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Antidiabetic effect of oral supplementation with *Caulerpa racemosa* powder

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ABSTRACT: Algae are known for their high nutritional value and the presence of bioactive compounds with anti-diabetic activity. In this study, the effects of oral supplementation with the whole powdered green alga *Caulerpa racemosa* was assessed on biochemical and organic parameters in rat model of type 2 diabetes. Type 2 diabetes model (DM) was induced by high fat diet (HFD) (5.75 kcal/g) combined to streptozotocin injection (35 mg/kg). The DM-C500 and DM-C1000 groups were maintained on HFD and supplemented orally during four weeks with powdered *C. racemosa* at 500 and 1000 mg/kg of body weight, respectively. The DM-C0 group was fed with HFD without *C. racemosa* supplementation. All the experimental rats were maintained on HFD during the 30 days of experiment. *C. racemosa* at 500 mg/kg improved fasting glycaemia and glucose tolerance. The IPGTT test revealed a decrease ($p<0.05$) in the fasting glycaemia recorded at the 120th min from day 0 (534 ± 38.88 mg/dL) to day 30 (326 ± 63.05 mg/dL). *C. racemosa* supplementation prevented liver lipid peroxidation in DM-C500 and DM-C1000 group (12.94 ± 2.20 and 10.48 ± 1.15 nmol MDA/g, respectively) compared to DM-C0 group (35.49 ± 2.30 nmol MDA/g). *Caulerpa racemosa* at 500 mg/kg, and relatively at 1000 mg/kg, alleviated pancreatic, liver and renal tissue damages compared to DM-C0 groups which displayed injuries in their histological sections. *Caulerpa racemosa* oral supplementation could represent a possible natural approach to prevent organic and metabolic disorders related to type 2 diabetes.

Keywords: *Caulerpa racemosa*; Diabetes; Oral supplementation; IPGTT; Lipid peroxidation; Histopathology.

1. INTRODUCTION

Diabetes is a complex and life-threatening disease that causes 4.2 million deaths worldwide each year. Its prevalence has increased in recent decades in most developed and developing countries and the total number of diabetic will reach 700 million by 2045 if no effective prevention methods are adopted [1].

Type 2 diabetes mellitus, formerly known as non-insulin dependent diabetes mellitus, is the most common form of diabetes mellitus resulting from interaction between genetic, environmental and behavioral risk factors [2]. This metabolic disease is characterized by chronic hyperglycemia subsequent to a

defective insulin action, insulin secretion or both [3]. Chronic hyperglycemia induces numerous forms of both micro-and macrovascular complications [4], which often lead to premature death [2]. Even if type 2 diabetes has long been identified as an incurable chronic disease, the best outcome that has been expected is amelioration of diabetes symptoms or delaying its inevitable progression [5]. So, prevention and control of diabetes goes through the improvement of blood glucose, lipid profile and oxidative damages to ovoid organic disturbances related to diabetes complications.

Traditional medicine, using terrestrial and marine resources, continues to be used around the world to treat diabetes and delay its complications. The development of natural resources as supplement or alternative to drugs is a concern that is becoming increasingly important in many countries. However, it is recommended to evaluate the safety and efficacy of herbal medicines with a view to standardizing their use and integrating them into conventional care systems [6].

Like terrestrial medicinal plants, seaweeds are also prescribed for many diseases in different traditional Asian medical systems [7]. Algae are known as a potential source of bioactive compounds with anti-diabetic [8], antioxidant and anti-cardiovascular activities [9-10]. Moreover, epidemiological studies comparing South-East Asian and Western-style diets have reported an association between the dietary intake of marine algae and a reduced prevalence of chronic diseases, including diabetes, hyperlipidemia and cardiovascular diseases [11].

Among marine seaweed, the green algae *Caulerpa racemosa* is known for its high nutritional value, including polyunsaturated fatty acids (PUFA), essential amino acids, minerals, dietary fibers, vitamins and natural bioactive compounds. The presence of natural bioactive compounds in *C. racemosa* contributes to its antioxidant, anticoagulant, antibacterial, anticancer and even antidiabetic power [12]. Studies performed on the anti-diabetic effect of *C. racemosa* were conducted by testing the in vitro [13-14] or in vivo [12] hypoglycemic effect of its extracts, but to our knowledge, no studies have been conducted on the effect of oral supplementation with whole *C. racemosa* on type 2 diabetes and its complication prevention. Our objective is therefore to preliminary assess the effect of this supplementation on a possible prevention of type 2 diabetes complication through the evaluation of biochemical and histological parameters in induced-diabetes rats.

