EFFECT OF *AETHUSA CYNAPIUM* ON QUINPIROLE INDUCED COMPULSIVE CHECKING

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ABSTRACT

Disorders of anxiety vary in severity to a wide extent, of which obsessive compulsive disorder persists as the 4th most common form of mental illness and is reported to be associated with memory impairment, necessitating effective means of treatment. The present study includes the determination of effect of methanolic extract of *Aethusa cynapium* (MEAC) at 200 and 400 mg/kg in quinpirole (0.5 mg/kg) induced model of Obsessive-compulsive disorder (OCD) in rats i.e. exploration on large open field with four distinct objects and on memory retention using water maze apparatus. The role of monoamines in the etiology of OCD was acknowledged by estimating the dopamine and serotonin levels in rat brain. Various parameters such as frequency of stops, duration of stopping and number of visits to other objects on successive return to an object were observed in open field and delay in time to reach the escape platform was observed in water maze apparatus. A significant improvement from the obsessive-compulsive symptoms induced with quinpirole was observed in MEAC and paroxetine treated rats, MEAC, had also shown a protective effect on memory task. An increase in serotonin levels and a decrease in the dopamine levels were observed in *Aethusa cynapium* treated rats when compared with those treated with quinpirole, thus providing an evidence for the predictive and construct validity of the model.

KEYWORDS: Obsessive compulsive disorder, *Aethusa cynapium*, Quinpirole, Water maze apparatus, Dopamine, Serotonin

INTRODUCTION

Anxiety disorders are prevailing to be the major central nervous system disorders in the community, of which Obsessive Compulsive Diosorder (OCD) is considered to be the 4th most common form of mental illness. It is reported that 1 out of every 40 people suffer with this disorder, approximating 2.5 % of the world population (Robert, 2005). Obsessive Compulsive Diosorder (OCD) is a chronic neurotic disorder and is one of the 10 leading causes of disability. It is considered to affect people of different age groups (Murray and Lopez, 1996) causing uncontrollable obsessions and compulsions causing marked distress or significant impairment are reported to be associated with general memory deficit and reduced memory confidence (American Psychiatric Association, 2000) (Vanden and Kindt, 2003). Orbitofrontal cortex (OFC), caudate nucleus and anterior cingulated gyrus (ACG) are identified as the principle regions in the brain that exhibit abnormality during OCD (Swinson, 1998). Various clinical studies have reported either increased metabolic activity in these regions during OCD or increased blood flow to these regions. Apart from this the widely accepted mechanisms in the occurrence of OCD is that excessive stimulation of OFC/ACG, which are involved in detection of errors (Filiz, 2005), results in increase in the ability of error detection thus sending excessive erroneous messages to basal ganglia causing a feeling that something was wrong.

The occurrence of neurobioligical abnormality in the central serotonergic system is widely accepted as the cause of OCD (Park, 1997; Insel,1985), this is further supported by the beneficial effect of antidepressants such as selective serotonin reuptake inhibitors (SSRI's) in alleviating OCD symptoms and this beneficial effect is confined to potent SSRI's (Bystritsky, 2004). Various studies have reported that use of SSRI's show a decrease in 5-hydroxy indole acetic acid (5HIAA), the major metabolite of serotonin (5-HT) in cerebrospinal fluid, which are higher in OCD patients. Use of meta-chlorophenylpiperizine (mCPP) as a serotonergic probe which stimulates several 5-HT receptors had shown an exacerabation of OCD symptoms. Neuroimaging studies of the central neurotransmitter systems used to visualize 5HT in the brain have reported that an impaired function of 5-HT play a role in the pathogenesis of OCD. The other neurotransmitter that is considered to be widely involved in OCD is dopamine (DA). This is reinstated by the potential of various dopaminergic agonists such as Quinpirole in inducing the symptoms of OCD (Westenberg, 2007).

