

## Research Article

# Key Role of Carnosic Acid in the Anxiolytic-like Activity of *Rosmarinus officinalis* Linn. in Rodents

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## Abstract

*Rosmarinus officinalis* Linn. has several therapeutic applications in folk medicine and carnosic acid is one of the major chemical constituents in this plant. Standardized extract of *Rosmarinus officinalis* (ROE) and carnosic acid (CA) isolated from *Rosmarinus officinalis* were evaluated for anxiolytic-like activity. Methods used were elevated plus maze, open field and social interaction tests. Charles foster albino rats were treated with ROE at the doses of 30, 100 and 300 mg/kg/day and CA at the doses of 5, 15 and 45 mg/kg/day, p.o. for seven consecutive days respectively. In all tests, ROE and CA exhibited significant anxiolytic activity. ROE and CA significantly increased the level of 5-hydroxy tryptamine and dopamine concentration in rat brain while norepinephrine concentration was decreased. These results suggest that both ROE and CA has significant anxiolytic effect in dose dependent manner and qualitatively similar to standard anxiolytic agent lorazepam, which likely to regulated through monoaminergic system in the brain. These observations strongly suggest that carnosic acid seems to be major active constituent of RO for its observed anxiolytic activity in rodents.

## INTRODUCTION

Anxiety is a universal human emotion. It alerts us to potential threats and motivates us to prepare for challenges. Anxiety is a disorder characterized by a broad range of symptoms including altered mood, emotions and feelings. Clinical anxiety is a chronic disease that can interfere significantly in the individual's life quality. Cognitive-behavioral therapy (CBT), behavior therapy (BT), and medication have been found to be efficacious for the treatment of anxiety however it often takes more than 5-8 treatment sessions to make the person panic free [1,2]. Moreover, conventional treatment modalities are hindered by adverse effects and produce a partial remission [3,4]. Plants have been used for some psychiatric disorders, like St. John's wort that is largely studied for the treatment of depression [5-7], and Kava-kava for anxiety [8]. Because of the limitations of the anxiolytic therapy, there has been renewed interest in other alternative therapies with medicinal plants, which may have comparable efficacy to prescription medications while lacking their severe side effects [9,10].

*Rosmarinus officinalis* Linn. (syn: Rosemary, family: Lamiaceae) has several therapeutic applications in folk medicine

[11] in curing or managing a wide range of diseases. *Rosmarinus officinalis* L. is native to Europe, but has been cultivated in all Brazilian states. Some studies have reported that the extract of this plant exerts a number of pharmacological activities, such as hepatoprotective [12], antibacterial [13], antithrombotic [14], antiulcerogenic [15], diuretic [16], antidiabetic and antioxidant [17], antinociceptive [18] and anti-inflammatory [19]. An ethnopharmacological use of *Rosmarinus officinalis* in the treatment of depression, among other uses, was reported [20]. The most abundant phenolic diterpene compound in Rosemary leaves is carnosic acid [21,22], a labile abietane diterpene that undergoes an oxidative degradation and rearrangement cascade, producing a series of compounds, many with antioxidant activity [21,23], such as carnosol, rosmanol [24], rosmariquinone [25], and methyl carnosate [26]. Carnosic acid is a lipophilic antioxidant that scavenges singlet oxygen, hydroxyl radicals, and lipid peroxy radicals, thus preventing lipid peroxidation and disruption of biological membranes [27]. Its radical scavenging activity is caused by the presence of two O-phenolic hydroxyl groups formed at C11 and C12 of the molecule [28].

Considering the therapeutic applications of *Rosmarinus officinalis* in folk medicine in the management of neurological

and psychiatric disorders, the present study was planned to support scientifically for its traditional claim by using reverse pharmacology approach. In view of impaired monoamines level which is one of the major contributions to psychiatric disorders including anxiety [29-31], we conclude that the observed potential anxiolytic-like activity of *Rosmarinus officinalis* and carnosic acid likely to be due its effect monoaminergic system.

## MATERIAL AND METHODS

### Animals

Adult Charles foster rats of either sex (3 males and 3 females in each group) weighing  $180 \pm 20$  g were used for animal studies [32-35]. The animals were obtained from the Central Animal House, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India and were randomly distributed into different experimental groups. The rats were housed in polyacrylic cages with stainless steel wire lids and maintained under standard laboratory conditions (temperature  $25 \pm 1$  °C) and relative humidity ( $50 \pm 5\%$ ) with dark and light cycle (12:12 hr). The animals were fed with standard pellet diet and water *ad libitum* unless stated otherwise. Principles of laboratory animal care (NIH publication number # 85-23, revised in 1985) guidelines were followed. The experimental protocol was approved by the Central Animal Ethical Committee of the University (No. Dean/10-11/284 dated October 19, 2010).

