ADAPTOGENIC EFFECT OF CARVACROL IN AN ANIMAL MODEL OF CHRONIC UNPREDICTABLE MILD STRESS

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Abstract

Introduction: In recent years, natural products have gained increasing attention due to their favorable safety profile and diverse therapeutic properties. Among these, plant-derived secondary metabolites—particularly polyphenols, phenolic acids, and terpenes—are extensively studied. Carvacrol, a monoterpenoid phenol predominantly found in the essential oils of *Thymus vulgaris*, *Origanum vulgare*, and *Satureja hortensis*, is well-documented for its antioxidant and anti-inflammatory properties. However, its potential effects on the central nervous system remain underexplored. **Aim:** This study aimed to evaluate the adaptogenic potential of Carvacrol in a rodent model of chronic unpredictable mild stress (CUMS). **Materials and Methods:** Twenty-four male Wistar rats were randomly assigned to three groups (n = 8). The control group received olive oil, while the experimental group was administered Carvacrol (500 mg/kg body weight, orally). CUMS was applied to induce anxiety- and depression-like behaviors. Behavioral assessments included the Elevated Plus Maze (EPM) and the Forced Swim Test (FST). To assess GABAergic involvement, the Vogel Conflict Test was employed. Data were analyzed using one-way ANOVA via IBM SPSS 19.0. **Results:** Exposure to CUMS significantly increased anxiety- and depression-related behaviors. Carvacrol treatment resulted in increased open arm activity in the EPM and reduced immobility in the FST, indicating anxiolytic and antidepressant-like effects, though the latter did not reach statistical significance ($p > 0.05$). No significant differences were observed in the Vogel test. **Conclusion:** Carvacrol demonstrated anxiolytic and antidepressant-like effects in the CUMS model, potentially independent of GABAergic pathways. Further research is warranted to elucidate its mechanism of action.

***Key words**: Carvacrol, adaptogens, depression, anxiety, GABA system*

Introduction:

Chronic stress is a key factor disrupting homeostasis and significantly affecting behavior and cognitive function (*El Marzouki H et al*). Depending on their nature—physical, social, or psychological—and duration—acute or chronic—stressors contribute to the onset of mood and behavioral disturbances, and are commonly modeled in experimental research to mimic anxiety and depressive disorders (*El Marzouki H et al*). These conditions are associated with telomerase inhibition and telomere shortening, leading to DNA damage and symptom development, particularly with repeated stress exposure (*de Punder K et al; Hau M et al*). Neurobiological alterations include heightened glutamatergic activity, reduced serotonergic signaling, and diminished monoamine neurotransmitter synthesis (*Sen ZD et al*). Elevated stress hormones, such as norepinephrine and cortisol, further exacerbate oxidative stress by promoting reactive oxygen species (ROS) and downregulating antioxidant enzymes (*Hussain T et al*). Concurrently, pro-inflammatory mediators like IL-6, TNF-α, and CRP are upregulated, resulting in subclinical chronic inflammation, which amplifies oxidative stress and contributes to psychopathology (*Hussain T et al*).

Conventional anxiolytics and antidepressants primarily modulate neurotransmitter pathways but show limited efficacy against inflammation and oxidative damage. Moreover, these drugs often

present delayed therapeutic onset and adverse effects (*Muraro C et al; Fedotova J et al*). Consequently, there is growing interest in natural compounds with multi-target activity and favorable safety profiles, especially plant-derived secondary metabolites such as polyphenols, phenolic acids, and terpenes (*Khan MSA et al*).

Carvacrol, a monoterpenoid phenol found in essential oils of *Thymus vulgaris*, *Origanum vulgare*, and *Satureja* species (*Khazdair MR et al*), is known for its strong antioxidant, anti-inflammatory, antibacterial, antiviral, and antifungal activities (*Mohammedi Z*). In vitro, it downregulates iNOS and COX-2 expression, reducing nitric oxide and prostaglandin E2 levels, and inhibits NF-κB signaling (*Xiao Y et al*). It also lowers circulating levels of IFN-γ, IL-1β, IL-4, IL-8, and IL-17, while increasing TGF-β and IL-10, and modulates Th1 and Th17 lymphocyte activity (*de Carvalho FO et al; Mahmoodi M et al*). Carvacrol's LD_{50} is approximately 800 mg/kg body weight (*Ghorani V et al*), and previous research has shown that prolonged administration (90 days at 500 mg/kg) does not induce liver or kidney toxicity.