2. MATERIALS AND METHODS

2.1. *Caulerpa racemosa* harvesting

The green alga *Caulerpa racemosa* was collected from Salamandre station (Latitude N 35 54’37.94”, Longitude E0 3’17.37”) in the coast of Mostaganem (Algeria), at a depth of 2 meters by a professional scuba diver. After harvesting, the algae were washed and then shade-dried. Dried samples were powdered, sieved and then stored.

2.2. Induction of type 2 diabetes model in rats

Twenty-four female Wistar rats weighing between 95 g and 120 g were purchased from the National Pasteur Institute (Algiers, Algeria). Rats were installed in polypropylene cages in a room maintained at 24 ± 2°C and subjected to 12 h/12 h light/dark cycle. Rats had free access to standard chow and water for one week. The composition of the standard chow (EL Alef, Tlemcen, Algeria) was: carbohydrates 31%, crude protein 16%, crude fat 3%, crude cellulose 3.9%, crude ash 4.9%, moisture 14%, and vitamins 1.7%. After one week of adaptation, the rats were divided randomly into two groups with similar mean body weights: the control group (n=6) was maintained on standard chow (2.15 Kcal/g) and the experimental group (n=18) was

subjected for eight weeks to high fat diet (HFD) and then streptozotocin injection in order to induce type 2 diabetes model according to Parveen et al. method [15] with slight modifications. For making HFD, sheep tallow was incorporated at a rate of 40% to standard chow. The total caloric value of the HFD was 5.75 kcal/g with 43% of calories from fat. After this period, all rats were fasted and injected intraperitoneally with streptozotocin (STZ 35 mg/kg, dissolved in citrate buffer, pH 4.5). The rats of the control group were injected with a citrate buffer solution. After 7 days, fasting blood glucose levels were checked using glucometer (Vital Check, Korea). All rats in the experimental group had fasting blood sugar levels greater than 140 mg/dL and displayed impaired glucose tolerance by the intraperitoneal glucose tolerance test (IPGTT) (see section 3.3).

2.3. Oral supplementation with *Caulerpa racemosa*

The experimental group (DM: diabetes model) was divided into 3 groups:

- DM-C1000: DM rats maintained on HFD + oral supplementation with *Caulerpa racemosa* at 1000 mg/kg of body weight.
- DM-C500: DM rats maintained on HFD + oral supplementation with *Caulerpa racemosa* at 500 mg/kg of body weight.
- DM-C0: DM rats maintained on HFD without oral supplementation with *Caulerpa racemosa*.

The control group (CTRL) was maintained on the standard chow throughout the experiment.

This protocol was approved by the council of Food Technology and Nutrition Laboratory (The Faculty of Sciences of Nature and Life, University of Abdelhamid Ibn Badis, Mostaganem (Algeria). The animals were treated according to the instructions of the Council of the European Communities on the protection of animals used in scientific investigations (86/609/CEE) [16].

2.4. Intraperitoneal glucose tolerance test (IPGTT)

The intraperitoneal glucose tolerance test (IPGTT) was performed in all rats after an overnight fasting. A solution of D-glucose (20%, w/v) freshly prepared in distilled water was injected intraperitoneally in the experimental and control rats (2 g glucose/kg body weight). The glycaemia was measured with glucometer in the blood collected from the caudal vein before (0 min) and 30, 60 and 120 min after administration of D-glucose. The IPGTT test was performed 3 times: before the treatment (d0), 15th day (d15) and finally on the 30th day (d30) of the experiment. The total area under curve (AUC) was computed in each individual experiment.

2.5. Food intake and body weight

Food intake was monitored daily and body weight weekly.

