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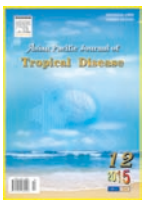

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Evaluation of phytochemical constituents by GC-MS and antidepressant activity of *Peganum harmala* L. seeds extract

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ABSTRACT

Objective: To identify the phytochemical constituents of *Peganum harmala* L. (*P. harmala*) seeds extract by gas chromatography-mass spectrometer (GC-MS) and evaluate their antidepressant activity.

Methods: The seeds extract was analyzed by GC-MS to identify their major chemical compounds. The antidepressant activity of *P. harmala* was demonstrated using the forced swim test and the adrenocorticotropic hormone analysis. After oral administration of the seeds extract and diazepam in rats, the immobility time in the forced swim test was measured and the defecation rate was estimated for all rats.

Results: GC-MS analysis showed the presence of indole alkaloids (β-carboline), for exmaple harmaline (48.009%), harmine (38.440%), tetrahydroharmine (8.513%), tetrahydroharman (0.061%) and 6-methoxytetrahydro-1-norharmanone (0.057%) were present in the extract. The results showed that the *P. harmala* extract (100 mg/kg and 300 mg/kg) were able to reduce the immobility time (80.20 ± 9.01, 46.80 ± 8.53; $P < 0.001$), the adrenocorticotropic hormone rate (131.33 ± 12.72, 49.72 ± 13.03; $P < 0.001$) and the defecation rate (1.400 ± 1.342, 0.600 ± 0.548; $P < 0.01$), respectively.

Conclusions: This indicates that the seeds extract can correct the depression and reach the normal state of the treated animals. The results demonstrated that seeds represented a source of indole alkaloids (harmaline, harmine and tetrahydroharmine).

1. Introduction

Depressive disorders are amongst the most severe and important illnesses globally. Up to 20% of people are affected by them in their various forms. They affect a person's thoughts, feelings, body and social relationships[1]. When a subject undergoes depression, a hyperactivity of the hypothalamic-pituitary-adrenal is observed. The hippocampus causes an activation of the hypothalamus which secretes the corticotropin-releasing hormone (CRH). This hormone in turn causes the hypophysis to produce the adrenocorticotropic hormone (ACTH)[2]. The increase in ACTH is clearly appeared in the blood as a marker of depression[3]. During the last two decades, new antidepressants appeared but that are not deprived of secondary effects. Therefore, a lot of patients refuse the treatment by synthetic psychotropics and prefer to the remedy of the medicinal plants that belongs to the phytotherapeutic products, the most used in the world and the most prescribed[4].

To this effect, we worked on *Peganum harmala* L. (*P. harmala*) that is used as a nervous sedative to treat depression[5]. This is because of its richness in alkaloids such as harmaline, harmine, harmidine and tetrahydroharmine[6]. The objective of this study is to identify the important alkaloids of *P. harmala* seeds extract by gas chromatography-mass spectrometer (GC-MS) and evaluate their antidepressant effect by forced swim test (FST) on rats, which allowed us to estimate the animal's behavior and their immobility time. We also evaluate the effect of *P. harmala* extract by analysis of ACTH.

2. Materials and methods

2.1. Plant material

P. harmala, spontaneous plant, was harvested from Ain El Roumiya

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region located about 20 km from Djelfa City, Algeria. *P. harmala* seeds were harvested on July 2014.

2.2. Animal material

For the pharmacological study, we used female Wistar rats weighing between 180 and 200 g. The rats were sheltered inside cages in polypropylene with a lid made of rustproof steel. The animals had water and food during one week. The litter was composed of wood sawdust, and renewed every two days. The animals were maintained under the laboratory conditions (20–24 ℃ and 50% humidity).

The experiment was conducted in the Pharmacotoxicology Laboratory of Development Research Center (DRC) of SAIDAL, Algiers. The experimental protocol was proven by the DRC.