Aethusa cynapium, has been reported in various studies to have an influential role on anxiety (Shri, 2010). Which was reported to be due to the presence of omega-3 polyunsaturted fatty acids (Mc Namaraa and Carlson,

2006), the involvement of these fatty acids primarily in the etiology of OCD and their potential to increase the monoamine levels by inhibiting monoamine oxidase has led to the evaluation of the effect of *Aethusa cynapium* in the treatment of OCD using quinpirole induced compulsive checking (Szechtman, 1998). The effect of OCD on acquired memory was studied using Morris water maze task of spatial learning (Morris, 1984). The effect of quinpirole and *Aethusa cynapium* on brain monoamine levels were studied to elucidate the mechanism involved, which also helps in determining the predictive validity of the model. There is a general consensus that deficiency of omega-3 fatty acids (which are reported to be present in Aethusa cynapium) results in impaired learning and memory in rodents and increased risk of mental disorders such as dementia, bipolar disorders and schizophrenia in humans (Freeman, 2006). Moreover, the existing treatment for OCD entail long term use of selective serotonin reuptake inhibitors (SSRI's) and other antidepressants which are associated with varied side effects such as loss of libido, nervousness, insomnia, anorexia, dyskinesia etc., (Tripathi, 2008). Hence *Aethusa cynapium* could be an effective natural alternative, devoid of side effects, in the treatment of OCD and associated memory disturbances.

Animals: Inbred Adult wistar rats (200 – 250 g) were procured from the animal house of Bapatla College of pharmacy (1032/ac/07/CPCSEA), Bapatla, India and were housed at a constant room temperature of 22 ± 1ºC, 40-50% relative humidity and 12 h light/dark cycles. Standard pellet feed (Rayan's Biotech, Hyderabad) and water was provided *ad libitum* throughout the experimentation period. Animals were acclimatized to laboratory conditions one week prior to initiation of experiments. The experimental protocol was approved by Institutional animal ethics committee (IAEC/III-4/BCOP/2011-2012) and all the experiments involved in this work were performed in accordance with CPCSEA guidelines for the use and care of experimental animals.

Drugs and drug administration: For oral administration, methanolic extract of *Aethusa cynapium* (MEAC) was mixed with 10% tween 20 and diluted to the desired concentration with the same, on the day of administration. Paroxetine 1.8 mg/kg, p.o. was suspended in 1 % carboxy methyl cellulose (CMC). The tween 20 and CMC were used as control treatments. Since the behavioural data did not differ between rats that received these vehicles peanut oil (vehicle) treated group is considered as control.

Training of rats for spatial learning: All the rats were trained to swim individually in Morris water maze (Hanish, 2009) that consists of circular water tank with a diameter of 100 cm and depth of 20 cm containing water at 25°C rendered opaque by adding milk powder. A platform (diameter 4.5 cm; height 19 cm) was submerged 1 cm below the water surface and is located at the centre of one quadrant. Each quadrant has a starting point, where the rats are placed in the maze and allowed to find the escape platform hidden under the water for 60-90 sec. 2 – 4 trials are performed a day for 4 -5 days until the latency to reach the escape platform was markedly reduced.

Grouping of animals: Rats were randomly assigned to 5 groups after training for spatial learning. Group I rats were treated with 10% tween 20 0.1 ml/100 mg and served as control, group II rats served as negative control, group III, group IV and group V rats were treated with 200 mg/kg, 400 mg/kg of MEAC and paroxetine 1.8 mg/kg p.o. respectively for 35 days. Quinpirole 0.5 mg/kg p.o. was administered to all the groups except control, twice in a week and 1 h before exposure to the open field on the last day of treatment.

Experimental investigations:
Quinpirole induced compulsive checking: Compulsive checking induced with quinpirole is tested by placing the rats individually on a large open field that consists of four objects with different shapes and colours fixed equidistantly at four corners of a flat wooden board. After 35 days of treatment with drugs, rats of each group were analyzed individually to obtain the behavioural measures such as 1. Frequency of stops in each locale, 2. Total duration of stopping in a given locale, 3. Number of visits to other objects in between returns to a given locale, 4. Occurrence and frequency of occurrence of ritualistic behaviour at various objects were observed and recorded for a period of 55 min (Szechtman, 2001). The open field was thoroughly cleaned to deodorise it after each observation.
Memory: After the behavioural measurements in quinpirole induced compulsive checking, rats of different groups were placed individually in the water maze to evaluate the effect of different treatments on retention of acquired task of identification of hidden platform under water. The latency to reach the hidden platform was observed, a cut-off time of 90 sec was followed after which the rats were returned to the cage.

