

# ANTIOXIDANT AND FERTILITY POTENTIALS OF A MARINE SULFATED POLYSACCHARIDE POTENTIEL ANTIOXYDANT ET DE FERTILITE D'UN POLYSACCHARIDE SULFATE MARIN

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## Abstract

The present study is the first to investigate and evaluate the health benefits of sulfated polysaccharide isolated from *Cymodocea nodosa* (CNSP) seagrass. The results revealed that CNSP had high activity in total antioxidant assay (59.03 mg ascorbic acid equivalents/g extract), with reducing power (OD= 0.3), a DPPH radical scavenging activity (IC<sub>50</sub>= 1.22 mg/ml) and an ABTS radical scavenging activity (IC<sub>50</sub>=1.14 mg/ml). The CNSP was able also to protect the testicular tissue of rats against lambda-cyhalothrin (LTC) induced damage, contributing to its effectiveness involve the quenching of free radicals, increasing the antioxidants status against testicular toxicity. Overall, the results presented in this study demonstrate that CNSP has several attractive antioxidant properties with the potential benefits towards fertility and spermatoc parameters.

**Key words :** Antioxydant ; Fertility ; *Cymodocea nodosa*

## Résumé

La présente étude est la première à étudier et à évaluer les effets bénéfiques pour la santé du polysaccharide sulfaté isolé à partir de la plante marine *Cymodocea nodosa* (CNSP). Les résultats ont révélé que le CNSP avait une activité antioxydante totale intéressante (59.03 mg d'équivalents d'acide ascorbique / extrait de g), avec un pouvoir réducteur (OD = 0,3), une activité de piégeage du radical DPPH (IC<sub>50</sub> = 1,22 mg / ml) et du radical ABTS (IC<sub>50</sub> = 1,14 Mg / ml) intéressantes. Le CNSP a également réussi à protéger le tissu testiculaire des rats contre les dommages induits par lambda-cyhalothrine (LTC) des rats, ce qui contribue à son efficacité implique la trempe des radicaux libres, augmentant le statut des antioxydants contre la toxicité testiculaire. Dans l'ensemble, les résultats présentés dans cette étude démontrent que le CNSP possède plusieurs propriétés antioxydantes attrayantes avec les avantages potentiels pour la fertilité et les paramètres du spermatoc.

**Mots clés :** Antioxydant ; Fertilité ; *Cymodocea nodosa*

## ملخص

هذه الدراسة هي الأولى من نوعها لدراسة وتقييم الفوائد من السكريات الكبريتية من نوع الأعشاب البحرية سيمودوسا نودوسا (س ن س ب). أظهرت النتائج أن لدى هذه الأعشاب نسبة نشاط عالية في مقياس الأكسدة الكلية (59.03 ملغم مكافئ الاسكوربيك حامض / غ)، وخفض القدرة (أود = 0.3)، و د ب ب ه الجذري (IC<sub>50</sub> = 1.22 ملغم / مل) و أ ب ت س جذري الكسح (IC<sub>50</sub> = 1.14 ملغم / مل). وقد تمكنت س ن س ب من حماية الأنسجة الخصية للفئران ضد الأضرار الناجمة عن لاميدا-سيهالوثرين (ل ت س)، والمساهمة في فعاليتها تنطوي على تبريد الجذور الحرة، وزيادة حالة مضادات الأكسدة ضد تسمم الخصية. وعموما، فإن النتائج المعروضة في هذه الدراسة تبين أن س ن س ب لديها العديد من خصائص مضادات الأكسدة مساعدة على احتمال الفوائد في الخصوبة وفي مؤشرات حيوية الحيوانات المنوية.

**الكلمات المفاتيح :** مضادات الأكسدة ; خصوبة ; سيمودوسا نودوسا

## INTRODUCTION

In recent years, there has been growing interest in the search for novel functional and bioactive compounds, particularly polysaccharides, from marine origins for application in the food and medical industries [1].

The search for novel nutritional antioxidants from marine plant sources has received increasing attention in recent research.

