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Research Article

Effect of Sesamol and Folic Acid on Behavioural Activity and Antioxidant Profile of Rats Induced With 6-Hydroxy Dopamine

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ABSTRACT
Parkinson's disease (PD), a neurodegenerative disorder, is caused due to the degeneration of dopaminergic neurons present in substantia nigra. Oxidative stress due to the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) within the nerve is one of the main etiologies of PD, which breaks down the neuronal integrity and threatens the neuronal survival. The deficiency of dietary folic acid also causes PD. This was experimented in male wistar rats. They were segregated into five groups (6 rats in each group: Group1-control; Group 2 – lesion; Group 3-lesion+sesamol; Group 4- lesion +sesamol+folic acid; and Group 5- lesion + L-Dopa). For lesion, the rats were induced once with 6-hydroxydopamine (10µg/2µl in 0.1% ascorbic acid-saline) by intrastriatal administration. After three weeks, each group was treated with Sesamol(SA), folic acid (FA) and L-Dopa. The behavioural tests, such as apomorphin-induced rotational test, grip test, ladder climbing test and swing and climb test were performed. The activity of Superoxide dismutase, Catalase, Glutathione peroxidiase and Glutathione reductase, and the levels of Glutathione, Vitamin-C, Vitamin- E, TBARS and Nitric oxide were estimated in the brain tissue. Disability was noted in the behaviour of rats induced with PD, and it was recovered by administering SA and FA. The activities and the levels of biochemical parameters were brought to near normal by administering SA and FA together. This study indicates that the combination of SA and FA is useful in treating PD and further investigation is required to prove the same.

Keywords: Sesamol, Folic acid, 6-Hydroxy dopamine, neurodegenaration, Behavioural study, Antioxidant

INTRODUCTION

Parkinson's disease (PD) is one of most prevalent neurodegenerative disorders. The prevalence of PD is being estimated approximately among 1% of sexagenarians, and it increases to 4% among octogenarians[1]. PD is the degeneration of dopaminergic neurons (DAneuron) present in substantia nigra pars compacta due to neuroinflammation, oxidative stress, accumulation of proteins (lewy bodies) and mitochondrial dysfunction[2,3]. Degeneration of DAneuron causes rigidity and disability in movement, which results in cognitive, behavioural problems and dementia. Symptoms of PD appear only after the depletion of 80–85% of nigral neurons. Striatal administration of 6- Hydroxydopamine (6-OHDA) was used as a Parkinsonian model, and it was considered as a tool for a model on nigral degeneration[4].

Sesamol (SA) is a natural organic compound present in sesame oil extracted from *Sesamum indicum* that belongs to Pedaliaceae family. Numerous wild relative species are found in Africa and a smaller number in India. *S. indicum* is widely cultivated in tropical regions around the world for its edible seeds. SA is a potent antioxidant[5] and inhibits UV- and Fe^{3+}/ascorbate-induced lipid peroxidation in the rat brain[6]. It has been demonstrated to inhibit several steps in the generation of neoplasia and mutagenesis[7]. SA has a neuroprotective effect on glioma-induced rats[8]. Hirose M et al. (1990) has linked the deficiency of folic acid (FA) and increased homocystine levels with various neurodegenerative conditions, including stroke, Alzheimer's disease and PD[9]. Dietary FA deficiency endangers dopaminergic neurons in models of PD induced with MPTP[10].

Therefore, we planned to assess the effect of SA in combination with FA on 6-OHDA-induced rats. This work focuses on the antioxidant defence system and the behaviour of rats induced with PD.

MATERIALS AND METHODS

Chemicals: 6-Hydroxydopamine, SA, L-Dopa, Apomorphin, FA and ascorbic acid were purchased from Sigma-Aldrich. All the other chemicals used for the study were of analytical grade.

Experimental protocol: Male Wister rats of weight 200–250 g were purchased and maintained at 25±2ºC in 12 h light/dark cycle with free access to food and water. The animal protocol was approved by the Institutional Animal Ethical Committee of Saveetha University (SU/BRULAC/RD/004/2013).

