Scopolamine-Induced Impairment in Conditioning and Exploratory Behaviours is Enhanced by Anacyclus Pyrethrum in Rats.

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Abstract—Context: For centuries, plants were used for food and medication. Medicinal plants serve as therapeutic alternatives, safer choices, or in some cases, as the only effective treatment. A larger number of medicinal plants and their purified constituents have been shown beneficial therapeutic potentials. Anacyclus pyrethrum (Compositae) is reputed to be useful as a pharmaceutical aid for learning and memory.

Objective: To evaluate the potential effects of ethanolic extract of Anacyclus pyrethrum (APE) on learning and exploratory deficits.

Material and methods: Wistar rats were used in the study. APE was administered in doses of 100 and 200 mg. Learning and exploratory deficits were produced by acute administration of scopolamine (1mg/Kg). The electric shock avoidance and the T-labyrinth tests are used to assess learning and exploration.

Results: In the group treated by orally injection of Anacyclus P (APE100 and 200mg), results show that 100% of rats are conditioned in the session six and five respectively. In the SCO group, neither rat was conditioned in all four sequences. At the tenth sequence, 7% of rats were conditioned. In the group receiving scopolamine and treatment by APE100, obtained results show that 26.6% of rats are conditioned in sequence seven and 63.3% in sequence ten. The same results are obtained with SCO + APE200, 40% of animals are conditioned in sequence seven and 83.3% in sequence ten. In exploratory test, scopolamine decrease exploratory activity of the novel environment and APE treatment enhances the exploratory behaviour in the rat. By comparison with controls, significant increase was observed (p<0.02). In rats receiving SCO and APE200, scopolamine does not disrupt this activity.

Conclusion: The combination of scopolamine and “APE” show that the APE reverses the power of SCO to decrease the number of conditioned responses and to disrupt exploration in the rat. These findings indicate that roots of Anacyclus Pyrethrum may contain effective compounds that stimulate learning and exploratory activities in rat.

Keywords—Anacyclus Pyrethrum; learning; exploration behaviour; scopolamine Introduction

I. INTRODUCTION

Learning and memory are two important functions of the brain. They appear to depend on the functions of multiple neural systems that process different attributes of an experience [16]. Exploration is a spontaneous activity which allows animals to sample information about their surrounding environment and the objects with which they are confronted.

In learning and conditioning experiments, it’s typical that relationships are arranged between discrete conditional stimulation (CS), such as light or tone and unconditional stimuli (US) such as food or foot-shocks. Although much of the learning in these situations reflects an association between the discrete CS and US, there is substantial evidence that the US also became associated with the training environment. Involvement of brain cholinergic system in learning and memory processes was reported [8, 9, 5]. It is in generally accepted that cholinergic stimulation facilitates learning and memory consolidation, while its blockade produces amnesia. Thus, administration of anticholinergic drugs, such as atropine and scopolamine, induces learning deficits in a wide variety of tasks [4, 18].

The use of plants in medicine dates from the earliest years of man’s evolution. Medicinal plants serve as therapeutic alternatives, or in some cases, as the only effective treatment. A larger number of medicinal plants and their purified constituents have been shown beneficial therapeutic potentials. Morocco is one of the largest producers and exporters of herbs and herb products.

Anacyclus pyrethrum (AP), (Compositae), commonly known (in Morocco) as ‘Tiguandiste’ is a perennial, plant which is largely distributed throughout Atlas Mountains of Morocco. It is a plant with 30 cm in height at most, leaves are finely and pinnately divided; roots are long, thick, fibrous, rough, brown in external part and white in the interior part. The use of Anacyclus pyrethrum is described in pharmacopoeias of numerous countries of the world. Roots are registered on the French pharmacopoeia since 1937, for their dialilogue properties [19]. AP is widely used in Moroccan traditional medicine. The infusion of AP roots is recommended in mouthwash, in treatment of teeth diseases and in treatment of memory disorders. A mixture of roots and milk, added by
honey is proposed as aphrodisiac, against the feminine infertility [12].

The roots of AP are regarded as a tonic to the nervous system. Extract roots is used as treatment to paralysis; hemiplegia, cephalalgia, epilepsy and rheumatism in Indian traditional medicine [15]. An infusion of the roots is used as cordial and stimulant and in treatment of fever. A decoction of the roots is used in treatment of pharyngitis, hemiplegia and in chronic ophtalmia [21].

Scopolamine, a muscarinic receptor blocker, causes impairment in memory tasks in animals and Humans [14].

In the present study, the main goal was to evaluate the effects of APE on scopolamine induced deficits in conditioned learning and exploratory behaviours in rats.

