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The Protective Effect of Sea Lettuce (*Ulva Lactuca*) Extract, on Fertility in Adult Male Rats Exposed to Polyvinyl Chloride

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ABSTRACT:

Polyvinyl chloride (PVC), a polymerized form of vinyl chloride that is extensively used in the plastics industry, has toxic effects through the induction of oxidative stress in the body and testicles. This study evaluated the effect of Sea Lettuce ethanolic extract, vitamin C, and zinc against testicular toxicity induced by PVC in male rats. Thirty male albino rats were divided into five groups of six rats. Group I (negative control group): received distilled water only. Group II (positive control group: were given PVC (1000 mg/kg b.w.). Group III, rats were given 100mg/kg Sea Lettuce extract. Group IV: were given 100 mg/kg of Vit C +and5 mg/kg of zinc. Group V: were given 50 mg/kg of Sea Lettuce extract + 50 mg/kg of Vit C + 2.5mg/kg of zinc. The doses were administered orally daily for 40 consecutive days. At the end of the experimental period, rats were sacrificed, and the weights of rats, feed intake, feed efficiency ratio, and testes weight percentage were recorded. Sperm characteristics count, serum sex hormone levels (testosterone, follicle-stimulating hormone (FSH), luteinizing hormone, (Lh)and oxidative enzyme including (catalase, superoxide dismutase, glutathione peroxidase, and glutathione) were estimated. Malondialdehyde was assessed in testicular tissue. The tumor necrosis factor **α**, interleukin-1 **β**, and interleukin-6 concentrations were analyzed. A histopathological examination was done on the testes. Results showed that treatments improved sperm parameters, antioxidant enzyme activity, and sex hormone levels. Therefore, the administration of (Sea lettuce extract, vit. C and zinc) is beneficial for improving male sexual performance.

Keywords: *Fertility, Polyvinyl chloride, Oxidative enzyme*

1- INTRODUCTION

Global concern about male infertility is growing, affecting approximately 50% of infertile couples[1]. Medical reports for the past 50 years have noted an increased incidence of male reproductive health issues, including a decline in sperm counts and an increased incidence of cryptorchidism, hypospadias, and other urogenital malformations in humans and wildlife, and these reproductive anomalies were linked to exposure to endocrine disruptors [2].

Polyvinyl chloride (PVC) is used as a raw material in the manufacturing chemical and plastic industries, including disposable containers and plastic tubes, cables and wires, floor coverings, film photography, automotive electronics, and toys [3]. Due to the increasing production of the products mentioned above, PVC production will also increase, resulting in more PVC exposure. Since PVC is not chemically bonded to the polymer, it is removed at the time of production and use of the polymer. Therefore, it is transmitted to humans through air, water, food, and medical devices[4]. One of the most critical uses of PVC is in the manufacturing medical devices and laboratory equipment. Blood storage bags, injection, and hemodialysis devices, and chip tubes contain large quantities of PVC. Many materials used to make PVC industries are found in the blood and tissues of patients having frequent transfusions. However, food is the primary source of human exposure to PVC [5].

It has been shown that PVC exerts hepatotoxic, teratogenic, mutagenic, and carcinogenic effects and produces renal, pulmonary, and reproductive dysfunctions. The liver and testes appear to be the target organs of PVC toxicity. PVC and other plasticizers have been reported to cause testicular atrophy associated with a decreased zinc concentration in the testes. Also, PVC can reduce the serum level of testosterone. Other studies have also shown that PVC causes apoptosis in the testis, induces oxidative stress, and reduces the activity of the antioxidant enzyme. Since oxidative stress in the testicle is one of the leading causes of apoptosis in the germ cells [6].

Today, due to the increasing use of plastic products, an increase in the consumption of PVC and more exposure to this environmental pollutant have become a matter of great concern. As a result, several treatments have been proposed to reduce the oxidative stress levels induced by PVC in testicular tissue. Oxidative stress is a well-known causative factor involved in the etiology of male infertility [7], which is a cellular event that occurs at the molecular level due to the imbalance between free radicals and antioxidants, leading to oxidative damage of proteins, fats, nucleic acids, and carbohydrates [8]. Antioxidants protect the body against the harmful effects of free radicals, which contribute to inflammation reactions [9].

In addition, natural antioxidants counteract the reactive oxygen species by neutralizing them and restoring the optimal balance [10]. *Ulva lactuca* is a widespread macro alga commonly known as "sea lettuce" occurring at all levels of the intertidal zone. In some regions, it is consumed for nutrition. Its nutrients include iron, protein (15%), iodine, vitamins (A, B1, and C), and trace elements. It has a high content of primary and secondary metabolites, including polysaccharides, phenolic compounds,

chlorophylls, carotenoids, flavonoids, alkaloids, terpenes, and phytosterols. These active compounds play an essential role in the higher antioxidant and anti-inflammatory activities of Sea Lettuce [11,12]. *Ulva lactuca* possesses high antioxidant potency, the total content of phenolic compounds in extracts, reducing activity, superoxide anion, scavenging activity, and inhibition of lipid peroxidation [13]. Moreover, *Ulva lactuca* methanolic crude extract had a significant effect on male infertility due to its high antioxidant activity and the improvement of fertilization by activating sperm enzymes. Therefore, the green algal extract could be used as a treatment for idiopathic male infertility and, thus, in reproductive technology [14].