The rats were segregated into five groups, 6 rats in each group. Group 1: (control) The rats were treated normally for 45 days. Group 2: (Lesion) The rats were infused with

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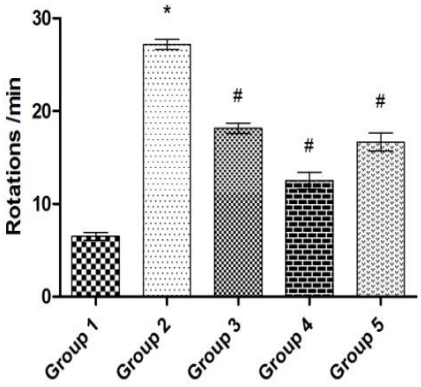

Figure: 1 Apomorphin induced rotation test: Group1-Control, Group2-Lesion, Group3-Lesion+SA, Group4-Lesion +SA+FA and Group5- Lesion +L-Dopa. The Data are expressed as Mean±S.E.M(n=6), *p<0.001 Lesion vs Control and #p<0.001 SA, SA+FA, L-Dopa vs Lesion.

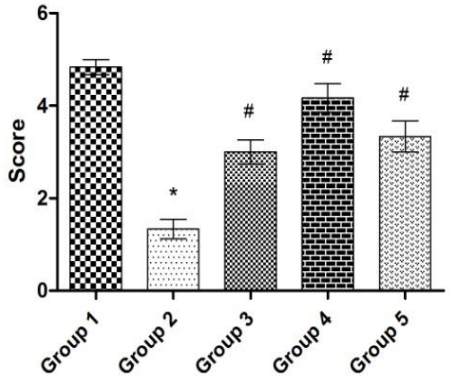

Figure2: Grip Test: Group1-Control, Group2-Lesion, Group3-Lesion+SA, Group4-Lesion +SA+FA and Group5- Lesion +L-Dopa. The Data are expressed as Mean±S.E.M(n=6), *p<0.001Lesion vs Control and #p<0.001 SA, SA+FA, L-Dopa vs Lesion.

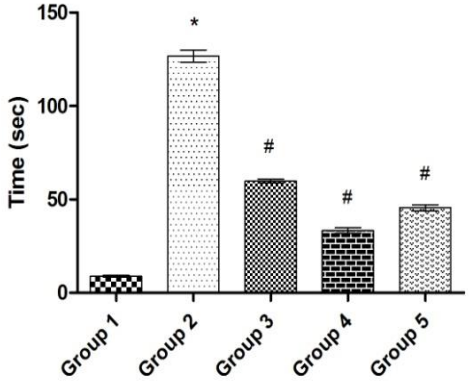

Figure: 3 Ladder Climbing test: Group1-Control, Group2-Lesion, Group3-Lesion+SA, Group4-Lesion +SA+FA and Group5- Lesion +L-Dopa. The Data are expressed as Mean±S.E.M(n=6), *p<0.001 Lesion vs Control and #p<0.001 SA, SA+FA, L-Dopa vs Lesion.

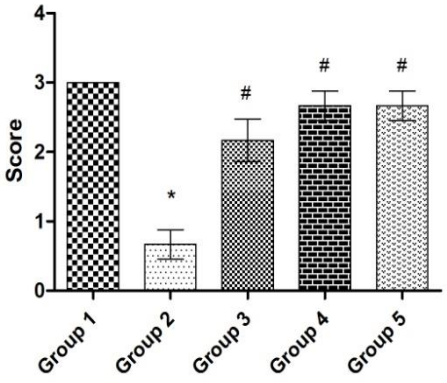

Figure: 4 Swing and Climb test: Group1-Control, Group2-Lesion, Group3-Lesion+SA, Group4-Lesion +SA+FA and Group5- Lesion +L-Dopa. The Data are expressed as Mean±S.E.M(n=6), *p<0.001 Lesion vs Control and #p<0.001 SA, SA+FA, L-Dopa vs Lesion.