2.3. Preparation of P. harmala extract

The seeds powder underwent a double ethanolic extraction by the Soxhlet. The extracts were combined and evaporated to dryness. The residue was dissolved in HCl (2%) then filtered. The filtrate was extracted two times with petroleum ether. The aqueous acid layer was basified (pH 10) with NH_4OH. Purification of the alkaloids was obtained by extraction four times with chloroform. The chloroform layer was combined and dried over anhydrous sodium spectrometry (GC-MS). About 0.05 g of the total alkaloids was diluted in 1 mL of chloroform then injected directly to the GC-MS and the remainder of the extract was diluted in the Tween 80 (1%) and used in the pharmacological test.

2.4. GC-MS analysis

The chemical analysis of the indole alkaloid was performed using Agilent Technologies CG7820 and MS5977, equipped with ZB-5MSi column (60 m × 250 μm × 0.25 μm). The carrier gas helium was used at a constant flow rate of 1.0 mL/min.

The thermal program was 60–300 ℃ at a rate of 5 ℃ per minute. The injected volume was 1.0 μL in split ratio 100:1. The amount of each compound was expressed as a relative percentage of the total area of the chromatograms.

2.5. Identification of compounds

Interpretation of GC-MS mass spectrum was conducted using National Institute of Standards and Technology database. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The name, molecular formula and structure of the components of the test material were determined.

2.6. Experimental protocol

Rats were divided into 4 groups of 5 rats each which were control group (G1), Group 2 (G2), Group 3 (G3) and Group 4 (G4).

2.6.1. FST

The FST is a method used for evaluating depression in animal models. The test was frequently used to measure the antidepressants effect on the animal behavior. The depressive animals began to float in the cylinders, demonstrating a behavioral despair. The time of floating or immobility during the FST was an accurate indication of antidepressant and anxiolytic effects.

A day before the test, we forced the rats to swim for 15 min in a vertical cylinder (height: 40 cm, diameter: 18 cm) full of water (25 ℃). Then, the rats were dried at a temperature of 32 ℃ and returned to their cages during 24 h. The rats were put on an empty stomach for 18 h before the test. The day of the test, and by oral way, we administered to all the rats, 1 mL of the following substances: Tween 80 (1%) to each rat (G1), phenylalanine (PHE) 100 mg/kg to each rat (G2), PHE 300 mg/kg to each rat (G3) and diazepam 2 mg/kg to each rat (G4).

After 1 h, the rats were placed in the cylinder and the period of immobility was recorded during 6 min. Rats were considered immobile when they ceased all activities. Meanwhile, we recorded the defecation rate for all rats during the 6 min of the test.

2.6.2. ACTH analysis

After the swimming session, we dried the rats for 5 min. Then, we conducted a blood sample at the rat's eye. A quantity of blood was collected for ACTH analysis.

2.7. Methods of statistical analysis

The immobility and ACTH data were treated by ANOVA, and Tukey and Dunnett. All analyses were performed using the software Minitab 16. The levels of statistical significance varied from $P < 0.05$ to $P < 0.001$.

3. Results

3.1. Chromatographic analysis of P. harmala seeds extract

Figure 1 represents the GC-MS chromatogram of *P. harmala* seeds extract, which showed a set of peaks indicating the presence of the phytochemical compounds. The active principles of the seeds with their retention time, molecular formula and peak area in percentage were presented in the Table 1.

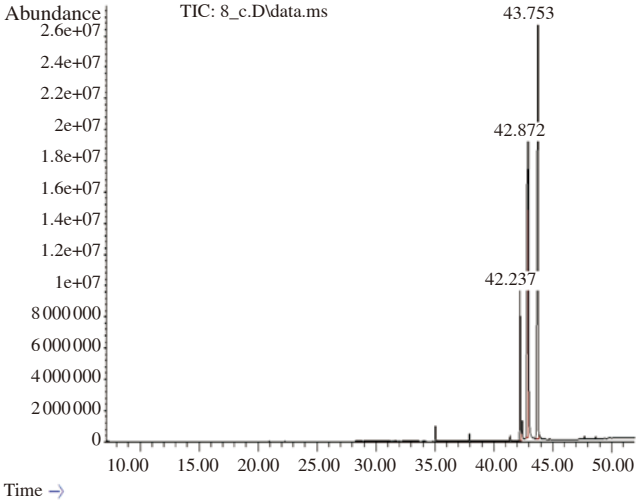

Figure 1. The GC-MS chromatogram of the *P. harmala* seeds extract.

