Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
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ORIGINAL ARTICLE

Effects of *Anacyclus pyrethrum* on Affective Behaviors and Memory during Withdrawal from Cigarette Smoke Exposure in Rats

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Submitted: 27-06-2019 Revised: 30-09-2019 Published: 31-03-2020

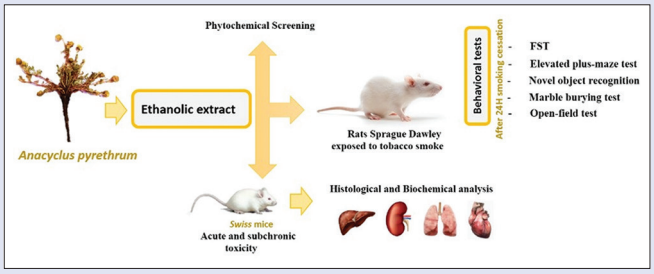
ABSTRACT

Background: During smoking cessation, nicotine withdrawal is associated with many symptoms including cognitive impairment and depressed mood, which lower the desire to quit smoking and predicts smoking relapse. Thus, pharmacotherapies that improve cognitive functions during nicotine withdrawal would be paramount in developing efficacious smoking cessation agents. Medicinal plants are currently considered as a promising source to identify new therapeutics. The roots of *Anacyclus pyrethrum* are used in traditional medicine to treat various diseases. **Objectives:** The aim of this study was to evaluate the behavioral effects of ethanolic extract of *A. pyrethrum* (EEAP) on smoking withdrawal in a rat model. **Subjects and Methods:** EEAP was administered orally at a dose of 200, 400, and 800 mg/kg. Forced swimming test (FST), open-field, marble-burying, and plus-maze tests were used to measure the level of depression and anxiety in animals. In addition, the novel object recognition test was used to test memory impairment. **Results:** The results showed that EEAP-treated animals had decreased immobility time in forced swimming test and they buried fewer marbles. The percentage of the time spent and the number of entries in the open arm within the elevated plus-maze test was increased in smoking withdrawn rat after treatment. On the other hand, EEAP increased the recognition of memory in the novel object recognition task. **Conclusion:** Taken together, our data indicated potential antidepressant-like and anxiolytic-like effects of EEAP in rats and the improvement of memory. Besides, this plant does not have any acute or subchronic toxicity effect.

Key words: Anacyclus pyrethrum, antidepressant-like effect, anxiolytic-like effect, improvement of memory, smoking cessation

SUMMARY

- *Anacyclus pyrethrum* did not produce any behavioral abnormalities and mortality in the acute and subchronic toxicity
- *A. pyrethrum* root extract has potential antidepressant and anxiolytic-like effects
- Ethanolic extract of *A. pyrethrum* (EEAP) (200, 400, and 800 mg/kg) produces an improvement of recognition memory following administration of EEAP in smoking withdrawn rats
- *A. pyrethrum* could provide potential novel agents for the prevention and treatment of smoking relapse due to withdrawals.



Abbreviations used: EEAP: Ethanolic extract of *Anacyclus pyrethrum;* FST: Forced swimming; AchEIs: Acetylcholinesterase inhibitors; CDC: The Centers for Disease Control and Prevention; WHO: World Health Organization; OECD: Organization for Economic Cooperation and Development; HPLC: High-performance liquid chromatography; PDA: Photodiode array detectors; HE: H and e; AST: Aminotransferase aspartate; ALT: Aspartate alanine transaminase; CPK: Creatine phosphokinase; LDH: Lactate dehydrogenase; ID: Discrimination index; GABA: Gamma-aminobutyric acid.

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DOI: 10.4103/pm.pm_279_19

Access this article online
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INTRODUCTION

Smoking addiction is now regarded as a chronic disorder.[1,2] When smokers attempt to quit, there are various withdrawal symptoms, such as smoking cravings, irritability, cognitive impairment, and depressed mood.[3,4] The disagreeable emotions, physiological, and cognitive symptoms of withdrawal primarily appeared during the 1st day of nicotine withdrawal,[5,6] In this study, we aimed to discover a new treatment to prevent smoking relapse.

Fiore *et al.* reported in their work that three-drug therapies are currently available to help people quit smoking: bupropion

Cite this article as: Bezza K, Laadraoui J, El Gabbas Z, Laaradia MA, Oufquir S, Aboufatima R, *et al.* Effects of *Anacyclus pyrethrum* on affective behaviors and memory during withdrawal from cigarette smoke exposure in rats. Phcog Mag 2020;16:S123-32.

hydrochloride (sustained-release), nicotine replacements, and varenicline tartrate.[7] As a potential treatment for nicotine addiction, acetylcholinesterase inhibitors (AChEIs) were recently proposed.[8,9] At the molecular level, these drugs acting as an AChEI would induce an increase in the extracellular level of acetylcholine in the brain, increasing cholinergic transmission.[10,11] The use of these drugs, which increased cholinergic transmission, could lead to several side effects such as malaise symptoms, nausea, and vomiting.[12-14]

There is, therefore, an urgent need to develop new and safer anti-smoking medicine. The World Health Organization supported the importance of medicinal plants for public healthcare in developing countries.[15]

A. pyrethrum (L.) Link (Asteraceae) is widely used for its variety of pharmacological actions. At the molecular level, the ethanolic extract of *A. pyrethrum (*EEAP) inhibited acetylcholinesterase activity.[16,17] A study done on pellitorine, which is among the major compounds of *A. pyrethrum* showed that this compound has good intestinal permeation and penetrates rapidly into the blood–brain barrier once in the blood, which points to a possible role in the treatment of central nervous system diseases.[18]

The aim of the present work was to evaluate the impact of the EEAP in withdrawn rats (24 h of smoking cessation) following exposure to cigarette smoke for 3 months. The memory and learning defects were evaluated by the novel object recognition test. The anxiolytic- and antidepressant-like activities were measured using plus-maze, open-field, marble burying, and forced swimming tests. Our data indicated that *A. pyrethrum* root extract has potential antidepressant, anxiolytic-like effects, and ameliorate the cognitive dysfunction in nicotine-withdrawing rats.