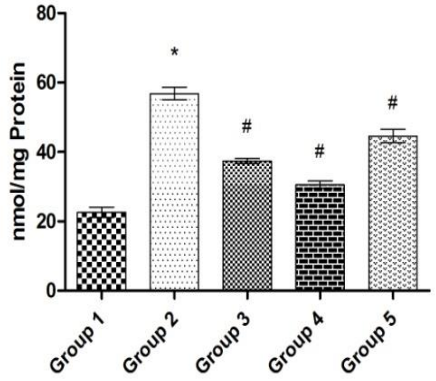

Figure: 5 TBARS Content in Brain: Group1-Control, Group2-Lesion, Group3-Lesion+SA, Group4-Lesion +SA+FA and Group5- Lesion +L-Dopa. The Data are expressed as Mean±S.E.M(n=6), *p<0.001 Lesion vs Control and #p<0.001 SA, SA+FA,L-Dopa vs Lesion.

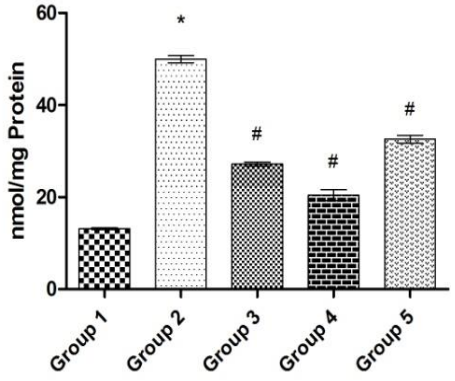

Figure: 6 Nitrite content in Brain: Group1-Control, Group2-Lesion, Group3-Lesion+SA, Group4-Lesion +SA+FA and Group5- Lesion +L-Dopa. The Data are expressed as Mean±S.E.M(n=6), *p<0.001 Lesion vs Control and #p<0.001SA, SA+FA, L-Dopa vs Lesion.

6-hydroxydopamine (10µg/2µl in 0.1% ascorbic acid-saline) in right striatum once for the development of PD and maintained for 45 days. Group 3: (Lesion+ SA) The rats were infused with 6-hydroxydopamine (10µg/2µl in 0.1% ascorbic acid-saline) in right striatum once. Then they were maintained for the development of PD for 21 days. On day 22, SA

Table 1: Enzymatic Antioxidant activity in Brain

Enzymes	Group 1 (control)	Group 2 (Lesion)	Group 3 (Lesion+SA)	Group 4 (Lesion+SA+ FA)	Group 5 (Lesion+L-Dopa)
SOD(µmol epinephrine oxidize/min/mg protein	3.22±0.05	1.73±0.05*	2.49±0.01 $^{\#}$	2.80±0.03 $^{\#}$	2.33±0.01 $^{\#}$
Catalase (µmol H_2O_2consumed/min/mg Protein)	3.50±0.02	1.93±0.08*	2.40±0.11$^{\#\#\#}$	2.76±0.08$^{\#}$	2.26±0.08^{NS}
GPx (nmol NADPH oxidize/min/mg Protein)	516.70±8.96	221.43±1.10*	349.20±6.46$^{\#}$	371.86±1.88$^{\#}$	320.50±7.11$^{\#}$
GRx (nmol NADPH oxidize/min/mg Protein)	220.50±0.72	104.63±1.80*	152.83±1.92$^{\#}$	171.20±1.70$^{\#}$	131.13±4.20$^{\#}$

*The value are expressed in mean ±S.E.M, *p<0.001 Lesion vs Control and #p<0.001, ###p<0.05 SA, SA+FA, L-Dopa vs Lesion. NS – non-significant*

