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Evaluation of the anxiolytic effect of rosemary in mice

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Abstract : Nowadays the occurrence of anxiety disorders are elevated resultant in an increase in the rate of morbidity. Commonly the most used drugs for anxiety disorders treatment are benzodiazepines but, their side effects limits their use. Traditionally a lot of herbs were used to treat many diseases and disorders for instance sleep disorders. The aims of this study to investigate the anxiolytic activity of *Rosmarinus officinalis* extract in mice. Two doses of *Rosmarinus officinalis* leaves watery extract (15 and 30 mg/kg) were compared to diazepam (1mg/kg) as a standard anxiolytic agent. Open field test was used to detect any behavioral changes due to stress. Results revealed thatthe numbers of both crossed squares and rearing were extremely significantly ($p<0.001$) increased in both diazepam and *Rosmarinus officinalis* (15 mg/kg) treated groups, whereas the numbers of crossed squares and rearing in *Rosmarinus officinalis* (30 mg/kg) treated group was increased highly significantly ($p<0.01$) and insignificantly ($p>0.05$) respectively. Also the period of time spend in the central squares was increased extremely significantly ($p<0.001$) and highly significantly ($p<0.01$) in diazepam and *Rosmarinus officinalis* (30 mg/kg) treated groups respectively, whereas in *Rosmarinus officinalis* (15 mg/kg) treated group the increase was insignificant ($p>0.05$). The number of grooming was extremely significantly ($p<0.001$) decreased in diazepam and *Rosmarinus officinalis* (15 and 30 mg/kg) treated groups. So**,** Watery extract of *Rosmarinus officinalis* leaveshave an anxiolytic like effect which more with the dose of 15 mg/kg.
Keywords: anxiolytic effect, *Rosmarinus Officinalis*, diazepam, open field test, mice.

Introduction:

Anxiety is an emotional state composed of two components; psychological and physiological which are responsible for the stimulating performance, the term of anxiety represent a feel of displease that is comprised of feelings of fear and concern, insecurity, or changes in the states of alert and awake[1]. Normal anxiety is a normal response ("flight or fight" response) which helps us how react to the dangers because it prompts humans either to escape from the danger or fight. It has been found that peoples with a reduced levels of anxiety are more susceptible to the risk of death comparing to those with a normal levels[2,3], but when it becomes excessive, it will be considered as a disorder of anxiety[4]. The anxiety disorders have found to be more in patients with a family history[5]. For example the generalized anxiety disorder is six times more likely to occur in the children from a parent with this disorder[6]. Anxiety affects about one eighth of the population all over the word, thus it is an important area for psychotherapy researches[7].

The modern society is associated with complexity of our daily life that can frequently results in a different degrees of anxiety. In developed and developing countries it has been found that anxiety disorders are accompanied by a chronic pain of the medical patients[8]. The anxiety disorders are of many types like generalized anxiety, phobias or post traumatic stress disorders, panic disorder, social anxiety disorder, and

obsessive compulsive disorder, which are the most common and major cause of disabilities[9]. The deficiency of the inhibitory neurotransmitter GABA, which reduce the CNS activity contribute to the development of anxiety. Therefore drugs modulating the receptors of GABA neurotransmitter will act as anxiolytic agents[10,11]. The serotonergic, adrenergic and dopamanergic systems could also involved in development of anxiety[12].

Selective serotonin reuptake inhibitors (SSRIs), are commonly the most used drugs for treatment of depression, they are often consider a first line therapy for anxiety disorders[13]. The SSRIs are the first-line recommended anxiolytic agents, although their use is associated with important side effects like sexual behavior changes (sexual dysfunction and decreased libido), nausea ,headaches, and insomnia, also SSRIs are effective in about 50% - 60% of cases only[14]. The second-line medical treatment used for anxiety disorders are benzodiazepines (BZDs) but, also their usage has been limited by some disadvantages such as cognitive and psychomotor impairments, development of dependence and potentiating the depressant effect of other CNS depressant agents[15,16]. Buspirone, is a non-benzodiazepines anxiolytic agent with no depressant effect, although it's onset of action is slow, and it is ineffective in a large percentage of cases, for example it is ineffective in children or adolescents with anxiety disorder[17], also buspirone use is associated with some side effects including gastric discomfort, tachycardia etc[18]. This has guide the scientists to seek out plants, that are usually employed in traditional and alternative medicine for treatment of sleep disorders and other related diseases[19]. Today, in the majority of the world medicinal plants are progressively more being used as herbs[20], and the interests on antioxidants from natural sources that are contained in vegetables and fruits, continuously increases[21].

