



## Toxicity assessment of the green *Dunaliella salina* microalgae

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### ABSTRACT

The chronic toxicity of the *Dunaliella salina* microalgae was examined to evaluate its toxicity by the exposure of laboratory animals to high doses of *Dunaliella salina* and to estimate the possibility of using it as a safe supplement. Different hematological and biochemical analysis including complete blood picture (CBC), liver function enzyme activities; aminotransferases (ALT and AST), alkaline phosphatase (ALP), total bilirubin, kidney function tests; urea, creatinine, and albumin, as well as blood glucose level, were measured. The histopathological investigation was also carried out on hepatic, renal and cardiac architectures to examine its safety. Treatment with the dose 100 mg/kg body weight of *D. salina* powder daily for three consecutive months did not show any signs of toxicity in both genders and in mice and rats (no mortality, no hair loss, no diarrhea, no patches of yellow color appearance, etc.....). Moreover, abnormalities on behavior, food and water intakes and health status among the treated animals were not observed. CBC profile revealed a significant increase in hemoglobin (Hb) level in treating male and female mice and rats compared to their related control levels. The biochemical analysis clearly showed an insignificant change in liver enzyme activities, blood glucose level at a dose of 100 mg/kg. Also, an insignificant reduction in total urea and creatinine levels in both genders of mice and rats were noticed. Histopathological investigation showed normal architectures of all organs. Hence we can conclude that *Dunaliella salina* has been proven a safe profile up to 100 mg/kg body weight, however, it succeeded to stimulate the Hb synthesis compared to control groups, showing its benefits to be used safely as food additives or protective and curative agent in different diseases in future.

### 1. Introduction

*Dunaliella salina* Chlorophyceae unicellular green algae are considered one of the most important species for commercial production due to the presence of  $\beta$ -carotene and other active compounds such as lutein zeaxanthin chlorophyll, and polyunsaturated fatty acids. *Dunaliella salina* because of  $\beta$ -carotene abundance, is seen as a potent antioxidant and vitamin A precursor so, it utilized as a health food supplement, food coloring agent, cosmetics additive [1]. There is a large body of evidence documented that *Dunaliella salina* may promote health-modulating effects and participate in diminishing the risk of developing different metabolic diseases [2].

It was reported that *Dunaliella salina* rich carotenoid as trans - $\beta$ -carotene and 9- or 9'- cis - $\beta$ -carotene, lutein, and zeaxanthin are considered the major identified carotenoids with the potent antioxidant efficiency [3].

No information is available in the literature up- till now for long-term toxicity study of *Dunaliella salina* (chronic toxicity). So, the present

work is regarded as the first study concerning the chronic study of *Dunaliella salina* using the dose 100 mg/kg body weight daily supplemented orally for three consecutive months to both genders of mice and rats. In this study, hematological and biochemical parameters including liver enzymes activity, kidney function tests, and blood glucose level were evaluated. Besides, histopathological examination of cardiac, hepatic and renal tissues of both male and female mice and rats was investigated.

### 2. Materials and methods

#### 2.1. *Dunaliella salina* cultivation in laboratory scale

*Dunaliella salina* species was isolated from water of River Nile using media named BG11 for isolation and purification of algae. *Dunaliella salina* was grown in 2 L of media for two weeks [4,5]. After the growth, the biomass of algae was harvested and grown for an additional two weeks. Then, the algal biomass was harvested again and grown in a

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**Table 1**  
Liver function enzyme activities in male and female mice post chronic administration of 100 mg /Kg *Dunaliella salina*.

Groups	ALT (U/l)	AST (U/l)	Bilirubin(mg/dl)	ALP(U/l)	Glucose (mg/dl)
Control male mice	30.11 ± 1.00a	73.65 ± 3.10a	0.79 ± 0.03a	41.90 ± 3.10a	100.50 ± 2.00a
Control female mice	32.19 ± 2.64a	71.80 ± 5.56a	0.89 ± 0.01a	42.00 ± 5.00 a	90.50 ± 7.00 a
<i>Dunaliella salina</i> male mice (100 mg /Kg)	31.00 ± 1.22a	72.34 ± 6.10a	0.84 ± 0.02a	40.56 ± 4.10 a	100.00 ± 4.90 a
<i>Dunaliellasalina</i> female mice (100 mg /Kg)	30.88 ± 3.45a	81.00 ± 2.89a	0.78 ± 0.03a	43.20 ± 1.19a	95.00 ± 6.30a

Data are Means ± SD of 15 mice in treated group. Statistical analysis is carried out using Co-state and SPSS computer programs (version 7), where unshared letter is significant at P ≤ 0.05.

**Table 2**  
Total urea, creatinine and albumin levels in serum of male and female mice post chronic administration of 100 mg /Kg *Dunaliella salina*.

Groups	Urea (mg/dl)	Creatinine (mg/dl)	Albumin (mg/dl)
Control male mice	31.00 ± 3.00 <sup>a</sup>	0.150 ± 0.01 <sup>a</sup>	4.80 ± 0.20 <sup>a</sup>
Control female mice	31.03 ± 2.92 <sup>a</sup>	0.144 ± 0.03 <sup>a</sup>	4.60 ± 0.3 <sup>a</sup>
<i>Dunaliella salina</i> male mice (100 mg /Kg)	26.00 ± 3.30 <sup>a</sup>	0.140 ± 0.01 <sup>a</sup>	4.9 ± 0.10 <sup>a</sup>
<i>Dunaliellasalina</i> female mice (100 mg /Kg)	26.30 ± 3.10 <sup>a</sup>	0.145 ± 0.02 <sup>a</sup>	4.60 ± 0.12 <sup>a</sup>

Data are Means ± SD of 15 mice in treated group. Statistical analysis is carried out using Co-state and SPSS computer programs (version 7), where unshared letter is significant at P ≤ 0.05.

plastic jar whose 17 L capacity and containing 15 L of culture media. The temperature of culture was 20 ± 3 °C with aeration. Stable fluorescent light was applied for the culture with intensity ≈ 2500 lx.

## 2.2. *Dunaliella salina* cultivation in the Photo-bioreactor

Post 10 days of growing the inoculums were put in a vertical photobioreactor with the capacity of 4000 L, automated and computerized.

## 2.3. Animals

Male and female Swiss mice with an average weight of 20–30 g as well as the male and female Wistar albino rats weighing 120–130 g were obtained from Animal House Lab, National Research Centre (NRC), Dokki, Giza were used in this study. Animals were acclimatized for one week before the starting of the experiment (adaptation period). The animals were housed with a well-ventilation (22 ± 20 °C) with a

cycle of twelve hours. Normal basal diets and water were supplied *ad libitum*. Animals were cared according to the Ethical Committee guidelines of NRC, Giza, Egypt for animal experiments, with ethical approval no: 13,115.

## 2.4. Experimental design

One hundred and twenty male and female mice and rats were used in this study; thirty mice and rats for each gender were supplemented orally with 100 mg /kg body weight of *Dunaliella salina* powdered and were divided into eight groups as follows:

Groups 1 and 2: Control male and female mice (15 mice each) were daily orally administered 0.9% normal saline solution for three consecutive months.

Groups 3 and 4: Male and female mice (15 mice each) were supplemented orally daily with 100 mg/ kg body weight of powdered of *Dunaliella salina* for three consecutive months.

Groups 5 and 6: Control male and female rats (15 rats each) as described aforementioned.

Groups 7 and 8: Male and female rats were administered orally daily with 100 mg/kg body weight of powdered of *Dunaliella salina* daily for three consecutive months (15 mice each). The animals were observed daily for behavioral, food and water intakes.

After the three months, all animals were sacrificed. Fasting blood samples were collected by puncture of the sublingual vein and left for clotting then, centrifuged at 3000 rpm for 15 min to separate serum for liver and kidney function tests [6–8]. Biochemical parameters were determined in serum using Biodiagnostic kits (Bio diagnostics Co., Egypt).

For hepatic, cardiac and renal tissues; the histopathological examination was carried out, tissue slices were fixed in 10% formaldehyde and embedded in paraffin wax blocks. Sections of 5 μm thick were stained with hematoxylin and eosin (H&E) then examined

**Table 3**  
Blood profile picture of male and female mice post chronic administration of 100 mg /Kg *Dunaliella salina*.

