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SPHAEROCOCCUS CORONOPIFOLIUS ALLEVIATES OXIDATIVE BRAIN INJURY ASSOCIATED TO DEPRESSION-RELATED BEHAVIOR INFEMALE WISTAR RATS

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ABSTRACT: Neuropathic pain is commonly comorbid with mood disorders, such as depression and anxiety. A state of depression may suggest biochemical imbalance that can increase the oxidative stress into the brain. The present study aimed to investigate the effect of aqueous extract of the red algae *Sphaerococcus coronopifolius* on depression-induced behavioral alterations and oxidative stress in female Wistar rats. Sciatic nerve injury was employed to induce depression in rats. Antidepressant-like effect of *S. coronopifolius* was investigated using two models: the forced swimming test (FST) and the open field test (OP). Three groups of rats were considered: The first group served as a control. The second group was subjected to sciatic nerve ligation (untreated group). The third group was subjected to sciatic nerve ligation treated daily by intraperitoneal injection of 25 mg algae extract kg⁻¹ body weight of rat during two consecutive weeks (treated group). At the end of the experiment, rats were sacrificed and brain tissues were isolated to determine the activities of catalase (CAT) and glutathione-S-transferase (GST) as well as the level of reduced glutathione (GSH). The results showed that the sciatic nerve ligation caused a depression-like behavior in female rats in contrast with the algae extract that demonstrated an antidepressant-like activity. The results obtained in the open field showed a preserved spontaneous locomotion. Untreated rats showed a significant decrease of GSH and antioxidant enzymes, and it seems that algae extract ameliorate CAT and GST activities.

INTRODUCTION: Neuropathic pain is commonly comorbid with mood disorders such as depression and anxiety ¹. Depression is one of the top five most prevalent diseases worldwide ². Furthermore, depression will be ranked second as measured by DALY (Disability Adjusted Life Years) for all ages by 2020. ³

A state of depression may suggest biochemical imbalance that can increase the oxidative stress into the brain ⁴. Many pathological conditions and diseases, such as infections, inflammatory disorders, aging, psychological stress, and psychiatric disorders can aggravate the biochemical imbalance in the body ⁵. Duda *et al.* ⁶ indicated an increase of oxidative stress into the brain of rats subjected to chronic mild stress (CMS)-induced depression in rodents.

In addition, unpredictable chronic mild stress (UCMS) induced a decrease in SOD and CAT activities ⁷. These observations may contribute to the development of new therapeutic agents based

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on antioxidant compounds. Recently much interest has been generated for a wide range of natural products with reports demonstrating their role in variety of clinical cases such as arthritis, rheumatism, pain, cancer and a vast range of other conditions⁸. In this context, biological activities of marine algae including antioxidant, anti-inflammatory, and neuroprotective effects have been characterized⁹.

Previous studies carried out with extracts from the red algae *S. coronopifolius* showed that it possesses several biological effects, such as antioxidant, antimicrobial, antitumor and antiviral potential due to the presence of active secondary metabolites^{10, 11, 12}. Furthermore, the effect of *S. coronopifolius* phenolic extracts on anxiety-like behavior in female Wistar rats was attempted¹³. Therefore, our interest was aroused to examine the status of the antioxidants in the brain tissue of rats suffering from pain-induced depression and to examine whether treatment with *S. coronopifolius* extract leads to change the depressive-like behavior and the oxidative status.

MATERIALS AND METHODS:

Algae Collection, Extract Preparation and Dose Determination: Algae collection, extract preparation and dose determination was carried out as described previously¹³. Briefly, *Sphaerococcus coronopifolius* was collected on submerged rocks at a depth of 6-7 m in Tiskerth, a small Mediterranean islet in the region of Boulimat, Bejaia, North-eastern Algeria. The Global Positioning Systems (GPS) location is 36°48' N, 4°58' E and the collected plant was identified in the Laboratory of Botany, University of Bejaia, Algeria. The collected algae were dried, ground and then subjected to extraction with distilled water (0.1 g/10 ml).

