Anxiolytic and hyperlocomotive effects of aqueous extract of *Nigella sativa* L. seeds in rats

Farhat Bano¹, Afrinah Ahmed², Tahira Parveen³ and Saida Haider³

¹Department of Biochemistry, Jinnah Medical and Dental Collage, Karachi, Pakistan
²Karachi Medical and Dental Collage, Karachi, Pakistan
³Department of Biochemistry, University of Karachi, Karachi, Pakistan

**Abstract:** Use of the herbal drugs increasing all over the world due to its minimum side effect. *Nigella sativa* black seeds used in folk medicine for the promotion of good health and for the treatment of many diseases. The present study is designed to investigate the neurochemical and behavioral effect of aqueous extract of *Nigella sativa* L. seeds in rats. Neurochemical studies were performed for DA and DOPAC levels in whole rats’ brain. Locomotive behavior was observed in novel environment and familiar environment. Elevated plus maze and light dark behavioral modules were used to monitor anxiety in rats. The oral administration of AENS for six weeks increased time spent in open arm of elevated plus maze and light compartment in light dark box. Increased locomotors activity in novel environment (open field) was noticed suggesting that increased in DA level may be related to increased locomotive activity in rats

**Keywords:** DOPAC: Dihydroxy Phenyl acetic acid, DOPA: Di-hydroxy phenylamine, AENS :aqueous extract of *Nigella sativa* L. seeds.

**INTRODUCTION**

*Nigella sativa* is also known as black seed or black cumin Kalonji and Habal-ul-Sauda (Sharma *et al*., 2009). It belongs to the Ranunculaceae family (Andersson, 2005) having historical and religious background (Huffman, M.A., 2003). Kalonji seeds were used for hundreds of years for medicinal purposes and mentioned a number of pharmacological properties, including antioxidant (Nahla *et al*., 2008), anti-hypertensive (Dehkordi *et al*., 2008), anti-inflammatory (Mohammad *et al*., 2011), potent analgesic (Bashir *et al*., 2010), anti-tumor (Ait *et al*., 2007), hypoglycemic and anti-hyperlipidemic properties (Farhat *et al*., 2011). Raza *et al*., 2006 reported that *N. sativa* seeds constituents produced anti-anxiety effect in different test used as modules for exploring anxiety.

Parkinson’s disease is a chronic progressive neuro degenerative movement disorder characterized by tremors, rigidity bradykinesia, poor balance, difficulty in walking and loss of dopamine neuron in nigrostriatal pathway.(Schrag and Pauline 2006; Carod *et al*., 2008; Mckinlay *et al*., 2008).

Dopamine has been suggested to be involved in behavioral changes. Ken *et al*., 2008 reported that the decreased concentration of DA reduce locomotors activity in honey bees. Deficits of DA are associated with Parkinson’s disease (Juan Andres *et al*., 2010). Neural systems of the brain use dopamine as a principal neurotransmitter to mediate locomotion (nigrostriatal system), motivated behavior (mesolimbic system).

Impairments in the nigrostriatal pathway contribute to dysfunctional movement, a common symptom in Parkinson's disease (Anh *et al*., 2002). Both dopamine 1 receptor (D1R) and dopamine 2 receptor (D2R) are involved in control of locomotors behavior, and they function in a synergistic interaction manner (David, *et al*., 2002; Tomiyama *et al*., 2001). Herbal preparations are getting popularity nowadays due to their beneficial effects in various conditions without the adverse effects. In the present study aqueous extract of *N. sativa* was used to monitor the anxiolytic and locomotors activity in rats.

**MATERIAL AND METHOD**

**Preparation of extract**

*Nigella sativa* seeds were purchased from local market. Identification was provided by the Agriculture Department of University of Karachi. The 50 gm seeds were crushed in blender. The crushed seeds was dipped in 150 ml of water and left for 24 hour at 4°C. The mixture was filtered and the filtrate was stored until ready to use.

