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Salicornia ramosissima J. Woods seeds affected the normal regenerative function on carbon tetrachloride-induced liver and kidney injury

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ABSTRACT

The growing importance of *Salicornia* plants as bioactive agents and health promoters associated with the continuous demand for alternative treatments for liver disorders, has stimulated us to evaluate the renal and hepatic effects of *S. ramosissima* seeds in mice under normal conditions and exposure to toxic products as carbon tetrachloride (CCl_4). Thus, histopathological and lipid peroxidation evaluations of the liver and kidneys were performed. Powdered dried seeds of *S. ramosissima* (SRS) were administered orally for 22 days at a dose of 2000 mg/kg/day to male mice in three different settings: 1) seed effects, 2) protection against CCl_4 acute toxicity (0.2 mL/kg) and 3) regeneration after acute exposure to CCl_4 (0.2 mL/kg), each study being performed with appropriate control animals. Mice treated with SRS *per se* had slightly enlarged hepatic sinusoids and noticeable renal inflammation.

SRS did not show effective protection against mice exposed to CCl_4 and had no positive influence on liver and kidney recovery after CCl_4 administration. These results demonstrated that SRS failed to improve hepato- and nephrotoxicity, in addition to the apparent synergism between CCl_4 and SRS under these experimental conditions. Although the biological mechanisms of *S. ramosissima* are not fully understood, the evidence suggests further research to elucidate its adverse biological effects.

1. Introduction

Several pharmacological drugs such as alcohol and environmental toxicants are major hazards to liver and kidney, disrupting their physiological functions and posing a serious public health risk. By 2015, liver diseases (chronic and cancer) have affected more than 3 million people worldwide [1]. Therefore, the search for reliable agents that can alleviate this burden is highly desirable. Conventional drugs have limited efficacy and availability, as well as side effects associated with prolonged use [2], highlighting the need for alternative treatments. Many plant extracts and plant-derived compounds are often tested using animal models for their potential in hepato- and nephroprotective activities against hepatotoxic agents such as the well characterized carbon tetrachloride (CCl_4) [3–5].

There has been a growing interest in sapal plants among scientific community, since halophytes have evidenced strong potential as a source of new compounds with therapeutic activity [6]. The annual genus *Salicornia* (Chenopodiaceae) is widely distributed in saline environments and is tolerant to water stress and climate variations, being able to thrive in extreme conditions. Following the above, it has been identified as a promising crop for human and animal farming purposes [7]. In the traditional medicine, *Salicornia* is known for its beneficial properties against diseases such as obesity, nephropathy, hepatitis, cancer, hypertension, headache and scurvy, with experimentally proven biological effects [7,8].

Salicornia ramosissima J. Woods is considered a substitute for green salt and the aerial parts have a nutritional profile suitable for human consumption, and are currently used as salads, pickles and gourmet

Abbreviations: BHT, butylated hydroxytoluene; CYP2E1, cytochrome P450 2E1; GFR, glomerular filtration rate; GSH, glutathione; MDA, malondialdehyde; SRS, *Salicornia ramosissima* seeds; TBA, 2-thiobarbituric acid; TCA, trichloroacetic acid; TMP, 1,1,3,3-tetramethoxypropane

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cuisine. It has been recently shown to be especially prolific in the production of structurally diverse secondary metabolites with antioxidant action [9,10], and to have a rich lipid profile with fatty acids and fatty alcohols in their composition [11]. Similarly, *Salicornia* seeds are rich in nutrients and varied minerals such as potassium, sucrose, glycerol, oleic and linoleic unsaturated fatty acids [12], and used as tea material [13]. Experimentally, it has been demonstrated that *Salicornia herbacea and Salicornia bigelovii* seed extract exhibit antitumor, antioxidant, antibacterial and hypolipidemic activity [13–16].

Although the aerial parts are the most studied, little is known about the bioactivity of *S. ramosissima* seeds (SRS). Considering the recognition of *Salicornia* seed oil as a dietary source with higher nutritional value and significant socioeconomic impact in the future [14], the aim of this study was to assess the preliminary influence of raw SRS on mice under acute toxicological conditions of CCl_4, with a focus on hepatic and renal failure and recovery.

