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Research Paper

Psychostimulant activity of *Rosmarinus officinalis* essential oils

Rachad Alnamer[1], Katim Alaoui[2*], El Houcine Bouidida[3], Abdelaziz Benjouad[1], Yahia Cherrah[2]

[1]Department of Biology, Faculty of Science, Laboratory of Genetic Immunology and Biochemistry, Mohammed-V Agdal University, Rabat, Morocco.
[2]Department of Drugs Sciences, Laboratory of Pharmacology and Toxicology, Faculty of Medicine and Pharmacy, Mohammed-V Souissi University, ERTP, Rabat, Morocco.
[3]National Laboratory of Drugs Controlled, Rabat, Morocco.
* Corresponding Author

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ABSTRACT

We evaluate the psychostimulant activity of *Rosmarinus officinalis* (*lamiaceae*) on central nervous system (CNS). The effects of the essential oil of the stems and leaves of this plant was investigated in three behavioral models including, observation of animals in free situation, search of stereotypes movement in rats and interaction with thiopental in mouse. The essential oil extracted from stems and leaves by hydrodistillation were characterized by means of gas chromatography-mass spectrometry (GC-MS). *Rosmarinus officinalis* contained α-pinene (15.82%), camphene (6.80%), β-pinene (4.75%), myrcene (1.70%), *p*-cymene (2.16%), 1, 8-cineole (50.49%), camphor (11.61%), broneol (2.58%), and broneol acetate (2.08%). We observed that essential oil (100mg/kg) of *Rosmarinus officinalis* significantly increased the locomotors activity and induced hypermobility and hyperexcitability, as compared to a control group. Treatment with *Rosmarinus officinalis* essential oil at the dose of 100 mg/kg, significantly decreased the duration of thiopental-induced sleeping time from 34 min ± 0.2 to 2 min ± 0.2 (P<0.001). These results suggest that, the essential oil from stems and leaves of *Rosmarinus officinalis* possess potential psychostimulant activity. This may justifies its use in traditional medicine as general stimulant.

Keywords: *Rosmarinus officinalis* L; Psychostimulant activity; Central nervous system; GCMS; Stereotypes movements; Psychiatric disorders.

INTRODUCTION

The varied climate and heterogeneous ecologic condition in Morocco have favoured the proliferation of more than 42,000 species of plants; divided into 150 families and 940 geniuses (Alnamer, et al., 2011). The term “psychostimulant drugs” refer to a diverse class of psychoactive compound that shares in common the capacity to activate the central nervous system, and subsequently behavior. Physiologically psychostimulants typically increase the functional activities of central monoaminergic and cholinergic systems (Dalfrerera, et al., 2000). Recently, the search for novel pharmacotherapy from medicinal plants for neurological and psychiatric diseases has progressed significantly owing to their less side effects and better tolerability (Zhang, 2004). Plant extract have been used for the treatment of some psychiatric disorders. Some studies have reported that the extract of *Rosmarinus officinalis* L have a number of pharmacological activities, such as hepatoprodective (Sotelo, et al., 2002), antibacterial (Gachkar, et al., 2007), antithrombotic (Yamamoto, et al., 2005), antiulcereogenic (Dias, et al., 2000), diuretic (Haloui, et al., 2007; Bakirel, et al., 2008), antioxidant (Gonzalez-Trujano, et al., 2007), antinociceptive (Altinier, et al., 2007) and anti-inflammatory (Heinrich, et al., 2006). An ethnopharmacological use of *R. officinalis* in the treatment of depression, among other uses, was reported (Zhang, 2004; Fibiger, et al., 1988; Machado, et al., 2009). However the psychostimulants properties have not yet been studied. Thus, the aim of this study is to evaluate the psychostimulant activity of *Rosmarinus officinalis* essential oil in animal models.

MATERIALS AND METHODS

Plant material: *Rosmarinus officinalis* was collected based on ethnopharmacological information, from villages around the region Rabat-Salé-Zemour-Zaers, with the agreement from the authorities and respecting the United Nations Convention of Biodiversity and with assistance of traditional medical practitioner. The plant was identified with botanist of scientific institute (Pr. M. Ibn Tatou). A voucher specimen (RAB12560) was deposited in the Herbarium of Scientific Institute, University Mohammed V–Rabat–Morocco.