Biomarkers	Control male	Control Female	Treated male mice	Treated female mice
HB (g/L)	12.20 ± 1.00 <sup>a</sup>	11.10 ± 1.20 <sup>a</sup>	15.90 ± 1.29 <sup>b</sup>	14.80 ± 1.98 <sup>b</sup>
RBCs(million cells/uL)	6.30 ± 0.91 <sup>a</sup>	6.00 ± 0.90 <sup>a</sup>	6.80 ± 0.98 <sup>a</sup>	6.89 ± 0.98 <sup>a</sup>
PCV (%)	41.80 ± 4.10 <sup>a</sup>	40.90 ± 3.50 <sup>a</sup>	41.00 ± 2.97 <sup>a</sup>	41.65 ± 4.00 <sup>a</sup>
WBCs(× 10 <sup>9</sup> /L)	9.97 ± 112.50 <sup>a</sup>	10.00 ± 120.50 <sup>a</sup>	10.500 ± 110.50 <sup>a</sup>	10.067 ± 100.00 <sup>a</sup>
Neutrophils (× 10 <sup>9</sup> /L)	20.69 ± 1.00 <sup>a</sup>	19.65 ± 1.20 <sup>a</sup>	18.00 ± 1.00 <sup>a</sup>	19.00 ± 1.00 <sup>a</sup>
Eosinophil (x100 cells /ul).	1.69 ± 0.07 <sup>a</sup>	1.59 ± 0.05 <sup>a</sup>	1.65 ± 0.02 <sup>a</sup>	1.66 ± 0.04 <sup>a</sup>
Lymphocyte(10 <sup>9</sup> /L)	79.00 ± 8.20 <sup>a</sup>	78.00 ± 3.50 <sup>a</sup>	77.00 ± 7.10 <sup>a</sup>	75.62 ± 7.10 <sup>a</sup>
Monocyte(10 <sup>9</sup> /L)	1.66 ± 0.05 <sup>a</sup>	1.63 ± 0.14 <sup>a</sup>	1.60 ± 0.12 <sup>a</sup>	1.59 ± 0.15 <sup>a</sup>
Platelets (x 10 <sup>9</sup> /L)	740.00 ± 50.00 <sup>a</sup>	770.00 ± 65.00 <sup>a</sup>	800.00 ± 60.00 <sup>a</sup>	780.00 ± 45.00 <sup>a</sup>

Data are Means ± SD of 15 mice in treated group. Statistical analysis is carried out using Co-state and SPSS computer programs (version 7), where unshared letter is significant at P ≤ 0.05.

**Table 4**  
Liver function enzyme activities in male and female rats post chronic administration of 100 mg /Kg *Dunaliella salina*.

Groups	ALT(U/l)	AST(U/l)	Bilirubin (mg/dl)	ALP(U/l)	Glucose (mg/dl)
Control male rats	35.10 ± 1.44 <sup>a</sup>	76.70 ± 6.17 <sup>a</sup>	0.80 ± 0.01 <sup>a</sup>	44.00 ± 3.10 <sup>a</sup>	80.50 ± 5.00 <sup>a</sup>
Control female rats	33.22 ± 2.09 <sup>a</sup>	76.88 ± 4.20 <sup>a</sup>	0.82 ± 0.03 <sup>a</sup>	46.00 ± 3.00 <sup>a</sup>	77.90 ± 3.00 <sup>a</sup>
<i>Dunaliella salina</i> male rats (100 mg /Kg)	34.00 ± 2.00 <sup>a</sup>	75.60 ± 3.76 <sup>a</sup>	0.84 ± 0.03 <sup>a</sup>	42.56 ± 2.67 <sup>a</sup>	82.00 ± 6.00 <sup>a</sup>
<i>Dunaliella salina</i> female rats (100 mg /Kg)	34.88 ± 3.00 <sup>a</sup>	71.80 ± 7.00 <sup>a</sup>	0.78 ± 0.05 <sup>a</sup>	44.00 ± 1.19 <sup>a</sup>	81.00 ± 2.30 <sup>a</sup>

Data are Means ± SD of 15 mice in treated group. Statistical analysis is carried out using Co-state and SPSS computer programs (version 7), where unshared letter is significant at P ≤ 0.05.

**Table 5**  
Total urea, creatinine and albumin levels in serum of male and female rats post chronic administration of 100 mg /Kg *Dunaliella salina*.

Groups	Urea (mg/dl)	Creatinine (mg/dl)	Albumin (mg/dl)
Control male rats	30.00 ± 2.90 <sup>a</sup>	0.110 ± 0.19 <sup>a</sup>	3.85 ± 0.60 <sup>a</sup>
Control female rats	31.11 ± 5.90 <sup>a</sup>	0.089 ± 0.36 <sup>a</sup>	4.00 ± 0.90 <sup>a</sup>
<i>Dunaliella salina</i> male rats (100 mg /Kg)	27.10 ± 1.30 <sup>a</sup>	0.088 ± 0.20 <sup>a</sup>	4.20 ± 0.80 <sup>a</sup>
<i>Dunaliella salina</i> female rats (100 mg /Kg)	28.30 ± 2.00 <sup>a</sup>	0.085 ± 0.08 <sup>a</sup>	4.18 ± 0.12 <sup>a</sup>

Data are Means ± SD of 15 mice in treated group. Statistical analysis is carried out using Co-state and SPSS computer programs (version 7), where unshared letter is significant at P ≤ 0.05.

under a light microscope for determination of pathological changes.

2.5. Hematological parameters

Erythrocytes count, hemoglobin (Hb) levels and hematocrit (PCVt) were determined immediately in blood samples [9]. White blood cell (WBC) count was determined as described by Schaperclaus et al. [10].

2.6. Biochemical parameters

Liver function enzyme activities, alanine and aspartate aminotransferases (AST and ALT), as well as alkaline phosphatase (ALP), were estimated in mice and rat sera [[11] [12], respectively]. The levels of total urea and creatinine were determined according to the methods of Bartles et al. [13] and Fawcett and Scott [14]. The level of bilirubin was also determined [15]. Glucose level was measured using colorimetric kits according to the method of Trinder [16].

**Table 6**  
Blood profile picture of male and female rats post chronic administration of 100 mg /Kg *Dunaliella salina*.

Biomarkers	Control male	Control Female	Treated male rats	Treated female rats
HB (g/L)	11.20 ± 1.23 <sup>a</sup>	11.00 ± 1.980 <sup>a</sup>	15.40 ± 1.20 <sup>b</sup>	14.99 ± 1.00 <sup>b</sup>
RBCs (million cells/ul)	6.00 ± 0.91 <sup>a</sup>	5.50 ± 0.60 <sup>a</sup>	6.90 ± 0.68 <sup>a</sup>	5.80 ± 0.56 <sup>a</sup>
PCV (%)	33.50 ± 4.10 <sup>a</sup>	33.00 ± 3.50 <sup>a</sup>	34.00 ± 3.90 <sup>a</sup>	33.00 ± 3.00 <sup>a</sup>
WBCs(× 10 <sup>9</sup> /L)	7.600 ± 66.50 <sup>a</sup>	7.400 ± 70.50 <sup>a</sup>	7.600 ± 65.00 <sup>a</sup>	7.00 ± 54.00 <sup>a</sup>
Neutrophils (× 10 <sup>9</sup> /L)	21.00 ± 1.20 <sup>a</sup>	19.95 ± 1.70 <sup>a</sup>	19.00 ± 1.10 <sup>a</sup>	19.45 ± 1.50 <sup>a</sup>
Eosinophil (x100 cells /uL).	1.60 ± 0.07 <sup>a</sup>	1.60 ± 0.05 <sup>a</sup>	1.66 ± 0.05 <sup>a</sup>	1.69 ± 0.07 <sup>a</sup>
Lymphocyte(10 <sup>9</sup> /L)	72.00 ± 5.26 <sup>a</sup>	73.00 ± 5.80 <sup>a</sup>	74.00 ± 4.90 <sup>a</sup>	74.00 ± 7.10 <sup>a</sup>
Monocyte(10 <sup>9</sup> /L)	1.55 ± 0.06 <sup>a</sup>	1.53 ± 0.05 <sup>a</sup>	1.50 ± 0.11 <sup>a</sup>	1.53 ± 0.10 <sup>a</sup>
Platelets (x 10 <sup>9</sup> /L)	740.00 ± 50.00 <sup>a</sup>	770.00 ± 65.00 <sup>a</sup>	800.00 ± 60.00 <sup>a</sup>	780.00 ± 45.00 <sup>a</sup>

Data are Means ± SD of 15 mice in treated group. Statistical analysis is carried out using Co-state and SPSS computer programs (version 7), where unshared letter is significant at P ≤ 0.05.