II. MATERIALS AND METHODS

A. Plant Material

*Anacyclus pyrethrum* roots were collected in June-July from Oukaimiden, a high Atlas Mountains region of Morocco. The plant material was botanically classified and authenticated for their correct botanical identification by professor Ouhammou. A voucher specimen of plant has been deposited at the herbarium of Semlalia sciences faculty, Cadi ayyad University.

Preparation of extract

The root of *Anacyclus pyrethrum* was powdered and ethanolic extract was prepared by simple maceration process. The ethanolic extract was evaporated, using rotavapor apparatus, under reduced pressure. The suspension was prepared and administered orally.

B. Animals

Male and female rats of the Wistar strain weighing about 200 - 250 g were used in these experiments. They were acclimatized at laboratory conditions for a week before starting the experiments. The experiment was conducted in accordance to internationally accepted standard guidelines for use of animals. The rats have free access to food and water and were kept under a controlled 12 h light/dark cycle at 22 ±2 °C.

C. Conditioned learning

All rats were trained initially in a wood box that was divided into two compartments (right and left) (30 x 25 x50). An opaque wood door (with one hole) separated the two compartments. The box was covered by a hinged roof of clear Plexiglas with numerous holes to allow ventilation. The floor consisted of metal rods, spaced 1 cm apart. The box was placed on table, 1 m above the floor. Training and testing were conducted in a quiet and illuminated room. The sound (CS) stimulation was an 80 db tone presented through a bell that was centrally mounted about 20 cm above the box. The electrical stimulation (US) was 1.2 mA foot-shock delivered by a shock generator.

- Procedure

The rats were handled daily about 10 min on 2 consecutive days and familiarised with both compartments in order to establish their natural preference for each compartment. On day 1, each rat was placed in the left or the right compartment of the box. The hole of the door (that divided the two compartments) was open and the rat was free to wander throughout the box. On day 2, the procedure was repeated except that each rat was placed in the other compartment of the box. On the following day the were assigned to the control group (CO), scopolamine group (SCO), *Anacyclus pyrethrum* group (100mg) (AP100), *Anacyclus pyrethrum* (200mg) group (AP200); scopolamine + *Anacyclus pyrethrum* (100mg) group (SCO + AP100) and Scopolamin + *Anacyclus pyrethrum* (200mg) group (SCO + AP200).

- Test

The test was initiated on day 3 and was identical for all groups. For the test, each rat was placed individually in the right compartment of the box with the hole open to prevent access to the left compartment. After about 30 minutes, a series of conditioned stimulus CS (tone) and unconditioned stimulus US (foot-shock) paired trials was initiated. For each paring, the tone was presented for 3 seconds, along with a 1.2 mA foot shock which was delivered 1s after the end of the CS period. The US was immediately stopped when the rat enters in the left compartment, or 30s after (cut of time) when the rat rests in the right of the compartment. After the last CS-US pairing, the rat remained in the right compartment for 2 minutes during which neither the CS nor the US was administrated. Each rat received ten presentations (associations) of the CS-US (in this experiment, 10 associations = 1 sequence). After each sequence, the rat remain in the right compartment for 15 minutes during which neither the CS nor the US was administrated. Each animal was tested along ten sequences. When the rat runs out the right compartment with CS alone, this was considered a conditioned response (CR). The increase or decrease in number of conditioned response was considered as an enhancement or impairment in learning, respectively. For each group of rats, means number of conditioned responses are scored in each sequence.

- Exploration

All animals were tested in wood T labyrinth that was formed of 3 compartments (hands). Each rat is placed in compartment 1(H1) and can explore this and the compartment 2 (H2) while the door of the third compartment (H3) is closed. The third compartment of the labyrinth is whit, the 1 and 2 are wood colour. 40 minutes following, the door of the hand 3 is open and each rat can explore the third hand that is the novel environment for 10 minutes. The time (seconds) spent by each animal in each hand of labyrinth is noted a long 10 minutes of the test.

Animals were assigned to the control group (CO), scopolamine group (SCO), *Anacyclus pyrethrum* (200mg) group (AP200) and Scopolamin + *Anacyclus pyrethrum* (200mg) group (SCO + AP200).
D. Statistical analysis

The results of sequences for all groups expressed as the means ± Standard error mean (SEM). The ability of scopolamine to alter classical conditioning, the effect of APE and the results of combination of this two treatments was studied by a One-way ANOVA or Krushal-Wallis one-way analysis of variance followed, when significant, by the post hoc Tukey’s test to determine statistical differences between groups.

III. RESULTS

Pre-training preferences for the compartments of test box were established by comparing the cumulative time spent in each compartment. There was no effect relative to the compartment in wish rats were placed first. (The right and the left compartments are identical).