2. Materials and methods

2.1. Chemicals

Carbon tetrachloride (CCl_4, 99.9% purity), butylated hydroxytoluene (BHT), 2-thiobarbituric acid (TBA) and 1,1,3,3-tetramethoxypropane (TMP) were obtained from Sigma Aldrich. Trichloroacetic acid (TCA) was purchased from Panreac. The remaining chemicals were of analytical grade.

2.2. Plant processing

Senescent specimens of *S. ramosissima* (Fig. 1) were collected in November 2012 at Troncalhada saltworks, in the Ria de Aveiro (40° 38′ 43.38″ N°, 8° 39′ 44.59″ W). A voucher specimen was identified by Prof. Helena Silva and deposited in the Herbarium of the Department of Biology, University of Aveiro, Portugal (AVE), under the reference ″AVE 6606″. Samples were further air dried at room temperature for 1 week. To recover the seeds, the dried plants were shaken vigorously into a plastic tray and the remains was passed through 2 and 1 mm sieves, and then through 0.5 and 0.355 mm sieves to clean the sample from residues. The resulting seed quantity (115 g) was homogenized with a blender to a fine powder and stored at room temperature.

2.3. Animals and experimental design

Five-week-old male CD-1 mice, supplied by Harlan laboratories (Barcelona, Spain) and weighing 34.71 ± 3.83 g, were housed in polycarbonate cages placed in a heated chamber suitable for small rodents under standard conditions: constant temperature of 22 ± 2 °C, relative humidity of 40–60%, and light/dark photoperiod of 12 h. Mice were fed with rodent chow (A04, SAFE diets, France) and water *ad libitum*, and were acclimatized to laboratory conditions for one week, prior to the 23 days experimental study.

Animals were randomly divided into 6 experimental groups (n = 4), as follows: Group I (W): control mice, orally treated with water for 22 days; Group II (SRS): exposed orally with SRS suspension (2000 mg/kg b.w./day) for 22 days; Group III (W + CCl_4): exposed orally with water for 22 days, followed by a single subcutaneous injection (250 µL) of CCl_4 (0.2 mL/kg b.w.) in olive oil on day 22; Group IV (SRS + CCl_4): orally exposed with SRS suspension (2000 mg/kg b.w./day) followed by administration of CCl_4, as mentioned above, on the 22nd day; Group V (CCl_4 + W): treated with a single injection of CCl_4 on the first day and water; Group VI (CCl_4 + SRS): treated with a single injection of CCl_4 on the first day, followed by oral SRS suspension (2000 mg/kg b.w./day).

Groups I and II refer to studies of SRS *per se*, while groups II and IV relate to protection studies and groups V and VI to regeneration studies, respectively, before or after administration of CCl_4 (Fig. 2).

The limit test dose of 2000 mg/kg b.w. for SRS was used, based on the OECD-423 guidelines [17] and previous studies [18,19]. The selected dosage of CCl_4 was made according to the study of Irie and colleagues [20] as sufficient to cause acute and non-lethal liver injury. Mice were euthanized by cervical dislocation 24 h after the last assigned treatment. The animals were handled as humanely as possible and all experiments followed the national guidelines and the European Directive (2010/63/EU) on the care and use of laboratory animals.

2.4. Body and organ weight measurement

Body weights were recorded at necropsy. The liver and kidneys were quickly removed, washed with phosphate buffer saline, and weighed. Liver and kidney index was calculated as gram per 100 g of body weight.