After shaking for 1 h at room temperature and centrifugation at 2220 rpm during 10 min, the obtained extract was collected and stored for the biological tests on animals. Dose determination was performed using the forced swimming test. Three doses of algae extract were tested: 15, 25 and 35 mg / kg of body weight (by intra-peritoneal injection). The group with the lowest immobility time was that treated with a dose of 25 mg/kg of body weight.

Animals: Healthy, female, Wistar albino rats (150 ±15g) were obtained from the Pasteur Institute of Algiers. Animals were maintained under standard environmental conditions (24 ± 1 °C, 12:12 h dark/light cycle) and had free access to tap water and food. Animals were handled according to the recommendation of the International Ethics Committees Directive 2010/63/EU, which updated and replaced the 1986 Directive 86/609/EEC and the ethical exigencies of the Faculty of Life and Nature Sciences of the University of Bejaia, Algeria. After three weeks of acclimatization period, female rats were randomly divided into three groups (five rats per group) as follows:

The first group served as a control. The second group was subjected to sciatic nerve ligation under general anesthesia (untreated group). The third group was subjected to sciatic nerve ligation under general anesthesia and treated with an intraperitoneal injection of 25 mg algae extract kg⁻¹ body weight for two consecutive weeks (treated group). During the experiment, animals were weighed every 10 days.

Depression Induced by Sciatic Nerve Ligation:

The sciatic nerve ligation was performed following the protocol of Bennett and Xie¹⁴ modified by Zaafour *et al.*¹⁵ Briefly, the animals were deeply anesthetized with a ketamine (0.3 ml/100 g)/largactil (10%) mixture (intra-peritoneal) and were fixed in the prone position. Tight ligation of sciatic nerve was performed with 6-0 silk suture. The incisions were sutured and animals were then allowed to recover from the anesthesia. Intra-peritoneal injection of an antibiotic in a volume of 0.3ml/250g of body weight was administered for five days after surgery to avoid the development of infection or other adverse outcomes.

Behavioral Assessments:

Forced Swimming Test: Rats were placed in an aquarium (30 cm large; 40 cm high) containing water at 23 ± 1 °C. This dimension ensures that the rats can't escape by climbing to the edges of the device. Two swimming sessions were conducted as described by Estrada-Camarena *et al.*¹⁶: an initial 15-min pre-test, followed by a 5-min test 24 h later. The second session was videotaped. After each swimming session, rats were towel-dried, placed into heated cages for 30 min and then returned to

their home cages. The time of immobility, climbing, and swimming was calculated.

Open Field Test: Each animal was placed in the center of the apparatus and was allowed to explore the arena for 5 min. The 5 min test was videotaped. The open-field arena was cleaned with 70% ethanol after every trial. Time spent in the central area, total distance traveled and the number of rears was calculated.

Organs Weights: After conducting depression-like behavior tests, rats were sacrificed under ether anesthesia, and organs (brain, spleen and adrenal glands) were harvested, rinsed with 0.9% saline solution at 4 °C and weighted. The weight of each organ was standardized to 100g per body of animals.

Biochemical Assays:

Preparation of Tissue Homogenate: The whole intact brain tissue was homogenized in 0.15 M Tris buffer (pH 7.4) and centrifuged at 3000 g at 4 °C for 30 min. An aliquot of the supernatant was collected and stored at -20 °C for the determination of reduced glutathione (GSH), protein levels, catalase (CAT) and glutathione-S-transferase (GST) activities.

Estimation of GSH Level: The level of GSH was estimated using a colorimetric technique, as mentioned by Ellman¹⁷, modified by Jollow *et al.*¹⁸ For that, 0.8 ml of brain supernatant was mixed with 0.3 ml of sulfosalicylic acid (0.25%) and the reaction mixture was centrifuged at 2500 g for 15 min. Afterward, the supernatant (0.5 ml) was put in reaction with 0.025 ml of DTNB (0.01 M) and 1 ml of phosphate buffer (0.1 M, pH 7.4). The absorbance was recorded at 412 nm and the total GSH content was expressed as nanomoles/mg protein.