The essential oil was produced from stems and leaves of *Rosmarinus officinalis* by hydrodistillation method. Plant materials (100g) cut into small pieces were placed in distillation apparatus and hydrodistilled for 3h after the oils was dried over an hydrous K_2 CO_3, they was stored at +4˚C until used for GC-MS analysis. The yield of extraction (ratio weight of EO/weight of dry plant) was 0.256%.

Phytochemical analysis of Rosmarinus officinalis essential oil by combined gas chromatography-mass spectrometry (GC-MS): The essential oil was submitted to quantitative analysis in a Hewlett-Packard 575, GC condition: carrier gas N_2 (0.5 bar) at flow rat at 1.0m/min, sample size, 0.2µl injected, capillary column (30m siloxane 5% HP EM). The temperature of the injector and detector was set at 250°C. The oven temperature was programmed from 50ºC to 250°C (5min). The MS was taken at 70eV. The components of the essential oil were identified by comparison of their mass spectra with those in the Wiley- NIST 7th edition library of mass spectral data. The percentage composition on the oil sample was calculated from GC-MS peak areas (Wiley and Sons, 2008).

Animals: Male Swiss mice (20-25g) (IOPS Offa) were used in pharmacological test and females of the same strain in the LD_{50} calculation. Animals were obtained from the animal experimental centre of Mohammed V- Souissi University, Medicine and Pharmacy Faculty – Rabat. They were housed three per plastic cage, photoperiod (one light from 6:00 to 18:00h); air changes and room temperature (22±1°C) were controlled. All animals had free access to tap water and at *ad-libitum* feeding; except for short fasting period before the treatment with the single dose of the essential oil. The general behavior of mice was observed continuously for 1h after treatment, intermittently for 4h and over period of 24h (Twaij, 1983). The mice were observed for 14days following treatment, and all signs of toxicity and deaths and their latencies were recorded. All experiments were conducted in accordance with the Official Journal of the European Committee in 1991. The experiment protocol was approved by the Institutional Research Committee regarding the care and use of animals for experimental procedure in 2010; CEE509. The particular treatment doses were selected depending on the LD_{50} results. The therapeutic doses should be very lesser than the LD_{50} value.

Acute toxicity: LD_{50} (median lethal dose) values were determined as described by Litchfield and Wilcoxon (Leitchfield and Wilcoxon, 1949). Seven groups of mice of both sexes (n =10; 5 males and 5 females) received or not oral single doses at different concentration. The control group received only the water or saline solution. After a single dose administration, mice were placed in individual clear plastic boxes and continuously observed for 24h at 6h time interval to detect any eventual side effect. The number of animals, which died during this period, was expressed as percentile, and the LD_{50} was determined by probit test using death percent versus doses log (Alaoui, et al., 1998). Of note, drugs used as control were given to mice in similar conditions.

Drugs: Thiopental (60mg/kg, i.p.) and Apomorphine (16mg/kg, s.c.) were used as reference drugs (positive control). It was dissolved in distilled water prior to administration.

Pharmacological evaluations: The psychostimulant activity of essential oils from *R. officinalis* on the central nervous system (CNS) was then studied, using a battery of behavioral tests used in psychopharmacology.

For testing psychostimulant effects, the effect of *R. officinalis* eeesntial oils on mice was qualified in one of the following tests:

Observation of animals in free situation: Detailed behavioral observations were made on lots of 3 mice per dose, for 15, 30, 60, 120, 180 min and 24h after administration of substances, based on the method that described by Irwin (Axiotis, et al., 1987; Petit, et al., 1990). Especially, the effect of psychostimulant is evaluated after hypermobility and hyperexcitability.

Interaction with thiopental in mice: Mice (6 per dose) were placed in individual boxes and given the substance (essential oil) 30 min before administration of thiopental (a sub-hypnotic dose, 60mg/kg, i.p.). The mice were treated with different dose of essential oil (50 and 100mg/kg, p.o., n = 6), the control group (n=6) was treated with distilled water (10ml/kg, p.o.). The effects was recorded for disappearance (latency) and reappearance (duration) of the righting reflex (Axiotis, et al., 1987; Petit, et al., 1990).

Search for stereotypes movements in rats: The stereotypes movements, characterized by movements of the head and buckle, sniffing, licking or chewing are scored from 0 to 3 according to the method described by Simon and Chermat (Simon, and Chermat, 1977), every 10 min until their disappearance in all animals.