SUBJECTS AND METHODS

Animal

At the beginning of smoking exposure, adult male, Sprague Dawley rats weighing 180–230 g were used for *in vivo* bioassays. The animals were provided by the Animal Care Facility of the Faculty of Sciences Semlalia, Cadi Ayyad University, Marrakech, Morocco. They were housed in a conditional room maintained at 22°C ± 2°C temperature under a 12:12 h light: Dark cycle with free access to water and food. Animals are housed in groups of six rats per standard Makrolon cage. They were allowed to adjust to the housing room conditions for 24 h before starting behavioral procedures. All experiments were carried out between 1100 and 1700, and each animal was used only once.

The experiments reported in this study were performed by following the guidelines care of laboratory animals and the ethical guidelines investigation of experimental pain in conscious animals. These are in strict accordance with the guidelines of the European Council Directive (EU2010/63). All efforts were made to minimize animals suffering and to reduce the number of animals used in the experiments.

Plant material and preparation of extracts

Plant material

Anacyclus pyrethrum was collected in May 2014 in the area of Bin El Ouidan in Morocco. The plant identification was performed by Professor Ouhammou from the Biology Department, Faculty of Sciences, Semlalia, Cadi Ayyad University. A voucher specimen MARK-10003 was deposited in the Herbarium of the Biology Department.

Preparation of ethanolic extract of Anacyclus pyrethrum

The roots of the plant were collected, dried, and cut into small pieces, then cleaned and ground in grinding machine to produce a fine powder. Then, 600 mL of a solvent (ethanol) was added to 200 g of powder to obtain the ethanol extract using the Soxhlet apparatus. The mixture of the powder and the solvent was then incorporated in the rotary evaporated to evaporate the ethanol and precipitate the extract. The evaporation conditions were at reduced pressure and at a temperature under 60°C. The weight of the concentrated extract was 34.1 g, which represents 17.05% yield.

Phytochemical screening of ethanolic extract of Anacyclus pyrethrum

Ethanol extract obtained from *A. pyrethrum* was analyzed for the detection of flavonoids, terpenoids alkaloids, saponins, and tannins. These phytochemicals were qualitatively determined using previously reported methods.[19,20]

Test for flavonoids

Flavonoids are revealed by the Cyanide reaction: To 1 mL of the ethanol extract, 5 mL of alcohol (50%), 1 mL of concentrated hydrochloric acid, and small quantities of magnesium chips were added. The appearance of the red color indicates the presence of flavonoids.

Test for tannins

The addition of a few drops of a 9% of ferric chloride solution to our extract produces a blue (Gallic tannins) or dark green (catechin tannins) color in the presence of the tannins.

Test for alkaloids

A total of 10 g of the sprayed plant are agitated in 50 ml of sulfuric acid (H_2SO_4) (0.1 N) for 15 min. After filtration, the solution obtained was alkalinized with 5 mL of ammonia solution (25%) and diluted with 50 mL of distilled water. Following extraction of the alkaloids with dichloromethane and vacuum evaporation, the residue obtained was taken up again with a (10%) sulfuric acid solution. The presence of alkaloids causes the formation of a precipitate after the Dragendorff's reagent was added.

Test for saponins

The presence of saponins in our plant was tested on a 20% decoction. After stirring, the reaction is said to be positive when it foam was formed, 1 cm high, stable for 10 min.

Test for anthocyanins

The addition of concentrated hydrochloric acid to 10% of the infused plant gives a red color in the presence of anthocyanins.

Characterization of *Anacyclus pyrethrum* extract

The high-performance liquid chromatography (HPLC) (Knauer) equipped with a (K-1001) pump and a photodiode array detector (200–700 ultraviolet–visible [UV/Vis]) was used to separate and characterize phenolic compounds present in *A. pyrethrum* extract. Chromatography separations were performed on a Reversed-Phase (RP-18) Columns, Agilent Technologies (250 mm × 4.6 mm, 5.0 µm), protected by Agilent technologies RP-18 (10 mm × 4.6 mm) pre-column. Both columns were placed in a column oven set at 25°C. The HPLC system consisted of Shimadzu SCL-10A pumping system, SIL-10AD automatic injector, and the Shimadzu SPD 10A UV/Vis detector (wavelength scanning range 200–700 nm). Data collection and analysis were performed using SHIAMDU LabSolutions software. Two solvents were used with a constant flow rate of 1 ml/min gradient program, and the sample volume injection was 10 µl. Solvent A consisted of 5% acetonitrile and 95% water. Solvent B is a phosphate buffer dissolved in water (pH 2.6).[21,22]

The identification of major compounds was made by comparison of retention time and spectra with those of commercially available standard compounds.

Drugs administration

Rats were divided into 12 groups (six animals per group). The animals were randomly divided into control and experimental as follows:

1. Control group received vehicle (distilled water)
2. Control group treated with EEAP (200 mg/kg; 400 mg/kg; 800 mg/kg); clomipramine 15 mg/kg and diazepam1 mg/kg. Each group contained six rats. Animals received one dose of the indicated drugs intraperitoneally (i. p.), except for the EEAP that was administered orally by gavage
3. Smoking withdrawn group (24 h smoking cessation) after 3 months of cigarette exposure
4. Smoking withdrawn treated group (24 h smoking cessation) with (EEAP 200 mg/kg; 400 mg/kg; 800 mg/kg; clomipramine 15 mg/kg; diazepam 1 mg/kg).

All drugs were orally administered between 09:00 and 12:00.