The protective effects of antioxidants derive from their abilities to (I) scavenge free radicals by acting as hydrogen/electron donors or by directly reacting with them; (II) chelate transition-metal ions; (III) inhibit free radical-producing enzymes, such as cyclooxygenase, lipoxygenase and NADPH oxidases or increase the expression of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) [2, 3].

In traditional practice, marine plants are used in many countries to control a lot of diseases. Today, polysaccharide fractions or molecules isolated are largely used to treat or relieve different aspects of male infertility such as: absence of libido, sexual asthenia, erectile dysfunction, ejaculatory and relaxation dysfunctions, loss of orgasm, and sperm abnormalities [4].

Considering the promising opportunities that seagrasses species might open for the development of efficient, safe and cost-effective bioactive compounds, the present study aimed to investigate and evaluate, for the first time, the biological and nutritional properties of sulfated polysaccharide from the leaves of seagrasses species grown in the coast of Chebba, Tunisia. Although this marine species is widely distributed in the Mediterranean region, little work has been performed to explore its promising potential as a source of natural bioactive compounds for use as food additives and preservative agents in the nutraceutical and functional food industries. Accordingly, this study aimed to investigate the antioxidant properties and benefit effect against reproduction of CNSP.

## MATERIALS AND METHODS

### 1. Plant material and extraction of sulfated polysaccharide

*Cymodocea nodosa* (CN) marine plant was collected from coast of Chebba. It was identified at the National Institute of Science and Technology of the Sea (INSTM), Sfax-Tunisia and the Stazione Zoologica 'A. Dohrn', Functional and Evolutionary

Ecology Laboratory, Punta S. Pietro, Ischia, Italy. The epiphytes on the leaves were removed with paper towel without damaging the organs.

The sulfated polysaccharide was isolated from dried Leaves according to our previous study described by Ben Abdallah Kolsi et al [5]. Briefly 300 g of sample was depigmented with acetone, ethanol followed by hot water extraction at 90–95°C for 3–4 h 3 times. The brown colored syrup was then filtered through a Whatman No 3 filter paper concentrated to 1/4 of the original volume, cooled and precipitated with three volumes of ethanol overnight at 4°C. The precipitate was collected by centrifugation and washed 3 times with 75% ethanol, dehydrated and lyophilized to get a dried brown crude sulfated polysaccharide (CNSP).

### 2. Determination of *in vitro* antioxidant activities

#### 2.1. DPPH radical scavenging activity

The DPPH free radical-scavenging activity of CNSP was evaluated according to Hsu. [6], with minor modifications. Various extract concentrations of 0.125, 0.25, 0.5, 1, 2 µg/ml were prepared in methanol. A 50 µl aliquot was mixed with 200 µl of 0.1 M DPPH dissolved in methanol and 100 µl of Tris-HCl buffer (50 mM, pH 7.4) at room temperature for 30 min. The decrease in DPPH radical was measured by recording the absorbance at 517 nm with L-ascorbic acid as positive control. Assays were performed in triplicate. The inhibition ratio was calculated from the following equation:

$$\text{Scavenging activity (\%)} = ((A_0 - A_1)/A_0) \times 100$$

Where, A<sub>0</sub> is the absorbance of the blank and A<sub>1</sub>, the absorbance of the extract or of L-ascorbic acid.

#### 2.2. ABTS radical-scavenging activity

ABTS was dissolved in water to make a concentration of 7 mM. ABTS was produced by reacting the ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. For the test of samples, the ABTS stock solution was diluted with phosphate-buffered saline 5 mM (pH 7.4) to an absorbance of 0.70 at 734 nm. After the addition of 1.0 ml of diluted ABTS to 20 µl of sample, the absorbance reading was taken 5 min after the initial mixing [7]. Data for each assay was recorded in

triplicate. Ascorbic acid was used as positive control.

This activity is given as percent ABTS scavenging that is calculated as

$$\% \text{ABTS scavenging activity} = ((A_0 - A_1)/A_0) \times 100$$

Where,  $A_0$  is the absorbance of the blank and  $A_1$ , the absorbance of the extract or of L-ascorbic acid.

