

# Antidepressant-like effect of *Rosmarinus officinalis* extract in male mice

Entisar J. Al Mukhtar\*, Selman M. Selman \*, Zena Hasan Sahib\*, Hamid Naji\*

\* = Lecturer in Pharmacology department. College of Medicine. University of Babylon. Babylon-Iraq. Email: [ejhas28@yahoo.com](mailto:ejhas28@yahoo.com)

## التأثير المضاد للكآبة لمستخلص إكليل الجبل في ذكور الفئران.

انتصار جواد المختار\*، سلمان محمد سلمان\*، زينا حسن صا حب\*، حامد ناجي\*

\* = مُحاضر في فرع الأدوية / كلية الطب/ جامعة بابل – العراق.

### الخلاصة

هدفت هذه الدراسة لمعرفة تأثير المستخلص المائي لأوراق نبات إكليل الجبل كمضاد للكآبة من خلال اختبار السباحة الإجبارية لذكور الفئران. لقد تم إعطاء المستخلص للفئران بجرعتين ١٥ ملغم/كغم و ٣٠ ملغم/كغم يوميا لمدة خمسة أيام متتالية حيث تم مقارنتهما مع عقار الامبرامين مرة ومع مجموعة لم تأخذ أي علاج مرة أخرى. إن نتائج هذه الدراسة بينت حصول زيادة بالغة الأهمية في وقت السباحة ( $p < 0.01$ ) في حين قل وقت عدم الحركة بنسبة بالغة الأهمية ( $p < 0.01$ ) مما يدل على إن لمستخلص إكليل الجبل تأثير مضاد للكآبة.

### Abstract

**Background:** Depression is the most common of the mood disorders. There are many types of antidepressant drugs which have various unwanted effects and interactions.

**Aim of the study:** to investigate the antidepressant-like effect of water extract of *Rosmarinus officinalis* (*R. officinalis*).

**Animals, materials and methods:** twenty eight male Swiss albino mice were divided into four groups (A, B, C, D), each group received distilled water 0.3 ml as a control; imipramine 15 mg/kg; *R. officinalis* 15 mg/kg and *R. officinalis* 30 mg/kg PO, once daily for 5 successive days respectively. On the fifth day and after thirty minutes of the treatment administration the mice were tested by using the forced swimming test (FST) and the immobility and swimming times were measured.

**Results:** Water extract of *R. officinalis* extract (15 mg/kg and 30 mg/kg) high significantly reduced the immobility time and increased the swimming time in the FST in comparison to untreated group, but when compared to imipramine (15mg/kg) it produced similar results. Therefore, water extract of *R. officinalis* had antidepressant effects in mice.

**Keywords:** *Rosmarinus officinalis* extract; Imipramine; Antidepressant effect; Mice.

## **Introduction:**

Depression is the most common of the mood disorders; it may range from a very mild condition, on bordering on normality, to severe (psychosis) depression accompanied by hallucinations and delusions. The main biochemical theory of depression is the monoamine hypothesis, which states that depression is caused by a functional deficit of monoamine transmitters at certain sites in the brain.<sup>(1,2)</sup> Initially the hypothesis was formulated in terms of noradrenaline, but subsequent work showed that most of the observations were equally consistent with 5-hydroxytryptamine (5-HT).<sup>(3)</sup>

There are many types of antidepressant drugs which have various unwanted effects and interactions. Therefore, our aim, in this study, was to explore the potential effects of plants in the treatment of depression in comparison to imipramine.

*Rosmarinus officinalis*, commonly known as Rosemary is a powerful herb belonging to the family Lamiaceae that originates from the Mediterranean region. The name "rosemary" derives from the Latin "dew" (*ros*) and "sea" (*marinus*), or "dew of the sea".<sup>(4)</sup> *R. officinalis*, is a common medicinal and aromatic plant, grown in many parts of the world. Rosemary is indigenous to southern Europe, particularly on the dry rocky hills of the Mediterranean region. Traditionally, rosemary is used as a spice in foods and beverages and as a herbal medicine for various spasmodic conditions such as renal and biliary colic.<sup>(5)</sup>

In folk medicine, rosemary is known for memory improving and treating cognitive decline. It is also used as sedative and relaxant, against headaches, epilepsy, and depression.<sup>(6)</sup> Additionally, various pharmacological studies have demonstrated the analgesic<sup>(7)</sup>, anti-inflammatory<sup>(8)</sup>, anti-tumor<sup>(9)</sup>, anti-ulcerogenic<sup>(10)</sup>, anti-bacterial<sup>(11)</sup> and hepatoprotective<sup>(12)</sup> properties of rosemary.