Table 1
Phytochemical compounds identified in the *P. harmala* seeds extract.

Peaks	Name of the compound	Molecular formula	Retention time (min)	Peak area (%)
1	Indole	C_8H_7N	22.263	0.044
2	Quinoline, 2,3,4-trimethyl-	$C_{12}H_{13}N$	35.023	0.935
3	Tetrahydroharman	$C_{12}H_{14}N_2$	37.140	0.061
4	4-Amino-2-ethyl-3 methylquinoline	$C_{12}H_{14}N_2$	37.935	0.238
5	Oleanitrile	$C_{18}H_{33}N$	39.868	0.033
6	Tetrahydroharmine	$C_{13}H_{16}N_2O$	42.237	8.513
7	Harmaline	$C_{13}H_{14}N_2O$	42.912	48.009
8	Harmine	$C_{13}H_{12}N_2O$	43.770	38.440
9	9-Octadecenamide, (z)-	$C_{18}H_{35}NO$	44.759	1.021
10	6-Methoxytetrahydro-1-norharmanone	$C_{13}H_{14}N_2O_2$	47.238	0.057

3.2. Antidepressant activity of P. harmala extract

3.2.1. Forced swim

Figure 2 shows the effect of the PHE and the diazepam on the immobility time in the FST. In G2 and G3, the rats treated with PHE doses (100 and 300 mg/kg), showed a decrease in their immobility time, with a very highly significant difference (80.20 ± 9.01, 46.80 ± 8.53 respectively, $P < 0.001$). Similarly, the rats dealt with diazepam (2 mg/kg) showed a very highly significant decrease in the immobility time (59.80 ± 9.98, $P < 0.001$). While, the G1 presented an increase in the immobility time (167.60 ± 11.46). Tukey's test showed a significant homogeneity only between the immobility time of the rats of G3 ($\bar{x}_3 = 46.8$) and G4 ($\bar{x}_4 = 59.8$), which indicated that the rats of G3 and G4 behaved in the same way.

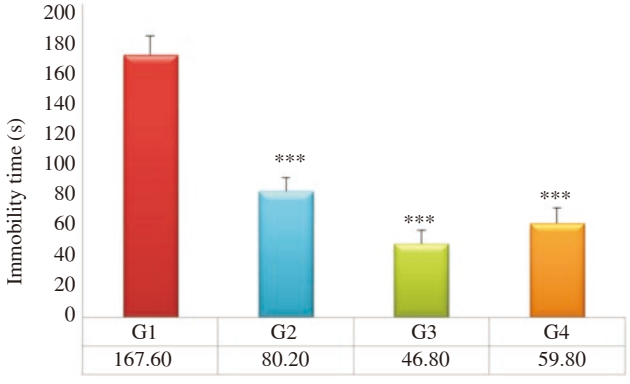

Figure 2. Effects of PHE (G1 and G2) and diazepam (G3) on immobility time in the FST.
***: $P < 0.001$ as compared to respective G1 using Dunnett's test.

3.2.2. ACTH analysis

Figure 3 shows the effect of the PHE and the diazepam on the ACTH levels. The results revealed that the ACTH was low in rats of G2 and G3 treated with both PHE doses (131.33 ± 12.72, 49.72 ± 13.03; $P < 0.001$), and those treated with the diazepam G4 (75.93 ± 17.11; $P < 0.001$), with a very highly significant difference in comparison with the control (457.10 ± 29.14). Tukey's test showed a significant homogeneity just between G3 ($\bar{x}_3 = 49.72$) and G4 ($\bar{x}_4 = 75.93$).

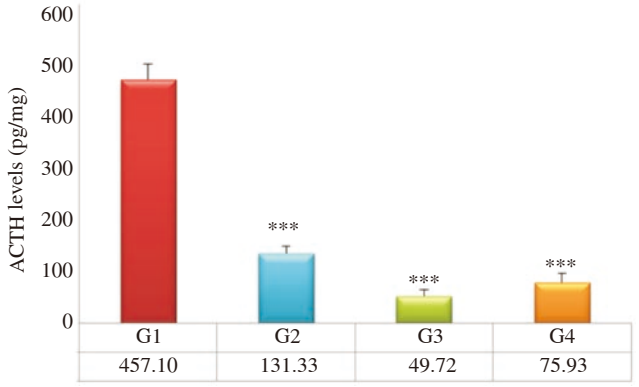

Figure 3. Effects of PHE (G1 and G2) and diazepam (G3) on ACTH levels.
***: $P < 0.001$ as compared to respective G1 using Dunnett's test.