Estimation of GST Activity: GST (EC 2.5.1.18) activity was measured according to the method of Habig *et al.*¹⁹ It depends on measuring the conjugation of the reaction of 1-chloro-2, 4-dinitrobenzene (CDNB) with reduced glutathione. The change in the absorbance was recorded at 340 nm, and enzyme activity was calculated as nanomoles CDNB conjugate formed/ min/mg protein.

Estimation of CAT Activity: CAT (EC 1.11.1.6) activity was assayed by the method of Aebi²⁰. The reaction mixture for the test contains 20 µl of homogenate mixed with 1255 µl of PBS, and the reaction was started by the addition of 725 µl of H₂O₂ (54 mmol/l). Change in absorbance is recorded at 240 nm and the catalase activity is calculated as nanomoles/min/mg protein.

Protein Estimation: The amount of protein was measured according to the method of Bradford using bovine serum albumin as a standard²¹.

Statistical Analysis: Results were expressed as Mean ± standard deviation (SD). Statistical analysis was performed by One-way and Two-way analysis of variance (ANOVA). Tukey test was used to evaluate differences between the groups at P < 0.05. All statistic tests were conducted using Graph Pad Prism (version 7) software.

RESULTS:

Behavior Analysis: Results of the forced swimming test are given in **Table 1**. A significant increase in immobility behavior [F (2, 10) = 22.24, P = 0.0002] against significant decrease in climbing behavior [F (2, 12) = 19.01, P = 0.0002] were observed between untreated and control groups. Whereas, no significant changes in immobility time were observed between treated and control groups after fourteen days of treatment. While, the Tukey test indicated a significant decrease in immobility time (q = 8.747, p = 0.0003) in treated rats compared to the untreated rats. There was also a significant increase in climbing time in treated rats compared with untreated and control groups (q = 8.72, p = 0.0001; q = 4.277, p = 0.0266, respectively). Significant changes in swimming behavior were also observed [F (2, 8) = 6.822, P = 0.0187]. The Tukey test revealed a significant decrease in swimming time in treated rats compared to the control (q = 5.014, p < 0.05). Values with the same letter in each separate parameter are insignificant (One-way ANOVA, p < 0.05, n = 5)

According to the open field test, no statistically significant differences in the total distance traveled had been found among the studied groups [F (2, 9) = 3.995, P = 0.0573] **Table 2**. Accordingly, the locomotor activity of rats was not altered by either

the surgery or the treatment with algae extract. Nevertheless, the Tukey test showed a significant decrease in the number of rearing in untreated group compared with control group ($q = 4.411$, $p = 0.03$). Whereas, rearing number significantly increased in treated rats when compared to the

untreated rats ($q = 7.092$, $p = 0.0019$). No significant changes were found in the center time in all groups. Values with the same letter in each separate parameter are insignificant (One-way ANOVA, $p < 0.05$, $n = 5$).

TABLE 1: MEANS AND STANDARD DEVIATIONS OF BEHAVIORAL PARAMETERS IN THE FORCED SWIMMING TEST

Groups	Immobility time (s)	Swimming time (s)	Climbing time (s)
Control	111.8 ± 21.86 ^b	95.63 ± 19.5 ^a	90.80 ± 32.8 ^b
Untreated group	188.25 ± 8.46 ^a	66.25 ± 6.65 ^b	45.40 ± 6.19 ^c
Treated group	95.63 ± 28.05 ^b	64.25 ± 9.91 ^b	134.50 ± 21.27 ^a

TABLE 2: MEANS AND STANDARD DEVIATIONS OF BEHAVIORAL PARAMETERS IN THE OPEN FIELD TEST

Groups	Time in the center (s)	Total distance traveled (cm)	Number of rearings
Control	5.75 ± 1.50 ^a	1492.26 ± 242.76 ^a	21.75 ± 2.63 ^a
Untreated group	3.50 ± 1.29 ^a	2081.31 ± 469.63 ^a	15.20 ± 2.95 ^b
Treated group	7.33 ± 3.06 ^a	2331.21 ± 527.06 ^a	26.67 ± 4.04 ^a

Animal Body and Organ Weights: As shown in Fig. 1, the bodyweight of rats increased over time during the experimentation (before and after ligation) in all groups. Administration of algae extract during 14 days did not significantly affect the body weights of animals.