Table 2: Non-Enzymatic Antioxidant levels in Brain

Antioxidant	Group 1 (control)	Group 2 (Lesion)	Group 3 (Lesion+SA)	Group 4 (Lesion+SA+ FA)	Group 5 (Lesion+L-Dopa)
GSH (µmol /mg protein)	1.63±0.08	0.80±0.05*	1.30±0.11 $^{\#\#}$	1.93±0.08 $^{\#}$	0.90±0.06 ^{NS}
Vitamin-C (ng/mg Protein)	335.89±4.60	180.90±8.58*	252.77±5.25$^{\#}$	274.35±6.43$^{\#}$	223.47±6.33$^{\#}$
Vitamin-E (ng/mg Protein)	251.98±5.04	110.83±6.42*	222.75±10.23$^{\#}$	245.35±8.51$^{\#}$	161.98±3.23$^{\#}$

*The value are expressed in mean ±S.E.M, *p<0.001 Lesion vs Control and #p<0.001, ##p<0.01, SA, SA+FA, L-Dopa vs Lesion. NS – non-significant*

(30mg/kg body weight) dissolved in saline was given intra peritoneally (i.p) for next 24 days. Group 4: (Lesion + SA+ FA) The rats were infused with 6-hydroxydopamine (10µg/2µl in 0.1% ascorbic acid-saline) in right striatum once. Then, they were maintained for the development of PD for 21 days. On day 22, SA (30mg/kg body weight (i.p)) and FA (5mg/kg body weight (orally)) were given for the next 24 days. Group 5: (Lesion+ L-dopa) The rats were infused with 6-hydroxydopamine (10µg/2µl in 0.1% ascorbic acid-saline) in right striatum once, and then they were maintained for the development of PD for 21 days. On day 22, L-dopa (100mg/kg) was given orally for the next 24 days.

Intra striatal administration of 6-OHDA: The rats were anesthetized intra peritoneally (i.p.) and fixed to a stereotaxic apparatus. A Hamilton syringe was attached to the apparatus with an infusion pump. The skin on the head was removed to expose skull, and the regions were located according to rat brain atlas (antero-posterior 0.5 mm, lateral 2.5 mm, dorso-ventral 4.5mm relative to bregma and ventral from dura) to make a hole. At the rate of 1µl/min, 2µl of 6-hydroxydopamine (10µg/2µl in 0.1% ascorbic acid) was injected intrastriatally.

Post operative care: Recovery from anaesthesia took approximately 4–5 hrs. The rats were kept in a well-ventilated room in individual cages to gain full consciousness; they were then housed together in groups of 2 animals per cage. For the following 2 days, 0.89 ml of Ibuprofen was added to 100 ml of water in drinking bottles to serve as a post surgery analgesic. Nutrient and water were kept inside the cages for the first week, leaving animals to easy access, without physical trauma due to overhead injury. The rats were then treated as normal, that is, food, water and the bedding of the cages were changed per week as usual.

Behaviour study: The development of PD was indicated by apomorphin-induced rotational test[11]. The test was performed after 3 weeks to confirm the disease development. After treatment, the behavioural tests, such as Apomorphin induced rotational test, Grip test, Ladder climbing test and Swing and climb test were performed.

Apomorphin-induced rotational test: The animals were administrated with apomorphin (0.5mg/kg body weight) subcutaneously to monitor contra lateral rotation. The rotation per minute was calculated. The rats were monitored for contralateral rotation before and after surgery to confirm the lesions and the recuperation of the disease, respectively[11].

Grip test: Grip strength was performed according to Moran PM et al. (1995) with some modification[12]. The apparatus consists of a rod and a string at 30 cm and 20 cm height, respectively, and pulled out between two vertical supports. The rats were allowed to hang onto the string first, and they were evaluated according to the scale given below: 0- fall off, 1- hangs on to the string by two fore paws, 2- hangs on to the string by both fore paw and hind limbs, 3-hangs on to the string with tail wrapped, 4-trying to climb on to the rod and 5- reaches the rod and escapes. The highest of three successive trials was taken for each animal.

Ladder climbing test: The ladder climbing test was done to check catalepsy of the rats. The ladder with 1 meter length was inclined at 45º. The rats were allowed to climb on the ladder and the time taken for the rats to reach the top was counted.