2.6. Sacrifice and sample collection

After 30 days of supplementation (experiment period), the rats were sacrificed after an overnight fasting, under anesthesia provoked by the intraperitoneal injection of ketamine. The blood samples were obtained from the heart and placed in EDTA tubes. The blood was centrifuged and the plasma was used to determine biochemical parameters. The pancreas, liver, and kidney were collected and a portion of each organ was fixed for a histological study or kept at -20°C for further analyses.

2.7. Biochemical measurements

Fasting blood sugar (GODPOD method), total cholesterol (CHOD-PAP method) and triglycerides (GPO-PAP) were determined using Biomaghreb kits (Tunisia). Creatinine (Jaffe kinetic reaction), urea (urease

and glutamate dehydrogenase methods), alanine transaminase (ALT), aspartate transaminase (AST), gamma-glutamyl transferase (GGT) and alkaline phosphatase (ALP) activities were measured using COBAS systems (Roche Diagnostic, Germany).

2.8. Estimation of lipid peroxidation in the liver by the Tbars method

Lipid peroxidation in liver was assessed according to Genot method [17]. Briefly, 2 g of the liver was placed in a tube containing 16 ml of 5% trichloroacetic acid and 100 µl of vitamin C. The mixture was homogenized and then filtered. From this filtrate, 2 ml were added to 2 ml of thiobarbituric acid. The tubes were immersed in a water bath at 70°C for 30 min and then placed in a cold water bath. The assay absorbance was measured at a wavelength of 532 nm. The results were obtained by the following formula: MDA rate (nmol/g of liver tissue) = [(0.72/1.56) × (A532 × Solvent volume × Filtrate volume) / sample weight (g)] / 72.0636.

2.9. Histological study

The organs (pancreas, liver and kidney) were fixed in a 10% (w/v) formaldehyde solution and embedded in paraffin. Sections (5 µm) were deparaffined and rehydrated for hematoxylin-eosine staining (H&E). The organ slides were examined under an optical microscope (Olympux CX22LED, Tokyo) at x40 magnification.

2.10. Statistical analysis

All results were expressed as a mean ± standard deviation (SD). The data were compared using a unidirectional variance analysis (ANOVA) followed by a post-hoc Duncan test. The statistical significance of the differences between the dependent mean values (IPGTT and AUC) was evaluated using the paired Student t test. $P<0.05$ were considered significant. All data were statistically evaluated using the SPSS Statistical Software version 20.0 (Chicago, IL, USA).

3. RESULTS AND DISCUSSION

This study is a preliminary study that assessed the effects of oral supplementation with *Caulerpa racemosa* on biochemical and organic parameters in type 2 diabetes induced by high-fat diet and a single low-dose of streptozotocin. In the present study, the supplementation with sheep tallow in experimental groups at a rate of 40% (w/w) combined to streptozotocin injection induced hyperglycemia exceeding 140 mg/dL. This fasting blood glucose criterion was reported in some studies [15, 18-19] that induced type 2 diabetes in Wistar rats by the combination of high fat diet consisting in 40% calories from fat and low dose streptozotocin. It reported that phenotype and the pathogenesis of Human type 2 diabetes could be mimed in rats by means of a HFD to induce peripheral insulin resistance followed by low dose STZ injection which targets the pancreatic β-cells [20-21]. The diabetes model induced in these rats was a good tool to assess the effect of *C. racemosa* supplementation on delaying diabetes complications.

Weight gain in orally supplemented rats with whole powdered *C. racemosa* indicates that this alga was well tolerated by rats and did not interfere with their growth (Fig. 1). Nevertheless, it should be noted that supplementation at 500 mg/kg was effective in slow down weight gain (12.5 ± 4.18 g) (Fig. 1). Moreover, the food intake (Table 1) of DM-C500 group decreased markedly from day 1 to day 30 of the experiment ($p<0.05$). It is well known that algae are a good source of dietary fibers that prolong the rate of gastric emptying and thus increase satiety and reduce food intake [11].