2.5. Histological studies and semi-quantitative analysis

Portions of hepatic and renal tissue were used for histopathological

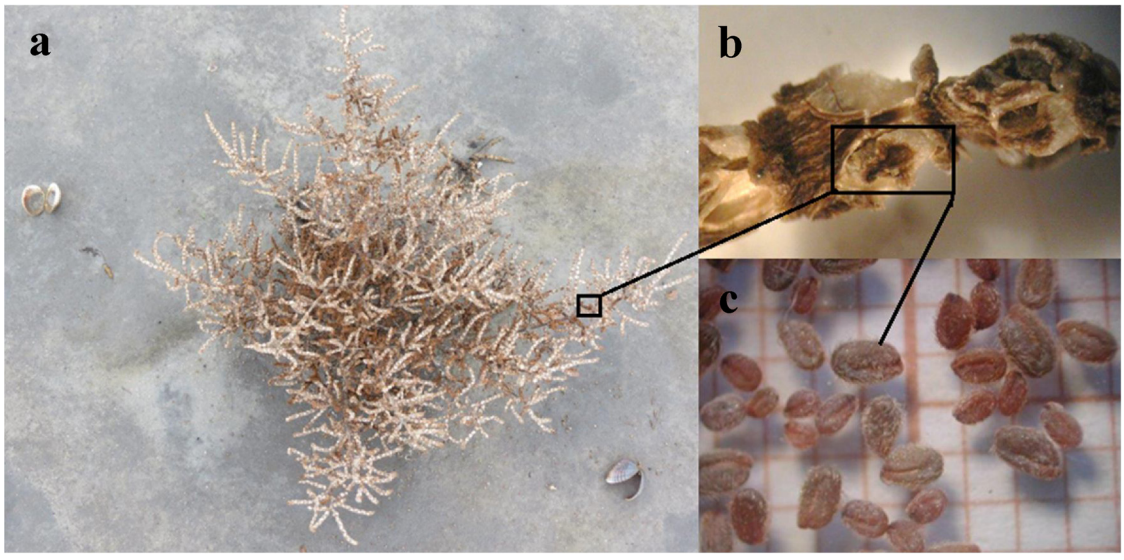

Fig. 1. *Salicornia ramosissima* in natural habitat (a); fructiferous branches (b) and seeds (c).