In several plants are alkaloids the most important secondary metabolites which are responsible for their sedative and anxiolytic actions[22]. Flavonoids were attractive for interest of researchers since they appeared a promising role as powerful anti-oxidants that can offer body defense against free radicals and oxidative stress[23]. Human body is unable to produce flavonoids, thus they are taken within daily meal. Flavornoids have vital bioactive functions, including ROS scavenging[24]. Fruits and vegetables provide the body with phenolics which are also efficient antioxidants[25]. The antioxidative activities of phenolics are attributed to (1) their ability as donors to hydrogen or electron (2) to the capability of polyphenol-derived radicals for stabilization and dislocation of the unpaired electron or (3) to their ability to chelate transition metal ions[26].

Rosmarinus officinalis(Rosemary):

R. officinalis is an evergreen plant, it's leaves characterized by a fragrant and needle-like shape[27]. Rosemary is a spices herb related to the Labiatae family. It is one of the widely used herb in traditional medicine[28], for example rosemary is known to be used as memory enhancer and to treat decline in cognitive. Also it is used as a remedy against headaches, epilepsy, and depression , it has relaxant and sedative effects[29]. *R. officinalis* essential oil play an important role in flock medical therapy because it has great antioxidant, cytotoxic, antimutagenic, antibacterial, acetylcholinesterase inhibitory activity, and chemoprotective properties[30,31,32]. Rosemary's leaves have many beneficial effects, such as antitumor[33], antidiabetic, antioxidant[34],stimulate growth of hair[35], anti-inflammatory[36], and antibacterial activities[27,30]. R officinalis have a toxicity effect against E. kuehniella larvae [37].

The activities of the rosemary leaves extract are equivalent to the component of the identified antioxidants, such as rosemarinic acid, carnosol, carnosic acid, ursolic acid, butylated hydroxytoluene and butylated hydroxyanisole, with an additional advantages as they loss the risk of carcinogenicity or cytotoxicity associated with the use of the artificial antioxidants[38,39].

Aim of the study: To investigate the possible anxiolytic effect of *R.O.*

Material and methods:

Animals: Twenty eight adult male Swiss mice (25- 30 g) were used. Mice were kept in a standard cages in the animal house of Medical College of Babylon, and the temperature was controlled on 25 °C and 12 hours light-dark cycles. Standard diet with a tap water *ad libitum* were provided.

Plant Extract Preparation:

The leaves of R.O. were brought from the local market of Hilla city and the was identified by a botany specialist at the girls's collage of science in Babylon university, Iraq. Leaves were carefully washed, then dehydrated with air in shade, and grinded very well to powder. Plant extract was prepared by adding 40 gm of the plant powder to 80 ml of distilled water and extracted by using soxhlet for thirty six hrs at the temperature of 50-60 ºC. The extract's pellets of the plant were obtained by using an incubator to evaporate the liquid from the extract. The desired amount of the plant was prepared by using distill water to dissolve the pellets and then administered orally by using gastric tube at two doses 15 and 30 mg/kg body weight daily for fourteen successive days[40].

