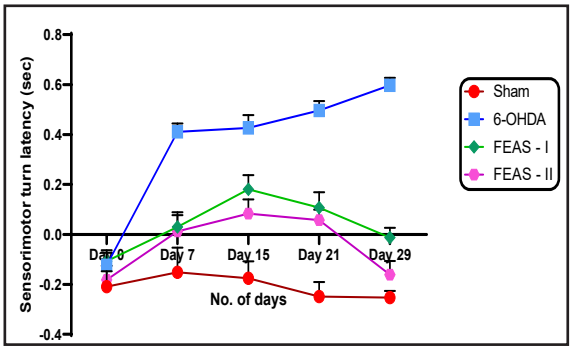


Fig 11. Effect of 6-OHDA and FEAS post-treatment on the sensorimotor turn latency (Mean ± SEM) of each group of rats were recorded. Data are shown as Mean ± SEM; n = 06 rats for each group. *p ≤ 0.05, **p ≤ 0.01, **p ≤ 0.001, ***p ≤ 0.0001, as compared with sham groups. Statistical analysis was done by one-way ANOVA and Dunnett's post hoc comparison test.

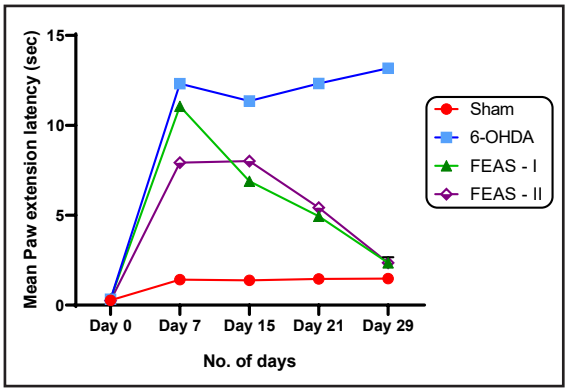


Fig 12. Effect of 6-OHDA and FEAS post-treatment on the forelimb paw extension latency (Mean ± SEM) of each group of rats were recorded. Data are shown as Mean ± SEM; n = 06 rats for each group. *p ≤ 0.05, **p ≤ 0.01, **p ≤ 0.001, ***p ≤ 0.0001, as compared with sham groups. Statistical analysis was done by one-way ANOVA and Dunnett's post hoc comparison test.

Effect of FEAS on 6-OHDA induced changes in brain antioxidant levels in rats

Systemic administration of 6-OHDA had a significantly elevated level of oxidative stress parameters, i.e.,TBARSand nitrite levels in the striatum with fall of SOD, CAT, GRH, GPx levelsas compared to the sham-operated group ($p < 0.0001$). However, treatment with FEAS-I & II (200 and 400 mg/kg) significantly ameliorated the oxidative stress with restoration of SOD, CAT, GRH, GPxlevels as compared to 6-OHDA treated group ($p < 0.0001$). (Fig. 13).