## **Materials And Methods:**

### **1. Preparation of Plant Extract:**

Leaves of *R. officinalis* were purchased from the Hilla local market and identified by a competent botanist at the collage of science for girls, at Babylon university, Iraq. The leaves were washed carefully, then air dried in shade at room temperature, then grinded to fine powder. The plant extract was prepared by extracting 40 gm of leaves powder with 80 ml distilled water by refluxing for 36 hrs at 50-60 °C. Pellets of the extract were obtained by evaporation of its liquid contents in the incubator. The required dose for treatment was prepared by dissolving the pellets in distilled water and administered by stomach tube at a doses of 15 mg/kg and 30 mg/kg body weight (B.W.) daily for 5 consecutive days.<sup>(13)</sup>

## 2. **Animals:**

Twenty eight male Swiss Albino mice (weighting 25 – 30 g) were used in this study. The mice kept in the animal house in the college of medicine in Babylon university under constant conditions of temperature ( $22 \pm 2$ ) °C and lighting (12:12hr light: dark cycle) for at least two week before and through the experimental work, being maintained on a standard commercial mice chow and water were available *ad-libitum* with tap water.

## 3. **Drug:**

Imipramine hydrochloride ( Tofranil 10 mg, NOVARTIS ) was dissolved in normal saline ( 10 mg in a final volume of 10 ml ).

## 4. **Model for testing antidepressant activities:**

**Forced swimming test:** Forced swimming test was proposed as a model to antidepressant activity by Porsolt et al <sup>(14)</sup> . Mice were forced to swim individually in glass jar (12 x 30 cm) containing fresh water up to 15 cm height and maintaining at 25 °C ( $\pm 3$  °C ). A mouse was considered to be immobile when it remained floating in the water without struggling, making only minimum movements of its limbs, necessary to keep its head above the water. The total duration of immobility was recorded within the total test duration of five minutes. Each mice was used only once.

## 5. **Experimental protocols:**

Animals were randomly divided into 4 groups (each group consist of 7 mice):

Group A(control): received 0.3ml distilled water, PO, once daily for 5 days.

Group B: received imipramine 15 mg / kg, PO, once daily for 5 days.

Group C: received *R. officinalis* 15 mg / kg PO, once daily for 5 days.

Group D: received *R. officinalis* 30 mg / kg PO, once daily for 5 days.

On the fifth day and after thirty minutes of the dose administration, immobility and swimming times were measured within 5 minutes for each mice by using FST. All behaviors of the mice in the test were recorded by video camera.

## 6- **Video Camera:**

Video Camera ( SONY- SD / DIGITAL VIDEO CAMERA ) was used to record all behaviors of the animals during the test.

## 7- **Statistical analysis:**

The data expressed as mean  $\pm$  SD, SPSS version 17.0 was used for the statistical analysis, ANOVA test was used in this study. P- values less than 0.05, 0.01 and 0.001 were considered as statistically significant, high significant and extremely significant respectively. <sup>(15)</sup>