Ascorbic acid contributes to the support of spermatogenesis, at least in part through its capacity to maintain this antioxidant in an active state. Vitamin C is kept in a reduced form by a GSH-dependent dehydroascorbate reductase, which is abundant in the testes. Vitamin C has been shown to improve sperm motility and enhance semen quality and fertility in rats. In recent years, vitamin C supplements have been widely used in rats' diets, and the levels for improving production and reproductive performance have increased severalfold. Supplementation with Vitamin C has also been shown to increase total sperm output and sperm concentration [15].

Zinc, the second-most necessary trace element, acts as an effective antioxidant, anti-apoptotic, and anti-inflammatory agent, and zinc supplementation decreases oxidative stress and apoptotic biomarkers as well as inflammatory cytokines[16,17]. It has beneficial effects on the antioxidant status of the reproductive tissues and may protect the male reproductive system against apoptotic and oxidative damage [18]. It also plays a vital role in the initial stages of germ cell development, spermatogenesis, and fertilization [19,20]. Therefore, the current study investigated the potential prophylactic effect of Sea Lettuce, vitamin C, and zinc on infertility induced by PVC in male albino rats.

2. Materials and Methods

2.1. Plant material and animals

Specimens of green algae (*Ulva lactuca*) were collected from Abu-Quir Beach, Alexandria Coast, and identified in the Microbiology and Botany Department, Faculty of Science, Alexandria University, Egypt. Thirty male albino rats of (150±10) g were obtained from the animal colony Helwan Farm, Vaccine and Immunity Organization, Cairo, Egypt. Animals were clinically healthy. They were acclimatized to the experimental conditions for one week before the start of the experiment. During this period, the rats were housed in plastic cages with galvanized iron filter tops and placed in a quiet room with natural ventilation and a 12:12-hour light-dark cycle. According to Reeves et al., [21], the rats were fed on a basal diet and

water ad-libitum throughout the experimental period.

2.2. Chemicals, kits, and other required materials

Casein, vitamins, minerals, cellulose, choline chloride, DL-methionine, and other required chemicals were obtained from El-Gomhoreya Company for Trading Drugs,Chemicals,and Medical in strument, Cairo, Egypt. Kits used for biochemical determinations were obtained from Gama Trade Company for Chemicals, Cairo, Egypt. Corn starch and corn oil were obtained from the local market, Tanta City, Al-Gharbia Governorate, Egypt. PVC was purchased from Sigma-Aldrich, USA. Dietary Supplement Zinc tablets 50 mg and vitamin C 500 mg were purchased from NOVARTIS Pharmaceuticals (Cairo, Egypt).

2.3. Extract preparation

Ulva lactuca was washed several times with both filtered and tap water, and after the excess water was removal, it was dried in a ventilated drying oven at 50 °C for 48 hours. It was then milled on a commercial mill and vacuum-packed. The biomass was stored in our laboratory facilities under dry and dark conditions, avoiding direct contact with sunlight [12]. The obtained powder was soaked in 70% ethanol at room temperature for 48 hours. The hydroethanolic extract was filtered and concentrated under vacuum by a rotary evaporator. *U. lactuca* hydroethanolic extract was dissolved in 1% carboxymethylcellulose (CMC) at 2% concentration (100 mg/5 ml) and was daily administered 100 mg /kg b.wt by oral gavage [22].

2.4. Determination of phenolic compounds

The polyphenolic compounds of U. lactuca hydroethanolic extract were fractionated and identified for phenolic compounds and flavonoid compounds by HPLC, according to the method described by [23]. The phenolic acid standard was dissolved in a mobile phase and injected into HPLC. The retention time and peak area were used to calculate the phenolic compound concentration.

2.5. Study design

The rats were assigned randomly into five groups; each group had six rats. Group I, Control rats, received only distilled water. In Group II (PVC), rats were gavaged with 1000 mg/kg b.w. PVC dissolved in corn oil, according to [24]. In Group III, rats were given 100 mg/kg of *Ulva* extract [25]. Group IV rats were gavaged 100 mg/kg Vit C (vitamin C was dissolved in distilled water) [26] and 5 mg/kg zinc [27]. Group V rats were gavaged with 50 mg/kg *Ulva* extract, 50 mg/kg Vit C and 2.5 mg/kg zinc. The doses were given via gastric tube daily for 40 consecutive days.

2.6. Sacrifice and biochemical analysis

At the end of the experimental period, feed intake, body weight gain, relative organ weight, and feed efficiency ratio were calculated according to Chapman et

al., [28]. The rats were fasted overnight before being sacrificed. The blood samples were collected from each rat and centrifuged for 10 minutes at 3000 (r.p.m) to separate the serum. The serum was carefully separated and transferred into dry clean Eppendorf tubes and kept frozen at-20°C for analysis as described by Schermer [29]. The testes were removed from each rat by careful dissection, cleaned from the adhesive matter with a saline solution (0.9%), dried with filter paper, and weighed. The left testis was fixed in 10% formalin for histopathological examination, and the right testis was homogenized for antioxidant analysis.

