Psychostimulants activity of Rosmarinus officinalis L Methanolic and Aqueous Extracts

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We evaluate the psychostimulants activity of Rosmarinus officinalis L. (lamiaceae) on central nervous system. The effects of the methanolic and aqueous extracts of the stems and leaves of this plant were investigated in three behavioral models including, observation of animals in free situation, search of stereotypes movement in rats and interaction with thiopental in mouse. We observed that extracts of R. officinalis increase the locomotors activity and induced hypermobility and hyperexcitability, as compared to the control group. Treatment with R. officinalis methanolic or aqueous extracts at the dose of 200 mg/kg, significantly decreased the duration of thiopental-induced sleeping time from 34 min ±0.2 to 3 min ± 0.1 and 2 min ±0.1, respectively (P<0.001). Thus, we can conclude that the methanolic and aqueous extracts of R. officinalis possess potential psychostimulants activity.

Key words: Rosmarinus officinalis L., psychostimulants activity, central nervous system, stereotypes movements, psychiatric disorders.

INTRODUCTION

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 (Coury et al., 1992; Mank et al., 1999; Nesthby et al., 1997). 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 hepatoprotective (Sotelo-Félix et al., 2002), antibacterial (Gachkar et al., 2007), antithrombotic (Yamamoto et al., 2005), antiulcerogenic (Dias et al., 2000), diuretic (Haloui et al., 2007; Bakirel et al., 2008), antioxidant (Gonzalez–Trujano et al., 2007), antinociceptive (Altitier 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 (Fibiger and Phillips, 1988; Machado et al., 2009). However the psychostimulant properties have not yet been studied. Thus, the aim of this study is to evaluate the psychostimulant activity of R. officinalis methanolic and aqueous extract in animal models.
MATERIALS AND METHODS

Plant material

R. 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 Pr. M. Ibn Tatou of Scientific Institute of Rabat. A voucher specimen (RAB12560) was deposited in the Herbarium of Scientific Institute, University Mohammed V–Rabat–Morocco.

Preparation of extract

**Methanolic extract**

Stems and leaves of R. officinalis were successively extracted with methanol by maceration at room temperature (25°C) over period of 48 h. 500 g of plant material and one liter of methanol were used in the extraction. Methanol containing the extract was then filtered through Whatman paper and the solvent was vacuum-distilled at 65°C in a rotary evaporator. The remaining extract was finally dried in the oven at 30°C for 2 h to ensure the removal of any residual solvent. Final extract was a yellow powder in percentage dry weight 22.8%; this methanolic extract was kept in deep freeze at -20°C until use.

**Aqueous extract**

500 g of plant material was extracted by infusion in boiled water (500 ml) for three days. The respective aqueous extracts were separated from its residues by gravity filtration and then lyophilised (Free Zone® Dry 4.5, USA). For each study, the lyophilized aqueous extract was carefully prepared under the same condition used throughout the studies (time, temperature and the amount of plant material and water used for extraction under reflux and lyophilization) and each time the quality of extraction was checked by the yield of the lyophilization material. The final crude extract was obtained as dark brown powder in percentage from dry weight (18.7%). For assuring stability, the lyophilized material was stored at -20°C.

**Animals**

Male Swiss mice (20 to 25 g) (IOPS Offa) were used in pharmacological test and females of the same strain in the LD50 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, centre of Mohammed V- Souissi University, Medicine and Pharmacy Faculty Rabat. They were housed three per plastic cage.
Table 1. Mortality according to the administration doses of *R. officinalis* methanolic and aqueous extracts.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Dose (mg/kg, p.o.)</th>
<th>Mortality (%)</th>
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<tbody>
<tr>
<td>EM</td>
<td>500</td>
<td>0</td>
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<tr>
<td></td>
<td>1000</td>
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<td></td>
<td>2000</td>
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<td>EA</td>
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<td></td>
<td>5000</td>
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</table>

Mice of each group (n= 10) were received single dose and they were carefully examined during 14 days to determine LD$_{50}$ and any signs of toxicity and behavioral changes. The lyophilized aqueous and methanolic extracts dissolved in distilled water were administered by oral route (p.o.); EM: mean methanolic extract; AE: mean aqueous extract.

every 10 min during 210 min. The intensity of stereotypes was assessed according to the quotation of Simon (Simon and Chermat, 1977). 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 et al., 1982).