The essential oil was tested at doses of 50 and 100mg/kg. The control groups have received the solvent under the same conditions. After administration of essential oil of *Rosmarinus officinalis L*, each animal was isolated in individual cages to be observed every 10 min during 210 min. The intensity of stereotypes was assessed according to the quotation of Simon**.** For each lot the sum of individual quotations obtained by fraction of 10 min is calculated. The total number of stereotypes observed during 210 min was also taken into account (Simon and Chermat, 1982).

Results are expressed as mean ± S.D (standard deviation). Statistical analysis of data was done using one-way analysis of variance (ANOVA) followed by student's *t*- test. A level of significance (P<0.05 or 0.01) was considered for each test.

RESULTS

Chemical composition of the essential oil: The results obtained by GC-MS analyses of the essential oils of *Rosmarinus officinalis* are presented in table 1. Thirteen compounds were identified in this essential oil by GC-MS analyses (Fig 1); *Rosmarinus officinalis* contained three major compounds *i.e.* 1, 8-cineole (50.49%), α-pinene (15.82%) and camphor (11.6%).

Acute toxicity of Rosmarinus officinalis essential oil: Following oral administration of *R. officinalis* essential oil at the dose 500 mg/kg, neurological deficit and abdominal contraction were observed by 20-25 min after the administration in 60% and might be due to the route of administration of *R. officinalis* essential oil. In addition to the abdominal contraction; the essential oil reduced spontaneous motor activity and exploratory behavior. The mortality was recorded 24h after administration at the doses 850, 900, 950, and 1000mg/kg. The severity of these effects was increased within 6h, and mortality was 100% (Table 4). Boniface et al., have established a program that calculate the percentage of mortality according to the administration dose (Boniface, et al., 1972). This calculation gives the following results: LD_{50} = 897.85mg/kg, 885.90 < LD_{50} < 909.97, with confidence limits at 95%. This result indicates that this essential oil had low toxicity (Table 2).

Psychostimulant activity of the essential oil on central nervous system: The results of psychostimulant effects of essential oil were determined by comparison with control group. Pharmacological tests were then performed at non toxic doses *i.e.* 50 and 100 mg/kg. These doses did not induce severe neurological side effects.

Observation of animals in free situation: We observed that oral administration of the essential oil of *Rosmarinus officinalis* L at the dose of 100mg/kg caused the appearance of rapid movements of the head, sniffing the buckle (gnawing, biting the cage walls) suggesting an increasing locomotors activity and curiosity, induction of spontaneous aggression, hypermobility and hyperexcitability of the central nervous system (stereotypes movements).

Interaction with thiopental in mice: The administration of the therapeutic doses (50 and 100mg/kg, p.o.) of the essential oil of *Rosmarinus officinalis*, 30 min before the injection of the hypnotic dose of thiopental (60mg/kg, i.p.), significantly increased sleep latency and caused a decrease in sleep time. The effect was significantly higher at the dose 100mg/kg than at the dose of 50mg/kg (Table 3).

Search for stereotypes movements in rats: From Table 4, Apomorphine (16mg/kg, s.c.) induces on the control group the appearance of stereotypes movements after just 10 min, after the end of 80 min, the total of quotation being 18. The stereotypes movements decreased gradually until disappear at 210 min. The stereotypes Phenomena are increased in duration and intensity. However, the essential oil (100mg/kg) produced a significant (P<0.001) potentiating of the effects of Apomorphine, but at the dose 50mg/kg the agonist of stereotypes movements was partial in duration and intensity compared with control group. Indeed, the stereotypes movements appeared at 30 min, reaching a peak at 40 min (total quotations = 18) remain at 260 min for disappearance at 280 min (Table 4).

DISCUSSION

It should be noted that, this type of work has not been done on same plant species or family. Also the papers that published previously are very few.