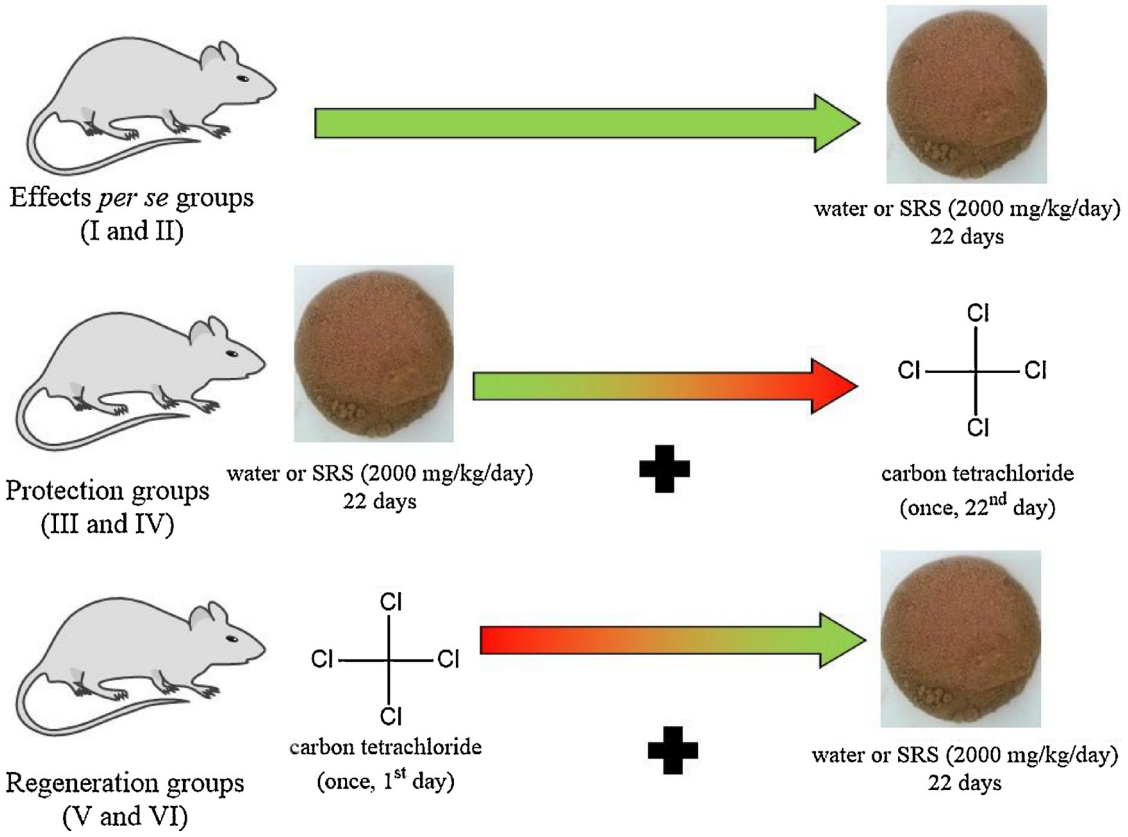

Fig. 2. Schematic representation of the animal study design.

studies. Sections (5 μm thick) were prepared, stained with hematoxylin and eosin dye and examined microscopically for cellular abnormalities. Pearse's Ziehl-Neelsen long staining method was used when appropriated. Histological lesions of liver and kidney were classified according to a previously described semi-quantitative score [21]. One section of each organ for each group was considered. The lesion's score (percent of damaged tissue) was evaluated as follows: 0: absent, 1: minimal (< 10%), 2: mild (10–39%), 3: moderate (40–79%), 4: marked (80–100%).

2.6. Determination of lipid peroxidation in mouse tissues

Immediately after collection, portions of liver and kidney were frozen in liquid nitrogen and stored at −80 °C. Lipid peroxidation was estimated on its malondialdehyde (MDA) content, using a third order selective derivative spectrophotometric method [22], with slight modifications. Therefore, samples (200 mg) were homogenized in the presence of 0.8 ml of 5% aqueous trichloroacetic acid (TCA) and 0.5 ml of 0.8% butylated hydroxytoluene (BHT) in hexane, and the mixture was centrifuged at 3000 x g for 3 min. The top layer was discarded and the bottom layer was prepared to 2.5 ml volume with 5% TCA in a screw capped tube, in which a volume of 1.5 ml of 0.8% aqueous 2-thiobarbituric acid was also added.

After incubation at 70 °C for 30 min, the mixture was cooled at room temperature and submitted to conventional spectrophotometry (Shimadzu, model UV-2501PC) in the range of 400–650 nm with a scanning speed of 562 nm/min. The third order derivative spectra were produced by electronic differentiation of the normal absorption spectrum of the analyzed samples, using a derivative configuration derived from 20 nm. The concentration of MDA (ng/g wet tissue) was calculated on the peak height of the third order derivative at 541.5 nm, referring to slope and intercept data of the calculated least squares fit of a standard curve of freshly prepared 1,1,3,3-tetramethoxypropane. The data were recorded with UV Probe software (Shimadzu Corp., Kyoto, Japan).

2.7. Statistical analysis

All values are presented as mean ± standard error of mean (SEM). The experimental data were computed statistically and tested for normality using the Shapiro-Wilk test prior to any further statistical analysis. Two-way ANOVA using oral treatment (with or without SRS) and toxic exposure (absence or presence of CCl_4) as factors and followed by Sidak multiple comparisons test were used to analyze organ indexes and MDA levels among groups. In addition, groups I, II and V were tested by one-way ANOVA, followed by Dunnett's test. Survival was analyzed by the log-rank test and expressed as Kaplan-Meier curves. A value of $p < 0.05$ was considered significant.

3. Results

3.1. Mice survival and behavior

The experimental period revealed no mortality from group I to V (100% survival), while the survival rate was reduced to 25% in group VI (CCl_4 + SRS) at the end of the study ($p < 0.05$ vs CCl_4 + W). Deaths occurred between 24 h and 8 days after administration of CCl_4 (Fig. 3). Although no significant changes in the behavior and physical appearance of groups I and II were observed, mice exposed to CCl_4 presented, 24 h after the injection, lusterless fur and pilo-erection, less activity and poor response to handling. Group V and the remaining mice from group VI recovered gradually until the end of the experiment.