2.7. Semen Analysis and Morphology

Semen was determined according to Bearden and Fuquay [30] and Van der Horst and Maree [31]. The sperm samples obtained from the cauda epididymis were spread as two slides for each rat. The samples were dried with an air drier and stained using the Diff-Quik staining method for morphological evaluations. The slides were then examined under the light microscope at 100 magnifications. A total of 200 sperm cells were examined for each slide, and the proportions of normal, head and tail abnormalities were determined, as given in Equation (1) according to Ulfanov et al. [32]:

Sperm morphology (%) Abnormal (head; tail) sperm count × 100/200

2.8. Preparation of testis homogenates and biochemical analysis

The testis was kept at -80°C for the preparation of tissue homogenate for the determination of antioxidant parameters. The homogenate was centrifuged at 10000 rpm for 20 min. The supernatant was used to assay malondialdehyde (MDA) as an indicator of lipid peroxidation according to Satoh [33] and the antioxidant enzymes catalase (CAT) [34] and superoxide dismutase (SOD) according to Paoletti and Mocali, [35] and glutathione peroxidase (GPx) according to Placer et al., [36], while the concentrations of reduced glutathione (GSH) were determined according to the methods described by Beutler et al., [37]. In addition, serum luteinizing hormone(LH) was carried out according to Loraine and Bell [38], and Folicle stimulating hormone (FSH)was carried out according to McCarm, [39]. The concentrations of TNF-**α**, IL-1-**β**, and IL-6 were also measured on incubated samples using ELISA kits and determined according to Bergmeyer et al., [40].

2.9. Histopathological examination

Samples taken from the testis of the studied rats in different groups were fixed in formalin (10%). Afterward, they were washed under tap water and introduced in a bath containing serial dilutions of graduated alcohol (methyl, ethyl, and absolute ethyl) used for dehydration. Samples were cleared in xylene and embedded in liquid paraffin at 56 °C. Next, sections of 4µm of thickness were cut, deparaffinized, and stained with

Hematoxylin /Eosin stains for histopathological examination under a light microscope [41].

2.10. Statistical Analysis

Statistical analysis was conducted using SPSS software (Version 20; Untitled ; SPSS Data Editor). The results were expressed as the mean ± standard deviation (mean ± SD). Data were analyzed using a one-way classification and analysis of variance (ANOVA) test. The differences between means were tested for significance using the Duncan test at $P<0.05$.

3. Results and discssion

HPLC identified the phenolic compounds of U. lactuca hydroethanolic extract against standard compounds, and the results are listed in Table 1. The most abundant components of U. lactuca were p-hydroxybenzoic acid, Catachin, Ferulic acid, and Quercetin (33.23, 22.72,2.03 and 2.27µg/ml).respectively These results agree with Zaatout et al.[42] who found that the most abundant components of U. lactuca were p-hydroxybenzoic acid. The extract had a high content of polyphenols with remarkable differences ranging from 0.19 to 0.38 mg gallic acid eq/mg, while the content of flavonoids ranged from 3.75 to 9.64 mg catechin eq/g. The hydroalcoholic extract with a polarity index of 5.8 displayed the best anti-oxidant, radical scavenging, and anti-inflammatory activities [63].

Table 1Phenolic compounds of U. lactuca hydroethanolic extract (µg/ml)

Samples / Compound	U. lactuca (µg/ml)
p-hydroxybenzoic acid	33.23
Catachin	22.72
Ferulic acid	2.03
Qurecetin	2.27

Data presented in Table 2 shows the effect of PVC, *Ulva* extract,(vitamin C + zinc), and (*Ulva* extract + vitamin C+ zinc) on weight gain, feed intake, feed efficiency ratio, and testis %. The results showed that there was a significant decrease in weight gain, feed intake, and feed efficiency ratio in the PVC(+ve) control group compared with the (-ve) control group. All treated groups show a significant increase in all the above parameters compared with the positive group ($P<0.05$). The treated group with *Ulva* extract + vitamin(C + zinc) is the best result compared with the negative group. The present results indicate that exposure to PVC at a dose level of 1000 mg/kg bw/day for 40 days caused changes in body weight %, feed intake, FER, and testis % of the treated animals, suggesting that the exposure had a systemic toxic effect. Also, the treated group improved the weight, feed intake, FER, and testis %. Also, our results agree with Archana et al., [44], who found that PVC treatment caused a marked decrease in the weights of the testis, epididymis, and other reproductive organs compared to controls. A decrease in testis and different reproductive organ weights in treated rats is not surprising because these organs require continuous

androgenic stimulation for their average growth and physiology [64-67]. U. lactuca has a tremendous potential antioxidant activity because it is considered a potentially rich source of natural colorants like chlorophyll a, chlorophyll b, 9-cis b-carotene, b-carotene, and trans-b-carotene [68]. In addition, ascorbic acid (C) is a known antioxidant present in the testis with the precise role of protecting the latter from oxidative damage. It also contributes to the support of spermatogenesis, at least in part through its capacity to maintain antioxidants in an active state [49,50]; had been demonstrated that zinc ion assists immune function, helps cells to grow and increase healthily and preserves prostate and sexual health [51] showed that co-treatment with Zn improved the reproductive organs' weights.

Table2. The protective effect of U. lactuca extract on FI(g/d), BWG%, FER, and Testis weight % levels in PVC-treated male rats

Parameters / Groups	BWG%	Feed intake (g/day)	FER	Testis %
Group I	79.85±3.41a	16.86±0.08a	0.18±0.006a	1.26±0.05a
Group II	16.48±1.64d	11.8±0.17e	0.05±0.005c	0.91±0.008d
Group III	56.59±3.23c	14.36±0.17d	0.16±0.011b	1.061±0.03c
Group IV	64.15±1.26bc	15.12±0.13c	0.17±0.012ab	1.14±0.01bc
Group V	67.64±5.42b	15.63±0.17b	0.16±0.004ab	1.182±0.03ab

*Data were expressed as mean ±SD. The different superscripts significantly differed within the same column. * Indicates significant p-value < 0.05.*