Preclinical data suggest Carvacrol may influence central neurotransmission by antagonizing norepinephrine receptors and inhibiting serotonin reuptake, thus elevating synaptic levels of both neurotransmitters. Despite these encouraging findings, its therapeutic potential in anxiety and depression remains underexplored and warrants further investigation.

Aim:

This study aimed to evaluate the adaptogenic potential of Carvacrol in a rodent model of chronic unpredictable mild stress (CUMS).

Materials and Methods:

Experimental animals

Twenty-four male Wistar rats were randomly assigned to three groups (n = 8 per group). Both control groups received olive oil, while the experimental group was administered Carvacrol (Sigma Aldrich) at a dose of 500 mg/kg body weight via oral gavage. The rodents were accessed from the Vivarium of Medical University Plovdiv and were raised under standard laboratory conditions.

Experimental protocol

To induce anxiety- and depression-like behaviors, a chronic unpredictable mild stress (CUMS) protocol was implemented. Starting from day one of the experiment, rodents in the positive control and Carvacrol-treated groups were exposed to a series of mild stressors for a period of 60 days. The CUMS protocol included the following stressors: 24-hour food or water deprivation, exposure to an empty water bottle for one hour, tilting of the home cage at a 45° angle for three hours, continuous illumination for 24 hours, bedding contamination (200 mL of water at 25°C per 100 g of bedding material), and exposure to predator sounds (two sessions of 20 minutes each). Each stressor was applied once per week, one hour after daily treatment. The order was randomized weekly to minimize habituation. On day 61, one hour after daily treatment, all Wistar rats underwent behavioral assessment using the Elevated Plus Maze (EPM) and the Forced Swim Test (FST). To evaluate potential GABA-ergic involvement, the Vogel Conflict Test (VCT) was employed. The experimental design is shown on Figure 1.

Figure 1. Experimental design

Elevated plus maze

The test is conducted in a single session without prior training. The duration of the test is 5 minutes, measured using a stopwatch. The Wistar rats are positioned at the center of the apparatus at the start. Throughout the test, the following variables are recorded: duration spent in the open and closed arms, the number of entries into each arm, the total number of arm visits, and the proportion of open arm entries relative to the total number of movements. As an indication of reduced anxiety the time spent in both the open and closed arms was recorded, with an increased duration in the open arms are interpreted. The apparatus was cleaned with 70% alcohol after each animal.

Forced swim test

The FST was carried out over two days. On the first day, during the habituation session, the animals were placed in plastic cylinders measuring 35 cm in height and 20 cm in diameter, filled with 18 cm of water maintained at 25°C, for a duration of 15 minutes. After a 24-hour interval, the test session was conducted, during which the rats were placed back into the same cylinders for 5 minutes. The immobility time (T_0) was measured via chronometer. Its reduction was interpreted as an indication of antidepressant activity of the tested substance.

Vogel Conflict test

The test lasts also two consecutive days three hours after FST. All Wistar rats were subjected to 24 hours of water deprivation prior to the first trial. 24-hours later they were placed in a Vogel apparatus (Ugo Basile) to become familiar with the testing environment and were allowed to drink water during a 3-minute training session without use of electric shock punishment. Test trial was followed by another 24-hour period without access to drinking water. The actual test session was conducted on the next day, also for 3 minutes. During the test, the rodents received a 2-second, 300 µA electric shock after every 15 licks. All measurements were automatically recorded by the Vogel software. An increase in the number of shock licks was interpreted as an anxiolytic response.

Statistical analysis

Observed results in all tests were analyzed using One Way ANOVA (ANOVA) with IBM SPSS Statistics version 19.0. Results were expressed as arithmetic mean and standard error of the mean (mean ± SEM). A p value ≤ 0.05 was considered statistically significant.