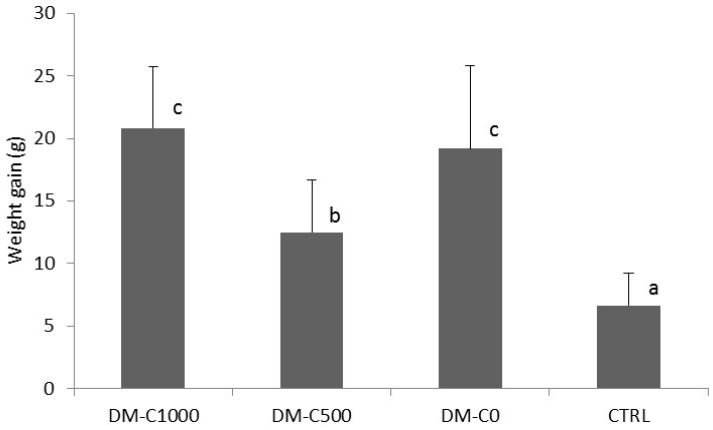

Figure 1. Body weight gain of rats from different groups over the 30 days of experimental period (mean ± SD, n=6).

Besides, γ-sitosterol which has been identified and for the first time in the invasive *C. racemosa* from the west coast of Algeria (Mostaganem coast) [22], is reported to improve body weight and reduce dietary intake in diabetic rats [23]. Surprisingly, at 1000 mg/kg, *C. racemosa* failed to slow the body weight gain of rats (20.83 ± 4.91 g) and their food intake failed to differ significantly between the beginning and the end of the 30 days experiments ($p>0.05$) (Table 1). The weight gain observed in this group could be explained by the relative toxicity of *C. racemosa* at the dose of 1000 mg/kg (see below) which could interfere with body weight control probably by affecting hormonal and/or nervous system.

Table 1. Calorie intake (Kcal/day) of control and experimental rats at the beginning (day 1) and the end (day 30) of experiment period (mean ± SD, n=6).

Groups	Day 1	Day 30
DM-C1000	200.40 ± 32.87^{b}	190.80 ± 54.64^{b}
DM-C500	$202.80 \pm 40.23^{b*}$	$142.80 \pm 13.21^{a**}$
DM-C0	211.20 ± 6.78^{b}	218.40 ± 8.31^{b}
CTRL	107.50 ± 3.51^{a}	106.42 ± 4.11^{a}

Index letters (a, b, c) indicate a significant difference between different groups, * indicates a significant difference inside group ($p< 0.05$).

Decreased in dietary intake and body weight gain would contribute in reducing fasting blood sugar in DM-C500 rats to a level comparable to that of control rats (Table 2). In parallel, an improved glucose tolerance was observed in these rats after 30 days of *C. racemosa* supplementation. Indeed, the total AUC (Fig. 2) in the DM-C500 group tended to lower significantly ($p<0.05$) from d0 (574.86 ± 58.7 g/L/min) to d30 (474.98 ± 57.52 g/L/min), whereas this decrease was negligible ($p>0.05$) in the DM-C1000 group (574.87 ± 42.98 g/L/min and 542.77 ± 76.81 g/L/min, respectively at d0 and d30). So, it seems that *C. racemosa* at 500 mg/kg had a positive impact on glucose homeostasis and reversed hyperglycemia in DM-C500 group (Table 2).

This improvement is reflected by pancreatic islet structure (Fig. 3) which was preserved in DM-C500 group. Glucose intolerance was not well improved in DM-C1000 rats; nevertheless, their fasting blood glycaemia was lower than 300 mg/dL contrary to the DM-C0 (Table 2) whose pancreas slide (Fig. 5) revealed islet cell vacuolization, acinar cell degeneration and obvious accumulation of lipid droplets inside some islets.