### 2.3. Determination of total antioxidant activity

Total antioxidant activity of the CNSP was determined according to the method of Kumaran and Karunakaran [8]. In brief, 100  $\mu\text{g}$  of extract and 100  $\mu\text{g}$  of ascorbic acid (as standard) were taken in 0.1 ml of alcohol, combined separately in an eppendroff tube with 1.9 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a thermal block at 95°C for 90 min. After the samples were cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against a blank. A typical blank solution contained 1.9 ml of reagent solution and the appropriate volume of the same solvent used for the sample and it was incubated under the same conditions as the rest of the samples. For samples of unknown composition, water-soluble antioxidant capacities are expressed as equivalents of ascorbic acid. Ascorbic acid equivalents were calculated using standard graph of ascorbic acid. The experiment was conducted in triplicate and values are expressed as ascorbic acid equivalents in mg per ml of extract.

### 2.4. Measurement of reducing power

Reducing power of the extract was determined by the method of Oyaizu [9], 50  $\mu\text{l}$  of various concentrations of the sample were mixed with 50  $\mu\text{l}$  of phosphate buffer (0.2 M, pH 6.6) and 50  $\mu\text{l}$  of 1% potassium ferricyanide was added. After cooling, the reaction was stopped by the addition of 50  $\mu\text{l}$  of 10% (w/v) trichloroacetic acid (TCA) followed by centrifugation at 650 x g for 10 min. From the upper layer, 200  $\mu\text{l}$  were mixed with 200  $\mu\text{l}$  bidistilled water and 40  $\mu\text{l}$  of 0.1% (w/v)  $\text{FeCl}_3$  and incubated for 10 min at room temperature and absorbance measured at 700 nm. The reducing power of ascorbic acid was used as positive control. Increased absorbance indicated stronger reducing power. Assays were performed in triplicate.

## 2.5. *In vivo* fertility potential

### 2.5.1. Experimental animals

Adult male Wistar rats (weighing 160–180 g) were obtained from the Central Pharmacy, Tunis, Tunisia. They were maintained under standard laboratory conditions (temperature 21  $\pm$  5°C; 12 h light–dark cycle). The experimental protocol was approved by the local animal care committee, and all the experimental procedures were carried out in accordance with international guidelines for care and use of laboratory animals.

### 2.5.2. Experimental design

After 1 week of acclimatization, male rats were divided into four groups of eight animals each:

Group I (Cont): The control group, received ad libitum, water and 1 ml of vehicle solution given by intraperitoneal (i.p.) way.

Group II (LTC): The treated group with lambda-cyhalothrin, received through drinking water 668 ppm (61.2 mg/kg b.wt) of LTC.

Group III (CNSP): The treated group with CNSP, was given daily a single i.p dose of 200mg/kg b.wt/day.

Group IV (LTC+ CNSP): The treated group with LTC and CNSP were given a single i.p. injection of extract (200 mg/kg b.wt/day), 12h after the onset of LTC administration.

All animals were treated during 28 days (including the control group). At the end of the experimental period, the animals of different groups were sacrificed by cervical dislocation to avoid stress conditions. The caudae epididymis was removed for sperm analysis (counts, motility, viability and morphology). The right testis was utilized for the biochemical analysis and the left testis was used for histopathological examination.

### 2.5.3. Analysis of epididymal sperm characteristics

#### 2.5.3.1. Sperm motility and count

The cell suspension was obtained from each epididymis tail cut into a small piece and was placed in 0.05 M of Phosphate Buffer Saline medium (PBS, pH 7.4). The percentage of motile spermatozoa was determined by the progressive movements (slow and fast movements of spermatozoa) and non-progressive movements (any kind of flagellar or head movement). The sperm

concentration was determined under a Neubauer Haemocytometer.

### 2.5.3.2 Sperm viability test

The percentage of live spermatozoa was assessed using the Eosin/Nigrosin stain. 1 v of sperm is mixed with 1 v of eosin (1%) and 1 v of nigrosin (10%). Once the smear is performed, the spermatozoa are stained with an optical microscope using  $\times 100$  objectives and oil immersion. Live spermatozoa represent intact heads.