Open field test (OFT):

Rodents behaviors are changed when they are transferred into a new surroundings environment, and by using OFT under identical states situations any changes in the behavior such as anxiolytic or angiogenic activity can detected. Different type of open field device were used to investigate the behavioral changes in mice. The OFT device composed of a wooden chamber with a dimensions of 100 cm x 100 cm and 50 cm high walls. The floor of the chamber stained with a black color and by using a white lines it was divided into 100 equal (10 x10 cm^2) squares (picture 1). By sitting the animal at the middle of the field and left it for 5 minute, each animal were carefully monitored and the below parameters were recorded with a video camera: (1) Number of the squares crossed by the animal (2) Period of time spent in the central square (seconds) (3) Rearing beside the wall (number of the stands of the animal on the near paws) (4) Number and period of grooming. (washing of the face, grooming of the body and genital area, licking of the body and foot, scratching). Area of the open field was sterilized by ethanol (70%). Each mouse was monitored carefully and recorded by video camera (SONY/ Cyber-shot) for 5 minutes in order to record the main parameters mentioned above[41,42,43].

Experimental design: Animals were adapted for 2 weeks at the animal house before starting the experiment, then the mice were randomly divided into 4 groups (7 mice in each) as follows:

Group 1(negative control): Kept on standard feeding only for fourteen days. At day fifteen the mice were exposed to OFT.

Group 2: In addition to the standard feeding, this group received R.O. extract (15mg/kg), orally once daily for fourteen days. At day fifteen the mice were exposed to OFT.

Group 3: In addition to the standard feeding, this group received R.O. extract (30mg/kg), orally once daily for fourteen days. At day fifteen the mice were exposed to OFT.

Group 4(positive control): Kept on standard feeding only for fourteen days. At day fifteen and 30 minutes before they exposed to OFT the mice were intraperitoneally (i.p.) injected with 1 mg/kg[44] diazepam (ALSAVAL, Syria).

Statistical analysis:

Analysis of variance (ANOVA) was used for multiple sample analysis. Statistical analysis was done by using SPSS (version 17), our results were expressed as mean ± SD[45].

picture 1: open field test

Results :

(1) Number of the crossed squares: The results of this study revealed an extremely significant ($p<0.001$) increase in the number of squares crossed in groups 2, 3 and 4 in comparison to group 1 (negative control). Also the number of squares crossed in groups 2 (15mg/kg *R.O. extract*) were extremely significantly ($p<0.001$) increased in comparison to group 3 (30mg/kg *R.O. extract*). Although the number of crossed squares in group 2 was more than that in group 3 and 4 (diazepam) significant difference was not found ($p>0.05$). Also significant difference was not found ($p>0.05$) between group 3 and 4 (figure 1).

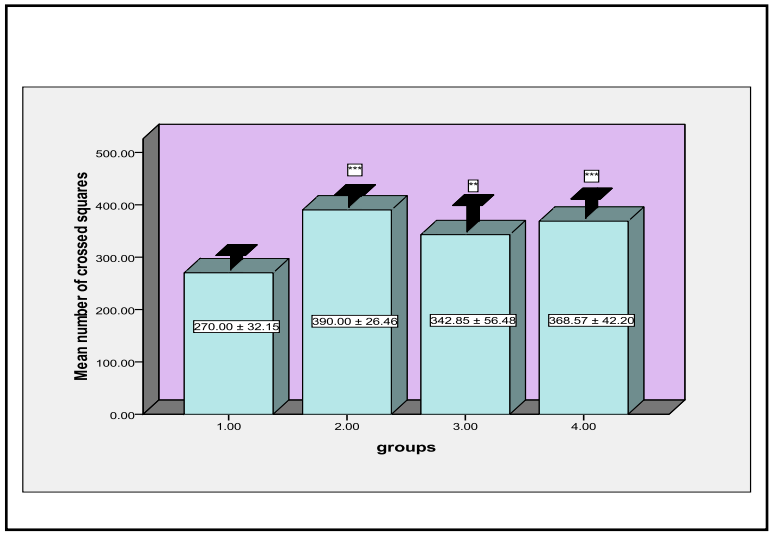

Figure (1) Number of crossed squares. Group 1 (negative control), group 2: received *R. officinalis extract* (15mg/kg), group 3: received *R. officinalis extract* (30mg/kg), group 4 (positive control): received diazepam 1 mg/kg