Cigarette smoke exposure

The rats were exposed to tobacco smoke as described[23,24] with slight modifications. Tobacco smoke exposure lasted 3 months in a special apparatus designed to expose the rats for 2 h to cigarette smoke. The exposure occurred in an inhalation chamber (50 cm × 30 cm × 24 cm). For 3 months, the smoker group was exposed to the smoke of six filtered commercial cigarettes per day (a total of 84.0 mg tar and 6 mg nicotine). A vacuum pump VP800 was used to generate and aspirate 100 mL of smoke in 1s. Tobacco smoke has been produced by burning filtered cigarettes; once, the cigarette was lit; the suction was activated at the same time. In the end, the suction pump was turned off.

Acute and subchronic toxicity

The acute and subchronic toxicity study was conducted according to the Organization for Economic Cooperation and Development (OECD) guideline no. 423 (OECD, 2001), where the 5000 mg/kg limit test dose was used. Mice were equally divided into nine groups (six animals per group). Six groups were orally treated by gavage with different doses (200, 400, 800, 1000, 2000, and 5000 mg/kg) of EEAP solution for the acute toxicity Whereas, in the subchronic toxicity, three groups were orally treated with three doses (200, 400, and 800 mg/kg) at 10 mL/kg. The group that received a vehicle (distilled water) was considered as a negative control. Mice were observed during the first 12 h after administration of the drug to detect signs of toxicity and death. Mice were observed daily and weighed for 14 and 90 days following treatment. After the 14- and 90-day period, mice were sacrificed by cervical dislocation, blood was collected, and vital organs were immediately collected then weighed to perform biochemical analysis and histological studies.

Histological study

For the histopathological study, all ten groups were used. Mice were sacrificed by cervical dislocation after the 24 h smoking cessation, and their vital organs such as the liver, heart, lungs, and kidneys were dissected and fixed in 10% formalin solution for one night. After, the organs are subjected to dehydration in a series of graded alcohols and incorporated into paraffin wax. Sections of 4–10-µm thickness were stained with hematoxylin and eosin for pathological studies, as described by Kiernan.[25]

Biochemical analysis

Biochemical analysis was carried out on the serum of mice outlined in the previous section. The serum was obtained by centrifugation of blood samples. Creatinine, aminotransferase (AST), aspartate alanine transaminase (ALT), creatine phosphokinase (CPK), and lactate dehydrogenase (LDH) levels were quantified in the control and treated groups, using the standard method with a biochemical automat (C 311 ROCH).

Behavioral tests

FST

FST was similar to that described by Porsolt *et al*.[26] Rats were individually required to swim in an open cylindrical container (diameter 21 cm, height 50 cm) containing 25 cm of water maintained at 25 ± 1°C. The total of time (in sec) that each animal stayed motionless during a 6-min session was recorded as immobility time. Immobility refers to the cessation of struggling and when rat remained floating motionless in the water with the absence of active behaviors such as swimming, jumping, rearing, sniffing, or diving, and they only moved to keep the head above the water. A decreased duration of immobility time is referred to as a behavioral profile with an antidepressant-like effect.

Open-field test

To evaluate the possible effects of EEAP on locomotor activities, a group of weaned rats was evaluated in the open-field test as previously described.[27] The rats were separately placed in a white arena, measuring (80 cm × 80 cm × 40 cm) which was divided into 25 squares of the same dimensions. Over a period of 5 min, the number of crosses representing the rectangles crossed by the animal with its four legs was registered. During the trial period, the animal's movements in the field were based on the number of crosses (at least three legs in a square), and the number of rearing is known as the animal standing on its hind legs (rear and free hind legs). The number of crossings was considered as indicative of locomotor activity. The floor of the open-field apparatus was cleaned with 10% ethanol at the end of each test to remove any olfactory cues.

Marble burying test

The marble-burying test is a valid model for anxiety.[28,29] It was suggested that the test could be useful in a predictable way for the identification of new antidepressants[30,31] or anxiolytics.[32] The protocol was the same as the procedure described by Deacon and Rawlins.[33,34] At a depth of 5 cm, the cage was full of wooden litter. Litter substrate can be used again if it is flattened and firmed down again between rats. A pattern of regular glass marbles was placed on a surface, which is uniformly spaced about 4 cm each. There was one animal in each cage that was placed and stayed there for 30 min. After the trial was ended, the rat was carefully pulled out of the cage. The number of marbles buried (to 2/3 their depth) with bedding was counted.

Elevated plus-maze test

To evaluate the anxiolytic-like effect of EEAP, the elevated plus-maze (EPM) was used. This test has been widely validated to measure anxiety in rodents.[35] The apparatus comprises two open arms (50 cm × 10 cm each), two enclosed arms (50 cm × 10 cm × 20 cm each) and a central platform (10 cm × 10 cm). The maze was raised 100 cm from the floor and was made of plywood.

Each animal was placed in the center of the labyrinth facing one of the closed arms. Over the 5-min trial period, the number of open and closed arm entries was recorded, as well as the time spent in open and closed arms.[36] Arm entry has been designated as the point at which the animal places its four legs on the arm. The percentage of open arm entries (100 × open/total entries) and time spent in open arms (100 × open/total times) was calculated for each animal. To record the animals' behavior, a video camera was placed above the maze. After the test, the maze was cleaned with a 10% ethanol solution.