### 2.5.3.3. Sperm morphology

The morphological abnormalities were evaluated as follows: the sperm suspension was stained with eosin. Smears were performed on slides observed under a microscope using the  $\times 100$  objective and immersion in the oil. The abnormal sperm morphology was counted and the percentage was calculated.

## 2.5.4. Biochemical assays

The cut of right testes were emerged into 1 ml ice cold Tris-Buffer Saline (TBS, Tris-HCl 20 mM, NaCl 150 mM, pH 7.4), centrifuged (5000 g, 30 min, 4°C) and the supernatants were stored at  $-80^{\circ}\text{C}$  until use.

### 2.5.4.1. Protein determination

Protein concentrations were evaluated according to Lowry et al [10] with bovine serum albumin as standard.

### 2.5.4.2. Lipid peroxidation

Lipid peroxidation was evaluated according to Yagi [11]. Briefly, 2 v of TBA reagent (15% v / v trichloroacetic acid and 0.25 N HCl) were mixed with 1 V of suspension and left for 15 minutes in a boiling water bath. After centrifugation at  $1000 \times g$  for 10 minutes, the supernatant was removed, and the absorbance of the samples was measured at 532 nm. The concentration of MDA was expressed in nmol of MDA / mg of protein.

### 2.5.4.3. Catalase activity (CAT)

The activity of catalase was measured as described previously by Aebi [12]. This technique is based on the dismutation of  $\text{H}_2\text{O}_2$  manifested by decreases in absorbance at 240 nm. A unit is defined as the amount of  $\text{H}_2\text{O}_2$  converted to  $\text{H}_2\text{O}$  and  $\frac{1}{2} \text{O}_2$  in 1

min and the specific activity is reported in units per milligram of protein.

### 2.5.4.4. Superoxide dismutase activity (SOD)

The total SOD activity was measured according to Marklund and Marklund [13]. One unit was defined as the amount of enzyme that inhibited oxidation of pyrogallol by 50%. The enzymatic activity was expressed in units per milligram of protein.

### 2.5.4.5. Glutathione peroxidase activity (GPx)

GPx was determined by the method of Mohandas et al [14]. Briefly, a mixture containing 1.59 ml of phosphate buffer (100 mM, pH 7.6), 100  $\mu\text{l}$  of EDTA (10 mM), 100  $\mu\text{l}$  of sodium azide, 50  $\mu\text{l}$  of glutathione reductase, 100  $\mu\text{l}$  of  $\text{H}_2\text{O}_2$  and 1 ml of enzyme source was prepared. The disappearance of NADPH was followed every 10 seconds for 3 min at 340 nm, against blanks containing all the components except for the enzyme source, the enzymatic activity was expressed as oxidized NADPH nanomoles / min / mg protein.

## 2.5.5. Histopathological examination of the testes

Left testis biopsies were removed and fixed in 10% formalin solution for 12 h. Dehydration in series of alcohols (methyl, ethyl and absolute) was used. The fragments were incorporated in paraffin. The paraffin blocks were sectioned at 5-micron thickness by slide microtome. The tissue sections obtained were collected on the glass slides and stained by hematoxylin / eosin staining for histopathological examination under light microscopy [15].

## 2.6. Statistical analysis

Values were expressed as mean  $\pm$  SEM. Analysis of variance (ANOVA) and the posthoc test were used to assess biological activities data, with  $P < 0.05$  accepted as statistically significant. Differences in the assay and between treatment and control were compared using an unpaired Student's t-test.

## RESULTS

The therapeutic benefits of natural extracts have often been attributed to their antioxidant properties. The results from the *in vitro* antioxidant scavenging activity analysis of CNSP are shown in Figure 1. However, the DPPH, ABTS, reducing power and total antioxidant assays showed that CNSP

exhibited an important antioxidant potential was concentration dependent, with IC<sub>50</sub> values closely similar to those of ascorbic acid (IC<sub>50</sub> = 0.6mg/ml, 0.5mg/ml, 0.6mg/ml and 0.5 mg/ml), respectively. Concerning the *In vivo* fertility study Treatment of male rats with LTC caused a significant decrease in sperm concentration, motility and viability (%), while abnormal sperm increased as compared to control. Treatment with CNSP alone or in combination with LTC caused a significant (P < 0:05) increase in semen quality, and minimized the toxic effects of LTC (Table I). The results presented herein clearly demonstrate that ingestion of LTC induced adverse effects on male fertility and reproduction.