Aromatherapy provides a potentially effective treatment for a range of psychiatric disorders (Perry and Perry, 2006; Sarris, 2007). In aromatherapy, the essential oils are believed to possess antidepressive effects and to be useful for treating nervous breakdown and depression (Tisserand, 1993; Fibiger, et al., 1988; Machado, et al., 2009). Oil compositions of *R. officinalis* have already been reported (Tisserand, 1993; Gachkar, et al., 2007; Costa, et al., 1972). Thus, it has been shown that α-pinene, 1,8-cineole, camphor, verbenone and broneol account for 80% of *R. officinalis* essential oils, but in our study, these compounds represent 97.6%. These differences in chemical composition of essential oil may be due to both developmental and environmental factors that influences plant metabolism. We analyzed the effects of different doses of essential oil from stems and leaves of rosemary (*Rosmarinus officinalis*) for their acute toxicity and psychostimulant activities. In the current study, the psychostimulant activity was investigated by recording the spontaneous locomotors activity in mice. In the test of observation in free situation, the essential oil (100mg/kg) produced an increase in motor activity and curiosity, by the induction of spontaneous aggression, hypermobility and hyperexcitability. These observations suggest that, the psychostimulant action of this plant is mediated by dopaminergic pathway, since dopaminergic transmission can produce profound stimulant effects in mice (Simon and Chermat, 1982; Boissier, et al., 1972). It is possible that, the dopaminergic transmission is due to the releasing of catecholamine (nor epinephrine and dopamine) neural storage vesicles (Costa, et al., 1972; Samanin, et al., 1977). The essential oil of *R. officinalis* has considerable dopaminergic potentiating mechanisms. In the test of thiopental-induced sleep, the essential oil (100mg/kg) produced a significant (P<0.001) highest increase in the time of onset of sleep as well as highest reduction of sleep induced by thiopental (Table 3).

The prolongation in the time of onset of sleep and the highest reduction in the sleep time and the induction of exploratory behavior indicate a central nervous system stimulant activity of *Rosmarinus officinalis* essential oil. Further chemical and pharmacological analysis of the essential oil will be conducted to isolate and characterize the active principles responsible for the psychostimulant effects.

Thus present study indicates that essential oil from stems and leaves of *Rosmarinus officinalis* possess potential psychostimulant activity. This may justifies its use in traditional medicine as general stimulant.

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Table -1: Chemical composition of *Rosmarinus officinalis* essential oil.

Peak N	Identified compound	Rt (retention time)	Relative percentage%
1	α-pinene	8.37	15.82
2	Camphene	8.83	6.80
3	β-pinene	9.79	4.75
4	Myrcene	10.40	1.70
5	p-cymene	11.54	2.16
6	1,8-cineole	11.80	50.49
7	Camphor	15.75	11.61
8	Borneol	16.57	2.58
9	Bornyl acetate	24.98	2.08
10	Tans-caryophyllene	29.85	Tr
11	δ-cadinene	35.59	Tr
12	Unknown	39.13	Tr
13	Bisabolol	43.35	Tr

Table -2: Mortality of *Rosmarinus officinalis* essentials oil by oral route (p.o.).

	Doses (mg/kg) p.o.	Mortality %
EO	500	0
	750	10
	850	30
	900	40
	950	60
	1000	100

- Mice of each group (*n*=10) were received single dose and they were examined for 14 days to determine LD_{50} and any behavioral changes.
- EO: mean essential oil; p.o.: mean oral route.

Table -3: Influence of essential oil of *Rosmarinus officinalis* on sleep latency and duration of sleep induced in mice by an hypnotic dose of thiopental (60mg/kg).

Groups	Dose (mg/kg)	Sleep latency (min)	Sleeping time (min)
Control	60	18 ± 1	34 ± 2
EO	50	20 ±1	6 ± 0.1*
EO	100	42 ±1*	2 ± 0.2*

- Data are expressed as mean ± SD; *n*= 5 per group; *P< 0.001 versus the control group.
- EO: mean essential oil; i.p.: mean intraperitonial route; n: mean number of animals; p.o.: mean oral

Table -4: In**fluence of essential oil of *Rosmarinus officinalis* L on stereotypes movements induced by Apomorphine (16 mg/kg, s.c.) in rats.**

	Sum of individual quotation stereotypic																											
Time (min)	10	20	30	40	50	60	70	80	90	100	110	120	130	140	150	160	170	180	190	200	210	220	230	240	250	260	270	280
AM 16 mg/kg	9	11	12	12	15	16	18	18	16	15	14	10	8	8	6	5	5	4	3	2	0	0	0	0	0	0	0	0
EO 50 mg/kg	8	10	12	13	15	16	16	14	14	12	11	10	10	8	8	6	5	3	2	1	0	0	0	0	0	0	0	0
EO 100 mg/Kg	15	15	16	18	18	18	16	16	14	15	15	15	14	13	12	10	10	9	8	8	6	6	5	5	3	2	1	0

- (n= 6)= number of animals per lots; EO= essential oils; AM: mean apomorphine; s.c. = subcutaneous.
- The individual quotation stereotypic was calculated as follows:
- 0 = absence of stereotypes movements and all animals were normal; 1 = rare presence of stereotypes movements; 2 = intense sniffing with rapid movements of the head; 3 = intense and continuous stereotypes movements (displacement of the head, licking) (P< 0.001).