3.2. SRS effects per se

Table 1 shows all the histopathological findings of this experimental

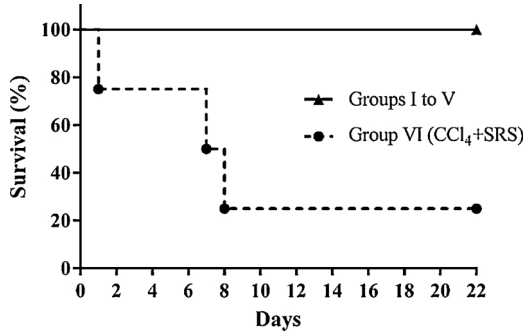

Fig. 3. Increased mortality in carbon tetrachloride (CCl_4)-treated mice following SRS administration. In the regeneration studies, mice were given a single subcutaneous injection of 0.2 mL/kg CCl_4 and treated with or without SRS for 22 days. Mice survival was monitored during the referred period. Notice that all animal deaths occurred within the first 8 days after intoxication. The difference in survival rate between the CCl_4 + W and CCl_4 + SRS groups was statistically significant ($p = 0.0401$).

study. The hepatic and renal control profile (group I) evidenced normal features (Figs. 4A and 5A). The liver index of animals treated with 2000 mg/kg b.w. of SRS *per se* (group II) significantly decreased in comparison to the control (Table 2). The levels of hepatic and renal MDA levels were not statistically significant (Table 3). Histologically, the hepatic parenchyma exhibited a nearly normal structure and organization, in addition to some sinusoidal spaces being dilated and microvesicular (Fig. 4B), being perceptible only in higher magnification. Significant renal alterations were found in the group exposed to SRS: cortical interstitial hemorrhages and infiltration of inflammatory cells (Fig. 5B), associated with surrounding glomerular areas. Glomerular integrity was slightly impaired.

3.3. Protection study

Concerning to group III (W + CCl_4), no significant changes were observed in organ weight and MDA level (Tables 2 and 3). The liver affected by CCl_4 displayed hepatocyte necrosis of the centrilobular zone as the predominant histopathological lesion, together with parenchyma disorganization and hepatocellular degeneration, as evidenced by histological analysis (Fig. 4C). Kidneys exhibited severe glomerular structural changes with different degrees of degeneration, and cellular degradation products and debris within the lumen of the tubules (Fig. 5C). Pre-administration of seeds (group IV) did not positively influence liver histological outcome, as it failed to reduce the extent of CCl_4-induced necrosis (Fig. 4D). Liver weight decreased significantly compared to group III (Table 2) and again MDA levels were not considered significant (Table 3). The renal tissue still evidenced CCl_4-features, such as degenerated glomeruli and impaired tubular epithelium, with additional cellular content within the tubular lumen, suggested as exfoliated tubule cells (Fig. 5D).

3.4. Regeneration study

No significant changes in organ weight and MDA level were observed in group V (CCl_4 + W) when compared to control. Histologically, lobular rearrangement of the liver and some degree of hepatocyte recovery were observed 22 days after centrilobular necrosis, although some cell degeneration still persisted (Table 1, Fig. 4E). Renal sections revealed no necrotic tubular injury, although significant bleeding and inflammatory foci were maintained after 22 days (Table 1, Fig. 5E). Post-treatment with SRS (group VI) caused a high mortality rate within the group (Fig. 3). Histologically, the liver from the remaining animals presented no additional recovery and there was a slight increase in the inflammatory response compared with those from group V (Table 1). In fact, some microgranulomas and yellow-brown pigmented cells were randomly distributed throughout the parenchyma (Fig. 4F). The nature of pigmentation, appearing mainly in Küpffer cells or macrophages, is suggested as lipofuscin or ceroid type, as demonstrated by the modified Ziehl-Neelsen long staining for lipofuscins (Fig. 6). The renal histological sections had several foci of tubular basophilia (Fig. 5F).