Novel object recognition

This test was carried out as described by Bevins and Besheer.[37] One day before testing, each rat was allowed to explore the apparatus which was an open field (80 cm × 80 cm × 40 cm) made of plexiglass for 2 min. After the administration of EEAP, two sessions were allowed for each rat. Two identical objects (F) were placed in the back corner of the box while training sessions. The experimental rat was placed in the box, and a 10-min total exploration time was recorded for both objects. At the retention session, the animal was placed back in the same box 60 min following the training session; a new object (N) replaced one of the familiar objects that were presented during the training session. The retention session lasts 5 min during which the rat is allowed to freely explore the two objects. The total time used to explore the two identical objects at the training session and the total time spent exploring two different objects, *F* and *N* at the retention session, was measured. The distinction between *F* and *N* in the retention session was calculated by comparing the time used to explore *F* with that used to explore *N*. The discrimination index (ID) was calculated according to the following formula:

$$DI = N - F/N + F$$

Statistical analysis

Comparisons between treatment groups and control were performed using one-way analysis of variance (ANOVA) followed by Tukey's HSD or Dunnett's test, only for responses that have produced significant treatment effects in the ANOVA test. A value of $P < 0.05$ was considered statistically significant

RESULTS

Acute and subchronic toxicity

No visible signs or symptoms of toxicity in mice were observed after oral administration of ethanolic extract of *Anacyclus pyrethrum*. This was observed for all doses up to 5000 mg/kg. Furthermore, no mortality or significant changes in body weight or organ weight were observed 14 days after the administration of EEAP.

In the subchronic study, mice received EEAP for 90 days with no resulting mortality or signs of toxicity. The daily intake of EEAP was 200, 400, and 800 mg/kg. There were no changes in body and organ weights [Table 1]. Creatinine, ALT, LDH, aspartate AST, and CPK levels were also not affected [Table 2].

Table 1: Effect of oral administration of ethanolic extract of *Anacyclus pyrethrum* on body weight and relative organ weights of mice

	Body weight	Brain	Liver	Kidney
Control	27.683±0.248	1.701±0.004	6.473±0.003	0.815±0.004
EEAP 200	27.900±0.214	1.703±0.008	6.468±0.004	0.813±0.004
EEAP 400	27.550±0.274	1.708±0.006	6.467±0.003	0.812±0.004
EEAP 800	27.950±0.067	1.710±0.007	6.463±0.004	0.813±0.004
EEAP 1000	27.416±0.221	1.713±0.004	6.458±0.004	0.817±0.003
EEAP 2000	27.783±0.207	1.708±0.006	6.457±0.005	0.815±0.005
EEAP 5000	27.7167±0.300	1.705±0.007	6.460±0.003	0.808±0.003

EEAP: Ethanolic extract of *Anacyclus pyrethrum*

Histological examination has not revealed any macroscopic or microscopic tissue lesions [Figure 1].

Phytochemical screening of *Anacyclus pyrethrum* extract

Qualitative phytochemical screening of EEAP revealed the presence of terpenoids, sapiens alkaloids, tannins, and flavonoids, and the absence of anthocyanins.

Characterization of *Anacyclus pyrethrum* extract

HPLC chromatogram analysis indicates the presence of Pellitorine in the *A. pyrethrum* namely as the major compound, Undeca-2E,4E-diene-8,10-diynoic acid IBA; Undeca-2E,4E diene-8,10-diynoic acid 2-phenylethylamide; Deca-2E, 4E-dienoic acid 4-OH phenylethylamide; Tetradeca-2E,4E-diene-8,10-diynoic acid IBA (Anacycline); Dodeca 2E,4E-dienoic acid 4-OH phenylethylamide; and Dodeca-2E,4E-dienoic acid IBA [Figure 2]. The concentrations of compounds identified in our extract are tabulated in Table 3.

Effects on immobility time in FST

The possible antidepressant effects of EEAP were assessed by FST. The immobility time was measured during 6 min of swimming [Figure 3]. There was no significant difference between the normal control group and the normal control group treated with different doses of EEAP (200, 400, and 800 mg/kg). The immobility time was significantly longer in a smoking withdrawn group than that for the normal control group ($P < 0.001$). A significant reduction of immobility time was observed in both EEAP and clomipramine groups as compared to the smoking withdrawn group.

Effects on the number of crossing and rearing in the open-field test

The open-field activity was measured after 3 months of smoking exposure. Our results showed that the treatment of the smoking withdrawn groups with EEAP or clomipramine had no effect on locomotor activity in the open-field test compared to the normal control group. *Post hoc* analysis indicated a significant reduction in the locomotors activity and in the vertical activity (number of rearing) in smoking withdrawn as compared to the normal control group $P < 0.001$. The smoking withdrawn rat treated by EEAP (200, 400, and 800 mg/kg) and clomipramine (15 mg/kg, i. p) significantly increased the movement distance, also increased the rearing [Figure 4a]. However, there was no significant difference between the normal control group and the normal control group treated with different doses of EEAP (200, 400, and 800 mg/kg) [Figure 4b].

Effect of *ethanolic extract of Anacyclus pyrethrum* in the marble-burying test

In the Marble-burying test, our results showed that there was no significant difference between the normal control group and the normal control group treated with different doses of EEAP (200, 400, and 800 mg/kg). Furthermore, smoking withdrawn rats buried the most marbles as compared

Table 2: Effect of ethanolic extract of *Anacyclus pyrethrum* on the enzymatic activities and levels of some metabolites in control and treated mice

	Urea (g/L)	Creatinine (g/L)	ASAT activity (U/L)	ALAT activity (U/L)	CK activity (U/L)	LDH activity (U/L)
Control	0.29±0.06	1.90±0.22	124.29±1.24	58.30±1.90	701.10±4.56	614.55±23.84
EEAP 200	0.28±0.03	1.70±0.21	134.80±2.03	59.80±2.05	730.90±11.83	609.45±34.56
EEAP 400	0.27±0.07	1.70±0.13	130.01±3.67	59.76±2.96	693.53±12.49	620.26±31.92
EEAP 800	0.29±0.02	1.80±0.15	129.57±2.66	56.07±1.09	698.70±9.48	593.77±52.32

Urea, Creatinine, ALAT, ASAT, CK and LDH activities. ASAT: Aminotransferase aspartate; ALAT: Aspartate alanine transaminase; CK: Creatine phosphokinase; LDH: Lactate dehydrogenase; EEAP: Ethanolic extract of *Anacyclus pyrethrum*