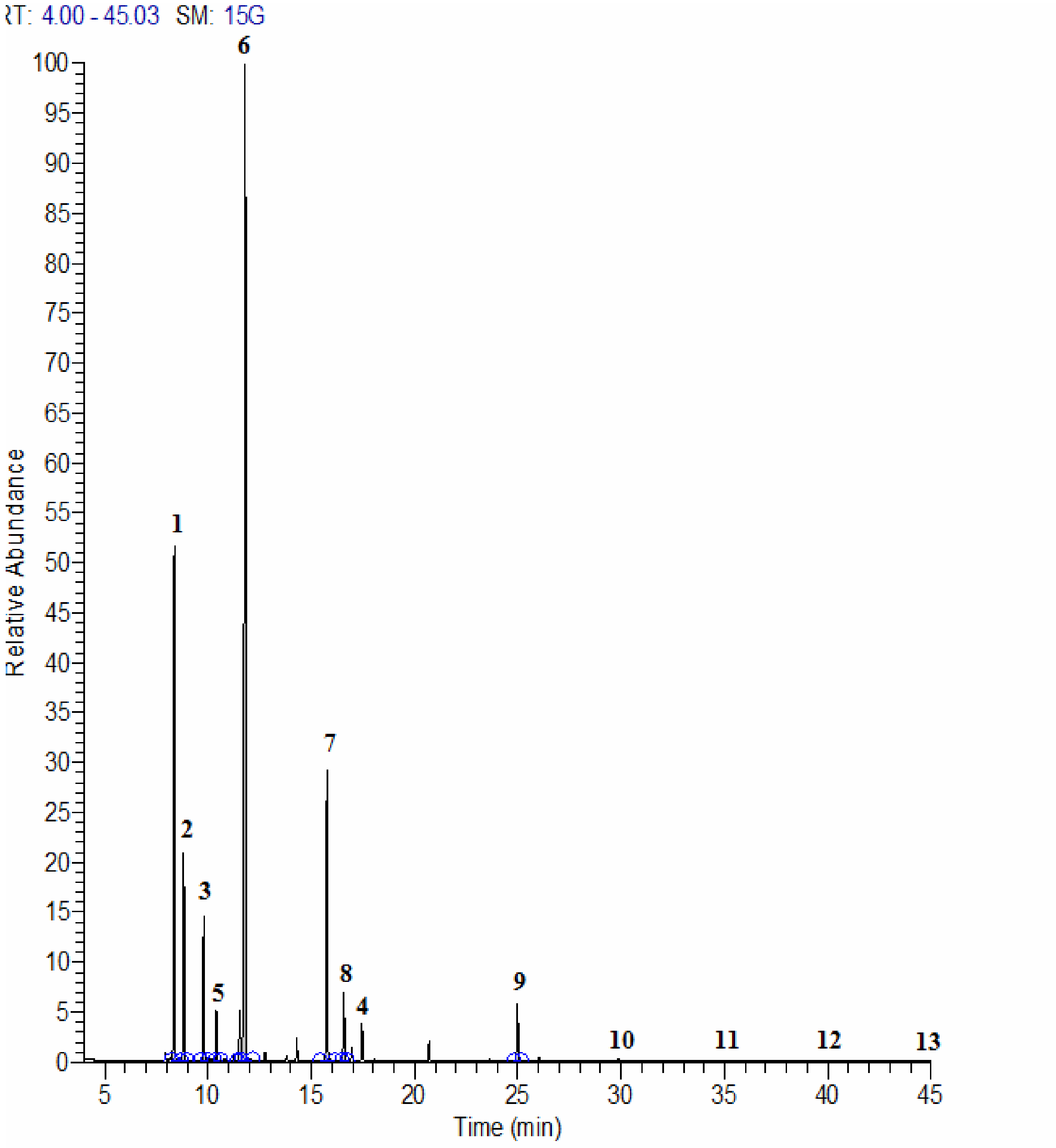

Figure-1: Gas chromatography- mass spectrometry (GC-MS) of *Rosmarinus officinalis* essential oil.