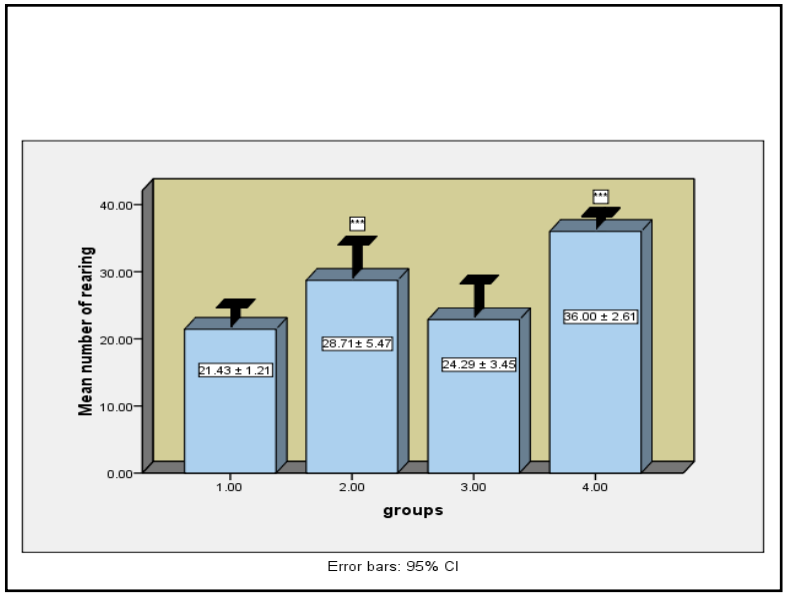

Figure (2) Number of rearing. Group 1 (negative control), group 2: received *R. officinalis extract* (15mg/kg), group 3: received *R. officinalis extract* (30mg/kg), group 4 (positive control): received diazepam 1 mg/kg.

(2) Number of rearing: In comparison to group1 the number of rearing was highly significantly ($p<0.01$) and extremely significantly ($p<0.001$) increased in groups 2 and 4 respectively, whereas no significant difference ($p>0.05$) was found between group1 and 3. Also the number of rearing was highly significantly ($p<0.01$) increased in group 4 in comparison to group 2, whereas rearing number was significantly ($p<0.05$)

increased in group 2 in comparison to group 3. Also rearing number was extremely significantly ($p<0.001$) increased in group 4 in comparison to group 3 (figure 2).

(3) Time spend in central squares: In comparison to group1 the time (seconds) spend in central squares was highly significantly ($p<0.01$) and extremely significantly ($p<0.001$) increased in groups 3 and 4 respectively, despite that the time spent in central squares in group 2 was more than that in group1, significant difference ($p>0.05$) was not found between these two groups. Although the time spend in central squares in group 3 was more in comparison to group 2, significant differences was not found ($p>0.05$). Also the time spend in central squares was extremely significantly ($p<0.001$) increased in group 4 in comparison to group 2 or 3 (figure 3).