### Drugs and treatment groups

The extract was standardized to contain carnosic acid (10% w/w) and carnosol (1% w/w) by HPLC. Assay of the isolated carnosic acid was found to be 95% when compared with the standard by HPLC. The extract of *Rosmarinus officinalis* (ROE) and carnosic acid (CA) were suspended in 0.5% Tween 80 in distilled water [18]. These suspensions were administered orally at three different doses (30, 100 and 300 mg/kg/day) of ROE and (5, 15 and 45 mg/kg/day) of CA respectively, for 7 consecutive days (day 1 to day 7) based on our pilot study. Behavioral experiments were performed 1 hour after administration of last dose (7<sup>th</sup> day) as per methods described somewhere [32-35]. The suspension of extract was freshly prepared immediately before its administration. A control group received 0.5% Tween 80 in distilled water as vehicle. Lorazepam (1 mg/kg, p.o.) was used as standard anxiolytic agent.

### Elevated plus maze

The elevated plus maze has been used as a model predictive of anxiolytic activity. The method of Pillow et al. was followed [36]. The plus-maze consists of two open arms,  $50 \times 10 \times 40$  cm, and two enclosed arms,  $50 \times 10 \times 40$  cm, with an open roof, arranged so that the two open arms are opposite to each other. The arms were connected to a central square,  $10 \times 10$  cm, giving the apparatus shape of plus sign. The maze was kept in a dimly lit room and elevated 50 cm above the floor. Rats were placed individually in centre of the maze, facing an enclosed arm. Thereafter, number of entries and time spent on the open and enclosed arms were recorded during the next 5 min by a blinded observer.

### Open field test

The method of Bronstein was followed [37]. The open field

apparatus was made of plywood and consisted of squares of ( $61 \times 61$  cm). The entire apparatus was printed black except for 6m thick white lines, which divided the floor into 16 squares. Open field was lighted by a 16 w bulb focusing on the field from a height of about 100 cm. The entire room, except the open field, was kept dark during the experiment. Each animal was centrally placed in the test apparatus for 5 min and the following behavioral aspects were noted:

Ambulation: This was measured in terms of no. of squares crossed by the animal, Rearing: Number of times the animal stood on its hind limbs, Self grooming: Number of times the animal groomed facial region and licked/washed/scratched various parts of the body, Activity in centre: Number of central square crossed by the animal and Fecal dropping: Number of fecal droppings excreted during the period.

### Social interaction test

The method of File was followed [38]. The rats were first housed individually for 5 days before testing. The apparatus used for the test was a wooden box ( $60 \times 60 \times 35$  cm) with a solid floor and was placed in a dimly lit room. On day 6, the rats were placed individually in the box and given to 7.5 min familiarization sessions at 2 hr interval. On day 7 rats were paired on weight and sex basis and place in the box for 7.5 min. During this time total time spent by the rat pair in "social interaction", included sniffing, grooming, kicking boxing, biting, and crawling under or over the partner, was recorded in real time scoring method by a neutral 'blinded' observer.

### Estimation of brain monoamines

Immediately after the last regimen, animals were sacrificed by decapitation. The brain was rapidly removed and left in the ice-cold isotonic saline for a few seconds to cool throughout, and then trimmed for consistent separation of the spinal cord. Simultaneous determination of dopamine (DA), 5-hydroxy tryptamine (5-HT) and norepinephrine (NE) in a single rat brain was done by the method described by Welch and Welch [39].

### Statistical analysis

All values were expressed as mean  $\pm$  standard error mean (SEM). Statistical significance between control and treatment groups was analyzed by one way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test. GraphPad Prism-6 software was used for all statistical analysis.

## RESULTS AND DISCUSSION

### Elevated plus maze test

Elevated plus maze (EPM) test is based on the principle that exposure to an elevated and open arm leads to an approach conflict that is stronger than that evoked by exposure to an enclosed arms of the maze. These responses are increased by anxiogenic agent and reduced by anxiolytics [40]. Both *Rosmarinus officinalis* extract (ROE) and carnosic acid (CA) treated rats exhibited significant dose dependent decrease in time spent in enclosed arms [ $F(7, 40) = 8.96$ ] and number of entries into enclosed arms [ $F(7, 40) = 16.91$ ] compared to control group. Also there was significant dose dependent increase in time spent in open arms [ $F(7, 40) = 46.88$ ] and numbers of entries into open

arms [F (7, 40) = 15.73] compared to control group. The observed anxiolytic-like effect of *Rosmarinus officinalis* and carnosic acid was qualitatively analogous to standard drug lorazepam (Table 1).