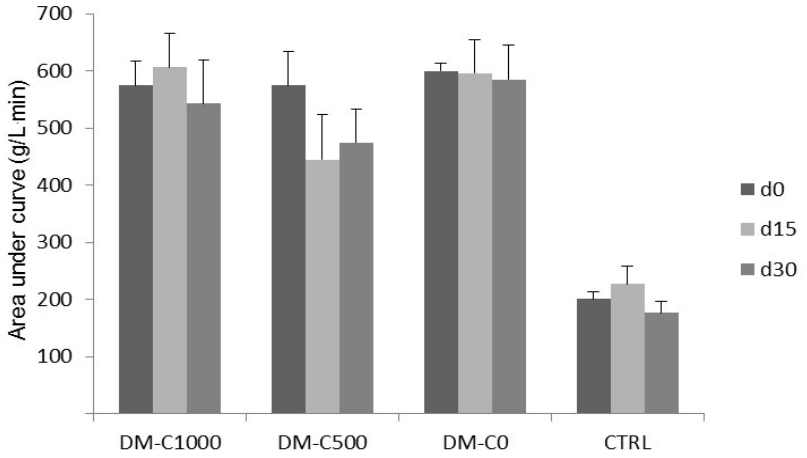

Figure 2. Area under the curve (AUC) during the IPGTT test performed on days d0, d15 and d30 of the experimental period (mean ± SD, n=6).

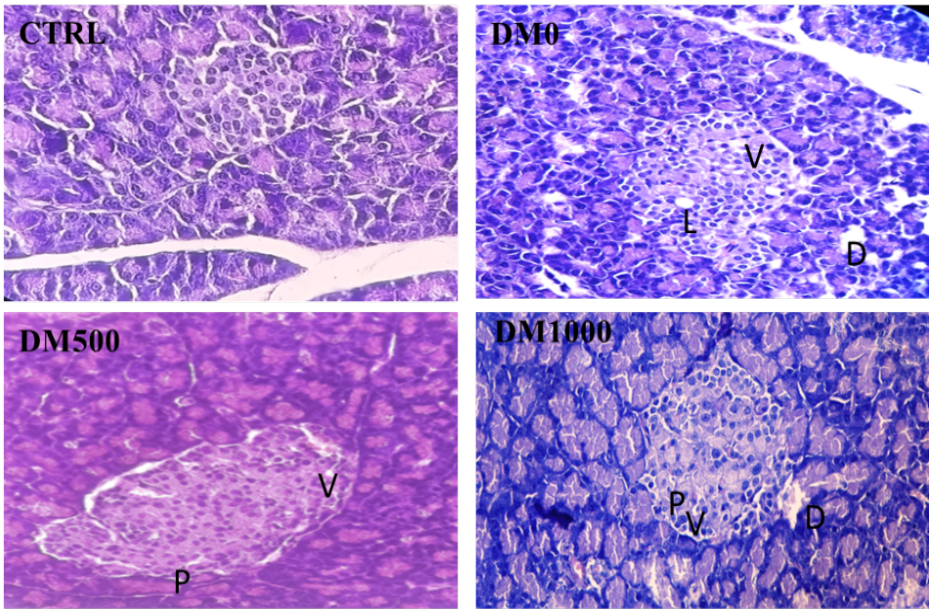

Figure 3. Photomicrograph of pancreas tissues from control and experimental groups rats (H&E staining, 400X). D: degeneration; L: lipid droplet; P: pycnotic nucleus V: vacuolization.

Besides improving impaired glucose homeostasis, *C. racemosa* supplementation seemed to overcome lipid metabolism disturbances induced by high fat diet. This seaweed supplementation resulted in a dose-dependent decrease in plasma total cholesterol and triglycerides. In fact, among the experimental groups, DM-C1000 displayed the lowest total cholesterol and triglycerides levels followed by DM-C500 than DM-C0 rats (Table 2). Dietary fibers from seaweed are known to decrease intestinal circulation of bile acids and thereby up-regulate bile acid synthesis from cholesterol, which results in a decreased serum cholesterol level [24]. Besides, phytosterols contained in seaweed contribute to decrease intestinal cholesterol absorption. Seaweeds are reported to alleviate liver steatosis and normalize liver enzymes activities [24-25]. At this fact, *C. racemosa* supplementation improved hepatic steatosis especially in DM-C500 group (Fig. 4).

Table 2. Plasma biochemical parameters of control and experimental groups (mean ± SD, n=6).