2.7. Statistical analysis

Statistical analysis for biochemical parts is carried out using the SPSS computer program (version 8) combined with a costate computer program, where unshared letters are significant at P ≤ 0.05.

3. Results

Treatment of both male and female rats and mice with the dose 100 mg /kg body weight of powdered of *Dunaliella salina* daily for three consecutive months did not show any toxicity sign in both sex in mice and rats (no mortality, no hair loss, no diarrhea, no patches of yellow color appearance etc.....). In addition, abnormalities on behavior, food and water intakes, and health status among the treated animals did not observe.

It has been clearly demonstrated that (Tables 1 and 4), no change in liver function enzyme activities and blood glucose level post chronic administration of *Dunaliella salina* to male and female mice and rats with 100 mg/Kg was observed. Additionally, the insignificant reduction was detected in total urea, creatinine and albumin levels post chronic administration of *Dunaliella salina* in both genders of mice and rats compared to their corresponding control levels (Tables 2 and 5). A significant increase in Hb level in treated male and female mice and rats while the insignificant change in the other parameters of CBC was detected as compared to their related control groups (Tables 3 and 6). Histopathological investigation revealed normal cardiac myocytes, normal histological structure of renal parenchyma as well as normal hepatic lobule in both sexes of mice and rats (Figs. 1–49).

4. Discussion

*Dunaliella salina* microalgae algae have been classified as sources of food and do not yield toxins. In this aspect, Cyanotech [17] announced

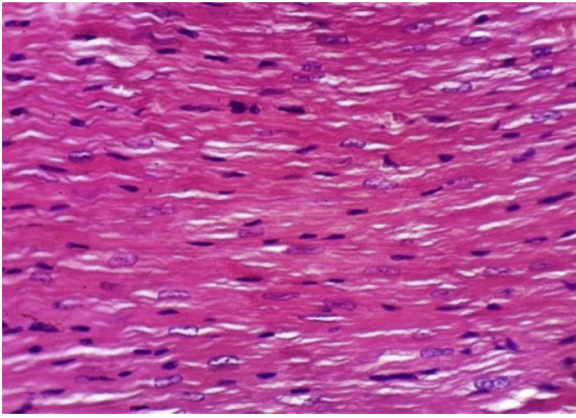


Fig. 1. Heart of mice from control female group showing normal cardiac myocytes (H & E X 400).

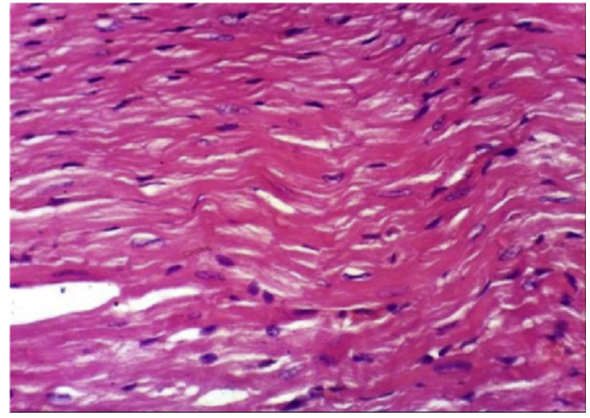


Fig. 4. Heart of mice from control male group showing normal cardiac myocytes (H & E X 400).

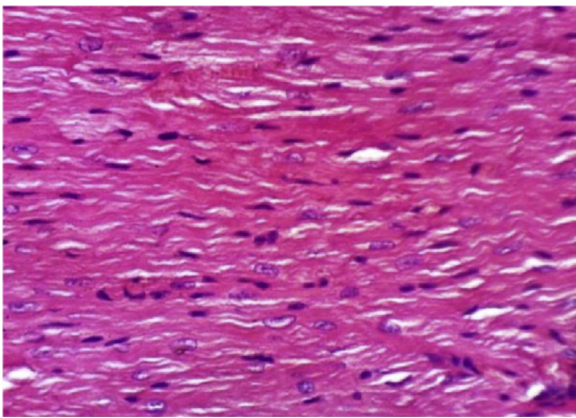


Fig. 2. Heart of mice from control female group showing normal cardiac myocytes (H & E X 400).

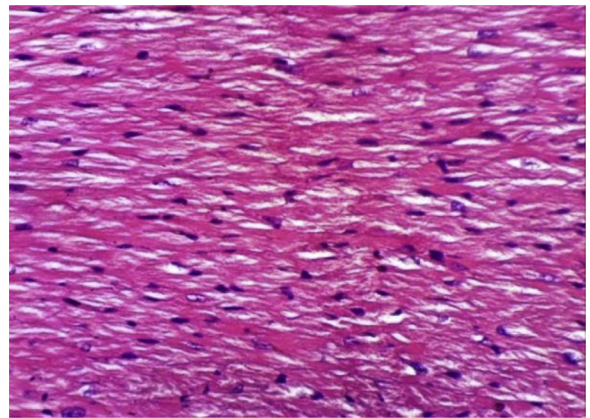


Fig. 5. Heart of mice from treated female group showing no histopathological alterations (H & E X 400).

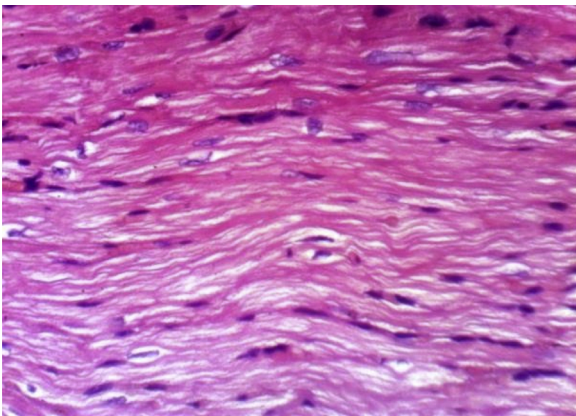


Fig. 3. Heart of mice from control male group showing normal cardiac myocytes (H & E X 400).

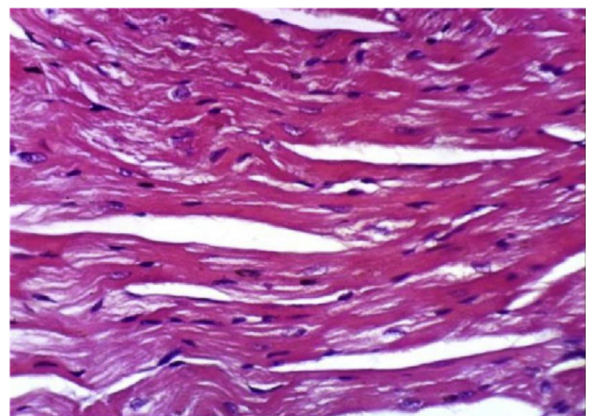


Fig. 6. Heart of mice from treated female group showing no histopathological alterations (H & E X 400).

that the uptake of *Dunaliella bardawil* does not show adverse effects. In a good parallel result; Mokady et al. [18], illustrated that the powder of the *Dunaliella bardawil* algae (which is similar to the *Dunaliella salina* species), supplemented to rats and chicken for two -eight weeks, has no signs of toxicity up to 0.1% of beta-carotene in the diet and was unable to stimulate mutation of a gene or aberrations of chromosomes in

lymphocytes of human. Further Mokady et al. [18], documented the safety of diets containing 0, 50 and 100 g/kg bw of *Dunaliella bardawil* ingested for one year and noticed, no significant differences in blood biochemistry and hematological parameters between the rats supplemented with algae and the control.

The current data showed insignificant difference in total urea and

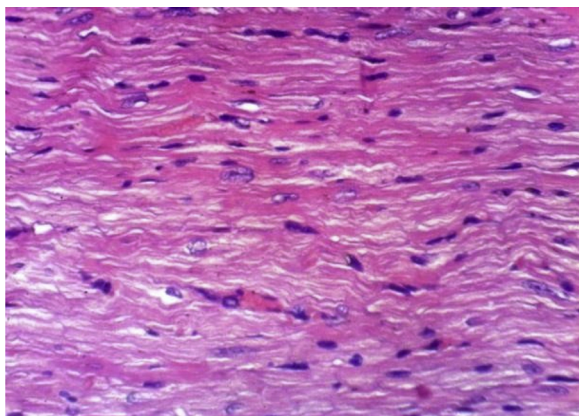


Fig. 7. Heart of mice from treated male group showing no histopathological alterations (H & E X 400).