The lipid peroxidation concentration was significantly increased in the testes of rats after administration of LTC (p < 0.05), while CNSP alone and LTC+CNSP cotreatment reversed this change to control values.

No significant change in TBARS level was found in rats treated with CNSP only in comparison with control group (Figure 2 A)

Results showed that treatment with LTC caused a significant decrease (p < 0.05) in the concentration of SOD, CAT and GPx levels in the testes.

No significant changes of testis antioxidants enzymes (SOD, CAT and GPX) concentrations in the rats treated with CNSP and LTC+CNSP in comparison to the control animals were observed (Figure 2 B, C, D).The protective effect of CNSP against testicular toxicity induced by LTC in male rats was studied, and it was confirmed by histopathological examination (Figure 3).

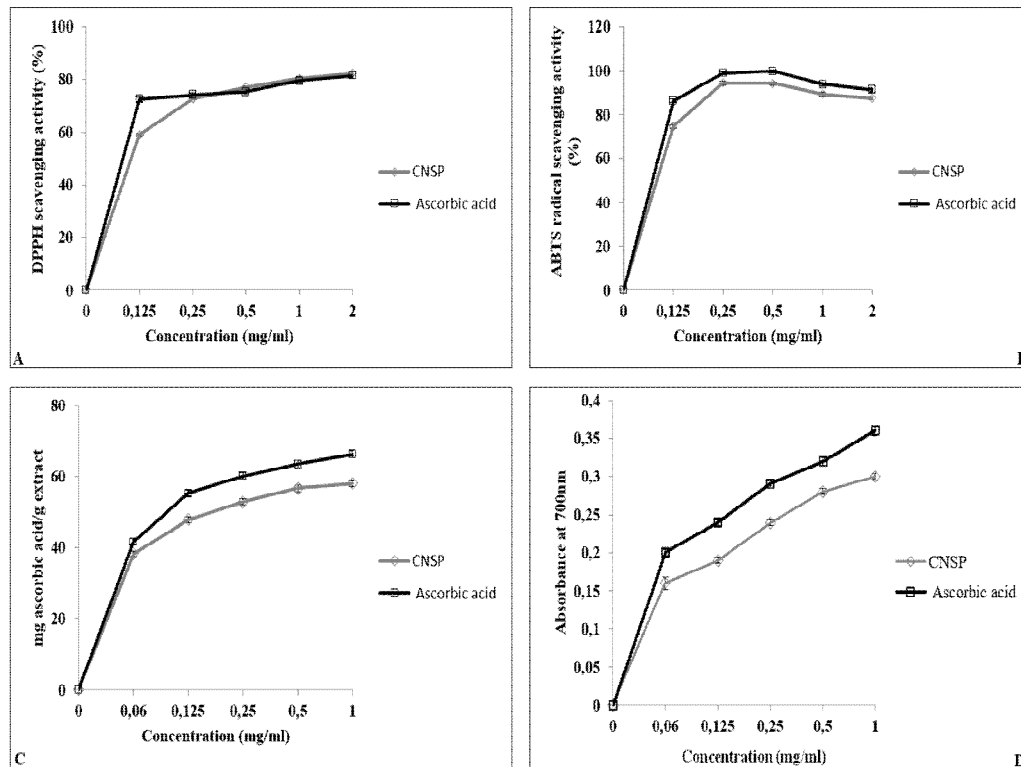
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**Table I:** Epididymal sperm characteristics of male rats as affected by treatment with LTC, CNSP and their combinations