However, there was a significant variation in body weight between the untreated and control groups from day 10, after ligation. Bodyweight gain was significantly different in untreated group compared to control group ($q = 4.208$, $p = 0.029$) Table 3.

Concerning the relative organ weights to the total body weight of rats, the results showed that both sciatic nerve ligation and treatment with algae extract, did not produce any significant effect on the weight of adrenals [$F(2, 6) = 3$, $P = 0.125$] and spleen [$F(2, 6) = 0.9855$, $P = 0.4265$]. However, brain weight/body weight ratio was significantly lower in the treated group when compared to the control group [$F(2, 6) = 11.85$, $P = 0.0082$]. Values

were expressed as mean ± SD. *Statistical significant at $p < 0.05$ as compared to control.

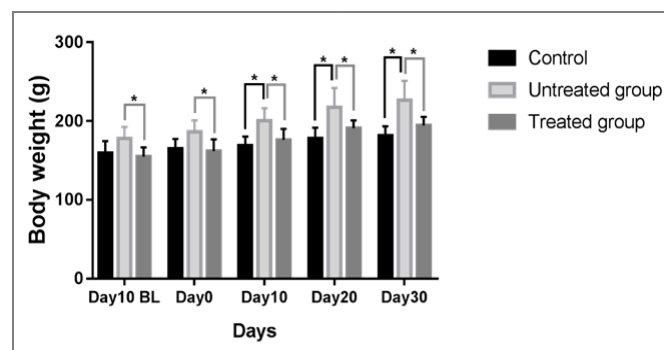


FIG. 1: BODY WEIGHT CHANGES OF RATS DURING THE EXPERIMENTATION. Results were expressed as mean ± SD (N=5). Two-way Anova, * $P < 0.05$.

Brain Antioxidant Status: The present study showed significant differences among the studied groups in terms of the mean level of GSH [$F(2, 15) = 6.95$, $P = 0.0073$] as well as GST [$F(2, 12) = 178.3$, $P < 0.0001$] and CAT [$F(2, 7) = 12.12$, $P = 0.0053$] activities Table 4.

TABLE 3: BODY WEIGHT GAIN AND RELATIVE ORGAN WEIGHTS OF RATS

Groups	Bodyweight gain (g)	Brain (g/100 bw)	Adrenals (g/100g bw)	Spleen (g/100g bw)
Control	22.0 ± 10.3	0.92 ± 0.05	0.06 ± 0.01	0.39 ± 0.07
Untreated group	48.2 ± 19.8*	0.73 ± 0.03*	0.07 ± 0.01	0.41 ± 0.06
Treated group	39.6 ± 9.13	0.73 ± 0.07*	0.05 ± 0.01	0.48 ± 0.1

Values were expressed as mean ± SD. *Statistical significant at $p < 0.05$ as compared to control.

TABLE 4: CHANGES IN THE LEVEL OF GSH AND ACTIVITIES OF GST AND CAT IN BRAIN HOMOGENATE OF EXPERIMENTAL GROUPS

Groups	GSH (nM/mg prot)	GST (nM/min/mg prot)	CAT (nM/min/mg prot)
Control	17.52 ± 2.14	1.42 ± 0.09	49.03 ± 14.38
Untreated group	14.48 ± 4.41	0.34 ± 0.05*	13.20 ± 2.94*
Treated group	10.92 ± 2.05*	0.42 ± 0.12*	23.27 ± 5.63*

Values were expressed as mean ± SD. *Statistical significant at $p < 0.05$ as compared to control.

GST and CAT were significantly decreased in the untreated group as compared to the control group. As well, GST and CAT significantly decreased in treated rats when compared to the control group but the values remained higher than that of untreated group.

DISCUSSION: The present study evaluates the antidepressant-like effect of *S. coronopifolius* aqueous extract by the virtue of its antioxidant potential. Depression was induced by sciatic nerve injury, which is a model of neuropathic pain. Neuropathic pain is present in most peripheral neuropathies and can be a causal or aggravating factor of psychiatric symptoms²². Accordingly, the results of the present study demonstrated a significant increase in the depression level in ligated female rats.