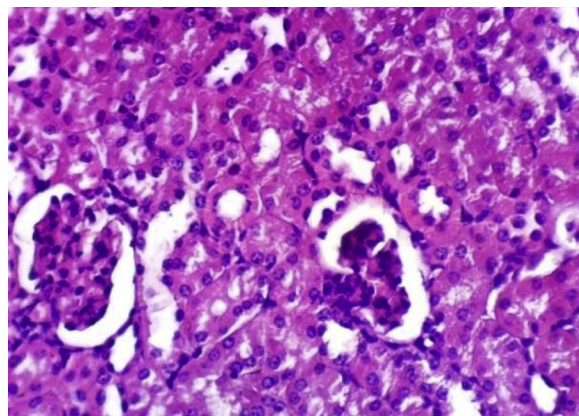


Fig. 10. Kidney of mice from control female group showing the normal histological structure of renal parenchyma (H & E X 400).

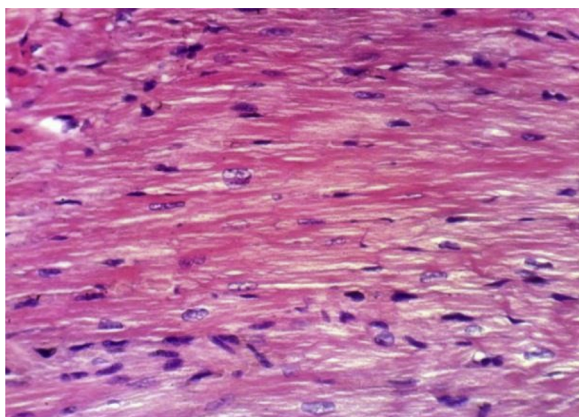


Fig. 8. Heart of mice from treated male group showing no histopathological alterations (H & E X 400).

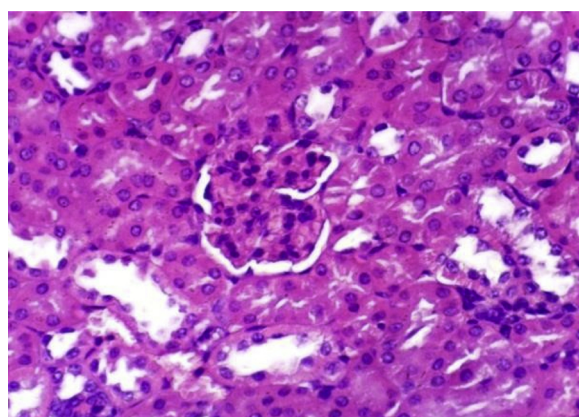


Fig. 11. Kidney of mice from control male group showing the normal histological structure of renal parenchyma (H & E X 400).

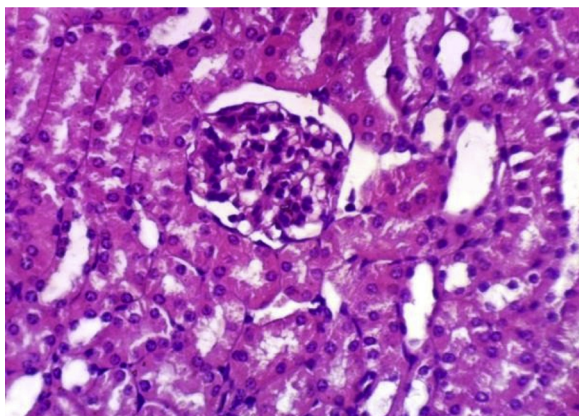


Fig. 9. Kidney of mice from control female group showing the normal histological structure of renal parenchyma (H & E X 400).

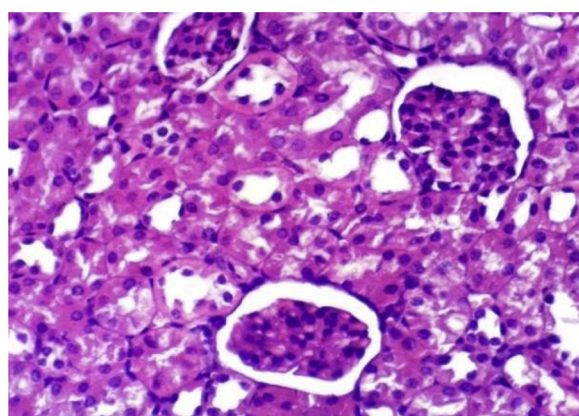


Fig. 12. Kidney of mice from control male group showing the normal histological structure of renal parenchyma (H & E X 400).

creatinine levels in mice and rats supplemented with *Dunaliella salina*. Nevertheless, a significant increase in blood hemoglobin level of both gender of rats and mice post chronic administration of 100 mg /kg body weight of *Dunaliella salina*, for three consecutive months. In this aspect, El-Baz et al. [19], demonstrated that *Dunaliella salina* rich with a high

antioxidant level of carotenoids such as  $\beta$ -carotene, astaxanthin, and fucoxanthin, causing an elevation in antioxidant enzyme activities, reduced inflammatory cytokines as a result of inhibition of oxidative stress (lipid peroxidation). So, *Dunaliella salina* powdered will preserve kidney function through normalization of urea and creatinine levels.

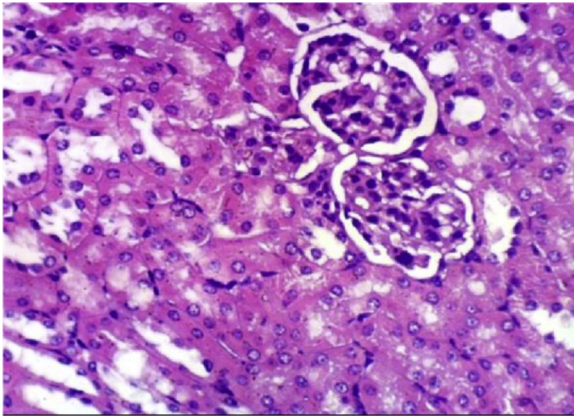


Fig. 13. Kidney of mice from treated female group showing no histopathological alterations (H & E X 400).

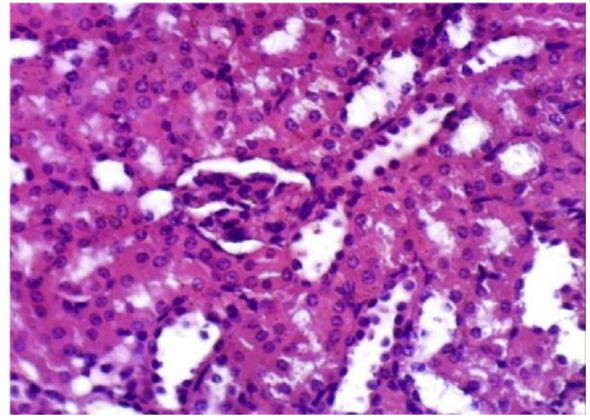


Fig. 16. no histopathological alterations of renal tissue (H & E X 400).

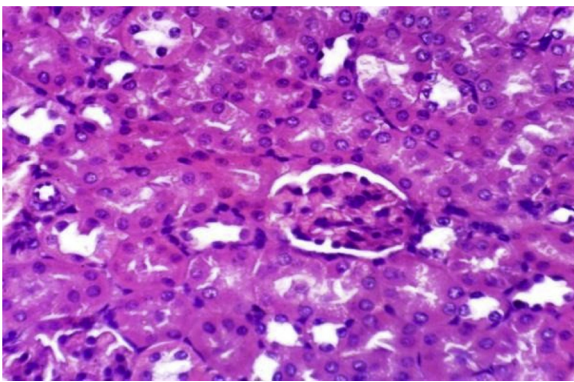


Fig. 14. Kidney of mice from control male group showing the normal histological structure of renal parenchyma (H & E X 400).