Rat groups	DM-C1000	DM-C500	DM-C0	CTRL
Fasting glucose (g/L)	2.44±0.59 ^{b}	1.05±0.25^{a}	3.34±0.65^{c}	1.21±0.2^{a}
Total cholesterol (g/L)	0.46±0.05^{a}	0.54±0.08^{ab}	0.61±0.06^{b}	0.52±0.03^{a}
Triglycerides (g/L)	0.41±0.04^{a}	0.51±0.13^{a}	0.64±0.2^{a}	0.47±0.02^{a}
AST (U/L)	126.50±27.25^{b}	102.25±13.07^{ab}	127.00±15.51^{b}	76.75±5.06^{a}
ALT (U/L)	43.00± 8.16^{b}	35.75±6.18^{ab}	41.25±3.77^{b}	28.75±2.87^{a}
GGT (U/L)	2.20±1.10^{b}	1.20±0.45^{ab}	1.15±0.82^{ab}	0.30±0.34^{a}
ALP (U/L)	134.00±14.69^{c}	75.75±9.53^{b}	283.50±22.17^{d}	34.50±4.04^{a}
Urea (g/L)	0.25±0.03^{a}	0.28±0.0^{a}	0.41±0.14^{b}	0.29±0.02^{a}
Creatinine (mg/dL)	4.95±0.28^{b}	4.88±0.28^{b}	5.59±0.27^{c}	4.11±0.29^{a}

The index letters (a, b, c, d) indicate a significant difference ($p<0.05$) between different groups of rats. ALT: alanine transaminase, AST: aspartate transaminase, GGT: gamma-glutamyl transferase, ALP: alkaline phosphatase.

In fact, lipid droplets faded, the liver tissue seems to recover its normal structure recalling that of the control animal and even binucleated cells were observed indicating possible cell regeneration. *C..racemosa* is a good source of Polyphenols [22] that act as antioxidants, reducing liver fat accumulation, mainly by inhibiting lipogenesis [26].

The decrease of food intake, the decrease the body-weight gain and the improved glucose homeostasis contributed to alleviate the liver steatosis and the disturbances of plasma liver enzymes activities in DM-C500 rats (Table 2). Besides, significant positive correlations were found between fasting glycaemia and AST ($r=0.669$, $p=0.005$), and ALP ($r=0.854$, $p=0.000$). However, no significant correlation was found between fasting glycaemia and ALT ($r=0.456$, $p=0.076$). Only some lipid droplets were depicted in DM-C1000 liver sections compared to those from DM-C0 whose liver parenchyma was disorganized and much disrupted hepatocyte structure associated with pycnotic nuclei was observed (Fig. 4). Furthermore, numerous lipid droplets, centrilobular mononuclear cells infiltration and numerous Kupffer cells were observed. According to Motshakeri et al. [27], Kupffer cells are activated in response to the high-fat diet. These histopathological evidences were in agreement with plasma levels of AST, ALT, and ALP which were higher in DM-C0 group (Table 2). The increase in these parameters reflects hepatocellular injury resulting in leakage of these cytosolic enzymes from damaged hepatocytes into blood [28] due to various reasons including liver cell necrosis, hepatitis, cirrhosis, and hepatotoxicity of some drugs [29]. At this fact, the high levels of these enzymes in the DM-C1000 group would reflect relative toxicity of *C. racemosa* at the 1000 mg/kg. Some algae at excessive dose could have a toxic effect on some organs. This fact was reported in Motshakeri et al. study [27] where oral administration of *Sargassum polycystum* extract at an excessive dose becomes toxic and has a deleterious effect on liver.

The hepatoprotective effect of *C. racemosa* at 500 mg/kg could be attributed to its positive impact on liver lipid peroxidation. Based on the obtained results, rats in the DM-C0 group who received only the high-calorie food without supplementation with *Caulerpa racemosa* had a lipid peroxidation almost 3 times higher (35.49 ± 2.30 nmol MDA/g) compared to DM-C500 (12.94 ± 2.20 nmol/g) and DM-C1000 (10.48 ± 1.15 nmol/g) and control group (11,02 ± 0.98 nmol/g) ($p<0.001$) (Fig. 5). Despite the fact that a long-term diet rich in saturated fats acts as an inducer of lipid peroxidation in the liver [30], this oxidation was prevented in orally supplemented rats with *C. racemosa*. This seaweed is known for its high content of phenolic compounds that have antioxidant power which would contribute in reducing lipid peroxidation. On the other hand, this

protective effect against lipid peroxidation could also be attributed to the presence of squalene in *C. racemosa* [22,31]. Squalene is recognized as a highly effective anti-radical agent, particularly against lipid peroxidation [31]. So, by reducing lipid peroxidation in liver, hepatocyte cell membrane structure and function were protected against free radicals that can be generated as a result of lipid peroxidation.