The forced swimming test (FST) is one of the most widely used tests across laboratories for assessing symptoms of depression²³. The FST is used both for screening of antidepressant drugs^{24, 25, 26} and for analysis of the neurobiological bases of depression²⁷. The results of the present study showed that sciatic nerve injury caused a significant increase in the depression, revealed by an increase in immobility time in the FST. The immobility behavior is considered as an index of 'behavioral despair' and constitutes a convenient animal model for the depression in human subjects²⁸. Interestingly, rats treated with *S. coronopifolius* extract exhibited a significant anti-depressive activity revealed by a decrease in immobility time accompanied by an increase in the climbing time. This behavioral pattern is a typical effect of selective noradrenergic reuptake inhibitors in rat-FST after chronic treatment. Indeed, it has been established that drugs affecting noradrenergic neurotransmission like imipramine decreased immobility and increase climbing²⁹.

The OFT is used in combination with the FST to discard non-specifications of antidepressant treatments^{30, 31, 32}. It was found that the ligated rats displayed no reduction in horizontal or vertical movements, which confirms the normal locomotion activity. Meanwhile, the effect of *S. coronopifolius* extract did not alter significantly locomotor activity indicating that the specifications of this extract on the behavioral model are predictive of anti-

depressant activity. Our results are in accordance with previous findings^{33, 34}. It is worth noting that the main difference between antidepressants and psychostimulants is that antidepressants do not increase general motor activity³⁵. Our results showed that *S. coronopifolius* administration increased rearing, which is driven by the instinct interests of rats to explore a novel environment as demonstrated by Zhou *et al.*³⁶

In the case of chronic stress, morphological changes may occur and body weight may be a useful indicator of the animal's response capabilities³⁷. Accordingly, the obtained results showed that depression increased body mass in female rats. A number of studies on animals under stress conditions have pointed out that food intake could be either stimulated or inhibited^{38, 39, 40}.

The brain is one of the most vulnerable organs to oxidative stress injury since is a major metabolizer of oxygen⁴. The present study revealed an important decrease in GSH as well as antioxidant enzyme systems including GST and CAT in brain tissue of depressive female rats. A decline in antioxidant enzymes activities associated with depression has been well documented^{41, 42, 43}. On the other hand, the low increase of CAT and GST activities after treatment with algae extract observed in this study may be a consequence of shorter observational period or maybe that the antioxidant activity of the studied extract acts via another system due to other enzymatic pathways. The mechanism of action of antioxidants on the central nervous system is not well-elucidated⁴¹.

Besides the fact that the algae extract did not provoke a statistically significant modification in antioxidant enzymes after two weeks of treatment, it promotes anti-depressive effect. In the same context, de Menezes da Silveira *et al.*,³³ indicated that ethanolic extract of Brazilian yellow propolis did not alter the inhibition of SOD and CAT activities induced by behavioral stress despite its anti-depressive effect. The classic hypothesis was formulated around the idea that the antidepressants restore noradrenergic and serotonergic neurotransmitter systems to normal levels as a primary or direct effect, while the antioxidant efforts are becoming a noted secondary effect⁴¹.

The antioxidant properties of *S. coronopifolius* extract may be related to their chemical composition, such as the content of phenolic compounds or other substances isolated like bromoditerpenes and sulfated polysaccharides^{11, 12}. Some studies have shown that terpene compounds are responsible for a strong antioxidant activity^{44, 45, 46}. Furthermore, de Menezes da Silveira *et al.*,³³ found that a sample of Brazilian yellow propolis, which is rich in triterpenes showed antidepressant-like activities.

CONCLUSION: In summary, our data seem to highlight the conclusion that the aqueous extract of *S. coronopifolius* has significant protective effect against depression, which may be due to antioxidant property of the extract. Considering that depression is associated with oxidative stress, the reported data suggest that the utilization of this extract may contribute to the protection of brain tissue, *via* the amelioration of CAT and GST activities. Advanced investigations of all oxidative parameters are required in order to understand the mechanism of action of antioxidant enzymes on the central nervous system. Furthermore, isolation and identification of major bioactive substances found in algae extract are needed to evaluate the contribution of each of them to the antidepressant-like activity.

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