Results:

Elevated Plus maze

In EPM rodents from positive control group had significant increase in the time spent in the enclosed arms of the apparatus while a statistically significant decrease in time spent in the open arms was measured. Administration of Carvacrol at dose at 500mg/kg b.w. resulted in statistically significant decrease in time spent in the enclosed arms, and increased time in the open arms, compared to the positive control. This results suggest anxiolytic effect of Carvacrol. Results are presented on Figure 2.

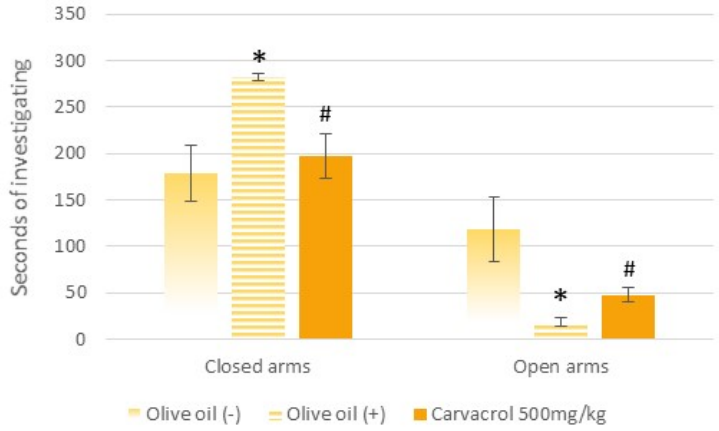

Figure 2. Anxiolytic-like effect of Carvacrol

* A significant increase in the time spent in the enclosed arms of the apparatus, and a statistically significant decrease in time spent in the open arms, compared to the negative control
# A statistically significant decrease in time spent in the enclosed arms, and increased time in the open arms, compared to the positive control

No statistically significant differences were observed between the Carvacrol-treated group and the negative control in terms of the total number of movements in both arms of the apparatus. This finding suggests that the tested substance does not exert any noticeable effect on locomotor activity. Detailed results not shown.

Forced Swim test

In FST a significant increase in the T_0 in the positive control was found when compared to the negative control group. Carvacrol treatment reduced statistically significant this parameter when compared to the positive control, indicating antidepressant effect. Results are presented on Figure 3.

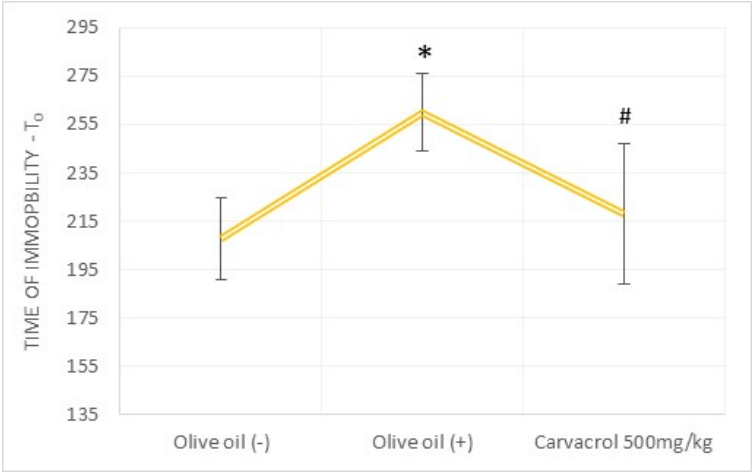

Figure 3. Antidepressant-like effect of Carvacrol

* A significant increase in the T_0, compared to the negative control.
# A statistically significant decrease in T_0, compared to the, compared to the positive control

Vogel test

No statistically significant differences were observed between the both control groups in Vogel test. Although the number of shock-induced licks increased in the Carvacrol-treated group, the change was not statistically significant ($p>0.05$). Results are presented on Figure 4.