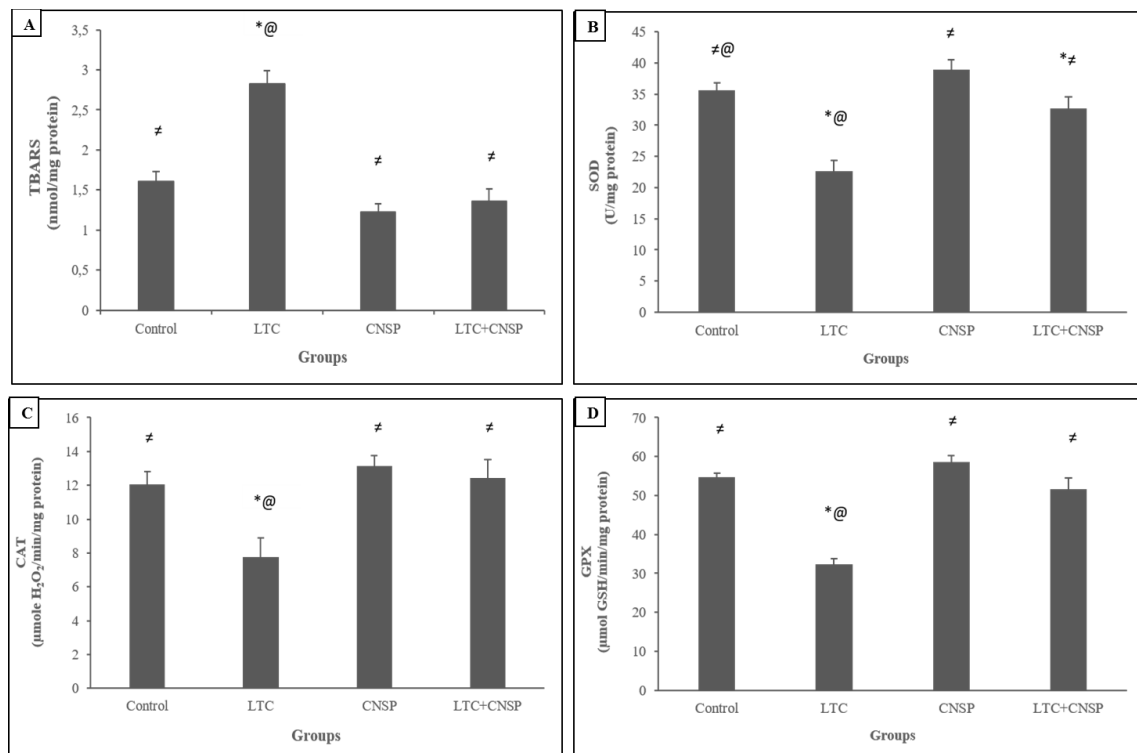
	Control	LTC	CNSP	LTC+CNSP
<b>Epididymal sperm characteristics</b>				
<b>Spermatozoa counts (10<sup>6</sup> /ml)</b>	152.67±27.03 <sup>‡@+</sup>	69.32±10.12 <sup>*@+</sup>	97.5±9.76 <sup>‡+</sup>	115.7 ±5.7 <sup>*‡@</sup>
<b>Motility (%)</b>	54.05±2.42 <sup>‡+</sup>	33.5±4.34 <sup>*@+</sup>	56.7±5.76 <sup>‡+</sup>	43.6±3.65 <sup>*‡@</sup>
<b>Viability (%)</b>	62.87±3.12 <sup>‡@+</sup>	42.5±1.67 <sup>*@+</sup>	65.6±2.5 <sup>*‡+</sup>	59.76±2.65 <sup>*‡@</sup>
<b>Abnormal morphology (%)</b>	22.12±2.12 <sup>‡@</sup>	56.23±3.43 <sup>*@+</sup>	28.3 ±1.65 <sup>*‡+</sup>	22.56±1.8 <sup>‡@</sup>

Data represent mean ± SD (n=8 for each group). Values differ significantly at P < 0.05. <sup>\*</sup>P < 0.05 compared with normal control rats ; <sup>‡</sup>P < 0.05, compared with LTC group and <sup>@</sup>P < 0.05 compared with CNSP treated group, <sup>+</sup>P < 0.05 compared with LTC+CNSP treated group.