4. Discussion

The liver is continually exposed to environmental toxicants, alcohol and drugs, which is a great concern worldwide. Although the current study was designed to investigate the protective and regenerative effects of *S. ramosissima* seeds, different results were obtained. The SRS itself generated slight changes in hepatic tissue and evident inflammatory reactions in the kidney. Although it comprises only a small portion of total body weight, the kidney receives from 20 to 25% of the circulating blood and has an extensive capacity of reabsorption and urinary concentration, which ensures the delivery, uptake and concentration of high levels of toxicants by renal cells, compared with other organs [23]. Contrary to our results, extracts of *Salicornia herbacea* seeds have been reported to have antioxidant activity and cytotoxicity against carcinoma cells *in vitro* [13]. In addition, there were no reports of renal or liver toxicity using *Salicornia herbacea* extract [19] or a *Chenopodium quinoa* (Chenopodiaceae) seed extract [18] at dosages of 2000 mg/kg. Therefore, we hypothesized that SRS may contain toxic substances, since no purification methods were used. *Salicornia* seeds contain several antinutritional factors (e.g. saponins, tannins) that may reduce growth and feed palatability [24], and possibly impair liver weight and histologic appearance of both organs. In fact, saponins have

Table 1
Frequency of hepatic and renal histopathological features.

Histopathological findings	Control (I)	SRS (II)	W + CCl_4 (III)	SRS + CCl_4 (IV)	CCl_4 + W (V)	CCl_4 + SRS (VI)
Liver						
Hydropic degeneration/vacuolar change	0	0	3	3	2	2
Disorganization of hepatic parenchyma	0	2	3	3	2	1
Inflammatory foci	0	1	1	1	1	2
Hemorrhages	0	1	2	2	1	1
Focal necrosis	0	0	3	3	0	0
Microgranulomas	0	0	0	0	0	2
Yellow-brown pigmented cells	0	0	0	0	0	3
Kidney						
Cellular debris within cortical tubular lumen	0	0	4	4	0	1
Glomerular structural changes	0	2	4	3	2	2
Inflammatory foci	1	3	2	2	3	3
Hemorrhages	0	3	1	2	3	3

Lesions were rated as 0: absent, 1: minimal, 2: mild, 3: moderate and 4: marked.