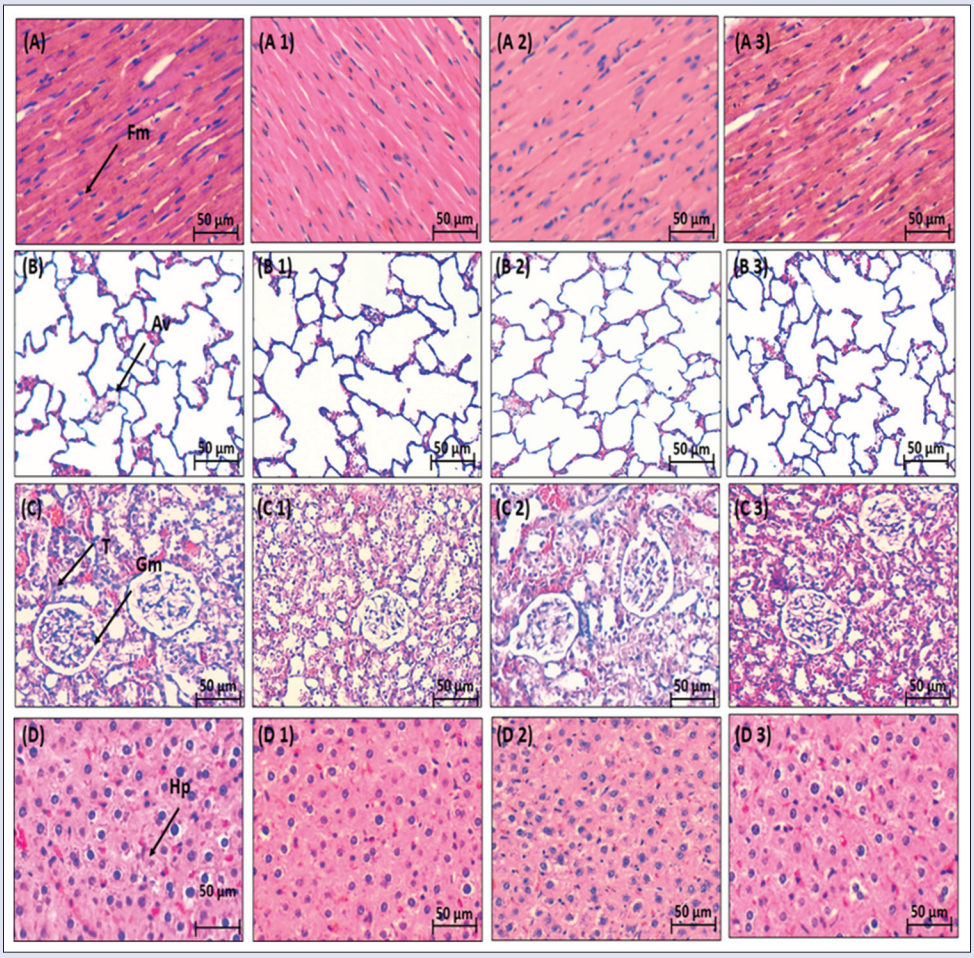

Figure 1: Histopathological observations of vital organs. Control mice injected with only saline and all treated organs showing normal tissue patterns in all vital organs: Heart (A); lungs (B); kidney (C) and liver (D). Hepatocyte (Hp), myofibril (Fm), alveoli (Av) and glomerulus (Gm)

to the normal control group, ($P < 0.001$). While, the treated group with EEAP (200, 400, and 800 mg/kg) buried fewer marbles than the smoking withdrawn group, ($P < 0.001$). The behavioral response was statistically significant between the smoking withdrawn group vs. normal control group and the smoking withdrawn group treated with EEAP [Figure 5].

Effect of *ethanolic extract of Anacyclus pyrethrum* in the elevated plus-maze test

The role of *A. pyrethrum* in the regulation of anxiety-like behaviors was assessed using the EPM test. The smoking withdrawn group enhanced anxiety-like behavior as indicated by the decrease in the percentage of entries in open arms and the time spent in open arms compared with the normal control group ($P < 0.001$) [Figure 6a]. Smoking withdrawn group treated with EEAP (200, 400, and 800 mg/kg) or diazepam exhibited a significant increase in both percentages of entries in open arms and the time spent in open arms compared to the smoking withdrawn group ($P < 0.001$) [Figure 6b]. Whereas, there was no significant difference between the normal control group and the normal control group treated with different doses of EEAP (200, 400, and 800 mg/kg).

Effect of *ethanolic extract of Anacyclus pyrethrum* in novel object recognition

Our results showed that there was no significant difference between the normal control group and the normal control group treated with different doses of EEAP (200, 400, and 800 mg/kg). This test indicated that the smoking withdrawn group explored the new object for significantly less time than control ($P < 0.001$) [Figure 7a]. Indeed, the statistical analysis indicated that the time spent in exploring the new object was significantly increased in the treated group compared to the smoking withdrawn group ($P < 0.001$) [Figure 7b]. The ID for the smoking withdrawn group was notably lower than that of the control group and the smoking withdrawn treated group ($P < 0.001$) [Figure 7c].

DISCUSSION

From the reported work in the literature, this is the first study of the effects of EEAP on the behavior of smoking withdrawn rat model by using open-field, EPM, marble burying, forced swimming tests, and novel object recognition. Our results suggested that EEAP exert antidepressant- and anxiolytic-like effects and improve memory in rats subjected to smoking withdrawal after 3 months of exposure to cigarette smoke.