Data recorded in Table 3 illustrated that PVC caused a significant decrease in (FSH, LH, and testosterone) hormones in the PVC (+ve) control group compared with the (-ve) control group. The other treated groups showed a significant increase in (FSH, LH, and testosterone) hormones compared with the (+ve) control group. The best result in test parameters was the rats treated with (50mg/kg *Ulva* extract + 50 mg/kg Vit C + 2.5mg/kg zinc).In men, testosterone is responsible for the development of male reproductive tissues and the spermatogenesis process; therefore, any reduction occurring in its level will decrease sperm production [68]. Our results showed that exposure to PVC resulted in a marked increase in serum levels of FSH, LH, and testosterone in treated rats compared to controls. Thus, it is reasonable to assume that the PVC-induced suppression of spermatogenesis in rat testes in the present study is caused by a deficiency of testosterone. In the present study, adult rats exposed to PVC exhibited a significant decrease in fertility hormones, including serum T, FSH, and LH, in comparison to the control group. Our results indicated that the male reproductive system is adversely affected upon exposure to PVC. Similar findings were obtained by Huang et al., [52] and Megahed et al., [53]. Also, Sadeghi et al.,

[24] found that oral administration of PVC for 40 days in adult rats decreased T levels. Despite the reduced serum T level, LH and FSH levels also decreased, which means that the negative feedback of the pituitary-testicular hormonal axis was interrupted.

Additionally, the decrease of LH and FSH secretion may be explained by their inhibition by T metabolites. This finding shed light on neuronal degeneration caused by PVC besides its toxic effect on the testicular tissue [54,55]. The PVC and other plasticizers have been reported to cause testicular atrophy associated with a decreased zinc concentration in testes.

Also, PVC can reduce the serum level of testosterone [24]. These results are in agreement with those of earlier reports [56], which showed that di(2-ethylhexyl) phthalate(DEHP) induced high levels of serum luteinizing hormone (LH) and testosterone caused by Leydig cell hyperplasia. DEHP is a high-production-volume phthalate and is used as a plasticizer or softener in polyvinyl chloride (PVC) products. Smith et al., [57] reported that the serum testosterone, FSH, and LH levels were significantly reduced in the PVC -intoxicated rats. The reduction in testosterone level might be attributed to the decrease in serum LH. This is because LH regulates the secretion of testosterone by the testicular Leydig cells. Previous studies have shown that the use of antioxidants in the culture medium improves the fertility ratio and implantation [58].

Ascorbic acid (vitamin C) has been considered an essential nutrient for animal species; it has been linked with fertility for many years; most consider the effect of ascorbic acid on fertility to be related to these principal functions: role in hormone production, promotion of collagen synthesis, prevention or protection against oxidation, disrupting both spermatogenesis and the production of testosterone caused by a lack of vitamin C which leads to a state of oxidative stress in the testes [59]. In addition, the previous study showed that the level of testosterone in plasma increased significantly in animals treated with ascorbic acid When compared with animal controls. This rise could be dependent on the ascorbic acid that activates LH release. Okon and Utuk, [60] found that ascorbic acid treatment elevated FSH in albino rats in the high-dose ascorbic acid group. Also, our study agrees with Salem et al., [61], who showed that using ascorbic acid caused improvements in the level of (FSH and LH via capturing the free radicals released.

Table 3. The effect of U. lactuca extract on testis hormones (FSH, LH, and testosterone) in PVC-treated male rats

Parameters / Groups	FSH (ng/ml)	LH (ng/ml)	Testosterone (ng/ml)
Group I	112.89±4.31 a	20.05±0.24 a	25.03±0.72a

Group II	8.27±0.54 e	3.39±0.059 e	3.28±0.32 e
Group III	30.47±0.87 d	8.11±0.38 d	10.85±0.16 d
Group IV	51.90±0.68 c	11.29±0.20 c	15.96±0.26c
Group V	71.52±2.91 b	14.23±0.24 b	18.97±0.16 b

*Data were expressed as mean ±SD. The different superscripts significantly differed within the same column. * Indicates significant p-value < 0.05.*

The results in Table 4 indicated that there was a highly significant decrease in antioxidant enzymes (CAT, SOD, GSH, and GPx) in the testicular tissue of the control positive group compared with the negative control group. (50mg/kg *Ulva* extract + 50 mg/kg Vit C + 2.5mg/kg zinc) treated groups showed significant increases in antioxidant enzymes compared with the positive control group. Malondialdehyde showed a significant increase in the positive control group compared with the negative control group. There were significant decreases in other treated groups as compared to the positive group. In the present study, we have observed a significant decrease in the activities of catalase SOD, GSH, GPx and concomitantly increased levels of MDA in the testis of rats exposed to PVC. The reduction in the activities of SOD, catalase, GSH, and GPx may reflect an inability of the testis to eliminate superoxide and hydrogen peroxide radicals produced by PVC or its metabolites or enzyme inactivation caused by excess reactive oxygen species (ROS) generated in testis. It is well known that SOD protects catalase against inhibition by the superoxide anion. Reciprocally, the catalase protects SOD against inactivation by H2O2. Thus, the balance of this enzyme system is essential to eliminating superoxide anions and peroxides generated in the testis. The reduction in the activities of antioxidant enzymes and the increase in lipid peroxidation could reflect an adverse effect of PVC on the antioxidant system in the testis. Lipid peroxidation can lead to irreversible cell damage. It may also be possible that the elevated levels of ROS in the testes of PVC-exposed rats may render the mitochondrial steroidogenic machinery more susceptible to oxidative damage, leading to their functional inactivation [62]. The present study agrees with Archana et al., [44] .