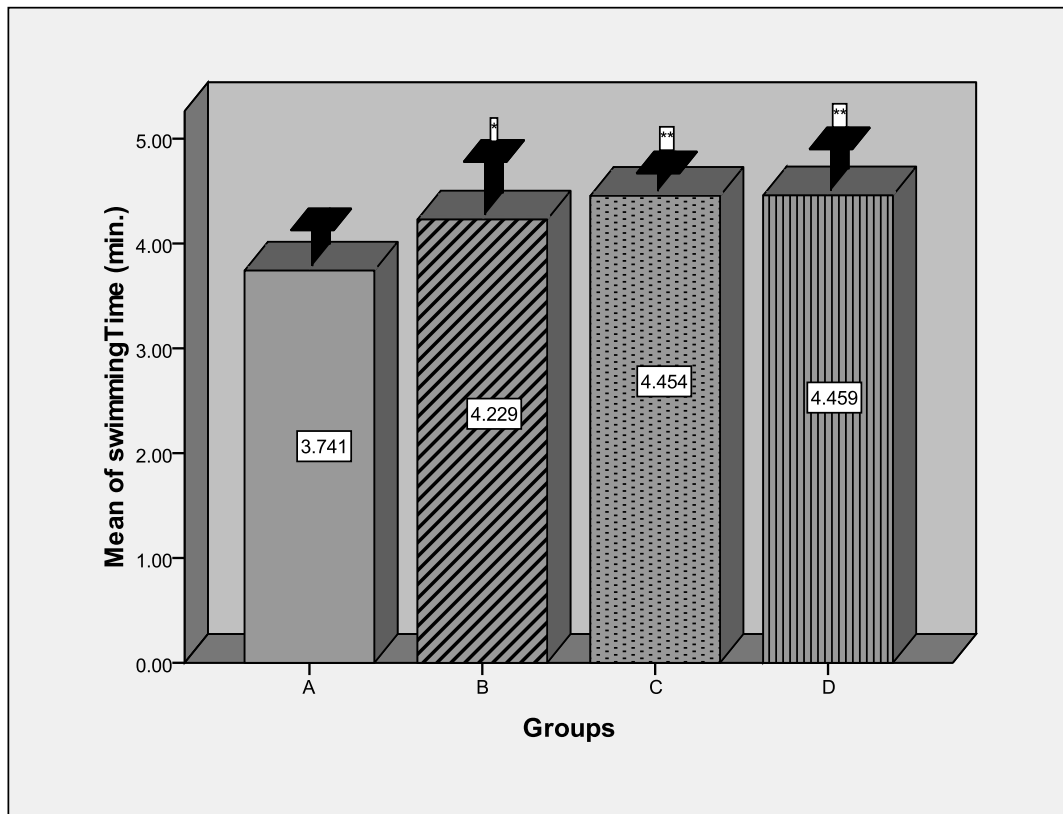
## **Results:**

Regarding the swimming time results of the present study showed a high significant increase ( $p < 0.01$ ) in swimming time through FST in both 15 and 30 mg/kg

*R. officinalis* treated groups compared to control group ( $4.45 \pm 0.19$  and  $4.46 \pm 0.43$  respectively vs.  $3.74 \pm 0.37$ ), while imipramine caused significant increase ( $p < 0.05$ ) in swimming time compared to control group ( $4.22 \pm 0.55$  vs.  $3.74 \pm 0.37$ ). Significant differences were neither found between imipramine and both doses (15 and 30mg/kg) of *R. officinalis* ( $4.22 \pm 0.55$  vs.  $4.45 \pm 0.19$  and  $4.46 \pm 0.43$  respectively) nor between 15mg/kg and 30mg/kg of *R. officinalis* ( $4.45 \pm 0.19$  vs.  $4.46 \pm 0.43$ ), as shown in fig. (1)

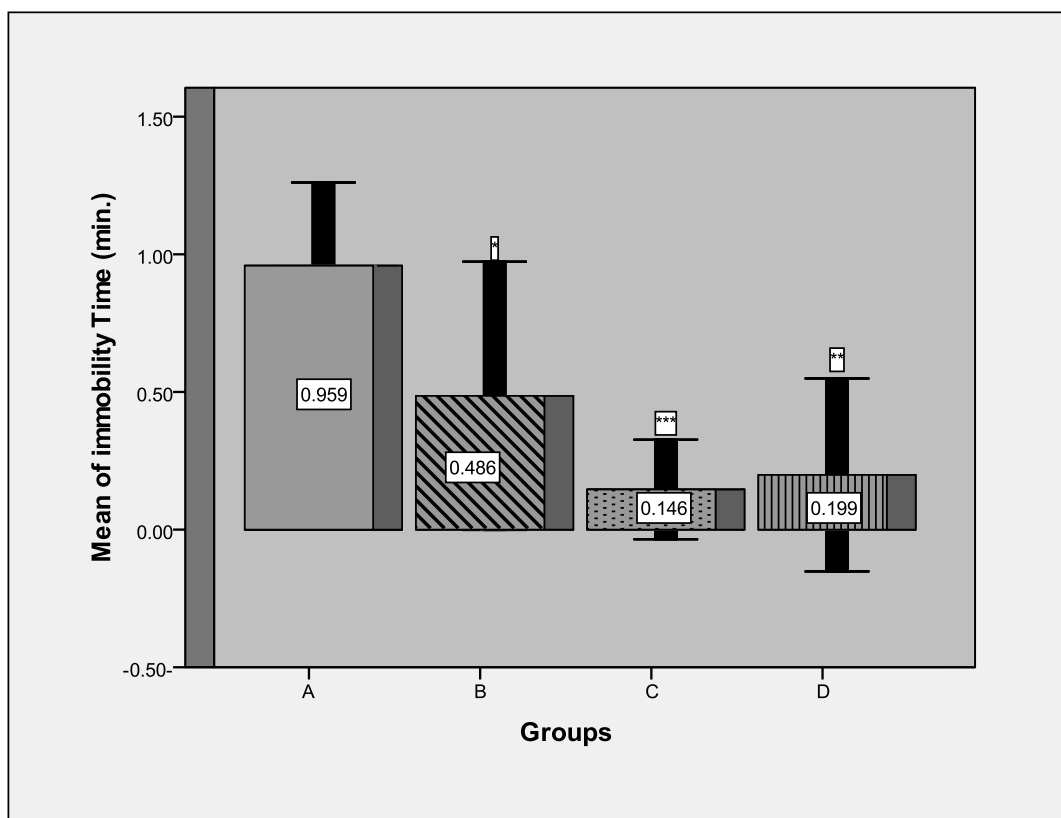
Regarding the immobility time it significantly decreased ( $p < 0.05$ ) in imipramine treated group compared to control group ( $0.48 \pm 0.52$  vs.  $0.95 \pm 0.32$ ), it also decreased high significantly ( $p < 0.01$ ) in 30mg/kg *R. officinalis* treated group compared to control group ( $0.19 \pm 0.37$  vs.  $0.95 \pm 0.32$ ), while it was extremely significantly decreased in 15mg/kg *R. officinalis* treated groups compared to control group ( $0.14 \pm 0.19$  vs.  $0.95 \pm 0.32$ ).