Estimation of Rat Brain Dopamine and Serotonin levels: Rats were sacrificed by cervical dislocation and the brains were isolated quickly. Anaesthesia was not used as it alters the brain amines (Ravindran, 2005). After sacrificing, the brains were rapidly removed and concentrations of DA and 5-HT were measured by fluorimetry.

The brain was weighed and homogenized with 6ml of cold acidified butanol at 800 x g. An aliquot from each homogenate served as a tissue sample. The internal standards were prepared by the addition of known amounts of standards (500μg each DA & 5-HT) to a portion of homogenate and processed in parallel with tissue samples. The reagent blanks and test samples for estimation were prepared following the same procedure described by Kari et.al., (1978). DA and 5-HT were read with an excitation and emission wavelength of 320/370 nm and 360/470 nm respectively with a slit width of 10/10 nm.

Statistical analysis: The data obtained from the performance on large open field was expressed as mean ± SEM and the results of each group were compared with negative control rats. The data obtained from frequency of stops and total duration of stopping at individual objects by different groups were categorized as - More (includes sum of scores attained at two objects where rats have shown more frequency of stops and duration of time spent) and were compared with that of less (includes sum of scores attained at two objects where rats have shown less frequency of stops and duration of time spent) using Bland-Altman analysis to determine the percentage difference and bias between the two categories at which rats have stopped and time spent at respective objects. The data of number of visits to other objects on successive return to the same object by different groups was analysed using one sample 't' test and are compared with the least number of visits for significance. One way ANOVA followed by Dunnet's 't' test was implied to report the effect of *Aethusa cynapium* and paroxetine treatment on data obtained from water maze and monoamine levels of brain.

Table.1. Effect of *Aethusa cynapium* on frequency of stops at different objects

Groups	Frequency of stops		Average	% Difference	Bias ± SD
	More	Less			
I	19.7 ± 1.5	15 ± 1.4	17.3	27.5	27.6 ± 14.9
II	12 ± 1.9	3.3 ± 0.7	7.6	116.9	116.9 ± 11.1
III	13.2 ± 2.5	6.8 ± 1.6	10.1	58.2	58.2 ± 28.9
IV	13.3 ± 2.4	9.2 ± 1.7	11.3	30.5	30.8 ± 14.1
V	14.3 ± 1.6	11.2 ± 0.6	12.6	26.5	26.5 ± 11.8

More and less denotes sum of two objects where rats have shown more or less frequency of stops respectively, SD = Standard deviation of Bias.

RESULTS AND DISCUSSION

Effect on frequency of stops at respective objects: Quinpirole treated rats had shown a marked discrimination in the frequency of stops at different locales, a bias (difference between sum of two objects where more number of stops are observed and sum of two objects where less number of stops are observed) of 116.9 ± 11.1 was observed. MEAC at a dose of 200 and 400 mg/kg had shown a bias of 58.2 ± 28.9 and 30.8 ± 14.1 respectively indicating a marked decrease in the development of compulsions (to visit specific objects). (Table.1)

Effect on total duration of stopping at respective objects: Control rats have shown a bias of 36.2 ± 37.4 whereas quinpirole treated rats have shown a bias of 181.91 ± 18.51, clearly indicating the development of obsessions towards specific objects. A significant reduction in the bias shown between objects was observed in MEAC 400 mg/kg treated rats (73.9 ± 34.8) and the results obtained are comparable with that of paroxetine treated group 58.1 ± 47.8. (Table.2).