In this study, the effect of "APE" and "sco" were evaluated using 10 sequences of classic conditioning. The results are presented in Table 1.

Results show that the control animals have learned to avoid the foot shock since the second sequence of conditioning. The Percentage of CR increases with number of trials. At the sixth session, 100% of the control rats were conditioned. The ANOVA (control compared to treated groups by APE or SCO association/sequences) showed a significant effect in the second and fifth sequence. In the 2nd sequence the analysis shows, a significant effect between control (1,5 ± 0,34) and treated group by APE 200 mg/Kg of APE (1 ± 0,25) (F(2,15)= 4,56 , P=0,028, Tukey test). However, in the 5th sequence of the treated group by 200 mg/Kg (10 ± 0), the performance is significantly higher than the control group (8,3 ± 0,49) (H(2,15)= 8,854, P= 0,012, Tukey test).– and also the treated group by 100mg/Kg of APE (8,33 ± 0,49). This results suggest that the involvement of a "APE" in acquisition of conditioned responses in behavioral test is important and shows a dose-response relationship of "APE" treatment (table1).

TABLE1: MEAN CONDITIONING RESPONSES SCORED IN EACH SEQUENCE OF CONDITIONING BEHAVIOUR.

<table>
<thead>
<tr>
<th>Sequences</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.33 ± 0.21</td>
<td>1.5 ± 0.4</td>
<td>4.5 ± 0.4</td>
<td>5 ± 0.36</td>
<td>10 ± 0</td>
<td>10 ± 0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Scopolamine</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0.33 ± 0.21</td>
<td>0.33 ± 0.3</td>
<td>0.33 ± 0.3</td>
<td>0.33 ± 0.3</td>
<td>0.33 ± 0.3</td>
<td>0.66 ± 0.21</td>
</tr>
<tr>
<td>APE (100)</td>
<td>0.5 ± 0.22</td>
<td>2 ± 0.25</td>
<td>4 ± 0.3</td>
<td>8 ± 0.33</td>
<td>3 ± 0.4</td>
<td>3 ± 0.4</td>
<td>0.66 ± 0.33</td>
<td>0.66 ± 0.21</td>
<td>0.66 ± 0.21</td>
<td>0.21</td>
</tr>
<tr>
<td>APE (200)</td>
<td>0.5 ± 0.25</td>
<td>2 ± 0.44</td>
<td>0 ± 0.36</td>
<td>0 ± 0.47</td>
<td>0 ± 0.36</td>
<td>0 ± 0.36</td>
<td>0 ± 0.36</td>
<td>0 ± 0.36</td>
<td>0 ± 0.36</td>
<td>0 ± 0.36</td>
</tr>
<tr>
<td>SCO + APE100</td>
<td>0.16 ± 0.16</td>
<td>2 ± 0.23</td>
<td>1 ± 0.23</td>
<td>1 ± 0.23</td>
<td>0 ± 0.16</td>
<td>0 ± 0.16</td>
<td>0 ± 0.16</td>
<td>0 ± 0.16</td>
<td>0 ± 0.16</td>
<td>0 ± 0.16</td>
</tr>
<tr>
<td>SCO + APE200</td>
<td>0.16 ± 0.22</td>
<td>2 ± 0.23</td>
<td>1 ± 0.23</td>
<td>1 ± 0.23</td>
<td>0 ± 0.16</td>
<td>0 ± 0.16</td>
<td>0 ± 0.16</td>
<td>0 ± 0.16</td>
<td>0 ± 0.16</td>
<td>0 ± 0.16</td>
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</table>

The results of administration of scopolamine in rats show a significant decreases in the acquisition of conditioned responses, compared to control, APE 100 mg/Kg , and 200 mg/Kg groups. In the SCO group, neither rat was conditioned in all fourth sequences; mean of conditioned response is < 1 in all the ten sessions of conditioning. At the tenth sequence, 7% of rats were conditioned. By comparison with control group, the difference is highly significant, the control group exhibits more ovoid behaviour than the scopolamine group in same conditions. Intraperitoneal administration of scopolamine impaired avoidance and learning processes.

In the group treated by orally injection of Anacyclus P (APE100), results show that 83% of rats are conditioned in the session five and 100% in the sequence six. In the AP200 group, 100% of animals are conditioned since the sequence five. In the group receiving scopolamine and treatment by APE100, obtained results show that 26.6% of rats are conditioned in sequence seven and 63.3% in sequence ten. The same results are obtained with SCO + APE200, 40% of animals are conditioned in sequence seven and 83.3% in sequence ten.