**Experimental protocol**

Albino Wistar male rats with weight between 280-320 grams were used. All animals were placed in single cages under 12 h light-dark cycle and controlled room temperature (23±2°C) with free access to specially prepared diet and normal water for one week, prior to starting the experiment so that rats could adept themselves to new conditions. The test group received 2mL*Nigella sativa* aqueous extract. The control group received normal water equivalent to aqueous extract of herb. Weighed amount of food was placed in the hopper of all cages. After the significant change in behavior of treated rats in

*Corresponding author: e-mail: farhatbano_2000@yahoo.com*
behavioral modules rats were decapitated. Brain and blood samples were harvested and preserved at -70°C for neurochemical and biochemical estimations. The untreated rats were also decapitated at the same time.

**Behavioral activity**

**Light dark box activity**
The apparatus used in this investigation consists of two compartments one compartment was dark and second was made up of transparent plastic. The compartments were of equal size (26*26*26cm) and had an access way (12*12 cm) between them that allowed the animal to pass through. Rats were introduced to the apparatus from the dark chamber. The number of entries and the time spent in the light compartment were measured for five minutes (Zanoli et al., 2000; Maribel et al., 2006; Crawley and Goodwin, 1980).

**Elevated plus maze activity**
The apparatus used in the present study consisted of two open arms (50*10cm) and two closed arms (50*10*25cm). The maze was elevated from the floor at a height of 60cm above the base level. Rats were entered in the middle of apparatus. Time spent and number of entries in open arm was monitored in a period of 5 minutes (Hogg, 1996; Pellow et al., 1986; Rodgers et al., 1998).

**Home cage activity**
Especially designed Perspex home cage (26*26*26cm) with saw dust covered floor was used for this purpose. The activity of rats was observed for 5 minutes (Darakshan, 1993).

**Open field activity**
The open field instrument used in this study consists of a square area 76*76 cm with opaque walls 42 cm high. The base was divided by lines in to 25 equal squares. To determine the activity, rat was placed in the middle of the open field. Number of square traversed and time spent in corner setting was monitored for a cut off time 5 minutes (Hmale, 1996).

**Estimation of monoamines**
Estimation of monoamines and their metabolites in the whole brain samples of rats was made by HPLC-EC method as reported by Haleem et al., (2004). It is a highly sensitive and sophisticated technique in which HPLC is associated with development of stationary phase in the form of small silica particles. In HPLC, the small particles of 5mm in diameter can resist the high pressure of the mobile phase which does not occur in the thin layer chromatography. All biogenic amines can be detected in a single sample by reversed phase HPLC with electrochemical detector. This technique separates biogenic amines and their metabolite on the basis of their hydrophobicity. The stationary phase used for the analysis of biogenic amines was 5mm octadecyl saline (ODS) column. The mobile phase was passed through this column under high pressure. A measured amount of sample (15ul or more) was applied through injector on top of the column. Biogenic amines were determined by using electrochemical detector (Schimadzu LEC 6A detector) at working potential of +0.8 to 1.0 V.

After detection, message from the detector was be recorded by an integrator. Each sample was identified by comparing with the retention time of standard (Haleem et al., 2004; Haleem and Khan, 2003).

**STATISTICAL ANALYSIS**
The significance differences between the mean of the treated and untreated groups were analyzed by student's t-test. Values of p<0.05 were considered as significant. Data were expressed as mean ± standard deviation (SD).

**RESULTS**

**Behavioral effect of repeated administration of AENS in rats**

**Effect of repeated administration of AENS on weekly open field activity in rats**
Significant decrease (p<0.01) in corner setting in open field were observed from 3rd week of the treatment while significant increase (p<0.01) in square crossed was observed at six week of treatment (figs. 1a,b) respectively as compare to control.

**Effect of repeated administration of AENS on weekly light dark activity box in rat**
Significant (p<0.01) increase in time pass in light compartment of light dark box were observed from 3rd week of repeated administration of AENS treated rats while no significant effect on number of entries in light box as compared to control (figs. 2 a, b) respectively.

**Effect of repeated administration of AENS on weekly elevated plus maze activity in rats**
Gradually increase in time pass in open arm of EP was observed from 4th to six weeks of the treatment (p<0.01, p<0.05and p<0.01) respectively in treated rats while no significant effect on number of entries in open arm as compared to control (figs. 3a, b) respectively.