Effect on number of visits to other objects in between returns to given object: One sample 't' test analysis between the number of visits to other objects of each object (Considered as B, C and D) with respect to the object for which the number of visits to other objects is less (considered as object A) did not show any significant difference between visits to other objects in control rats (Object A – 2.83, B – 3.83, C – 4.83, D – 5). Whereas quinpirole treated rats have shown a significant difference for two objects (Object A – 0.78, B – 1, C – 3.67, D – 4.23). MEAC at 200 mg/kg did not show any deviation when compared with that of quinpirole treated group but MEAC at 400 mg/kg had increased the number of visits, a significant difference was observed for one object only (Object A – 3.16, B – 3.83, C – 5.33, D – 5.67) and is comparable with that of paroxetine (Object A – 3.5, B – 3.66, C – 4, D – 4.5) (Figure.1).

Ritual like behaviours: A characteristic ritualistic behaviour, such as repeated grooming with hind paws and cleaning the snout with both fore paws, were exhibited by quinpirole treated rats at those objects where obsessions have been developed. No such behaviours were observed in control rats, the severity of these behaviours decreased markedly on treatment with MEAC and paroxetine.

Effect on spatial learning: After the training session of 4-5 days, a marked decrease in the latency to reach the escape platform was observed whereas quinpirole treatment did not show any retrieval of the learned task but MEAC at a dose of 400 mg/kg had shown a good retention of memory and was significant than that observed with paroxetine (Figure.2).

Effect of MEAC on brain dopamine and serotonin levels: A significant increase ($p < 0.001$) in the dopamine and a significant decrease ($p < 0.001$) in serotonin levels were observed in the rats treated with quinpirole i.e. negative control when compared with control. Rats treated with MEAC 200 and 400 mg/kg had shown a marked decrease ($p < 0.01$) in DA whereas a significant increase ($p < 0.05$) in the serotonin was observed only at 400 mg/kg when compared to that of negative control. Paroxetine treated rats had shown a significant increase in serotonin ($P < 0.05$) with minimal effect on DA levels (Figure.3).

**Table.2. Effect of *Aethusa cynapium* on duration of stopping at different objects**

Groups	Duration of stopping		Average	% Difference	Bias ± SD
I	More	Less			
II	29.8 ± 3.2	21.5 ± 4.3	25.6	36.2	36.2 ± 37.4
III	41.5 ± 6.3	1.6 ± 0.6	21.7	181.9	181.9 ± 18.5
IV	31.4 ± 4.2	11.2 ± 2.4	21.1	96.1	96.2 ± 17.6
V	26.5 ± 6.1	12.5 ± 3.1	19.2	73.8	73.9 ± 34.8
	25.5 ± 3.7	13.1 ± 1.3	19.2	58.1	58.1 ± 47.8

More and less denotes sum of two objects where rats have shown more or less duration of stopping respectively, SD = Standard deviation of Bias.
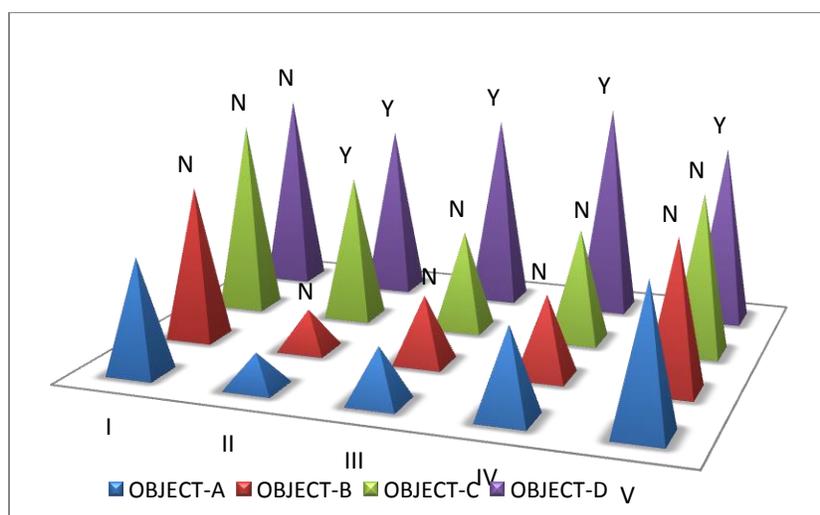