Significant differences were neither found between imipramine and 15 and 30mg/kg doses of *R. officinalis* ( $0.48 \pm 0.52$  vs.  $0.14 \pm 0.19$  and  $0.19 \pm 0.37$  respectively) nor between 15mg/kg and 30mg/kg of *R. officinalis* ( $0.14 \pm 0.19$  vs.  $0.19 \pm 0.37$ ), as shown in fig. (2).



**Figure (1) shows the changes in swimming time ( minutes).**

A (control group) received 0.3ml distilled water; B received imipramine 15 mg/kg; C received *R. officinalis* 15 mg/kg; D received *R. officinalis* 30 mg/kg Results express as mean  $\pm$  SD (\* =  $p < 0.05$ , \*\* =  $p < 0.01$ ).



**Figure (2) shows the changes in immobility time ( minutes).**

A (control group) received 0.3ml distilled water; B received imipramine 15 mg/kg; C received *R. officinalis* 15 mg/kg; D received *R. officinalis* 30 mg/kg Results express as mean  $\pm$  SD (\* =  $p < 0.05$ , \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$ ).

## Discussion:

The antidepressant action of *R. officinalis* extract found by the present study may be mediated by an interaction with the monoaminergic system as had been shown by Machado et al <sup>(16)</sup>, who also found that the pretreatment of mice with p-chlorophenylalanine (5-HT synthesis inhibitor), NAN-190 (5-HT<sub>1A</sub> receptor antagonist), ketanserin (5-HT<sub>2A</sub> receptor antagonist), biguanide (mCPBG, 5-HT<sub>3</sub> receptor agonist), SCH23390 (dopamine D<sub>1</sub> receptor antagonist) or sulpiride (dopamine D<sub>2</sub> receptor antagonist) was able to reverse the anti-immobility effect of the extract (10 mg/kg, p.o.) in the tail suspension test (TST). <sup>(16)</sup>

The combination of MDL72222, (5-HT<sub>3</sub> receptor antagonist) with a sub-effective dose of the *R. officinalis* extract (1 mg/kg, p.o.) produced an anti-immobility effect in the TST. <sup>(16)</sup>

In this study the antidepressant action of *R. officinalis* also may be attributed to the presence of the, luteolin, carnosic acid, and rosmarinic acid as these compounds

can cause upregulated of the two major genes (tyrosine hydroxylase and pyruvate carboxylase) involved in the regulations of dopaminergic, serotonergic and GABAergic pathway,<sup>(17)</sup>

Regarding the two doses of *R. officinalis* used in this study, up to our knowledge no study had found to which we can compare results of the present study.

### **Conclusions:**

The antidepressant like-effect of *Rosmarinus officinalis* extract is more than that of imipramine. Further studies including human trials are needed.

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