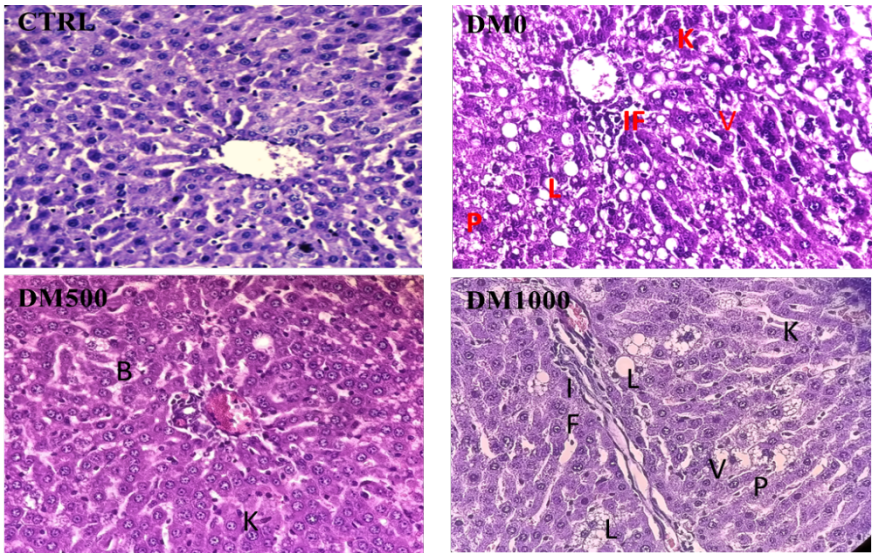

Figure 4. Photomicrograph of liver tissues from control and experimental groups rats (H&E staining, 400x).
B: binucleated hepatocytes; K: Kupffer cells; IF: cell infiltration; L: lipid droplet; P: pycnotic nucleus; V: vacuolization.

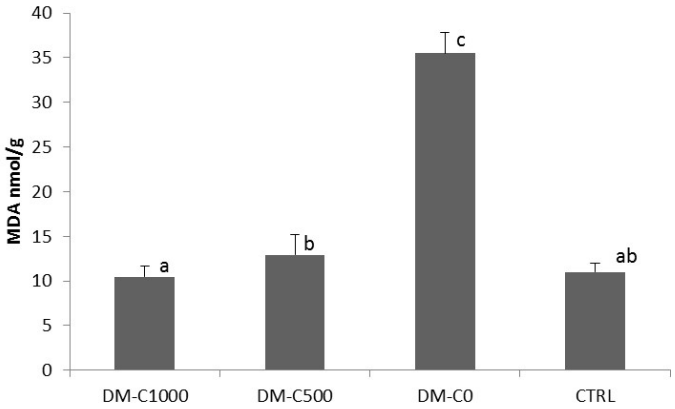

Figure 5. Lipid peroxidation in livers from control and experimental rats (mean ± SD, n=6). The index letters (a, b, c) indicate a significant difference ($p < 0.05$) between different groups of rats.

Examination of kidney histological slices was also performed in order to assess the effect of oral administration of whole *C. racemosa* on this organ. Renal histological sections revealed renal tissue injuries in DM-C0 group (Fig. 6). The animals of this group exposed to only high fat diet/low dose streptozotocin displayed disrupted structure of both proximal and distal tubules and even degenerated tubular cells. Hyperglycemia and hyperlipidemia contribute to renal tissues damages and brought about degenerations in convoluted tubules in the renal cortex [32]. These injuries seemed to be less extensive in DM rats orally supplemented with *C. racemosa* especially in those supplemented at 500 mg/kg. In fact, oral supplementation at 500 mg/kg did not affect renal cortex. The renal slides showed normal architecture with regular distal and