The HPLC analysis of the extract of U. lactuca revealed the presence of phenolic and flavonoid active constituents in high content. These compounds give the extract powerful antioxidant capacity [70,71], where the algae extract showed in vivo antioxidant effect appear in increased seminal plasma and spermatozoa SOD and CAT levels which is associated with an elevation in non-enzymatic antioxidant level (GSH) and enzymatic antioxidants activities (GPx) when compared to the control group. Also, Badade et al., [72] reported that high levels of MDA in oligoasthenospermic patients, as compared to normospermic, are

negatively correlated with sperm count and sperm motility. This means that a decrease in oxidative stress will be associated with an increase in sperm count and motility. Our study is in agreement with Gulbahce-Mutlu et al., [68], who found that being treated with zinc causes a decrease in MDA levels. This is because zinc may be effective in preventing tissue damage, and it is a powerful antioxidant. Also, vitamin C restored the activity and the content of the antioxidants [49]. UL plays an essential role against oxidative stress due to the presence of flavonoids, polyphenolic compounds, and carotenoids, which are known as antioxidant and anti-inflammatory substan wces [42].

Table 4. The Protective effect of U. lactuca extract on CAT, SOD, GSH ,GPX and MDA in PVC -treated male rats

Parameters / Groups	CAT(U/mg)	SOD(U/mg)	GSH (mg/mg)	GPx (U/gT)	MDA (nmol/mg)
Group I	4.53±0.05 a	3.64±0.08 a	5.16±0.03 a	6.09±0.08 a	0.87 ±0.006 d
Group II	1.02±0.02 e	0.93±0.01 e	1.26±0.08 d	1.76±0.04 e	5.25±0.19 a
Group III	1.95±0.017 d	1.72±0.06 d	2.53±0.03 d	2.67±0.05 d	3.78 ±0.098 b
Group IV	2.67±0.07 c	2.32±0.11 c	3.35±0.05 c	3.60±0.26 c	2.67±0.20 c
Group V	3.58±0.017 b	3.06±0.09 b	4.00±0.07 b	4.46±0.18 b	2.42 ± 0.25 c

*Data were expressed as mean ±SD. The different superscripts significantly differed within the same column. * Indicates significant p-value < 0.05.*

Statistical analysis of Table 5 showed a highly significant increase in IL-1b, IL-6, and TNF-**α** in testicular tissue of the control positive group compared with the negative control group. (50mg/kg *Ulva* extract + 50 mg/kg Vit C + 2.5mg/kg zinc) treated groups showed significant decreases in IL-1b, IL-6 and TNF-**α** compared with the positive control group. There were significant decreases in the other treated groups as compared to the positive group. The innate immune system plays a critical role in providing the host with an initial defense against infection. Antigen-presenting cells (APCs), such as dendritic cells (DCs) and macrophages, can stimulate innate immunity and bridge innate and adaptive immunity through T-cell activation [43]. Pro-inflammatory cytokine production, such as tumor necrosis factor (TNF)-**α**, interleukin (IL)-6, and IL-12, promote interactions between T-cells and APCs to upregulate their surface molecules and have been used to investigate the biological and immunological effects of many new compounds, such as drugs and extracts [44]. The results from our study showed a statistically significant ($P<0.05$) increase in the serum levels of the pro-inflammatory cytokine in rats treated with PVC. Similar outcomes have been reported in the study of Schwarz et al. [45]. U. lactuca algae extracts contain vital components that treat various diseases, such as tumors, obesity, and infectious diseases. This could be due to the immunomodulatory and anti-

inflammatory activities of the algae extract. Moreover, it exhibits strong antiviral activity both in vitro and in vivo [46]. The main biologically active component in the algae extract is carotenoids, which are commensurate with the levels of flavonoids and phenolic compounds. It is known to enhance cognitive functions by promoting antioxidant actions within the brain, which inhibits the expression of genes encoding TNF-**α** synthesis. This may be due to the antioxidant action of the substance and inhibition of NF-**κ**B activation, which in turn inhibits TNF-**α** production [47]. In addition, this study agrees with Gulbahce-Mutlu et al., [68], who found that zinc supplementation inhibits increased IL-6 synthesis. Zinc can reduce the levels of the proinflammatory cytokine IL-1B [73]. Emojevwe et al. [74] concluded that zinc decreases the release of TNF**α**, and this agrees with our study. Also El Kotb et al., [75] showed that Serum TNF-**α** levels in the treated groups with vitamin C were significantly lower when compared to the sodium glutamate group. Vitamin C can reduce the plasma levels of the inflammatory mediators TNF-**α** and IL-6 via downregulation of hepatic mRNA expression [76]. (Ellulu et al., 2015).

Table (5): The protective effect of U. lactuca extract on IL-1b, IL-6, and TNF-α in PVC-treated male rats

Parameters / Groups	IL-1B (pg/mg)	IL6 (Pg/ml)	TNF**α**(pg/mg)
Group I	15.68±0.71 e	101.25±0.72 e	50.82±0.64 e
Group II	139.84±3.29 a	525.0±14.43 a	182.18±4.04 a
Group III	109.047±1.13 b	495.25 ±10.54 b	116.10±2.08 b
Group IV	81.13±1.42 c	377.5±4.33 c	98.89±0.95 c
Group V	61.46±2.01 d	242.5 ± 1.44 d	80.33±0.84 d

*Data were expressed as mean ±SD. The different superscripts significantly differed within the same column. * Indicates significant p-value < 0.05.*