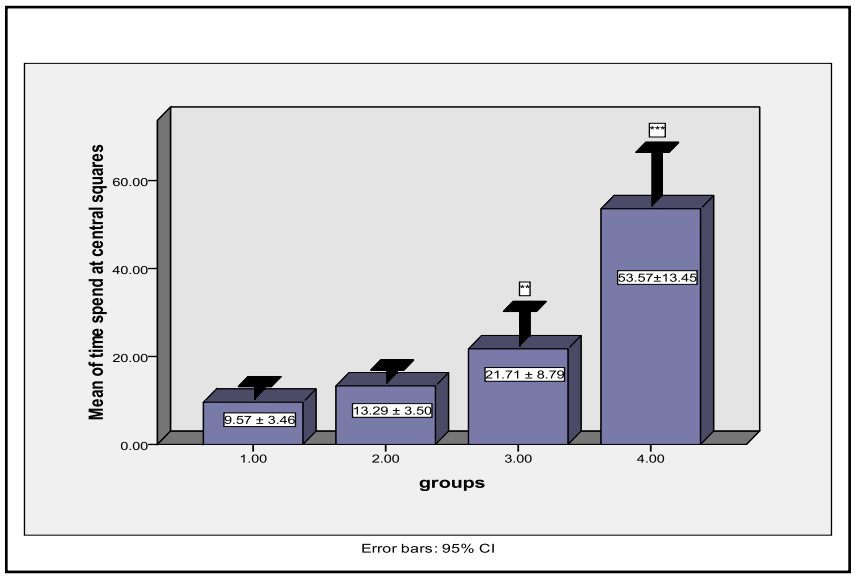

Figure (3) Time spend at central squares. Group 1 (negative control), group 2: received *R. officinalis extract* (15mg/kg), group 3: received *R. officinalis extract* (30mg/kg), group 4 (positive control): received diazepam 1 mg/kg.

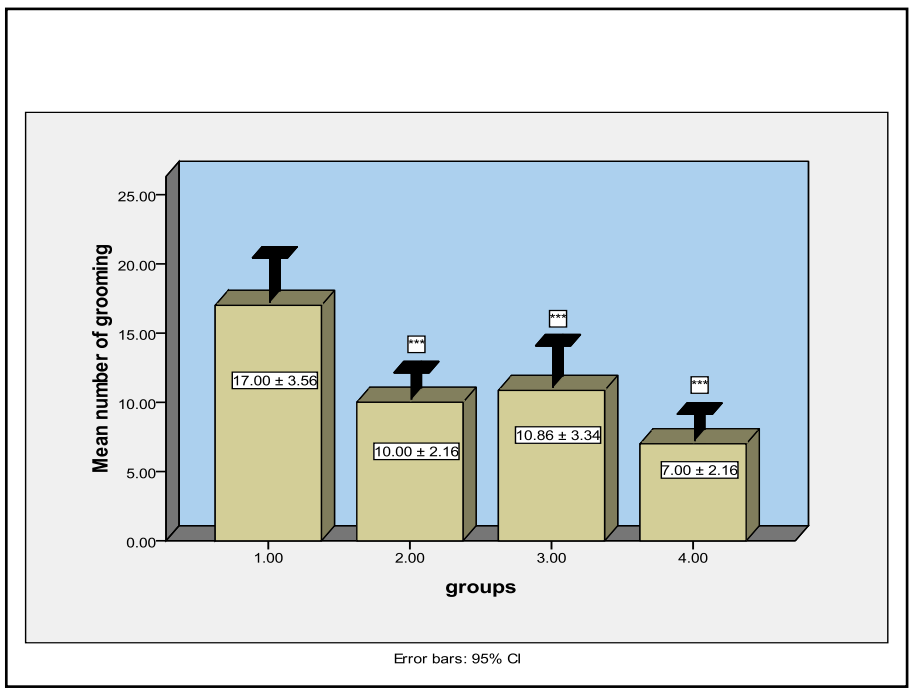

Figure (4) Number of grooming. Group 1 (negative control), group 2: received *R. officinalis extract* (15mg/kg), group 3: received *R. officinalis extract* (30mg/kg), group 4 (positive control): received diazepam 1 mg/kg

(4) Number of grooming: In comparison to group1 the number of grooming was extremely significantly ($p<0.001$), highly significantly ($p<0.01$) and extremely significantly ($p<0.001$) decreased in groups 2, 3 and 4 respectively. The significant differences was not found ($p>0.05$) between group 2 and 3 or 4, whereas number of grooming was significantly ($p<0.05$) decreased in group 4 in comparison to group 3 (figure 4).