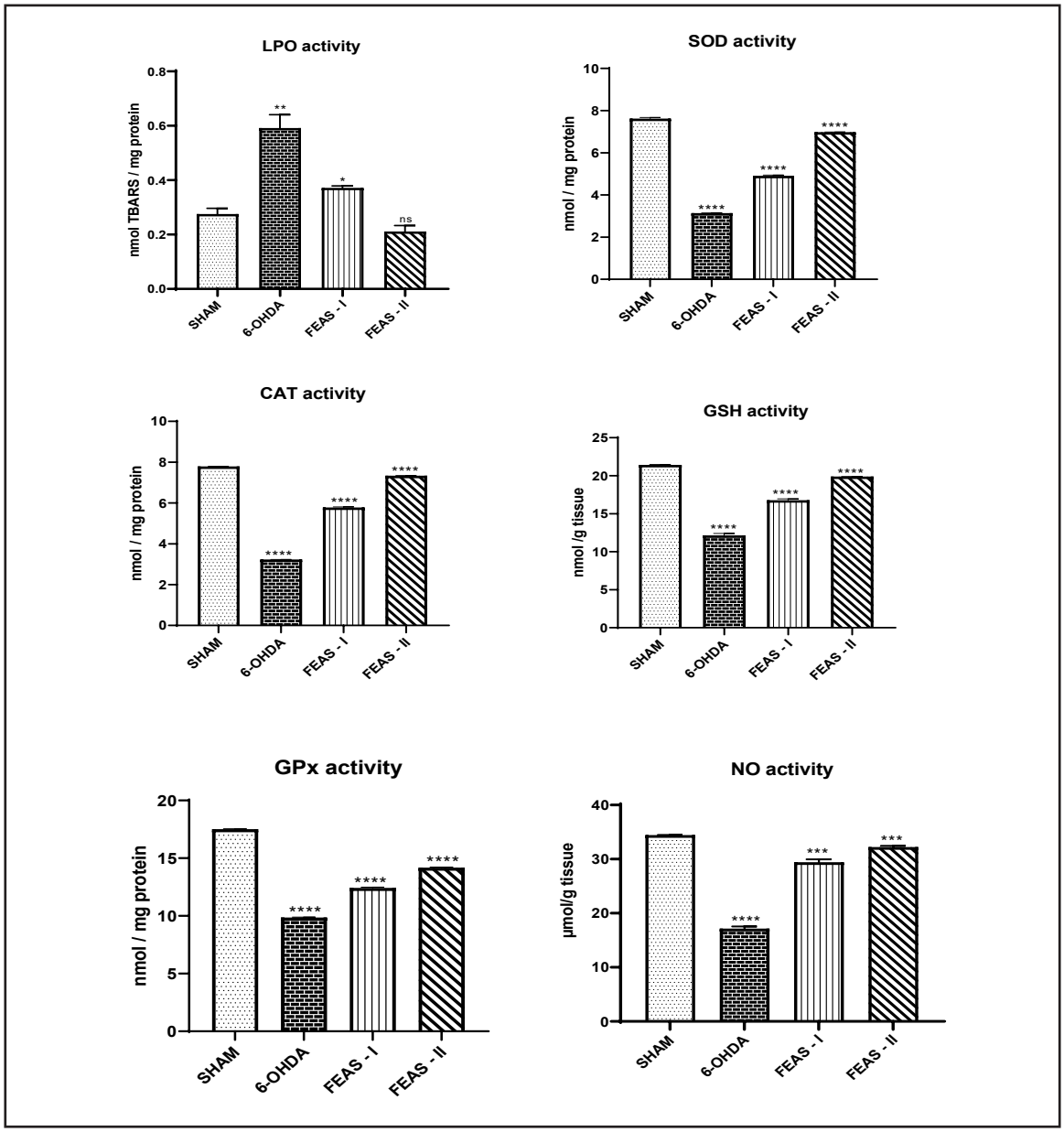
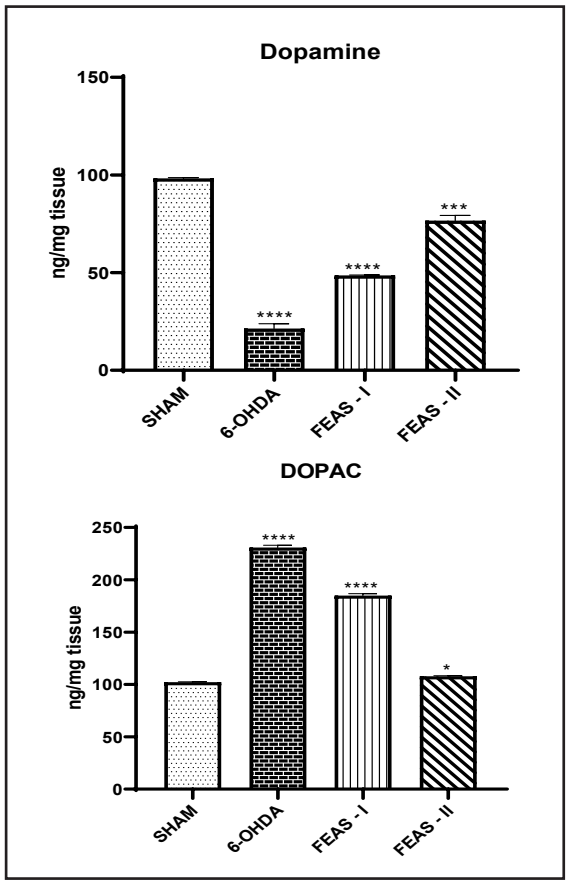


Fig 13. Effect of 6-OHDA and FEAS post-treatment on the brain antioxidant levels (Mean ± SEM) of each group of rats were recorded. Data are shown as Mean ± SEM; n = 06 rats for each group. *p

≤ 0.05, **p ≤ 0.01, **p ≤ 0.001, ***p ≤ 0.0001, as compared with sham groups. Statistical analysis was done by one-way ANOVA and Dunnett's post hoc comparison test.