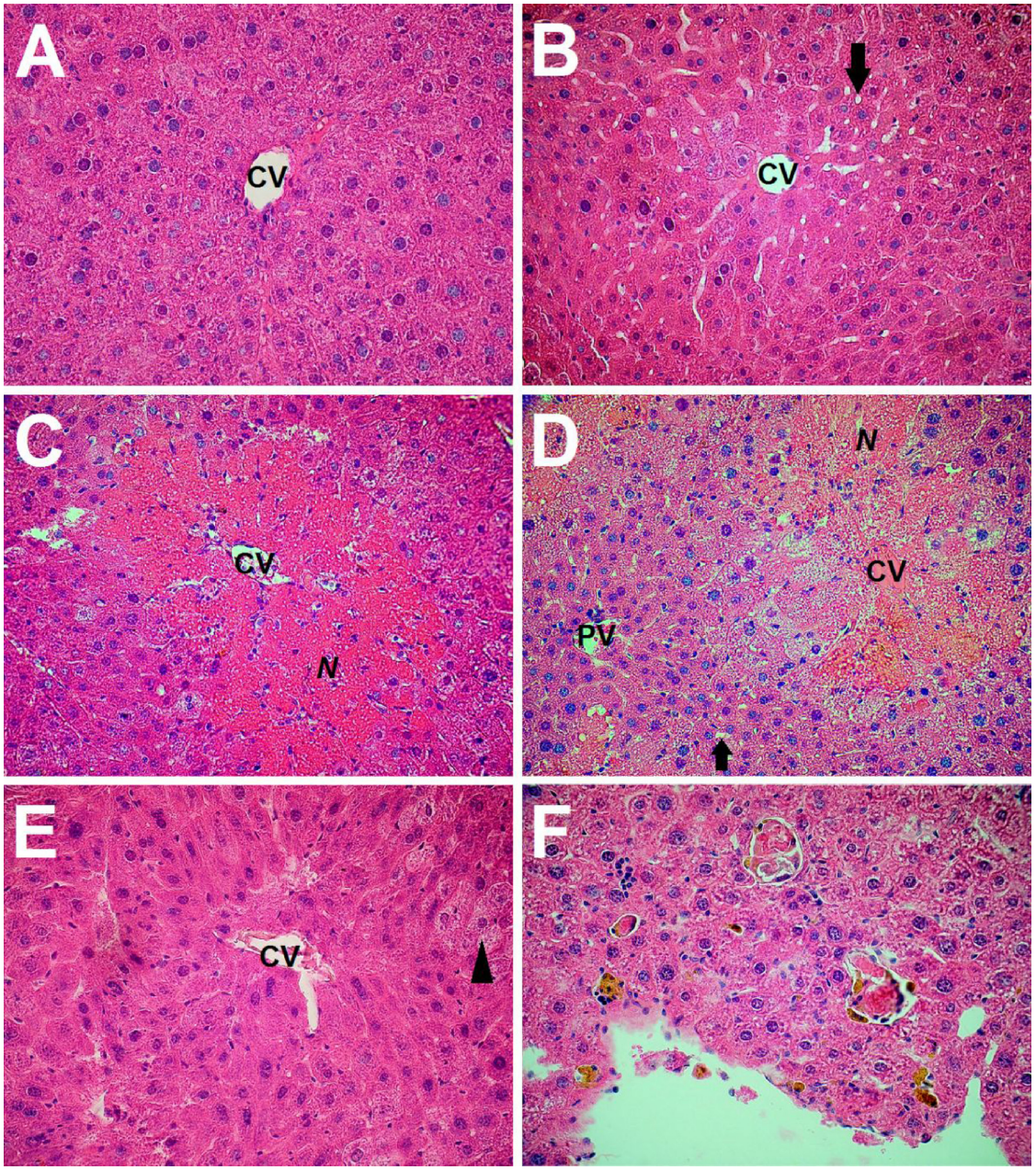

Fig. 4. Microphotographs of mouse hepatic sections: A) Control group; B) SRS-treated group; C) W + CCl_4; D) SRS + CCl_4; E) CCl_4 + W; F) CCl_4 + SRS. Necrosis (N), hydropic degeneration (arrowhead) and microvesicles (arrow) are shown. CV: central vein; PV: portal vein. (H&E stain, 400× original magnifications).

been previously shown to induce hepatorenal toxicity in mice [25]. Finally, the site of harvesting have been another factor that contributed to the toxic reactions, since Troncalhada salt pan is located near a highway route and the risk of contamination with pollutants is higher. Recently, other potential harmful effects of consuming sapal plants, such as *Salicornia* have been reviewed [8].

Regarding the protection study, the observed physical and behavioral response of mice to CCl_4 has also been described previously [26]. The hepatic and renal damage of CCl_4 are well documented and the histopathological findings present are comparable to the literature data [3–5]. Accordingly, hepatic centrilobular area and the renal proximal tubules are major xenobiotic transformation sites and particularly sensitive to chemical insults, since they contain biotransformation enzymes from the cytochrome P450 family, which mediate the formation of toxic metabolites and reactive intermediates [27,28]. CCl_4 is metabolized mainly through the action of the isozyme CYP2E1 in free radicals, which are reactive enough to covalently bind and inactivate CYP2E1. These radicals can react with several important biological substances, mainly fatty acids from membrane phospholipids, leading to lipid peroxidation [29]. MDA is a marker of lipid peroxidation most frequently used and increased levels can be interpreted as resulting from damage to the cell membrane damage due to the increased formation of radicals [30]. At the same time, it has also been proposed that the mechanism by which $CC1_4$ inactivates CYP2E1 is independent of lipid peroxidation [31]. Consequently, some early signs of liver injury such as swelling of hepatocytes, the induction of genes related to the stress/protection and reduction of CYP2E1 mRNA levels are mediated by other independent pathways [32]. Furthermore, dependent events of lipid peroxidation and depleted levels of glutathione (GSH), a molecule which preserves a reducing environment within the hepatocyte, can be restored 24 h after a single CCl_4 injection in CD-1 mice [32,33]. This, together with the low dose of CCl_4 in the present study, could explain