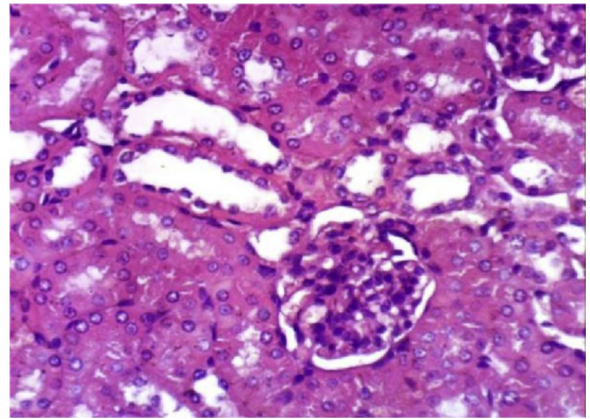


Fig. 17. Kidney of mice from treated male group showing no histopathological alterations (H & E X 400).

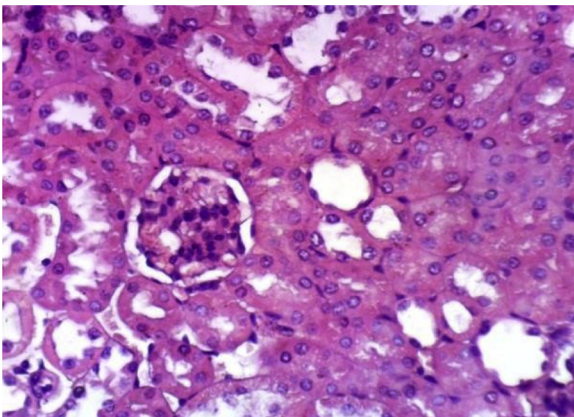


Fig. 15. Kidney of mice from treated male group showing no histopathological alterations (H & E X 400).

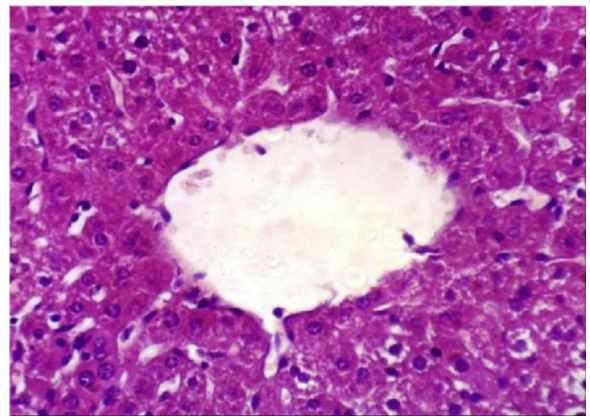


Fig. 18. Liver of mice from control female group showing the normal histological structure of hepatic lobule (H & E X 400).

This may be linked to the antioxidant properties of *Dunaliella*; since ROS may be implicated in the Glomerular Filtration Rate (GFR) impaired [20–22]. The increase in Hb level, which detected in the present study may be attributed to the  $\beta$ -carotene is considered as a precursor of vitamin A and a single oxygen quencher, so could affect the immune system through both pathways of antioxidant and retinoid [23]. Also,

*Dunaliella salina* contains a high percentage of 9-cis  $\beta$ -carotene, which has been detected to be more potent than all-trans  $\beta$ -carotene in modulating cell-cell interaction through gap junctions [24]. Hence, *Dunaliella salina* rich with  $\beta$ -carotene has been shown to be a potent immunostimulants, attenuating the erythropoietic tissue, resulting in the viability of the cells might be affected [25,26]. In opposite results, Nakano

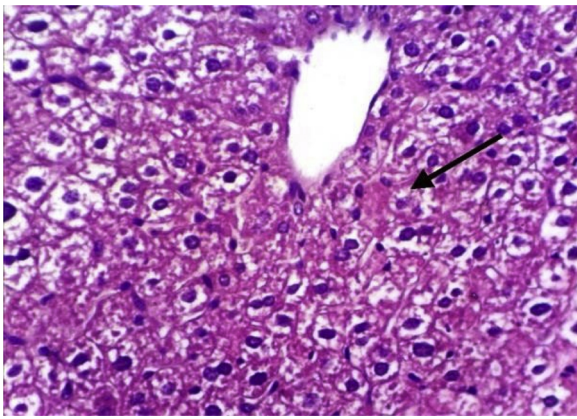


Fig. 19. Liver of mice from control male group showing slight hydropic degeneration of hepatocytes (H & E X 400). (mild +).

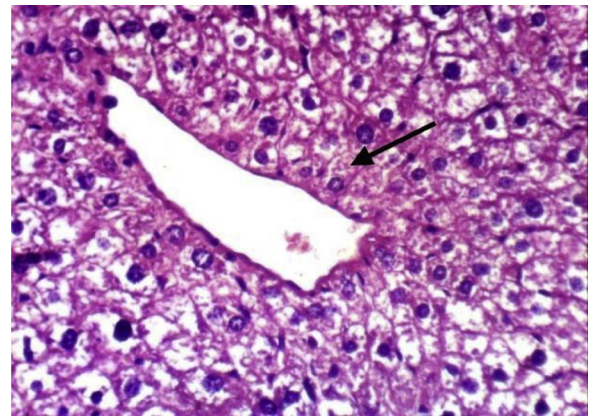


Fig. 22. Liver of mice from control male group showing slight hydropic degeneration of hepatocytes (H & E X 400). (mild +).

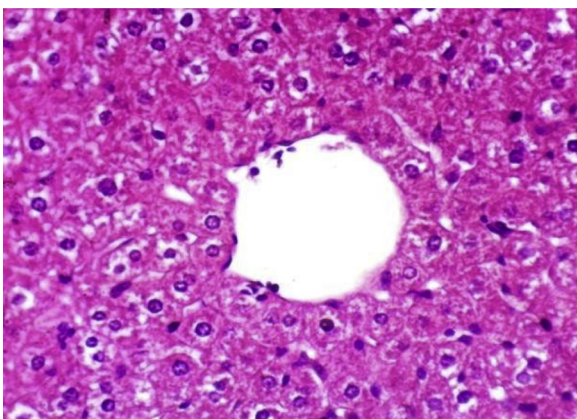


Fig. 20. Liver of mice from control female group showing the normal histological structure of hepatic lobule (H & E X 400).

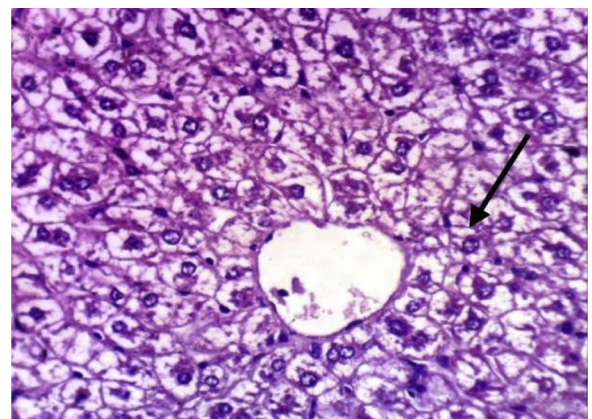


Fig. 23. Liver of mice from treated male group showing slight hydropic degeneration of hepatocytes (H & E X 400). (moderate ++).

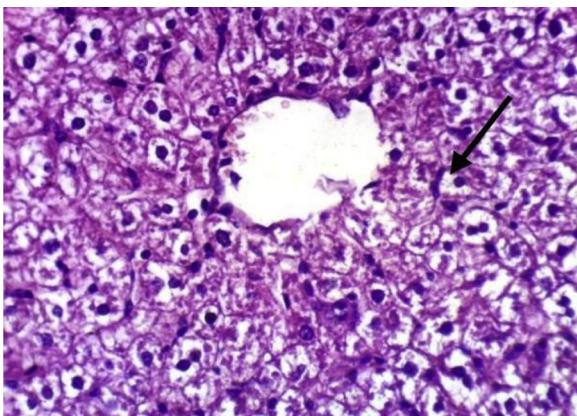


Fig. 21. Liver of mice from treated female group showing slight hydropic degeneration of hepatocytes (H & E X 400). (mild +).