Effect of FEAS with 6-OHDA on striatal dopamine and its metabolites

The level of catecholamine (dopamine) wasfound to be significantly decreased in the striatum after treatment with 6-OHDA as compared to sham operated group. But its metabolites (DOPAC and HVA) were observed to be elevated in the striatum after treatment with 6-OHDA compared to sham-operated group ($p < 0.0001$). Treatment with FEAS-I & II (200 and 400 mg/kg) significantly and dose dependently ameliorated the alteration in catecholamine and its metabolite level in the striatum as compared to 6-OHDA alone treated group. (Fig. 14)



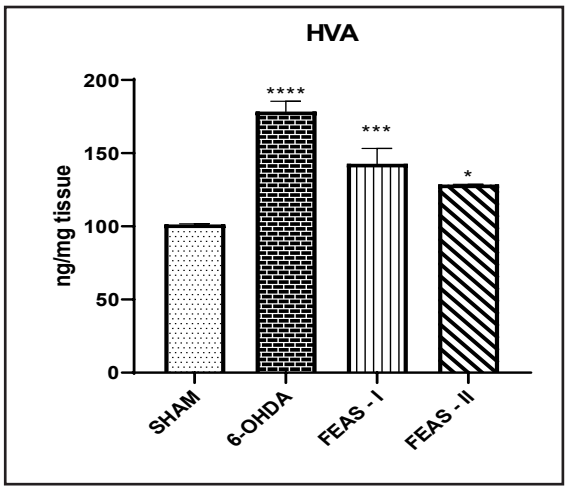


Fig 14. Effect of 6-OHDA and FEAS post-treatment on the neurochemicals (Mean ± SEM) of each group of rats were recorded. Data are shown as Mean ± SEM; n = 06 rats for each group. *p ≤ 0.05, **p ≤ 0.01, **p ≤ 0.001, ***p ≤ 0.0001, as compared with sham groups. Statistical analysis was done by one-way analysis of variance (ANOVA) and Dunnett's post hoc comparison test.

An investigation using UPLC-ESI-MS/MS sheds new light on the phenolic composition of Annona squamosa fruit pulp. And found a total of 16, 15 and 13 free, bound, and esterified phenolic compounds, respectively, in the extracts of Annona squamosa Linn.fruit pulp. From Fig.1, it is also confirmed that the IC50 value of the extract was found to be higher against DPPH, hydroxyl, and superoxide radical scavenging activity.

When 6-OHDA injected directly into the hypothalamus, it had a profound effect on behavioral studies even when injected in a volume smaller. But this study demonstrates the effect of Annona squamosa Linn. fruit pulp in 6-OHDA induced Parkinson disease, and it is confirmed from the behavioral analysis, biochemical and neurochemical analysis.

Treatment with FEAS-I & II (200 and 400 mg/kg) significantly attenuated the 6-OHDA induced motor deficit, oxidativedamage, neuroinflammation and alterations in striatal neurotransmitter

levels in rats. A single intranigral administration of 6-OHDA to experimental rat'scausePD like symptoms due to its selectivity for striatal dopaminergic neurons, serve as widely accepted experimental model of PD. Through the early onset of hyperkinetic movements followed by hypokinesia, it causes chronic motor deficit and replicates many characteristics of PD patients. [49] Similarly, in the present study treatment with 6-OHDA produces stable motor deficit as confirmed by increase in transfer latency in narrow beam walk, loss of grip strength, decrease in fall of time in rotarod indicate the motor impairment and dysfunctioning of striatal nuclei.

Additionally, earlier research suggested that the main pathophysiological causes of PD were oxidative stress, mitochondrial dysfunction, excitotoxicity, and neuroinflammation. Oxidative stress in neurons is further facilitated by faulty mitochondrial action and low ATP levels. This further contributes to excessive Ca^{2+} discharge via NMDA. Literature of this sort demonstrates that 6-OHDA injection considerably retards striatal cholinergic and dopaminergic neurons, which lowers catecholamine levels (dopamine) and increased the level of DOPAC and HVA inthe striatum nuclei.[50] This study found that acute 6-OHDA injection significantly increased levels of lipid peroxidation (LPO) and nitrite while depleting antioxidants like SOD, CAT, GSH, and GPx suggested that oxidative stress may play a role in PD. [51]

CONCLUSION

The results of the current studyindicate that FEAS-I & II (200, 400 mg/kg) corrected the behavioral, biochemical, neuroinflammatory, and chemical deficits brought on by 6-OHDA. FEAS-I & II have been shown to have neuroprotective effects, and these effects are related to their antioxidant and anti-inflammatory properties as well as their ability to modify neurotransmitter levels in the basal ganglia circuit. Also, it is confirmed that FEAS-II (400 mg/kg) is proved to have the therapeutic effect for the treatment of Parkinson's disease (PD), although more research is needed to delve into obscure targets.

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