Figure.1.Number of visits to other objects on successive return to each object

In the histogram, N and Y indicates non significant and significant difference respectively obtained by one sample 't' test comparision (n=6) of number of visits on successive return to the object with that of the object where less number of visits on successive return are shown.
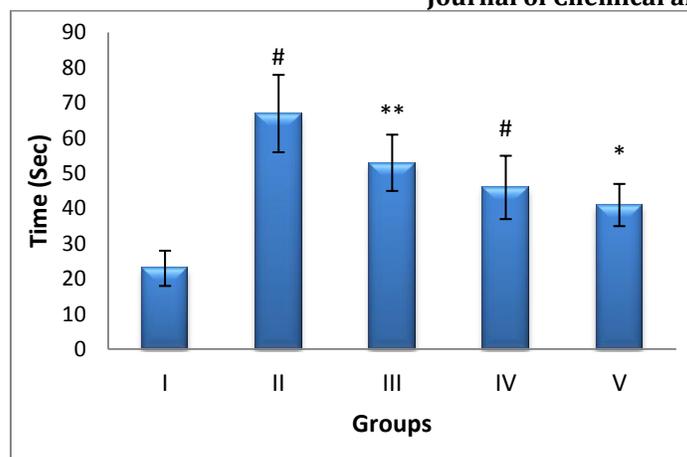
Figure.2.Effect on retention of learned task

Histogram showing the delay in time to reach the escape platform from the starting point on the last day of treatment after exploration on open field, each column represents mean ± SEM (n=6) of time taken by the rats to reach the escape platform from the starting point, Values of quinpirole treated are compared with that of control group, values of MEAC 200, 400 mg/kg and paroxetine treated were compared with that of quinpirole treated negative control rats # $p<0.001$, ** $p<0.01$, * $p<0.05$, ns – non significant.
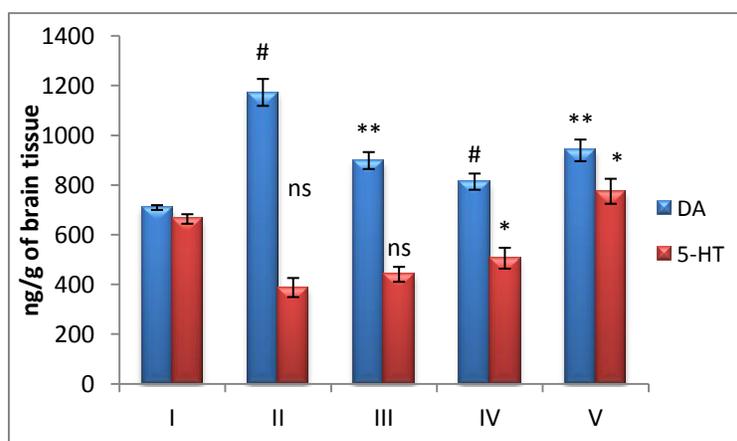
Figure.3.Dopamine and Serotonin levels of whole brain

The effect of MEAC and Paroxetine on Dopamine and serotonin levels (nanogram/gram of wet tissue) in rat brain, each column represents the mean ± SEM (n=6). Values of Control, MEAC 200, 400 mg/kg and Paroxetine treated rats were compared with negative control. All the groups except Control were treated with quinpirole # $p<0.001$, ** $p<0.01$, * $p<0.05$, ns – non significant.