proximal tubules; glomeruli structure and Bowman space did not differ from those of control rats. At 1000 mg/kg supplementation, glomeruli had a compressed Bowman's space and most convoluted tubules had small lumen. Nevertheless, these rats displayed with DM-C500 group the lowest plasma urea and creatinine ($p<0.05$) compared to DM-C0 rats. The serum levels of urea and creatinine are monitoring tests of renal function, however the creatinine is a much more reliable indicator of renal function than the urea because the urea is far more likely to be affected by dietary and physiologic conditions not related to renal function [33]. The renoprotective effect exerted by *C. racemosa* supplementation at 500 mg/kg could be attributed to their ability to inhibit oxidative stress induced by hyperglycemia. At this fact, Cao et al. [34] reported that polysaccharides of *C. racemosa* possess antioxidant and anti-inflammatory properties that can be applied to prevent progression of diabetic nephropathy. However, *C. racemosa* at 1000 mg/kg had a less improving effect on kidney and could even have a slight toxic effect on it like the one exerted on liver.

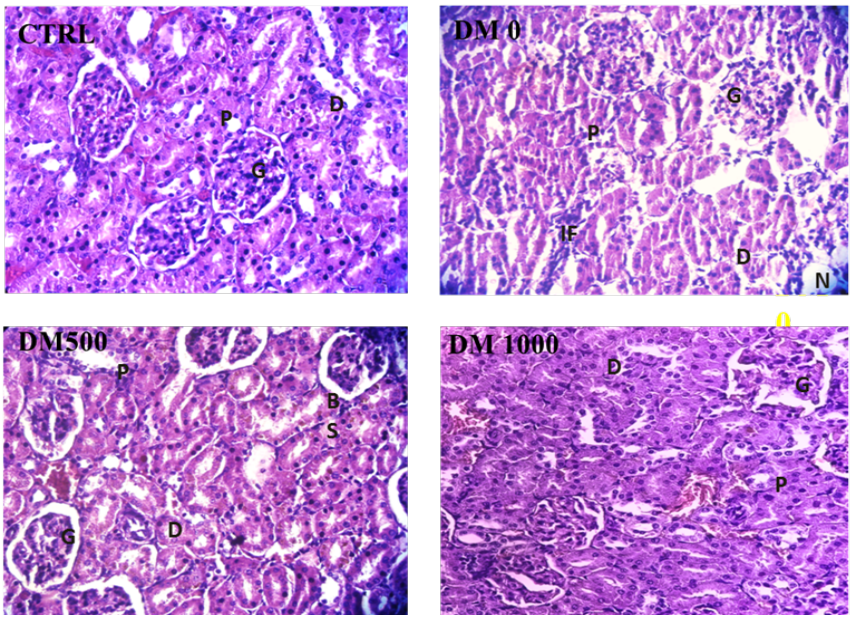

Figure 6. Photomicrograph of renal cortex tissues from control and experimental groups rats (H&E staining, 400x). BS: Bowman's space; D: distal tubule; G: glomeruli; IF: cell infiltration; P: proximal tubule; V: vacuolization.

4. CONCLUSION

The present preliminary study indicates that oral supplementation with powdered *Caulerpa racemosa* at 500 mg/kg in rats was well tolerated and did not cause any damage on the organic and biochemical parameters. The data suggest that at this dose, *C. racemosa* exerted beneficial effect on the body mass and glucose homeostasis by improving glucose tolerance and thereby hyperglycemia. Thus, the oral administration of *C. racemosa* can offer a new natural approach to the management of diabetes and the delay of its associated metabolic and organic disorders. Further investigations on the anti-inflammatory activity of *C. racemosa* and its effect on the antioxidant defense system are needed. Finally, the possible toxicity effect of excessive dose of *C. racemosa* on body weight control and different tissues should be explored.

Authors' Contributions: LB planned, conceptualized, designed the experiment. Both LB and NEH contributed to the animal experiment and histology. NEH carried out the blood and Tbars analyses. Data analysis and histology

interpretation were performed by LB. NEH wrote the draft of article and LB restructured and completed it. Both authors approved to the final version of the manuscript.

Conflict of Interest: The authors declare no conflict of interest.

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