The results in Table 6 indicated that there was a significant decrease in sperm count, sperm motility, and sperm viability in the group treated with PVC compared with the negative control group.(50mg/kg *Ulva* extract + 50 mg/kg Vit C + 2.5mg/kg zinc) groups showed a significant increase in sperm count, sperm motility, and sperm viability compared with the positive group. Sperm abnormality showed a significant increase in the group treated with PVC as compared to the negative group. All treated groups showed significant decreases in sperm abnormality compared with the positive control group. PVC, an environmental contaminant, has been shown to interfere with normal reproductive processes, leading to decreased sperm count, motility, and semen quality [52]. In the present study, PVC caused a significant decrease in sperm count, sperm motility percentage, Sperm Viability percentage, and an increase in Sperm abnormality. Our study agrees with Sadeghi et al., [24],

who found a decrease in sperm count and sperm motility %. Studies have shown that PVC induces oxidative stress, reduces the activity of antioxidant enzymes, and thus causes apoptosis in testicular tissue [77]. One possible mechanism is that ROS generation might be correlated with PVC-induced calcium (Ca) entry, potentially through the Ca-mediated activation of the nicotinamide adenine dinucleotide phosphate complex [24]. Conventionally, sperm quality is typically measured by assessing viability, motility, and membrane integrity. In the present study, PVC treatment also had adverse effects on the motility, viability, and membrane integrity of spermatozoa in the cauda epididymis. The reduction in the daily sperm production number of spermatozoa in the cauda epididymis of the treated rats is likely to have been caused by the suppressive effect of PVC on spermatogenesis. In contrast, the alterations in motility, viability, and membrane integrity of spermatozoa might have resulted from interference with epididymal function [44]. The improved effects of *Ulva lactuca* supplementation could be related to the higher zinc content and antioxidant capacity represented in vitamin A, vitamin E, selenium, and phenols, which are known to increase sperm percentage and improve semen quality through maintenance of the acrosomal membrane of the sperm head and enhancement of sperm motility [25]. In addition, Al-Mousaw, [78] showed the effect of ascorbic acid on sperm parameters, namely motility, viability, morphology, and concentration, against the adverse effects of stress. It is concluded that ascorbic acid plays a productive antioxidant role against the harmful effect of stress on tastes and therefore, sperm parameters and fertility. Moreover, zinc is an essential element in the male reproductive system, required for proper hormonal metabolism, sperm formation, and motility. Besides its potential role in the testicular development and maturation of secondary sexual characteristics, its deficiency has also been associated with impotence, hypogonadism, and reduced sexual performance [79].

Histopathological examination of testes tissue specimens from the normal control group (Fig. 1and2) revealed the normal histological structure of seminiferous tubules with active spermatogonial cells and numerous spermatids. The treated group (Fig. 3and4) showed testicular degeneration manifested by the presence of numerous seminiferous tubules with a marked reduction in spermatogonial cells and the absence of spermatids. The affected seminiferous tubules appeared with a corrugated basement membrane. The Ulva extract-treated group (Fig. 5and6) exhibited mild degeneration in some seminiferous tubules. Some tubules were missing the spermatids formed by vacuolation in Sertoli cells. Some sections were apparently normal. Vit C and Zinc

treated group (Fig. 7and8) was improved; most of the examined sections were apparently normal. A Marked improvement was observed in the Ulva extract & Vit C & Zinc treated group (Fig. 9&10) as all examined testes sections were free from any detectable histopathological changes.

53

Table 6. The effect of U. lactuca extract on Semen Analysis and Morphology in PVC-treated male rats

Parameters / Groups	Sperm count (×10⁶/ml)	Sperm Motility (%)	Sperm Viability (%)	Sperm Abnormality (%)
Group I	88.54±0.352a	86 ±3.055a	91.16±0.72a	2.56±0.2166e
Group II	53.94±0.816e	45.43±1.919e	57.69±0 .554e	20.66±0.284a
Group III	71.77±0.853c	58.86±0 .8875c	72.13±0 .583c	5.71±0.122c
Group IV	63.86±1.452d	52.25±0. 732d	62.39±0.485d	9.40±0.172b
Group V	77.86±0.9417b	68.86±0. 6064b	80.80±0 .301b	4.43±0.069d

*Data were expressed as mean ±SD. The different superscripts significantly differed within the same column. * Indicates significant p-value < 0.05.*

Histopathological examination of the testes in PVC-exposed rats confirmed our findings. Seminiferous tubules decreased in diameter with various degrees of atrophy; also, the basement membrane showed irregularity and reduced thickness. Leydig cells decreased in number and exhibited nuclei indentation and clumped chromatin. Similar findings are observed by Sadeghi et al., [80]. Moreover, zinc is an essential element in the male reproductive system, required for proper hormonal metabolism, sperm formation, and motility. Besides its potential role in the testicular development and maturation of secondary sexual characteristics, its deficiency has also been associated with impotence, hypogonadism, and reduced sexual performance [79].

Moreover, Zn can improve the testicular damage caused by many stressors, such as heavy metals, fluoride, and heat. Zn provides protective effects against testicular injury and maintains normal testicular functions. Zn is a cofactor of metalloenzymes that are involved in DNA transcription, gene expression of the hormone receptors, and protein synthesis [51].

54

Fig. A .Testes of control animal showing normal normal seminiferous tubules (H&E). bar= (50&100) µm, X200 (1,2). Testes of PVC Rats showing testicular degeneration with empty tubules (arrows) (H&E). bar= (50&100)µm, X200(3,4). Testes of animal of G3 showing mild degeneration in the spermatogonial cells (H&E)., bar= (50&100) µm, X200(5,6). Testes of animal of G4 showing apparently normal seminiferous tubules (H&E). , bar= (50 &100) µm, X200(7,8).Testes of animal of G5 showing apparently normal seminiferous tubules (H&E)., bar= (50 &100)µm, X200(9,10).