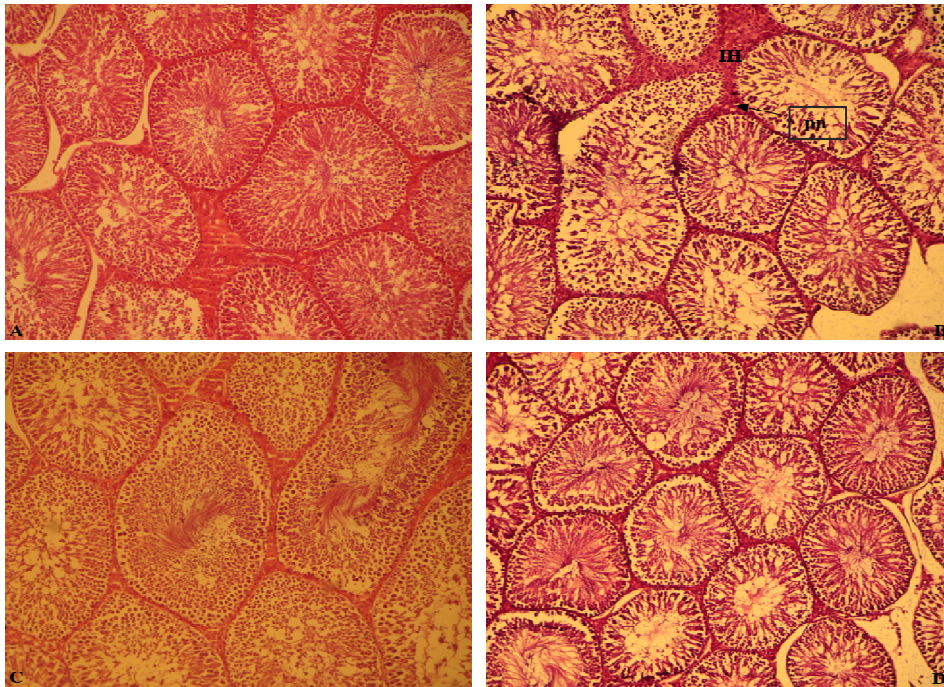
## ANTIOXIDANT AND FERTILITY POTENTIALS OF A MARINE SULFATED POLYSACCHARIDE



**Figure 1:** The *in vitro* antioxidant activities of CNSP at different concentrations. DPPH free radical-scavenging activity (A), ABTS radical-scavenging activity (B), Total antioxidant activity (C) and reducing power (D). Values are means of three replications  $\pm$  SD.



**Figure 2:** Activities of TBARS, SOD, CAT and GPX in testis of controls normal group, LTC treated group, CNSP treated group and LTC+ CNSP treated group. Data represent mean  $\pm$  SD (n=8 for each group). Values differ significantly at  $P < 0.05$ . \* $P < 0.05$  compared with normal control rats ; # $P < 0.05$ , compared with LTC group and @ $P < 0.05$  compared with CNSP treated group.



**Figure 3:** Photomicrographs of H&E stained sections of rat testis. (A) Control; showing normal testicular histology with evidence of spermatogenesis; (B) LTC treated group; (IH): intertubular haemorrhage; (Pn) pyknotic nucleus, (C) CNSP treated group and (D) LTC+ CNSP treated group.

## DISCUSSION

Polysaccharides, especially those extracted from marine origins, have attracted special interest in recent research. They have been demonstrated to play an important role as free radical scavengers and antioxidants for the prevention of oxidative damage in living organisms [16].

The results of the present study are in agreement with the findings previously reported on other marine polysaccharides exhibiting significant antioxidant properties [17] and showing high scavenging activity through the protective action of antioxidant molecules against various free radicals including singlet oxygen [18].

The scavenging effect increased with concentrations, the results presented above are in agreement with those described for other several marine alginate derivatives, sulfated fucoidans from the brown seaweed *Laminaria japonica*, agar-sulfated galactans from the red seaweed *Nori*, and sulfated polysaccharides from *Fucus vesiculosus*, which have been reported to exhibit significant antioxidant activities [19, 8].