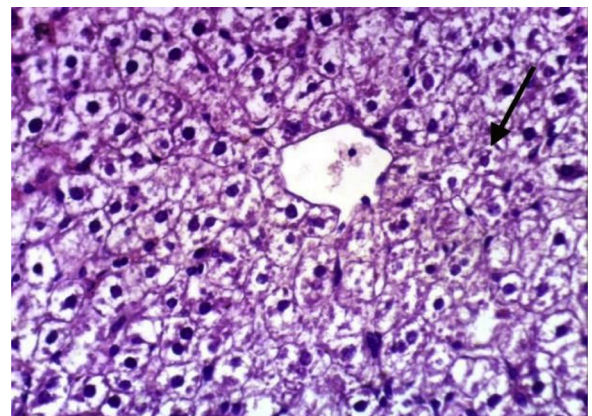


Fig. 24. Liver of mice from treated female group showing slight hydropic degeneration of hepatocytes (H & E X 400). (mild).

et al. and Rehulka [27,28], showed the interaction of carotenoids with hematological factors resulting in fluctuating in its concentrations compared to control. These controversial results may be attributed to, the route and dose of ingestion and physiological condition.

Histopathological examination revealed no differences in cardiac, renal and hepatic architectures between control and animals daily feed with 100 mg /kg bw of *Dunaliella salina* for three months. Similar results were obtained by Mokady et al. [18], who observed no difference in

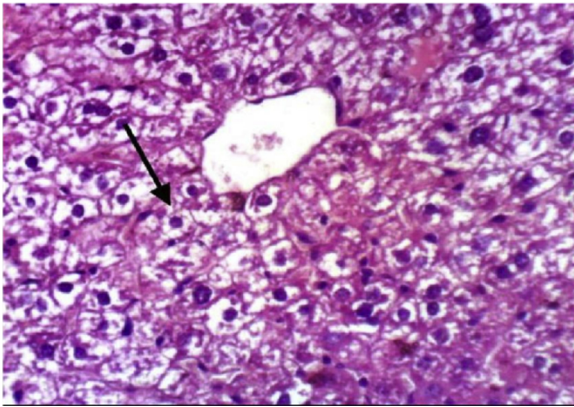


Fig. 25. Liver of mice from treated male group showing slight hydropic degeneration of hepatocytes (H & E X 400). (mild +).

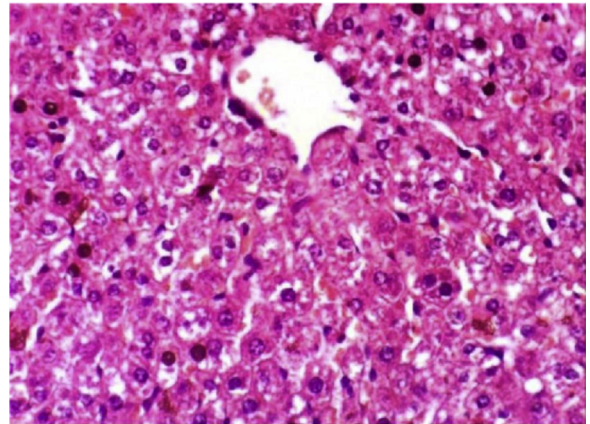


Fig. 28. Liver of rat from control male group showing the normal histological structure of hepatic lobule (H & E X 400).

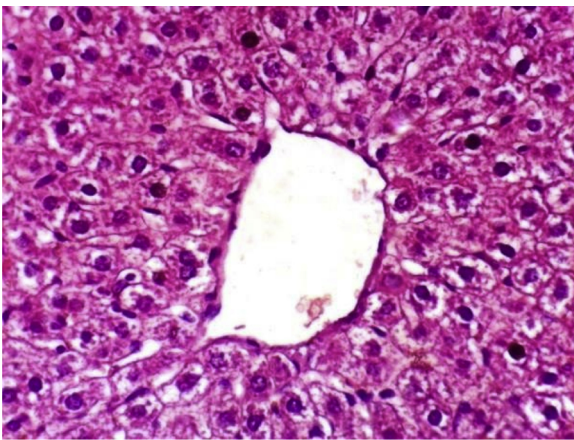


Fig. 26. Liver of rat from control female group showing the normal histological structure of hepatic lobule (H & E X 400).

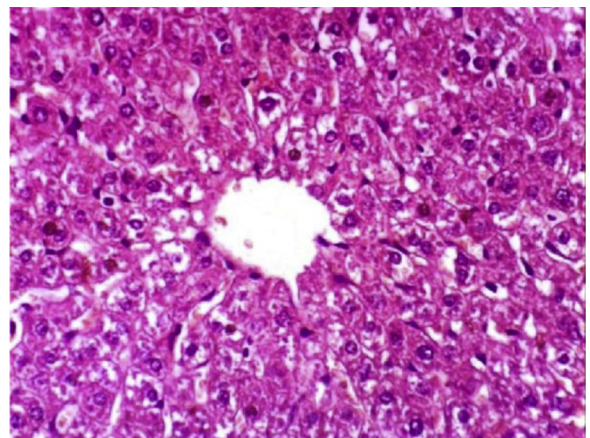


Fig. 29. histological structure of hepatic lobule (H & E X 400).

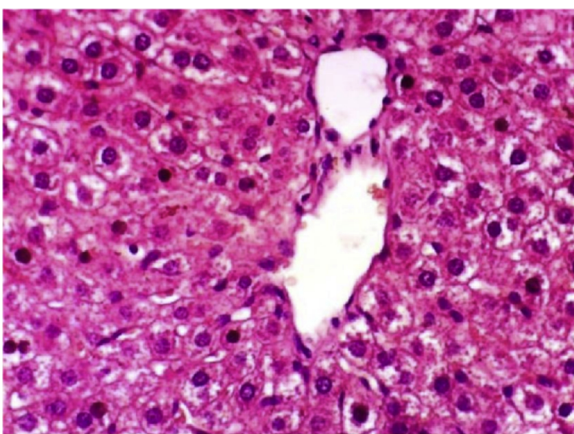


Fig. 27. Liver of rat from control female group showing the normal histological structure of hepatic lobule (H & E X 400).

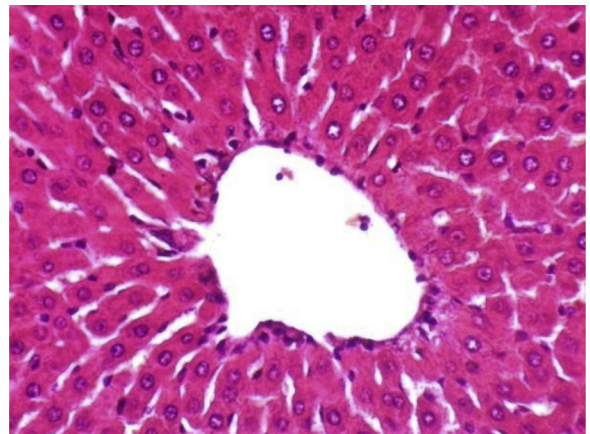


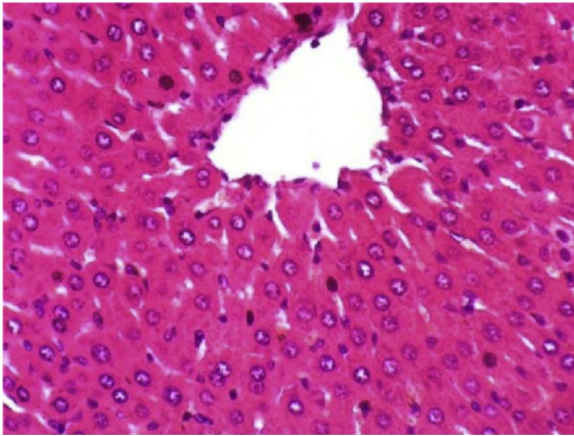
Fig. 30. Liver of rat from treated female group showing no histopathological changes (H & E X 400).

renal architecture post supplementation of rats for one year with 10 g /kg bw *Dunaliella bardawil*, suggesting the safety of consumption of *Dunaliella bardawil* for human [18].