Statistical analysis

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

Acute toxicity of *R. officinalis* methanolic extract

After oral administration of *R. officinalis* methanolic extract at the doses of 500, 1000, 2000, 3000, 4000 and 5000 mg/kg some signs of toxicity were produced. The mortality was recorded 48 h after administration at the dose 1000, 2000, 3000, 4000 and 5000 mg/kg, p.o. The severity of these effects was increased within 8 h and mortality was 100% (Table 1). The calculation of LD$_{50}$ by a program of Boniface et al. (1972) gives the following results: LD$_{50}$ = 2104.85 mg/kg., 1890 < LD$_{50}$ < 2343, with confidence limits at 95%. This result indicates that the methanolic extract had very low toxicity (Table 1).

Acute toxicity of *R. officinalis* aqueous extract

Following oral administration of *R. officinalis* aqueous extract at the doses 500, 1000, 1500, 2000; 3000 and 5000 mg/kg, p.o., no toxicity was observed at these doses. This result indicates that this aqueous extract is not toxic (Table 1).

Psychostimulants activity of the methanolic and aqueous extracts on central nervous system

The results of psychostimulants effect were determined by comparison with control groups. Pharmacological tests were then performed at non toxic doses that is, 100 and 200 mg/kg for methanolic and aqueous extract. These doses did not induce severe neurological side effects.

Observation of animals in free situation

It was observed that oral administration of the methanolic and aqueous extract of *R. officinalis* L. at the therapeutic dose (100 and 200 mg/kg), causes the appearance of rapid movements of the head, sniffing the buckle (gnawing, biting the cage walls) suggesting an increasing locomotor activity and curiosity, induction of spontaneous aggression, hypermobility and hyperexcitability of the central nervous system (stereotypes movements). This psychostimulant effect is appearing significant at the dose of 200 mg/kg. However, the aqueous extract was found to be more active than methanolic extract.

Interaction with thiopental in mice

The oral administration of the therapeutic dose (100 and 200 mg/kg, p.o.) of the methanolic and aqueous extracts
of \textit{R. officinalis} significantly increased sleep latency and caused a decrease in sleep time. The effect was significantly higher in the dose 200 mg/kg than at the dose of 100 mg/kg, but the sleep time reduction by the aqueous extract is longer than the sleep time reduction by the methanolic extract. The reduction of sleep time is significantly longer than the methanolic extract (Table 2).

**Table 2. Influence of the essential oil, methanolic and aqueous extracts of \textit{Rosmarinus officinalis} L on the onset of sleep (T.E) and sleeping time (T.S) induced in mice by a hypnotic dose of thiopental.**

<table>
<thead>
<tr>
<th>Time</th>
<th>Thiopental (mg/kg, i.p.)</th>
<th>ME (mg/kg, p.o.)</th>
<th>AE (mg/kg, p.o.)</th>
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<tbody>
<tr>
<td></td>
<td>60</td>
<td>100</td>
<td>200</td>
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<tr>
<td>T.E (min)</td>
<td>18 ±1</td>
<td>34 ± 1</td>
<td>58 ± 0.2</td>
</tr>
<tr>
<td>T.S (min)</td>
<td>34 ± 2</td>
<td>8 ± 1</td>
<td>3 ± 0.1</td>
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</table>

Mice of each group (n= 5); p< 0.001 vs the control group; p.o.: mean oral route; i.p.: mean intraperitoneal route; T.E: mean sleep latency; T.S: mean sleeping time. Data are expressed as mean ± SD.

**Search for stereotypes movements in rats**

From Table 3, Apomorphine (16 mg/kg, s.c.) induced on the control groups 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 methanolic and aqueous extract (200 mg/kg) produced a significant (P< 0.001) potentiating of the effect of Apomorphine, but at the dose 100 mg/kg the agonist of stereotypes movements was partial in duration and intensity compared with controls. For the methanolic extract, stereotypic movements reached a maximum at 80 min (quotation total= 16 and 18, for 100 and 200 mg/kg, respectively) and persist longer time for not disappearing after 210 min (some rats continued to exhibit stereotypic movements up to 270 min with the dose 200 mg/kg) (Table 3). For the aqueous extract of \textit{R. officinalis} L., after the administration of the therapeutic dose (100 and 200 mg/kg, p.o.), the stereotypes movements appeared at 10 min, reaching a peak at 40 min (total quotation = 18), remain at 270 min for disappearance at 280 min (Table 3).