Swing and climb test: Swing and climb tests were performed by holding the rats upside down[13]. The rats were lifted by holding their tail from the ground of about 5 inches and maintained for 1 minute. The score was given to the rats according to their activity. 0- not swinging, 1- When the rat swings, 2- when they turn themselves and

hold their tail and 3- when they climb on to the hand. According to the score, the rats were evaluated.

Biochemical analysis: The rats were sacrificed; whole brains were removed immediately for biochemical analysis. The brain was homogenised with 0.1M phosphate buffer, pH 7.4 for 10 minutes at 3000 rpm. The supernatant was used for the biochemical estimation. Enzymatic antioxidants, such as Superoxide dismutase[14], Catalase[15], Glutathion Peroxidase[16] and Glutathion Reductase[17] and non-enzymatic antioxidant like Glutathion[18], Vitamin-C[19] and Vitamin- E[20]. Level of TBARS was also estimated as specified by Ohkawa et al.[21]. Nitrosative stress was evaluated by estimating the Nitrite level following the method of Green et al.[22].

Statistical analysis: The results are expressed as Mean ±S.E.M. Analysis of variance and *t*-test were applied on the data of all the parameters to derive the significance of the results. The p value < 0.05 was considered statistically significance.

RESULTS

Behaviour study: The behaviour study was performed on all the groups. The significance was arrived by comparing the control group with the lesion group (*) and the lesion group with other groups (#).

Apomorphine-induced rotation test: The contra-lateral body rotation induced by apomorphine was monitored, and it was recorded as number of rotations/minute (Figure 1). There was a drastic increase in the number of rotations in Group 2 (lesion group) when compared with control group (p<0.001), and it was reduced on treatment with SA. The Group 3(SA+FA) had significantly restored the contra-lateral rotation than the standard group (L-Dopa) when compared with lesion group.

Grip test: The grip test was performed to evaluate the grip strength of the rats (Figure 2). According to their score, the grip strength of the lesion group has significantly decreased when compared with the control group, whereas the grip strength was increased in the Group 3 (SA), Group 4 (SA+FA) and Group 5 (L-Dopa). The Group 4 (SA+FA) showed better improvement in grip strength than Group 3 (SA) and Group 5(L-Dopa).

Ladder climbing test: In ladder climbing test, the lesion group showed more catalepsy effect as indicated in Figure 3. The control rats had climbed the ladder easily, but not the lesion group. They took more time to reach the top, some rats even failed to climb and fell off. The difficulty in climbing was significantly decreased in the Group 4(SA+FA) rats than Group 3(SA) and Group 5(L-Dopa).

Swing and Climb test: The swing and climb test was performed to evaluate the climbing strength of the rats by holding their tail. The control rats immediately swung and held their tail to climb up but Group 2 (lesion group) showed significantly poor performance in climbing. On comparing with lesion group, all the three groups showed better performance. Group 4(SA+FA) found to be significantly far better than Group 3(SA) and Group 5(L-Dopa) (Figure 4).

Activities of the enzymatic antioxidant (SOD, CAT, GPx and GRx): The activities of the enzymatic antioxidants of the rats were assessed (Table 1). The lesion group showed significantly less activity than the control group. The enzyme activity was restored by supplementing SA and FA. The Group 5(L-Dopa) has only a mere improvement whereas Group 3(SA) and Group 4(SA+FA) showed much better improvement. .

Non-enzymatic antioxidants (GSH, Vit-C and Vit-E): The various levels of non-enzymatic antioxidants are given in Table 2. The GSH, Vitamin-C and Vitamin-E levels of lesion group were found to be decreased when compared with the control group. It was brought to near normal by administering SA and FA significantly when compared with the lesion group.

Levels of TBARS and Nitrite: Figures 5 and 6 show the levels of TBARS and Nitrite, respectively. The administration of 6-OHDA increased TBARS and Nitrite levels. It was significantly brought to near normal by SA and FA administration.