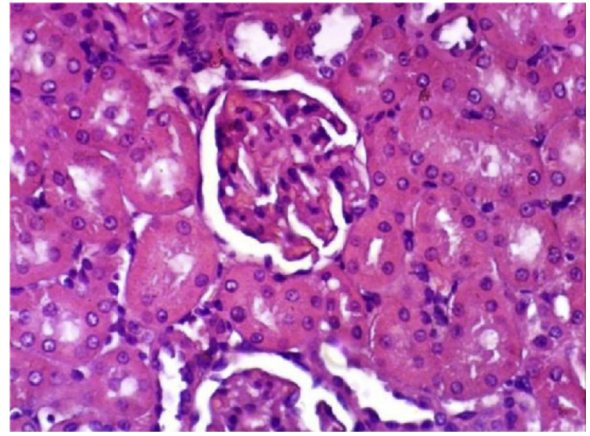
### 5. Conclusion

Marked elevation in Hb level was observed in both sexes of mice and rats compared to control which may be related to the potent

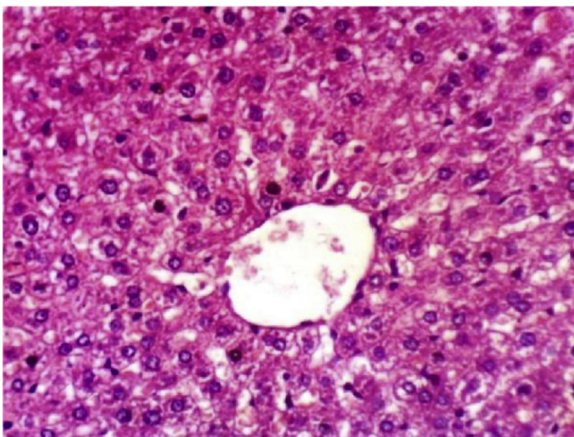




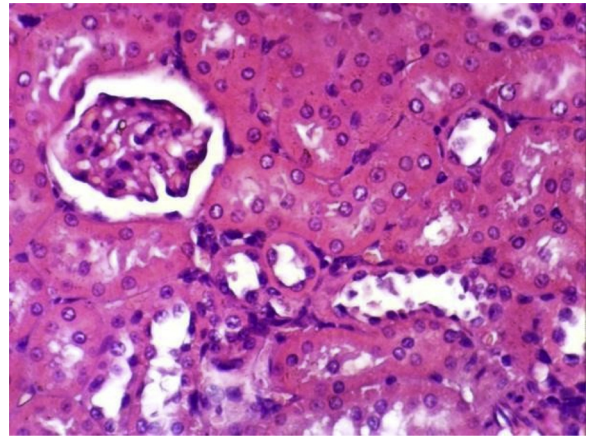
**Fig. 31.** Liver of rat from treated female group showing no histopathological changes (H & E X 400).



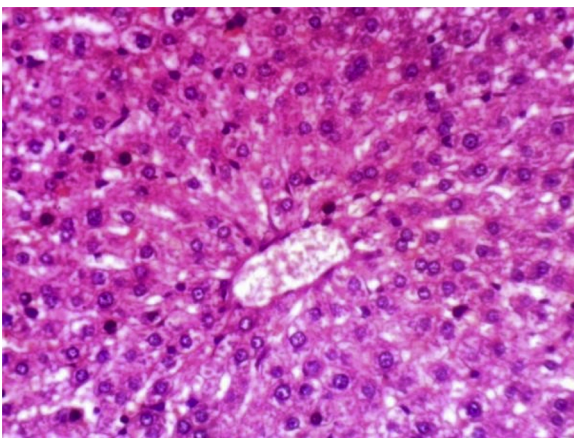
**Fig. 34.** Kidney of rat from control female group showing the normal histological structure of renal parenchyma (H & E X 400).



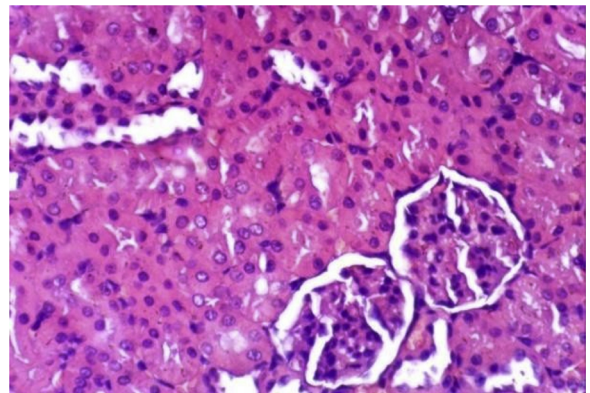
**Fig. 32.** Liver of rat from treated male group showing no histopathological changes (H & E X 400).



**Fig. 35.** Kidney of rat from control female group showing the normal histological structure of renal parenchyma (H & E X 400).



**Fig. 33.** Liver of rat from treated male group showing no histopathological changes (H & E X 400).



**Fig. 36.** histological structure of renal parenchyma (H & E X 400).

immune-stimulant activity from  $\beta$ - carotene.No significant differences were observed in all hematological and biochemical parameters as well as in histopathological examination of cardiac, renal and hepatic architectures of both gender of rats and mice post chronic administration

of 100 mg/kg bw of *Dunaliella salina* for three consecutive months.

**Declaration of Competing Interest**

The authors declared no conflict of interest regarding this study.

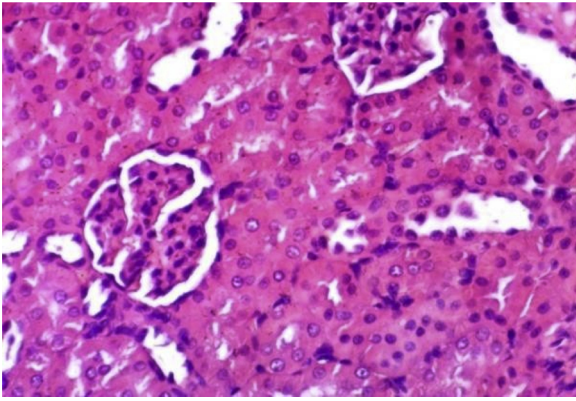


Fig. 37. Kidney of rat from control male group showing the normal histological structure of renal parenchyma (H & E X 400).

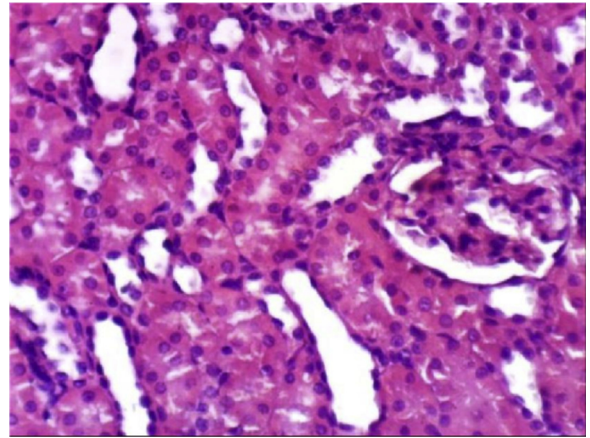


Fig. 40. Kidney of rat from treated male group showing no histopathological alterations (H & E X 400).

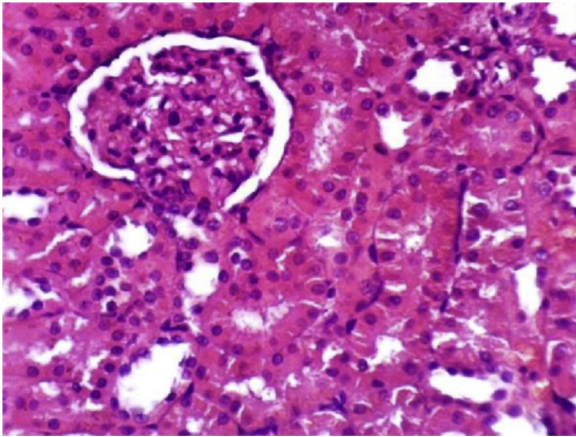


Fig. 38. Kidney of rat from treated female group showing no histopathological alterations (H & E X 400).

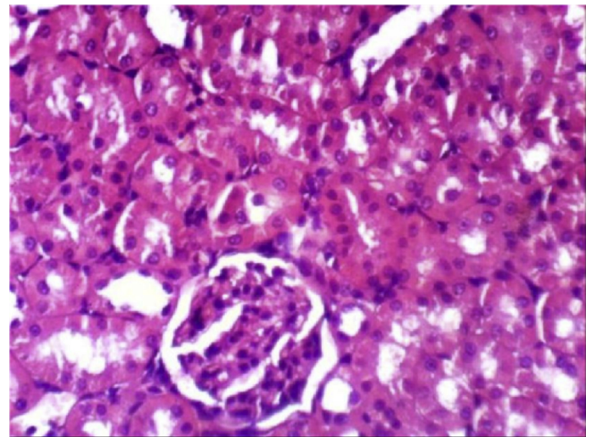


Fig. 41. Kidney of rat from treated male group showing no histopathological alterations (H & E X 400).