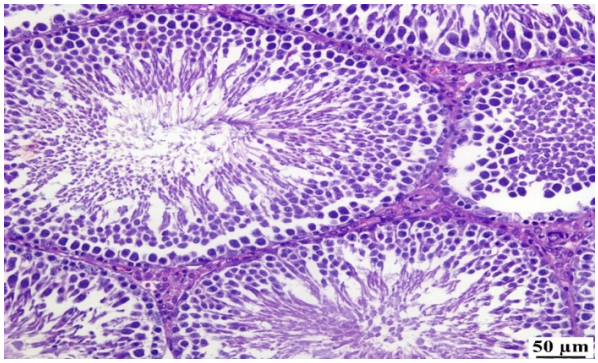

Figure 1

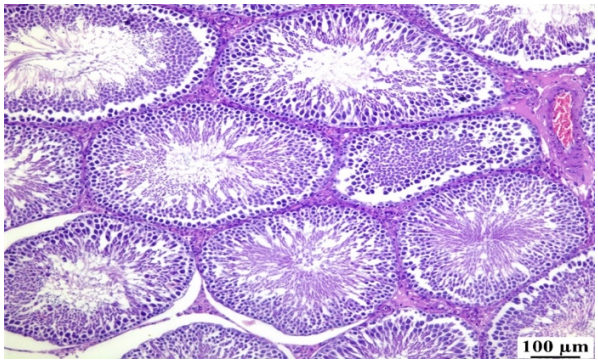

Figure 2

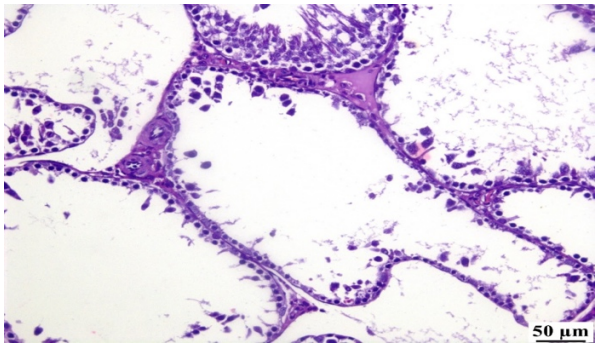

Figure 3

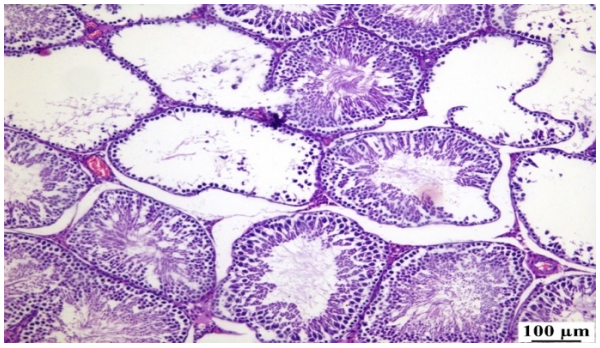

Figure 4

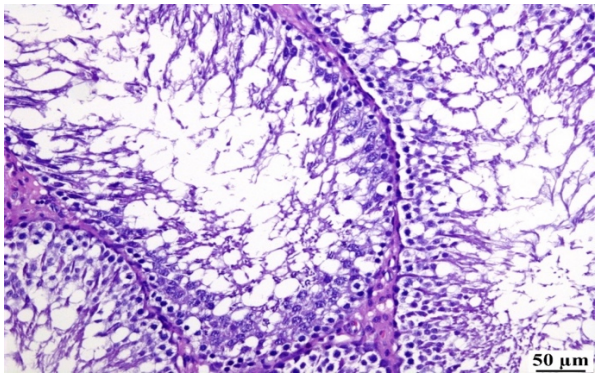

Figure 5

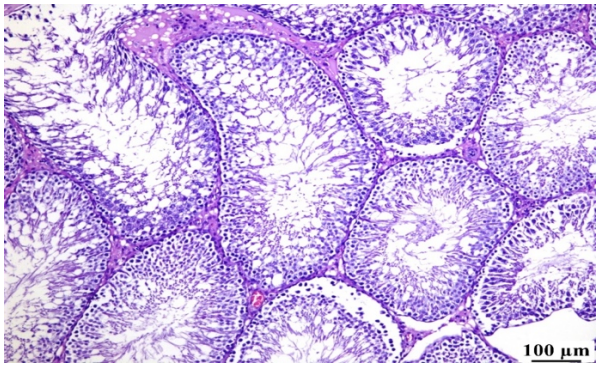

Figure 6

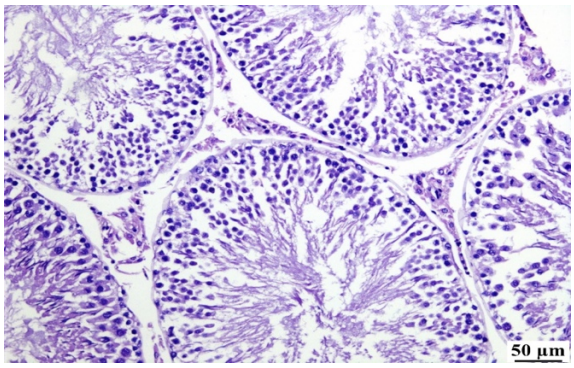

Figure7

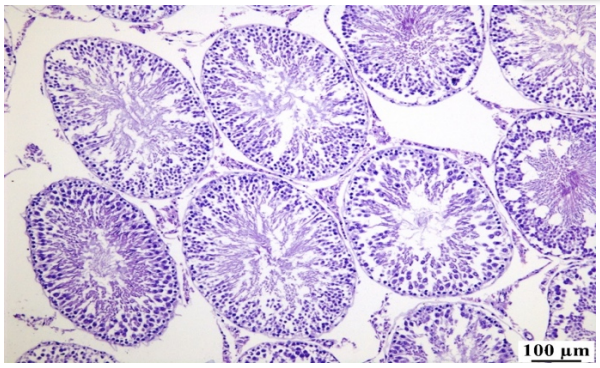

Figure 8

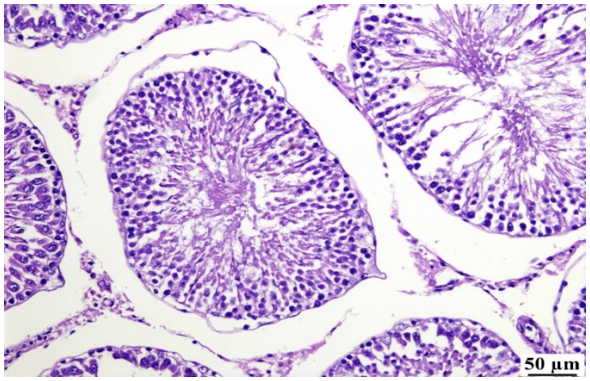

Figure 9

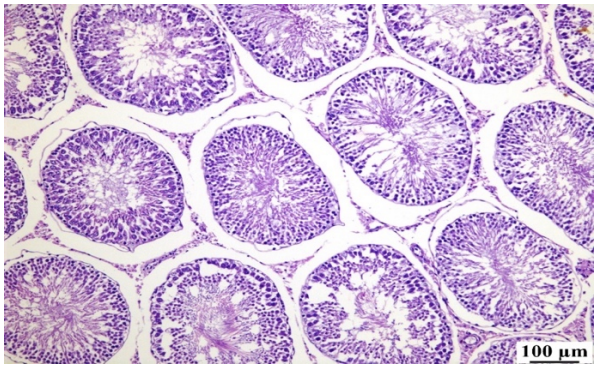

Figure 10

4. Conclusion

Biochemical and histopathological findings demonstrate that PVC has harmful adverse effects on experimental animals. The findings showed that the administration of Ulva extract + Vit C+ zinc improved the tissues and functions of the testes.

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التغذية وعلوم الأطعمة

التأثير الوقائي لمستخلص خس البحر على الخصوبة لدى ذكور الجرذان البالغة المعرضة لكلوريد البوليفينيل

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الملخص العربى:

كلوريد البوليفينيل هو شكل مبلمر من كلوريد الفينيل يستخدم على نطاق واسع فى صناعة البلاستيك، له تأثيرات سامة من خلال زيادة الإجهاد التأكسدي في الجسم والخصيتين. قامت هذه الدراسة بتقييم تأثير المستخلص الإيثانولى لخس البحر وفيتامين C والزنك ضد سمية الخصية المحدثة بواسطة كلوريد البوليفينيل فى ذكور الفئران. تم تقسيم ثلاثين من الفئران البيضاء