DISCUSSION

The striatal administration of 6-OHDA led to motor impairment and abnormal involuntary movement, and drastic change in antioxidant profile as evidenced by this study. Evaluating a drug that restores these defects is an important preliminary study in drug discovery for PD. Thus, with the help of the behavioural and biochemical study, the effects of SA, FA and L-Dopa on 6-OHDA-induced rats were evaluated. SA showed better antioxidant activity, and it crossed brain blood barrier[23]. SA has a therapeutic effect on diabetes-associated cognitive decline[24]. FA deficiency was observed in the PD-induced rat model[10]. With this fact, an innovative idea has aroused to evaluate the effect of SA in association with FA and compared with standard drug for PD (L-Dopa).

The apomorphine-induced contra-lateral rotation is a marker for dopaminergic neuron depletion. The disease development and recovery can be evidenced by this method. The circulating frequency due to 6-OHDA induction is positively correlated with the damage of dopaminergic neuron of substantia nigra[25]. The SA and FA supplement has significantly reduced the number of rotations/minute. The grip test was performed to indicate the grip strength of the rats. The 6-OHDA-induced rats were found to have less grip strength because the rats fell off immediately after hanging on string, whereas the SA- and FA-administered groups scored more near to control than other groups. One of the motor impairment was the difficulty in climbing the steps. Thus, the rats were subjected to perform ladder climbing test to show the difference between the diseased and recovering group and our results justified the same. On monitoring the rats in swing and climb tests, the control rats responded immediately . When the rats lifted from the ground by holding their tail, they immediately swung and held their own tail to climb up, whereas 6-OHDA-induced rats found difficulty even to swing. The present behavioural study is correlated with the earlier studies[26-28].

Oxidative stress is one of the main etiologies of PD. When the antioxidants present in the brain drain out, the supplement to antioxidants needs to be added. The

elevated levels of TBARS and nitrite were observed in PD-induced rats[28,29], and it was also evidenced in our study (Figures 5&6). The ROS and RNS generation collapsed the neuronal integrity and threaten the neuronal survival. SA and FA prevent the ROS and RNS generation and protect the neurons from degeneration. Catalase plays a vital role in removing the toxic peroxides that causes serious damage to the cells. Similarly, superoxide dismutase, an enzyme that eliminates super oxide anion attack of the cell. These enzyme activities were reduced by the administration of 6-OHAD, but supplemented with SA and FA to restore their action.

All the antioxidants are interconnected[30], hence disruption of any one would disturb the whole system. The presence of Glutathione in mitochondria is known to protect organelles from oxidative stress. GSH plays an important role in defence against endogenous membrane peroxidation and subsequent changes by reducing H_2O_2 through glutathione peroxidase (GPx). GPx is a hydroperoxidase that is essential for maintaining redox balance in a cell[31]. Most of the H_2O_2 in the brain is removed by GPx, which is used to oxidise GSH. GRx plays a major role in providing the pool for GSH which protects the membrane from toxification. The levels of GSH, vitamin-C and vitamin- E and the activity of GPx and GRx were compared and their levels were significantly decreased due to 6-OHDA induction. Various findings reveal that SA has modulatory effect on antioxidant defence system, lipid peroxidation and Nitrite generation after iron-intoxication[32], and our study also justifies the same. In addition to that, administration of FA along with SA shows better results. L-DOPA is a standard drug for PD. It can be called as universal antiparkinsonian drug as it improves all the manifestations of Parkinsonism. The prolonged medication of L-DOPA shows adverse effects, such as nausea, vomiting cardiovascular problems and so on. Hence, SA and FA may be considered as a therapy for PD, which has fewer side effects. From this study, it is observed that the administration of SA and FA provides better results in treating PD.

CONCLUSION

This study suggests that the change in behaviour and antioxidant profile of rats induced with PD is due to degeneration of DAneurons on striatal administration of 6-OHDA. SA and FA together normalise the rats induced with PD. Further investigation is required to justify the same.

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