Table 1 : Effects ofRosemaryon OFT parameters, results expressed as mean ± SD. Group 1 (negative control), group 2: received *R. officinalis extract* (15mg/kg), group 3: received *R officinalis. extract* (30mg/kg), group 4 (positive control): received diazepam 1 mg/kg

groups	Number of crossed squares	Number of rearing	Time spend at central Squares (seconds)	Number of grooming
1	270.00 ± 32.15	21.43 ± 1.21	9.57 ± 3.46	17.00 ± 3.56
2	390.00 ± 26.46 ***	28.71± 5.47 ***	13.29 ± 3.50	10.00 ± 2.16 ***
3	342.85 ± 56.48 **	24.29 ± 3.45	21.71 ± 8.79 **	10.86 ± 3.34 ***
4	368.57 ± 42.20 ***	36.00 ± 2.61 ***	53.57±13.45 ***	7.00 ± 2.16 ***

**= $p<0.01$, ***= $p<0.001$.

Discussion:

Anxiety is an emotional condition consisting of two components physiological and psychological, which comprising the different human being experiences, that can affect performance stimulation. When anxiety is not proportional to the exciting state, or directed to no certain object it will be converted into pathological condition[1]. Receptors of the inhibitory neurotransmitter GABA are included in the anxiety disorders, and[46] (Vogel, 2002) the pharmacological action of the anxiolytics agents are exerted as an increment in the level of GABA in the cerebral hemisphere[47]. By using the open field we can estimate the anxiety and fear in rodents[43].

Open field test measure effects of the therapeutic agents on unpleasant behavior and is used to evaluate the intensity of anxious excitability[48]. When an animal leave its acclimatized habitat cage and located in a new place, it express an anxiety with fear characterized by ambulate and explored decrement, restricted mobility or freezing, and the usual behaviors of rearing or grooming will be reduced or increased respectively. In addition, the stimulation of autonomic activity cause an increase in the micturation and defecation. The above mentioned signs and symptoms are augmented by anxiogenic agents or reduced by the typical anxiolytics drugs[49]. In comparison to a standard anxiolytic agent such as diazepam the OFT as a standard screening method was used to investigate the probable anxiolytics effect of ROE[41,50]. Stress induces an anxiety like behavior and by using the open-field this behavioral changes will be expressed as a decrease of locomotion and period spent in the central area of the field; whereas effective anxiolytic drugs can increase the locomotion which reflect a low level of anxiety[43,51].

Regarding our study, R.O extract resulted in a favorable effect on locomotor and exploration activity[43,51]which has been indicated by an increment in the number of the crossed squares in the tested groups (in comparison to group1 "control"), especially in group 2 (received 15 mg/kg R.O. extract) in which the number of the crossed squares was more even than that in diazepam (as a standard anxiolytic) treated group, this result demonstrating the anxiolytic activity of R. O. extract particularly with the lower dose (15 mg/kg), in addition the anxiolytic activity of R. O. *extract* was confirmed by the increment in the time spend in central squares. In group 2 (15mg/kg R. .O extract) the insignificant ($p>0.05$) increase of the time spend in central squares in comparison to group 3 (30mg/kg R. O. extract) this can be explained by the highly increased number of the crossed squares which was extremely significant ($p<0.001$).

Also the number of rearing was increased in both groups treated with ROE (it was more in group2 than that in group3) and it was less than that in diazepam group, this result also indicating the activity of R. O.extract

as anxiolytic agent. Whereas number of grooming (as a behavior appeared in a new surroundings) was decreased which represent a reduction in the stress and confirm the anxiolytic-like effect of R. O.[52].

Up to knowledge there is no similar study to which we can compare our results. In our study the anxiolytic activity of R. O. extract goes with what have been found by Alsumi and Tonosaki (2007) study in which the anxiolytic activity of R. O. scents (sniffed for 5 minutes) may be attributed to the ability of R. O. as a free radical scavenger decreasing cortisol (hormone of stress) level, thus it promotes body protection against oxidative and emotional anxiety[53,54]. The ability of rosemary to scavenge free radicals may be attributed to the presence of flavonoids which represent the largest group of phenol compounds found in the plants. These flavonoids are called bioflavonoids. Luteolin, genkwanina, diosmetin are in the free and glycosidic form in the rosemary[55].

Conclusions

Rosemary aqueous extract has anxiolytic effect especially with the dose of 15 mg/kg. Further studies especially clinical one are needed.

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