DISCUSSION

Obsessive compulsive disorder, resulting from the abnormality in signal processing by OFC, ACG and basal ganglia are currently treated with SSRI's, serotonin and norepinephrine reuptake inhibitors (Zohar and Judge, 1996). This apart from providing inadequate support is also involved in many adverse effects. Hence the current study recommends a treatment that reduces the symptoms, progression of OCD along with memory disturbances. Though mechanisms underlying the pathogenesis of OCD are widely understood, there exists a controversy in suggesting the cause. As mentioned previously one study suggests that the occurrence is due to excessive stimulation of OFC and ACG resulting in generation of excessive messages to basal ganglia other theory states that the damage to the OFC and ACG resulted in a loss of error detection abilities causing the brain to increase repetition of messages leading to OCD. The former was widely accepted because of its ability to explain various symptoms of OCD. This can be further substantiated by the present study that quinpirole, a D_2/D_3 agonist, is considered to increase the dopaminergic activity to produce the symptoms.

SSRI's are widely used in the treatment of OCD implicating that serotonin is involved in its etiology, dopaminergic over activity is also considered to be involved in OCD (Goodman, 1990) as basal ganglia, the structure of the brain considered to be malfunctioning in OCD, is innervated with dopaminergic fibres. The caudate nucleus, a structure of basal ganglia that prevents the initial signal of "error" from OFC to thalamus, does not function normally in those with OCD, and therefore does not prevent this initial signal from recurring. This causes the thalamus to become hyperactive and creates a virtually never-ending loop of worry signals being sent back and forth between the OFC and the thalamus (Huey, 2008). Hence OFC responds by increasing anxiety and engaging in compulsive behaviors in an attempt to relieve this apprehension. Individuals with OCD are also associated with memory disturbances (Tallis, 1997) which can be due to abnormality in the dopamine levels of hippocampus and amygdala, the two structures that actively participate in consolidation of recent and emotional memory respectively (Zola and Squire, 1993). Both these structures are widely innervated with dopaminergic fibres (Dahlstorm and Fuxe, 1964) further confirming the involvement of dopamine in the etiology of memory disturbances in OCD.

The increased duration of stopping, frequency of visits and decreased number of visits to other objects on successive visit to the object indicates the development of OCD symptoms. Performance of ritual like movements at these objects can be exemplified as compulsions developed in OCD patient for obsessions. Treatment with *Aethusa cynapium* had markedly reduced these observed changes indicating its therapeutic potential in the treatment of OCD. Water maze exploration had shown memory impairment in quinpirole treated rats and retrieval in *Aethusa cynapium* treated rats. The decrease in dopamine levels and increase in serotonin levels with *Aethusa cynapium* treatment explains the therapeutic effect of *Aethusa cynapium*, the predictive validity of this model can be explained based on the theory that a substantial interaction exists between the serotonergic and dopaminergic systems in two mid brain regions, ventral tegmentum and substantia nigra, with dopamine producing neurons being targets for serotonin cells has been explained as, $5HT_{1A}$ auto receptor activation inhibits dopamine release in the dorsal striatum of the mid brain and stimulating dopamine release in the nucleus accumbens (McManamy, 2008). This theory provides the key mechanism probably involved in the beneficial effect of SSRI's in the treatment of quinpirole induced OCD, since quinpirole acts as an agonist on the D_2 & D_3 receptors in the striatal region of the mid brain (Szechtman, 1998) thus increasing the dopamine levels but the increased serotonin concentration acts on $5HT_{1A}$ auto receptor inhibiting dopamine release. This can be further supported by a study that sertraline, an SSRI, has been reported to decrease the extracellular striatal levels of dopamine (Rocco, 1998), and this decrease in dopamine levels which is also exhibited by paroxetine in the present study, probably in the above said mechanism, might have contributed to the protective effect of paroxetine in the treatment of qunipirole induced OCD providing a clue for the construct validity of the model.

In conclusion, our results made it evident that *Aethusa cynapium* exerts anti OCD and memory protective effect in quinpirole induced model of OCD. The mechanism involved in its anti OCD effect might be due to accentuation of serotonin levels in various regions of brain as observed in the present study and decrease in dopamine levels due to activation of 5-HT_{1A} auto receptors, thus contributing to the predictive and construct validity of the model (Joel, 2006). However, further studies are essential to substantiate and validate the authenticity of these results in other models of OCD, which helps in investigating the role of *Aethusa cynapium* to emerge as an effective counterpart in the treatment of OCD.

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