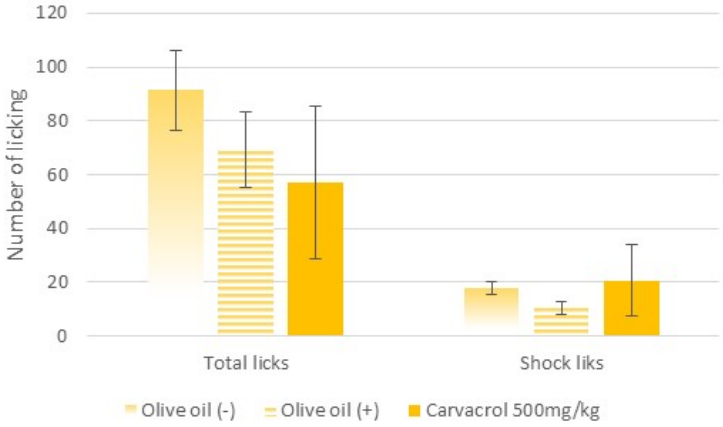

Figure 4. GABA-ergic effect of Carvacrol

Discussion

In rodents, anxiety is typically assessed using behavioral tests that involve conflict between exploratory drive and aversion to unfamiliar stimuli (*Fedotova J et al*). Literature suggests that no single test is sufficient to evaluate anxiolytic activity, and thus multiple behavioral paradigms are recommended (*Komada M et al*). Among the most commonly employed are the Elevated Plus Maze (EPM) and the Vogel Conflict Test (*Fedotova J et al*), both of which were utilized in the current study.

The EPM is a standard screening tool for assessing potential anxiolytic effects of novel compounds (*Fedotova J et al*). Anxious rodents tend to avoid the open arms, spending more time in the enclosed arms (*Tang M et al*). The Vogel Conflict Test, sensitive to GABAergic modulation, is frequently used to explore underlying mechanisms of anxiolytic activity (*Millan MJ et al*). In this test, anxiety manifests as reduced willingness to engage in punished drinking, reflected by a decreased number of shock-induced licks (*Fedotova J et al*).

For assessing antidepressant-like activity, the Forced Swim Test (FST) is widely used due to its reproducibility and predictive validity (*Slattery DA et al*). However, it may yield false positives or negatives, particularly for compounds not acting on core depressive mechanisms (*Slattery DA et al*). To mitigate this, some authors recommend complementary behavioral assessments (*Kraeuter AK et al*). In our study, total locomotor activity in the EPM was also evaluated to rule out confounding effects.

The behavioral results from our control groups align with previously reported stress-induced anxiety- and depression-like phenotypes (*Tang M et al; Fedotova J et al*). In accordance with findings by Naeem K et al., Carvacrol reduced anxiety-like behavior in the EPM without affecting locomotor activity (*Naeem K et al*). Other studies suggest that Carvacrol modulates multiple neurotransmitter systems, including GABA, norepinephrine (NE), and serotonin (5-HT) (*Melo FH et al*). However, in our study, Carvacrol did not significantly affect performance in the Vogel test, which may suggest its anxiolytic effect is mediated via NE and 5-HT rather than GABAergic pathways. Alternatively, the observed behavioral changes could stem from Carvacrol's anti-inflammatory and antioxidant properties (*Mohammedi Z*), both of which are implicated in the pathophysiology of anxiety disorders (*Costello H et al*).

Previous studies have also reported significant antidepressant-like effects of Carvacrol in the FST (*Polli FS et al*), findings corroborated by our own results. The proposed mechanisms include modulation of dopaminergic neurotransmission, via reduced neuronal degeneration in dopaminergic brain regions and inhibition of dopamine reuptake (Polli FS et al). Other potential mechanisms involve the regulation of ion channel expression and suppression of microglial activation (*Polli FS et al*), along with the compound's well-established anti-inflammatory and antioxidant effects.

Nevertheless, the present study employed general behavioral screening tools, which do not permit definitive conclusions regarding the specific mechanisms of action. While the findings are indicative of anxiolytic and antidepressant-like effects, they do not clarify whether these are mediated through monoaminergic, GABAergic, or other pathways. Further research using targeted, pathway-specific assays is required to elucidate the precise neuropharmacological mechanisms underlying Carvacrol's effects.

Conclusion

Carvacrol demonstrated anxiolytic and antidepressant-like effects in the CUMS model, potentially independent of GABA-ergic pathways. Further research is warranted to elucidate its mechanism of action.

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