Rats exposed to cigarette smoke showed behavioral defects, including reduction in locomotor activity and rearing behavior in open-field activity tests, increased immobility time in forced swimming tests and increased in the number of the buried balls in marble-burying test. In addition, our model showed impairment in memory retention in the novel object recognition task. In this work, *A. pyrethrum* was screened for its acute and subchronic toxicity; it did not produce any behavioral abnormalities and mortality. The

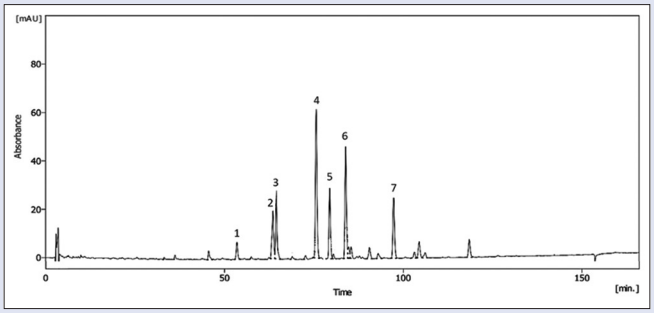

Figure 2: High-performance liquid chromatography chromatogram of *Anacyclus perythrum* extract

results of our study revealed that oral administration of EEAP produced a significant antidepressant-like response in FST on smoking withdrawn rat model. To exclude false-positive FST results, it is important to eliminate the possibility that a decreased immobility time is not simply the result of the psychostimulant effects of the extract, which might be considered a false-positive result in the FST.[38] FST, when used in combination with an open field test, may separate locomotor stimulants from antidepressants.[39] On the other hand, the extract had no significant effect on motor activity and rearing compared to the normal control group as assessed by the open-field test. Thus, the present finding is in agreement with the previous study.[40,41] This study also evaluated the anxiolytic-like effect of EEAP. Our results showed that EEAP significantly reduces the number of buried balls at the dose of (200, 400, and 800 mg/kg). *A. pyrethrum* has been shown to have an anxiolytic-like effect.[42] These findings were confirmed using the

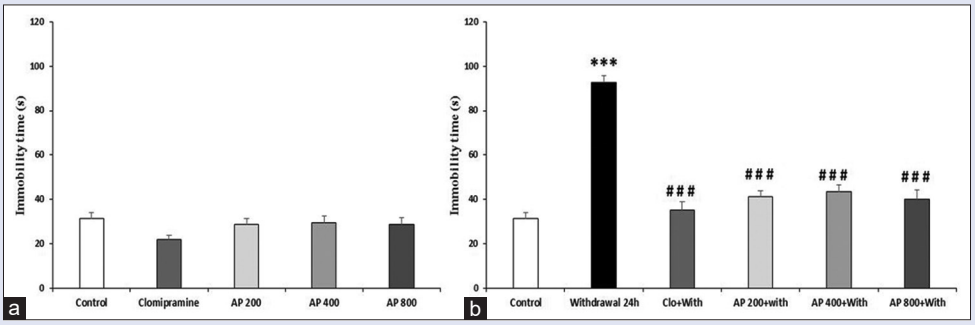

Figure 3: Effect of treatment with *Anacyclus pyrethrum* (200, 400 and 800 mg/kg, p. o) or clomipramine (clo 15 mg/kg, i. p) on rats withdrawn from smoking as assessed by the immobility time in the FST. (a) Immobility time in the normal control group and the normal control group treated with different doses of *ethanolic extract of Anacyclus pyrethrum* (200, 400, and 800 mg/kg). (b) Immobility time in the smoking withdrawn rat treated by *ethanolic extract of Anacyclus pyrethrum* (200, 400 and 800 mg/kg). Results are expressed as means ± standard error of the mean ($n = 6$ per group). ***$P < 0.001$ compared with the normal control group. ###$P < 0.001$, as compared to the withdrawal group

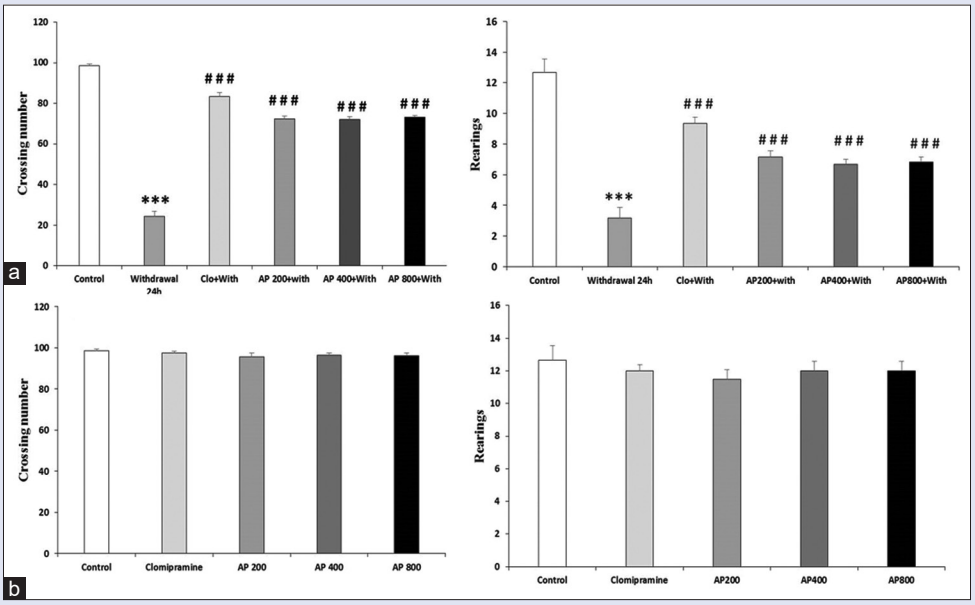

Figure 4: Open-field activity of rats. Animals withdrawn from smoking were treated with *Anacyclus pyrethrum* (200, 400, and 800 mg/kg p. o) or clomipramine (clo 15 mg/kg, i. p) after 3 months exposure to cigarette. The open-field test was carried out 24 h after smoking is stopped. (a) Number of crossings and rearings during the 5-min session in the smoking withdrawn rat treated by *ethanolic extract of Anacyclus pyrethrum* (200, 400 and 800 mg/kg). (b) Number of crossings and rearings during the 5 min session in the normal control group and the normal control group treated with different doses of *ethanolic extract of Anacyclus pyrethrum* (200, 400, and 800 mg/kg). Values are the means ± standard error of the mean ($n = 6$). ***$P < 0.001$ compared with the normal control group. ###$P < 0.001$, as compared to the withdrawal group