### Open field test

In open field test (OFT), when animals are taken from their home cage and placed in a novel environment, they express their anxiety and fear by decrease in ambulation and exploration freezing, rearing and grooming behaviors and increase in defecation due to heightened autonomic activity [41]. Likewise lorazepam treated animals; the ROE and CA treated rats were showed significant increase in open field ambulation [F (7, 40) = 86.19], activity in centre [F (7, 40) = 13.16], self grooming [F (7, 40) = 13.01] and rearing [F (7, 40) = 16.37] in comparison to vehicle treated rats indicating anxiolytic activity of *Rosmarinus officinalis* and carnosic acid. Additionally the fecal droppings were significantly decreased [F (7, 40) = 7.731] in dose dependent manner after treatment with both ROE and CA (Table 2).

### Social interaction test

The SIT is based on the fact that, an increase in social interaction without a concomitant increase in motor activity is indicative of anxiolytic activity [42]. Rats treated with ROE and CA spent significantly more time in social interaction [F (7, 40) = 217.3] in comparison to control rats in a dose dependent manner. Likewise, lorazepam also caused significant increase in social interaction in rats and its effect were also found to be qualitatively comparable to that of *Rosmarinus officinalis* and carnosic acid (Figure 1).

### Level of brain monoamines

Impaired monoamines level is one of the major contributions to anxiety. Various classes of anxiolytics exert their effect by achieving any of the monoamines processing mechanisms like synthesis, release and synaptic availability. In anxiety majority of evidence supports the brain deficiency of serotonergic [29] and dopaminergic activity [30], while over activation of noradrenergic system [31]. In the present study ROE and CA produced significant increase in 5-HT [F (7, 40) = 4.507] and DA [F (7, 40) = 13.90] levels in brain while NE level [F (7, 40) = 39.75] was decreased in a dose dependent manner compared to control group and qualitatively similar to standard benzodiazepine drug (Figure 2).

Many activities of the rosemary extracts were attributed to the carnosic acid content. So it is used for evaluation of its effect on the anxiety. Most of the animals models of anxiety now in use were developed for benzodiazepines (BDZ) and, since these compounds also exhibit significant muscle relaxant and anticonvulsant effects, evaluation of anxiolytic activity, even with non-BDZ compounds, invariably now includes test for these neuropharmacological actions [43]. The sedative, amnesic and ataxic effects of BDZ and non-BDZ anxiolytics are definite drawbacks when these drugs are used for the treatment of anxiety [36]. RO and its active chemical constituent CA have also reported to have antioxidant activity [17,27]. Oxidative stress involved in many acute and chronic diseases including cancer, cardiovascular disorders and neurodegenerative disease including anxiety. Bouayed et al. (2007) have studied the

**Table 1:** Effect of *Rosmarinus officinalis* extract and carnosic acid on elevated plus maze in rats.

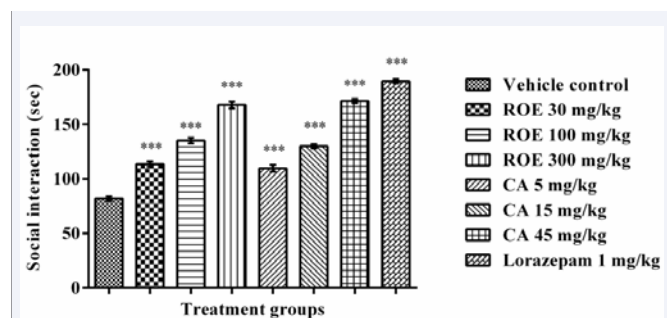
Treatment groups	Time spent (sec)		Number of entries (N)	
	Enclosed arms	Open arms	Enclosed arms	Open arms
Vehicle control	250.50±10.45	46.50±4.31	5.25±0.36	1.50±0.22
ROE 30 mg/kg	220.00±7.18*	59.16±1.53*	3.33±0.42**	2.00±0.25
ROE 100 mg/kg	211.50±12.69**	65.83±2.08***	2.16±0.30***	3.30±0.49*
ROE 300 mg/kg	202.50±2.61***	88.16±3.58***	1.50±0.22***	4.33±0.33***
CA 5 mg/kg	216.17±2.27**	62.83±2.27**	4.50±0.43	3.07±0.42*
CA 15 mg/kg	207.17±2.78***	79.35±2.78***	3.17±0.34***	4.20±0.43***
CA 45 mg/kg	192.50±2.34***	96.07±3.42***	2.67±0.33***	5.53±0.48***
Lorazepam 1 mg/kg	185.17±2.38***	105.86±2.76***	1.14±0.30***	6.02±0.48***

Values are mean ± SEM, n=6. ROE- *Rosmarinus officinalis* extract, CA- carnosic acid. \* = p < 0.05; \*\* = p < 0.01; \*\*\* = p < 0.001 vs. vehicle control.