Reducing properties are generally associated with the presence of reductones, which have been shown to exert antioxidant effect by breaking the free radical chain through donating a hydrogen atom. Reductones have also been reported to respond to

various precursors of peroxides, thus preventing their generation [20]. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant capacity. This antioxidant activity could be explained by the chemical composition and the high content of sulfate. This result is in agreement with previous studies in the literature [21, 22] reporting that the antioxidant activities of sulfated polysaccharides is related to the amount and distribution of the sulfate and hydroxyl groups in their structures with labile hydrogen atoms able to stabilize free radicals. Moreover, the sulfated polysaccharides molecular weight may affect the antioxidant activity proved by Liu et al. [22] mentions showing that the polysaccharide extracted from *Enteromorpha prolifera* with the lowest molecular weight has the largest antioxidant activity. However, that the chemical composition and structures of polysaccharides are important factors governing the efficacy of antioxidants from natural sources, such as marine flora and fauna.

Our study showed that rats treated with LTC significantly altered sperm quality by decreasing the number, mobility and viability of spermatozoa and by increasing the abnormal morphology of cells. The reduction in sperm quality may be due to

an adverse effect of LTC on spermatogenesis. These results are in agreement with the findings by Yousef MI [23] who mentioned that semen quality was deteriorated following treatment with LTC.

Some polysaccharides isolated from natural sources show various important biological activities which are strongly affected by their chemical structures. Results indicate that the exposure of male rat to CNSP alone or LTC followed by CNSP can be reducing the deleterious effects of LTC on functional spermatozoa parameters. We suggest that the beneficial effect of CNSP is mostly due to its antioxidant properties and that it could antagonize the toxic effects of LTC and improve semen quality of male rat.

Recently, oral administration of Peach gum polysaccharides can effectively protect the spermatogenesis of KKAY mice with impaired reproduction system [24]. Furthermore, *Lycium barbarum* polysaccharides (LBPs) have obviously protective effect on the spermatogenesis of rats with impaired reproduction system induced by cyclophosphamide. Indeed, it has improved sperm density, sperm movement and the rate of normal sperm morphology [24].

During pyrethroid metabolism, reactive oxygen species (ROS) are generated and result in oxidative stress. Our results showed that exposure to LTC increase significantly the TBARS levels and decrease significantly the antioxidant defenses such as CAT, SOD and GPx levels. Previous studies have shown that LTC induced oxidative damage of testes in male rat [25]. We suggest that oxidative damage induced by LTC may be due to their lipophilicity, wherein they could penetrate the cell membrane easily.

This damage could be translated by membrane lipid peroxidation and antioxidant defense defect. Studies concerning the effect of bioactive compounds from marine origins on the reproductive organs and fertility of male rats are limited. The natural bioactive compounds such as sulfated polysaccharide from *C. nodosa* may be helpful in preventing or reducing the harmful effects of ROS on testes and semen quality. In fact, we found that after exposure of male rat to CNSP alone or LTC followed by CNSP, the TBARS, SOD, CAT and GPx levels did not show any significant differences compared with control, this could be due to antioxidant properties characteristic of this sulfated polysaccharide from marine origin. The alteration of sperm function might reflect a direct effect of LTC on spermatogenesis processes. Indeed, testes of rats administered with LTC

showed many histopathological changes compared with control testes. These changes included degeneration of spermatogenic cells in seminiferous tubules, intertubular haemorrhage, and pyknotic nucleus (Figure 3B). This may be related to decreases in spermatid functional parameters such as mobility, vitality and normal morphology of spermatozoa. Observations made by earlier workers [45, 46] also state that LTC change in the histoarchitecture of testis and damage to seminiferous tubules in male rats. The protective effect of CNSP against testicular toxicity induced by LTC in male rats was studied.

Histopathological examination of the LTC- CNSP treated testes revealed that they caused attenuation of testicular degenerative lesions. We found that normal histological structure of most seminiferous tubules (Figure 3D). The CNSP possesses antioxidant properties which may be related to its cytoprotective action in testis rats.

## CONCLUSION

The present study is the first to investigate that *Cymodocea nodosa* exhibited attractive properties and promising therapeutic effects on oxidative stress and reproductive problem which make it a promising candidate for future application in various nutraceutical and functional food industries as well as in alternative medicine and natural therapies.

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