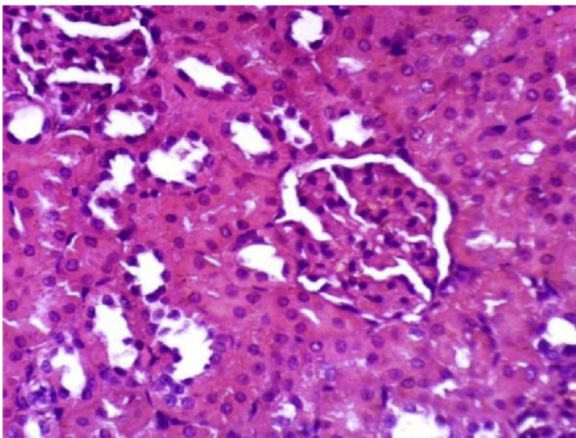


Fig. 39. Kidney of rat from treated female group showing no histopathological alterations (H & E X 400).

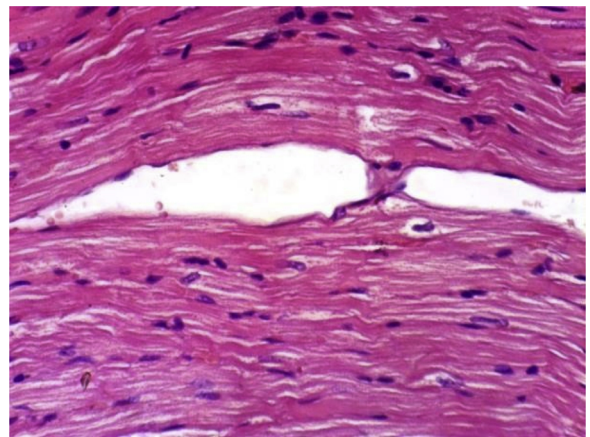
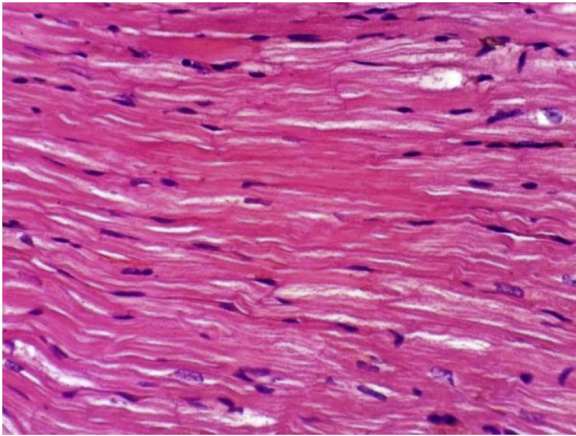
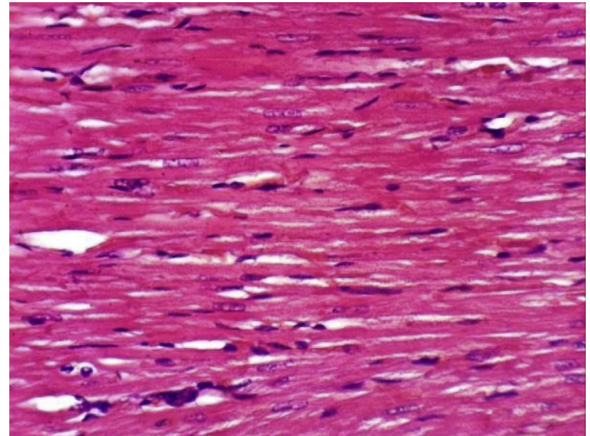


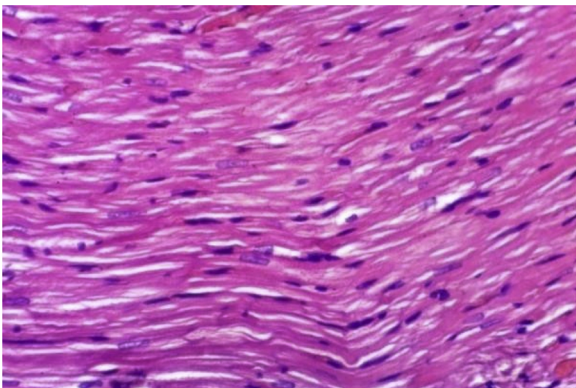
Fig. 42. normal cardiac myocytes (H & E X 400).



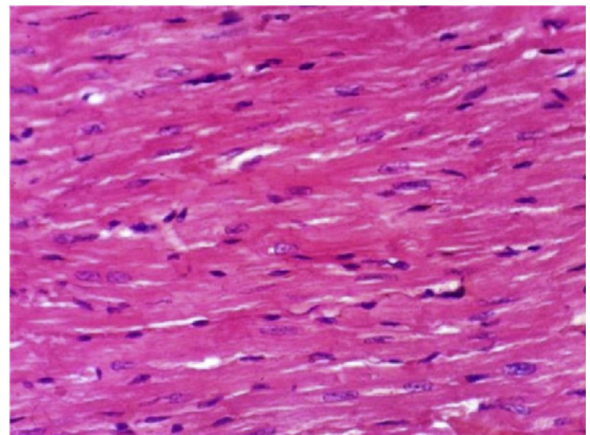
**Fig. 43.** Heart of rat from control female group showing normal cardiac myocytes (H & E X 400).



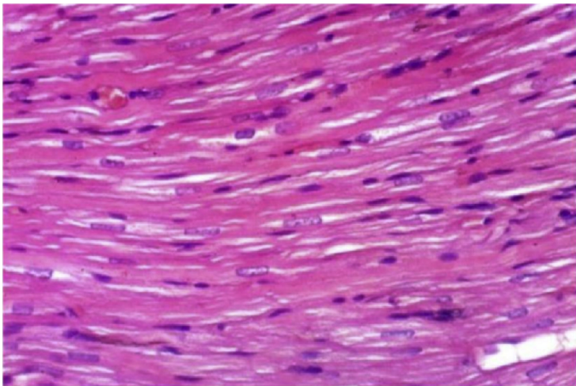
**Fig. 46.** Heart of rat from treated female group showing no histopathological alterations (H & E X 400).



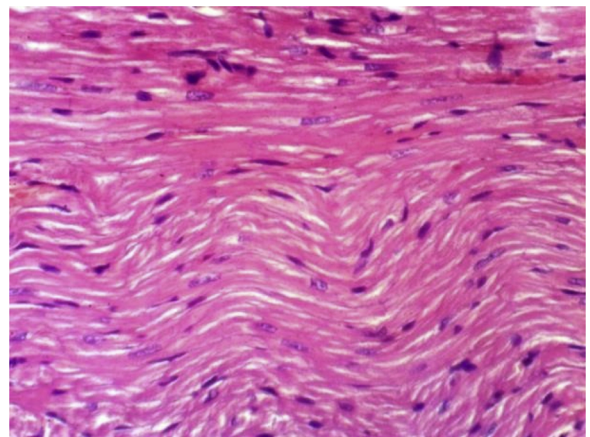
**Fig. 44.** Heart of rat from control male group showing normal cardiac myocytes (H & E X 400).



**Fig. 47.** Heart of rat from treated female group showing no histopathological alterations (H & E X 400).



**Fig. 45.** Heart of rat from control male group showing normal cardiac myocytes (H & E X 400).



**Fig. 48.** Heart of rat from treated male group showing no histopathological alterations (H & E X 400).

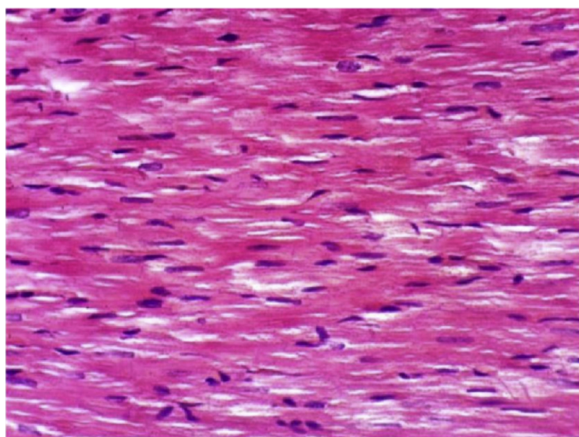


Fig. 49. Heart of rat from treated male group showing no histopathological alterations (H & E X 400).

### Author contributions

All authors contributed to collecting and analyzing data. All authors participated in writing every part of this study. All authors read and approved the final version.

### Acknowledgments

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