البالغة إلى خمس مجموعات مكونة من ستة فئران. المجموعة الأولى: تلقت الفئران الماء المقطر فقط كمجموعة ضابطة سلبية. المجموعة الثانية: تناولت الفئران كلوريد البوليفينيل بتركيز (1000 ملجم/كجم من وزن الجسم) كمجموعة ضابطة موجبة. المجموعة الثالثة: تناولت الفئران 100 ملجم/كجم من مستخلص خس البحرعن طريق الفم . المجموعة الرابعة: تناولت الفئران 100 ملجم/كجم من فيتامين ج و5ملجم/كجم من الزنك. المجموعة الخامسة: تناولت الفئران 50 ملجم/كجم من مستخلص خس البحر + 50 ملجم/كجم فيتامين ج + 2.5 ملجم/كجم من الزنك. تعطى الجرعات عن طريق أنبوب المعدة يوميا لمدة 40 يوما متتالية. وفى نهاية التجربة تم ذبح الفئران ، وتم تسجيل أوزان الفئران والغذاء المتناول ونسبة كفاءة الغذاء، ووزن الخصية %. تم تقدير خصائص الحيوانات المنوية، ومستويات الهرمونات الجنسية فى الدم (هرمون التستوستيرون، الهرمون المنبه للجريب، الهرمون اللوتينى، الانزيمات المضادة للأكسدة (الكتاليز، فوق أكسيد الديسموتيز، الجلوتاثيون بيروكسيديز، والجلوتاثيون).تم تقديرالمالوندىالدهيد فى أنسجة الخصية.عامل نخر الورم α، تم تحليل إنترلوكين -β1 والإنترلوكين-6، وتم إجراء الفحص النسيجي المرضى للخصيتين، وأظهرت النتائج أن المعالجات أدت إلى تحسين مؤشرات الحيوانات المنوية ونشاط الإنزيمات المضادة للأكسدة ومستويات الهرمونات الجنسية، وبالتالى فان تناول خليط من مستخلص خس البحر وفيتامين ج والزنك مفيدًا لتحسين الأداء الجنسي للذكور.

الكلمات الكاشفة: الخصوبة، كلوريد البوليفينيل، السوبر اوكسيد ديسميوتيز، الانزيمات المضاد للأكسدة