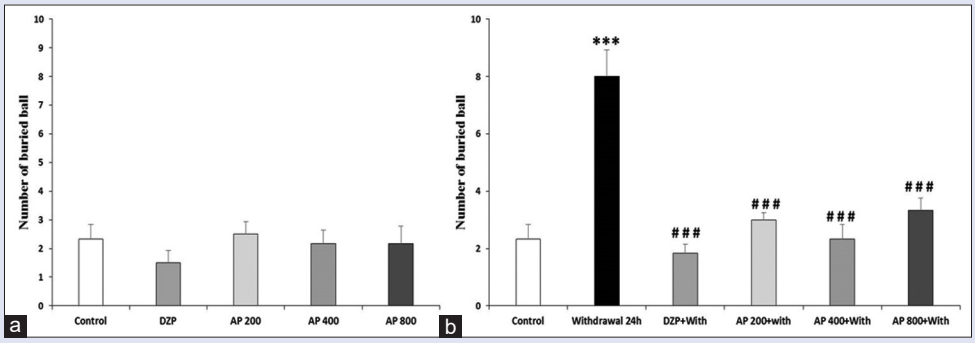

Figure 5: Effect of treatment with *Anacyclus pyrethrum* and diazepam on rats withdrawn from smoking using marble buried test for 30-min period. (a) Number of buried ball in the normal control group and the normal control group treated with different doses of *ethanolic extract of Anacyclus pyrethrum* (200, 400, and 800 mg/kg). (b) Number of buried ball in the smoking withdrawn rat treated by *ethanolic extract of Anacyclus pyrethrum* (200, 400 and 800 mg/kg). Data are expressed as means ± standard error of the mean ($n = 6$ per group). $^{***}P < 0.001$ compared with the normal control group. $^{###}P < 0.001$, as compared to the withdrawal group

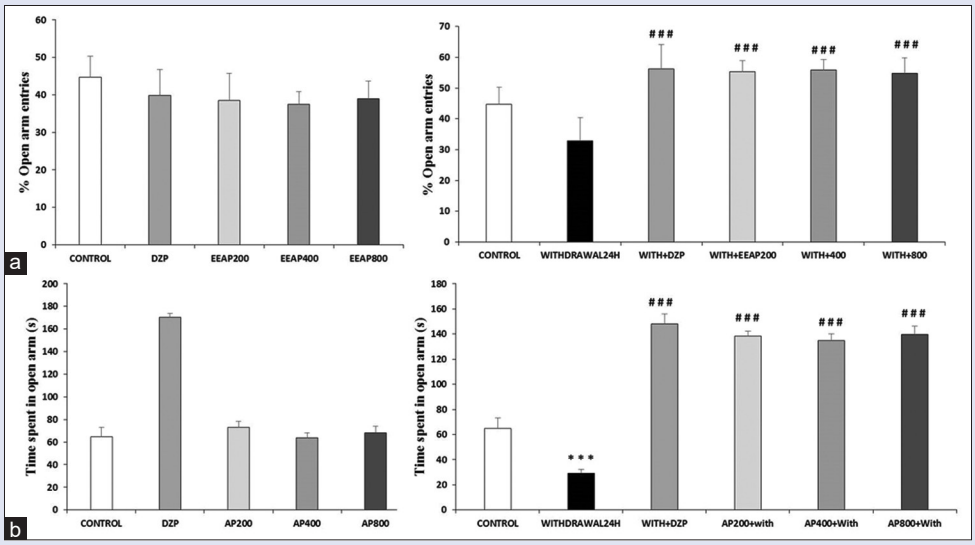

Figure 6: Effect of the treatment with *Anacyclus pyrethrum* (200, 400, and 800 mg/kg, p. o) and Diazepam (1 mg/kg) on rats withdrawn from smoking. The percentage of Open Arms entries and the time spent in the Open Arms in the normal control group and the normal control group treated with different doses of *ethanolic extract of Anacyclus pyrethrum* (200, 400 and 800 mg/kg) (a) was recorded. The percentage of open arms entries and the time spent in the Open Arms in the smoking withdrawn rat treated by *ethanolic extract of Anacyclus pyrethrum* (200, 400, and 800 mg/kg) (b) was recorded. Values are the means ± standard error of the mean ($n = 6$), $^{***}P < 0.001$ compared with the normal control group. $^{###}P < 0.001$, as compared to the withdrawal group

Table 3: Quantification of main compounds in *Anacyclus pyrethrum* expressed in mg of equivalent to gallic acid/100 g of dry matter

Concentrations (mg EGA/100 g DM)	Compounds
12,846	Undeca-2E, 4E-diene-8,10-diynoic acid isobutylamide
14,655	Undeca-2E, 4E-diene-8,10-diynoic acid 2-phenylethylamide
15,169	Deca-2E, 4E-dienoic acid 4-OH phenylethylamide
19,520	Deca-2E, 4E-dienoic acid isobutylamide (Pellitorine)
15,277	Tetradeca-2E, 4E-diene-8,10-diynoic acid isobutylamide (anacycline)
17,236	Dodeca-2E, 4E-dienoic acid 4-OH phenylethylamide
15,473	Dodeca-2E, 4E-dienoic acid

EGA: Equivalent to gallic acid; DM: Dry matter

plus-maze test. However, our study showed that EEAP caused an increase in the number of entries and the time spent on the open arm. The present study also reported an improvement of recognition memory following the administration of EEAP in smoking withdrawn rats. This result is in agreement with what was reported by Sujith *et al.*[16]

In chronic nicotine administration through smoking cigarettes, desensitization occurs at the nicotinic receptor level, which recovered at the end of the exposure.[43,44] Super-sensitivity of some nicotinic receptor subtypes due to nicotine withdrawal can increase the sensitivity of receptors to endogenous acetylcholine ligand.[45] This will enhance the normalization of neural circuits that regulate mood.