**DISCUSSION**

Aromatic and medicinal plants have been used for thousands of years in every part of the world by numerous civilizations. Driven by their intuition and their sense of observation, they were able to find answer to their health problems in the plant environment (Akhdzadeh, 2007; Stelfitsch and Steflitsch, 2004). Aromatherapy provides a potentially effective treatment for a range of psychiatric disorders (Sarris, 2007; Perry and Perry, 2006; Stelfitsch and Steflitsch, 2004). In aromatherapy, the essential oils are believed to possess antidepressive effect and to be useful for treating nervous breakdown and nervous depression (Fibiger and Phillips, 1985; Machado et al., 2009; Tisserand, 1985). We analyzed the effect of different doses of the methanolic and aqueous extracts from stems and leaves of rosemary (\textit{R. officinalis} L.) for their acute toxicity and psychostimulants 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 methanolic extract (200 mg/kg) produced an increase in motor activity and curiosity, by the induction of spontaneous aggression, hypermobility and hyperexcitability. It was also observed that, the aqueous extract (200 mg/kg) produced a significant (P< 0.001) increase in the spontaneous motor activity and induction of stereotypes movements. These observations suggest that, the psychostimulant action of this plant is mediated by dopaminergic pathway, since dopaminergic transmission can produce profound stimulant effect in mice (Simon et al., 1982). 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 methanolic and aqueous extract of \textit{R. officinalis} has considerable dopaminergic potentiating mechanisms. In the test of thiopental-induced sleep, the methanolic (200 mg/kg) extract 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 2). It was also observed that, the aqueous extract (200 mg/kg) not only induces highest decrease the sleeping time but also induces prolongation the latency of falling asleep and highest decrease in the rate of sleep onset (Table 2). 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 \textit{R. officinalis} extract. In the test of apomorphine-induced stereotypes movement in rats, the methanolic and aqueous extract (200 mg/kg, p.o.) produced a significant (P< 0.001) potentiating of the effect of Apomorphine, the stereotypes movements was increased in duration and intensity. In addition, the dose of 100 mg/kg of both extracts was partial increased the
Table 3. Influence of the methanolic and aqueous extracts of *Rosmarinus officinalis* L on stereotypes movement induced in rats.

<table>
<thead>
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<th>Dose (mg/kg)</th>
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</table>

Rats of each group (n= 6) were received the methanolic or aqueous extracts (100 and 200 mg/kg) by oral route (p.o.); Apomorphine (16 mg/kg) was injected via subcutaneous pathway (s.c.); ME: mean methanolic extract; AE: mean aqueous extract; AM: mean Apomorphine; individual quotation stereotypic was calculated as follows: 0 = absence of stereotypes movement 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 movement (displacement of the head, licking). *P* < 0.001 versus the control group.

In conclusion, the present study indicates that the methanolic and aqueous extracts from stems and leaves of *R. officinalis* possess potential psychostimulants activity. This may justifies its use in traditional medicine as general stimulant.

ACKNOWLEDGEMENTS

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REFERENCES


stereotypes movements in duration and intensity compared with controls (Table 3). Phytochemical studies have identified active components in the methanolic and aqueous extract of this plant, such as flavonoids including diasmetin, diasmin, luteolin, apigenin, quercetin and kaempherol, phenols such as caffeic and rosmanarinc acids and terpenoids like, carnosol, carnosic acid, rosmanol and oleononic and ursolic acids (Altinier et al., 2007; Al-Sereitia et al., 1999; Askun et al., 2009; Barnes et al., 2001; Bentayeb et al., 2007; Erkan et al., 2008; Frankel et al., 1996; Machado et al., 2009; Nolkemper et al., 2006; Okamura et al., 1994; Yesil-Celiktas et al., 2007a,b). Screening of the methanolic and ethanolic extract of *R. officinalis* aerial part has reported the presence of these compounds, but not the presence of alkaloids as detected in an aqueous extract (Hosseinzadeh and Nourbakhsh, 2003; Al-Sereitia et al., 1999; Askun et al., 2009; Barnes et al., 2001; Bentayeb et al., 2007; Erkan et al., 2008; Frankel et al., 1996; Machado et al., 2009; Nolkemper et al., 2006; Okamura et al., 1994; Yesil-Celiktas et al., 2007a,b).