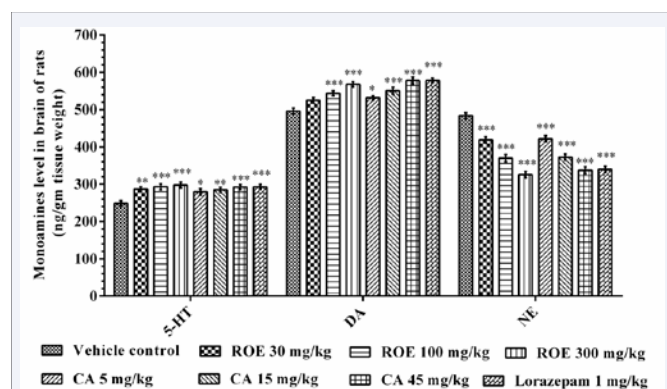
**Table 2:** Effect of *Rosmarinus officinalis* extract and carnosic acid in open field test in rats.

Treatment groups	Ambulation	Activity at centre	Self grooming	Rearing	Fecal dropping
Vehicle control	26.33±0.80	0.33±0.21	3.25±0.30	8.33±1.01	5.28±0.87
ROE 30 mg/kg	32.33±1.05*	1.00±0.36	5.00±0.36*	14.33±1.43***	4.66±0.21
ROE 100 mg/kg	45.66±1.30***	1.83±0.30	5.33±0.42**	17.33±1.30***	2.66±0.88**
ROE 300 mg/kg	54.16±1.30***	3.83±0.47***	5.16±0.30*	18.33±0.79***	1.67±0.55***
CA 5 mg/kg	34.50±1.43***	1.50±0.22	4.17±0.31	13.17±0.70**	4.17±0.30
CA 15 mg/kg	48.50±1.48***	2.83±0.31***	5.07±0.35*	15.83±0.48***	3.17±0.31*
CA 45 mg/kg	55.17±1.25***	3.83±0.60***	5.33±0.33**	18.33±0.61***	2.33±0.33**
Lorazepam 1 mg/kg	57.50±1.40***	4.16±0.54***	8.50±0.76***	20.83±0.94***	1.00±0.36***

Values are mean ± SEM, n=6. ROE- *Rosmarinus officinalis* extract, CA- carnosic acid. \* = p < 0.05; \*\* = p < 0.01; \*\*\* = p < 0.001 vs. vehicle control.



**Figure 1** Effect of *Rosmarinus officinalis* extract and carnosic acid in social interaction test in rats. ROE- *Rosmarinus officinalis* extract, CA- carnosic acid. Values are mean  $\pm$  SEM, n=6. \*\*\*=p<0.001 vs. vehicle control.



**Figure 2** Effect of *Rosmarinus officinalis* extract and carnosic acid on the monoamines level in brain. ROE- *Rosmarinus officinalis* extract, CA- carnosic acid, 5-HT- 5-hydroxytryptamine, DA- dopamine, NE- norepinephrine, Values are mean  $\pm$  SEM, n=6. \* = p < 0.05; \*\* = p < 0.01; \*\*\* = p < 0.001 vs. vehicle control.

relationship between the level of intracellular reactive oxygen species in peripheral granulocytes and estimated anxiety level of rodents. The results of this study suggest a positive relationship between peripheral oxidative status and level of anxiety another study shows that a high anxiety level significantly increased the generation of reactive oxygen species in the rodents [31,44]. In view of this, the reported antioxidant activity of RO and CA is likely to play a significant role in our observed anxiolytic effect of ROE.

The findings of the present study indicate that ROE and CA treatment caused significant dose related anxiolytic-like activity in rats tested on all the behavior paradigms viz. EPM, OFT and SIT and the results were qualitatively similar to standard anxiolytic agent lorazepam. Further, observed anxiolytic effect of ROE and CA seems to be regulated through monoaminergic system in the brain.

## CONCLUSION

*Rosmarinus officinalis* extract possesses promising anxiolytic activity regulated through monoaminergic system in the brain and carnosic acid is likely to be its active chemical constituent responsible for observed anxiolytic activity.

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