The combination of scopolamine and "APE" using two different doses (100 mg/kg and 200 mg/kg) show that the APE reverses the power of SCO to decrease the number of conditioned responses in all sequences of this behavioral test. The ANOVA (mean of association/sequences of control group compared to SCO and SCO+APE groups ) showed a significant effect on the number of conditioned responses in each sequence. ( sequence 2, p = 0.004; sequence 3, p = < 0.001; sequence 4, p = <0.001; sequence 5; p = <0.001; sequence 6, p = <0.001; sequence 7, p= <0.001); sequence 8, p = <0.001); sequence 9, p = <0.001; sequence 10, p = < 0.001). The effect of the APE at 200mg/Kg was significantly higher compared to that of APE at 100mg (Tukey’s test). Obtained results show that Anacyclus pyrethrum roots extract has inhibited the scopolamine effect on learning and conditioning processes.

Experiment 2:

Concerning the exploratory activity, results are expressed in seconds spent by each rat in each compartment of the labyrinth.

In controls, the time spent in each hand is identical; there is no preference for the new environment. In SCO group, results show that the visit of H3 is more less by comparison with controls (P<0,02), with APE200 group (p<0.005) or SCO + APE200 group (p<0.05) (Table 2). Intraperitoneal injection of scopolamine decrease exploratory activity of the novel environment and APE treatment enhances the exploratory behaviour in the rat. By comparison with controls, significant increase was observed (p<0.02). In rats receiving SCO and APE200, scopolamine does not disrupt the exploratory activity. In this group, time spent to explore H3, a novel compartment, is more important, by comparison with SCO group, a significant increase was observed (p<0.005). In this behaviour, obtained results show that the APE enhanced SCO impairment in exploratory activity in the rat.
IV. DISCUSSION

Deficits in memory are a neurodegenerative disorder without effective treatment. This cognitive trouble is associated with deficits in abilities; patients also have non cognitive symptoms such as depression, apathy and psychosis that impair learning [1, 7]. Progressive memory loss is currently seen as medical and social problems. The scopolamine amnesia test is widely used as primary screening test for anti amnesic drugs [6]. In the present study, we suggest that ethanolic extract of Anacyclus pyrethrum; a tonic to the nervous system can enhance scopolamine impairment in learning and exploratory behaviours.

APE treated rats, show a high performance in the acquisition of conditioned responses which is a cognitive sign improvement. Results have shown that scopolamine inhibited learning and conditioning behaviors. This finding is in agreement with previous studies reporting that scopolamine altered the active ovoidance behavior [2]. In our experiences, animals treated by this substance, had difficulties to make correlation between the CS and US. But SCO group treated by APE exhibited an important effect in reversal of scopolamine induced-impairment in learning and exploratory behaviours. These effects are revealed by increase in the number of conditioned responses in learning and increase in the time spent in exploring a novel environment. Badhe et al. (2010) [23] previously determined antidepressant activity of APE. Anacyclus pyrethrum root extract was found to be effective in reversing hypothermia produced by clonidine and reserpine. The AP root extract inhibited haloperidol-induced catalepsy. It suggests that, AP root extract might produce antidepressant effect by interaction with adrenergic and dopamine receptor thereby increasing the level of noradrenaline and dopamine in brains of mice. In the other hand, the implication of central cholinergic system in the regulation of the cognitive functions was also reported [20]. Thus, cholinergic stimulations facilitate learning and information consolidation while cholinergic blockade products the amnesia [22, 8, 9, 5]. Loss of cholinergic neurons in certain sits of the brain as nucleus basalis mango cellular of the cortex is the sign of feature memory disorders [10]. Scopolamine is a centrally acting cholinergic agent, which cause impairment in learning [24]. The treatments with drugs, which increase cholinergic transmission, cause an improvement in cognitive deficits [17]. In this study, Anacyclus pyrethrum extract enhance scopolamine impairment in learning and exploratory behaviours. The effect of APE could be attributed to the elevating acetylcholine concentration in the brain. In this way, [11], has reported that APE inhibited Acetylcholinesterase enzyme. These results suggest the possible neuroprotective effects of Anacyclus pyrethrum. These findings confirm the use of this plant as a tonic to the nervous system and as treatment of memory disorders in traditional medicine in North Africa.

In conclusion, this study shows that scopolamine acts to block exploratory, learning and conditioning processes, this blockage is reversed by Anacyclus pyrethrum treatment. Still more experiments are required with the Anacyclus pyrethrum to investigate the mechanism of action with others tests and other therapeutic activities.

ACKNOWLEDGMENT

We are very thankful to Pr. A. Ouhamou for helping with the plant specimen’s identification and to Mr. A. Regragui for his technical assistance.

REFERENCES