3.2.3. Defecation rate

The rats of G2 and G3 treated with PHE doses (100 and 300 mg/kg) showed a decrease in the defecation rate, with a highly significant difference (1.400 ± 1.342, 0.600 ± 0.548, respectively, $P < 0.01$) in comparison with G1 (4.600 ± 3.050). Similarly, the rats treated with the diazepam (G4) (2 mg/kg) showed a highly significant decrease in the defecation rate (0.600 ± 0.894, $P < 0.01$).

4. Discussion

The chromatographic analysis of *P. harmala* extract shows the presence of the alkaloids and in major part, this alkaloids contain the leptaflorin (tetrahydroharmine), the harmaline and the harmine. These are the indole alkaloids (derivative of the tryptophan amino acid) of β-carboline type[7]. We observed the presence of other β-carboline that are tetrahydroharman and 6-methoxy-tetrahydro-1-norharmanone. The β-carbolines are known by their neuropharmacological and toxicological effects. They have an antidepressant, narcotic and hallucinogenic activity, by the deactivation of the monoamine oxidase of type A responsible for the deterioration and the regulation of the rate of the serotonin and catecholamine in tissues[8].

In this work, it was demonstrated that the administration of different doses of *P. harmala* seeds extract in rats, could be able to decrease immobility time and induce antidepressant effects. This may be due to the presence of β-carboline in the seeds extract and the most important alkaloids are the harmaline, harmine and the tetrahydroharmine[9]. Harmaline alkaloids act as reversible monoamine oxidase inhibitors and with other β-carboline bind to 5-hydroxy tryptamine receptors[10,11]. It has been shown that shortening the immobility time in the FST depends principally on the improvement of the central 5-hydroxy tryptamine and catecholamines neurotransmission[12]. The same β-carbolines are present in the *Passiflora foetida* L., having neurosedative properties[13]. This β-carbolines are known by their neuropharmacological effects which are very powerful analgesics, antidepressants, narcotics and hallucinogens[14].

We observed that the immobility time is higher in the control group,

which could be explained by the fact that these rats are in a depressed state. The rats are considered immobile when they cease all activities[15]. The increase of the immobility time is an indicator of the depression state of the animals[16]. Stress represents the reaction of the body to stimuli that disturbs the normal homeostasis often with detrimental effects[17].

The depression is associated to the changes and hyperactivity of the hypothalamic-pituitary-adrenal. It causes an increase of blood glucocorticoids. They provoke, via specific receptors situated in the hippocampus, an activation of the hypothalamus that secretes the CRH. This hormone brings on its turn the hypophysis to produce the ACTH hormone (adrenocorticotropin) which circulates in the bloodstream[18]. Our results showed that the ACTH levels in the control group were higher than the levels recorded in G2 and G3. This could explain the PHE effect on the regulation of the ACTH levels and therefore on depression.

The result showed that the defecation rate in the groups treated with the seeds extract and diazepam was lower than the control group. This could be due to the PHE effectiveness that is able to reduce the anxiety. Stress influences most digestive functions (contractions, secretion and visceral sensitivity) through the release of CRH which acts on receptors colic. The brain has a role in triggering essential defecation[19].

GC-MS analysis of *P. harmala* seeds showed the presence of β-carboline like harmine, harmaline and tetrahydroharmine. The *P. harmala* extracts were summed with therapeutic benefit in the treatment of the depression and with a higher efficiency for the dose of 300 mg/kg. The significant difference of the ACTH levels confirms the efficacy and the therapeutic power of the plant on the depression.

In conclusion, a significant response was observed with an improvement in the animal's behavior receiving *P. harmala* seeds extract. In this view, the effective antidepressant activity of this plant extract is put in evidence. This can lead to the development of new diagnostic tools and alternative therapeutic strategies.

Conflict of interest statement

We declare that we have no conflict of interest.

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