**Effect of repeated administration of AENS on weekly home cage activity in rats**
Non significant effects were observed in home cage (fig. 4) activity after the six weeks treatment with AENS in treated rats as compared to control.

**Neurochemical effect of repeated administration of AENS on rats brain**
Effect of repeated administration of AENS (six weeks) on whole brain DA and DOPAC levels in rats: Significant increase (p<0.01) in DA and DOPAC levels in treated rats (fig. 5a, b) respectively as compared to control.
Figs. 1(a, b): Effect of repeated administration of AENS on open field activity of rats. Values are mean ± SD (n=12) significant difference by Student t-test **p<0.01 and *p<0.05 from respective controls.

Figs. 2(a, b): Effect of repeated administration of AENS on light dark activity of rats. Values are mean ± SD (n=12) significant difference by Student t-test **p<0.01 from respective controls.

Figs. 3(a, b): Effect of repeated administration of AENS on elevated plus maze activity of rats. Values are mean ± SD (n=12) significant difference by Student t-test **p<0.01 from respective controls.

Fig. 4: Effect of repeated administration of AENS on home cage activity of rats. Values are mean ± SD (n=12). Non-significant difference by Student t-test from respective controls.

DISCUSSION

Use of herb and other remedies is increasing nowadays as an alternative to prescription drugs for the managements of anxiety. Because several side effect, dependence and toxicity may be produced by these drugs.

The objective of this study was to determination of *Nigella sativa* L. seeds aqueous extract on animal behavior and DA and DOPAC levels in brain. In the present study AENS showing anxiolytic and hyper locomotive effect on rats.
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After the six weeks of treatment we observed significant effect in animals behavior in open field apparatus. AENS increases number of square crossed in open fields compared to control (fig. 1). However no significant effect of AENS observed on familiar activity (home cage) (fig. 4).

This shows that AENS increases locomotive activity moderately and expressed more in novel environment than in familiar environment.

We have also monitored increased DA and DOPAC level in whole brain in this study (fig. 5).

Numerous studies have shown that dopamine play vital role in locomotive behavior. Increase DA metabolism resulted in increased locomotive activity (Eun and Insop, 2011; Charntikov et al., 2011) while a decrease in DA levels decreases locomotive activity (Pulvirenti et al., 1991).

The present findings tend to demonstrate the hyperlocomotive effect of AENS possibly mediated via increase DA availability toward respective receptor. Increase in brain DA levels which in turn generate a neuro chemical signal for the increase locomotive activity in rats. Deficits of DA is associated with Parkinson's disease. The results of this study suggested that AENS may be effective to manage dysfunctional movement in Parkinson's disease.

Behavior in elevated plus maze and light dark box is well established animal model for evaluating anxiolytic and anxiogenic behavior (Dawson et al., 1995: Kulkarni et al., 1996: File, 1993 and Saleem et al., 2006).

Anti-anxiety drugs promote exploration of open arm in elevated plus maze (Maria et al., 2007). Light dark activity is useful to predict the anxiolytic and anxiogenic effect of different compounds (Altaf et al., 2008).

AENS increases exploratory activity in elevated plus maze (fig. 3a) and in Light dark box (fig. 2a). Increased exploration in open arm of elevated plus maze and light compartment of light dark box Suggested that AENS induces anxiolytic effects. Our finding is supported by our previous study that Nigella sativa oil exhibit anxiolytics effect (Praveen et al., 2009). Neeraj et al., 2011 demonstrated that thymoquinone is major constituent of Nigella sativa L. seeds exhibit anti-anxiety activity in mice through NO and GABA pathways.

CONCLUSION

Aqueous extract of Nigella sativa L. seeds could be used full in the management of dysfunctional movement in Parkinson's disease. Aqueous extract of Nigella sativa L. seeds might be effective to treat anxiety and related disorders. Further studies are desirable at indiviudal constituent of Nigella sativa L. seeds.

REFERENCES


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