Bupropion has been marketed for a long time as an antidepressant, given that a number of depressed patients who were receiving bupropion hydrochloride reported a sudden lack of taste for their cigarettes or even spontaneous cessation of smoking.[46,47]

The role and mechanism of bupropion in this indication are not yet fully understood and remains unknown, although its interaction with brain mediators such as noradrenaline and dopamine has been established.[48] It blocked norepinephrine preferentially as well as dopamine reuptake in the mesolimbic system and nucleus accumbens,[49] which may interfere

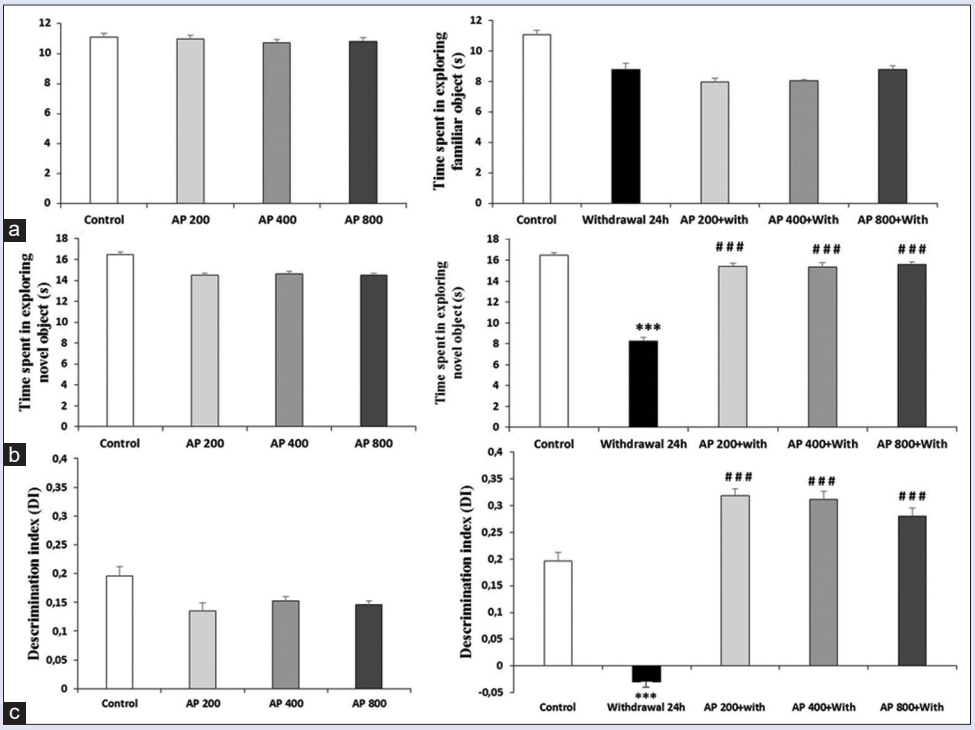

Figure 7: Effect of *Anacyclus pyrethrum* treatment of the normal control group and smoking withdrawn rat on recognition memory. (a) Time spent in exploring familiar object (s). (b) Time spent in exploring novel object (s). (c) Discrimination index. Values are the means ± standard error of the mean ($n = 6$), ***$P < 0.001$ compared with the normal control group. ###$P < 0.001$, as compared to the withdrawal group

with addiction mechanisms through modulation to the accumbens nucleus. Fryer and Lukas suggested that bupropion blocks certain nicotinic cholinergic receptors.[50]

Furthermore, in the Morris water labyrinth and the radial arm labyrinth, which are similar to working memory tasks in humans, the rats that were withdrawing from nicotine have performance issues.[51,52] Studies based on the microdialysis technique have shown an increase in extracellular acetylcholine levels over many cognitive functions, which included exposure to novelty,[53] attention,[54] spatial memory,[55] and functioning memory.[56] Inhibition of acetylcholine and cholinergic system dysfunctions commonly resulted in learning and memory defects.[57]

Galantamine, as an AChEIs, improved cognitive performances following nicotine withdrawal in mice.[58] Furthermore, AChEIs administration increased cognitive performance among healthy smokers.[59] AChEIs could also partially replaced the discriminative stimulus properties of nicotine in humans[8] and rats.[60] Taken together, these data suggested that AChEIs may prevent smoking relapse by alleviating withdrawal symptoms. Interestingly, the EEAP inhibited acetylcholinesterase enzymatic activity,[16,17] causing an increase in the level of acetylcholine in the brain. Indeed, EEAP would restore an imbalanced acetylcholine tone in smoking withdrawn rats. It has also been suggested that the presence of alkaloids and flavonoids in plants supported the acetylcholinesterase activity of plant extracts.[61] In addition, the phytochemical screening of EEAP revealed the presence of alkaloids and flavonoids.

A further suggestion was made that the anxiolytic properties of *A. pyrethrum* could be due to an agonist effect on the benzodiazepine/GABAA receptors, the 5-HT1A receptors, or with other unknown mechanisms.[41] Badhe *et al.* suggested that the *A. pyrethrum* root extract could have an antidepressant effect by interacting with the adrenergic or dopaminergic system, resulting in increased concentrations of norepinephrine and dopamine levels.[42]

CONCLUSION

Our results made evident that EEAP at doses of (200, 400, and 800 mg/kg) improved affective behaviors by possessing anxiolytic and antidepressant properties and have a beneficial effect on memory in rats during the smoking cessation period.

Our data indicated that *A. pyrethrum* offers new opportunities to develop new preventive therapeutics for smoking relapse due to withdrawals.

Acknowledgements

The authors would like to thank deeply E. Gaylard for her time and efforts for the revision of the scientific English.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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