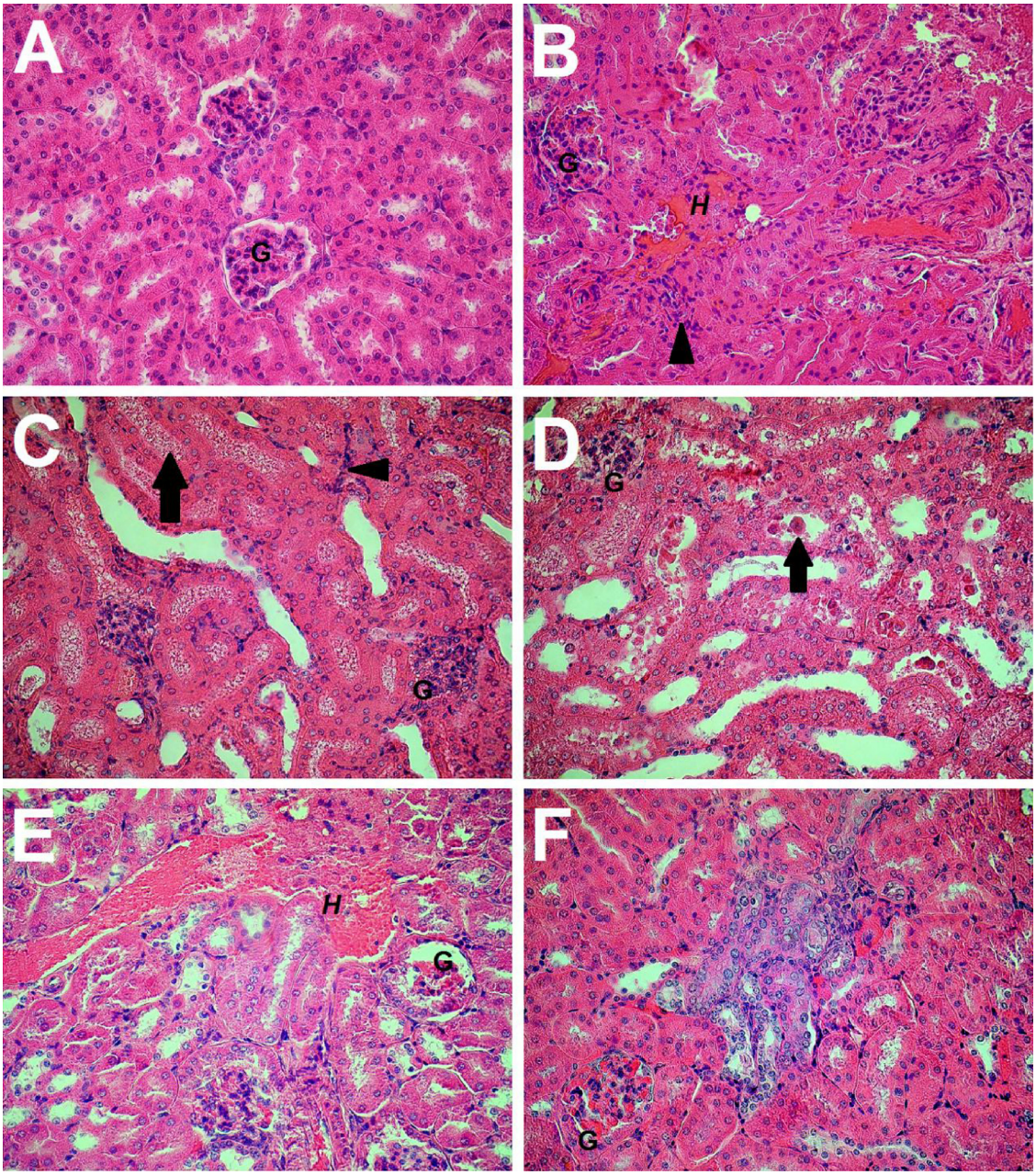

Fig. 5. Renal histological sections: A) Control group; B) SRS-treated group; C) W + CCl_4; D) SRS + CCl_4; E) CCl_4 + W; F) CCl_4 + SRS. Some lesions are presented: hemorrhages (H), leukocyte infiltration (arrowhead) tubular debris (thin arrow). G: glomerulus. (H&E stain, 400× original magnifications).

Table 2
Relative organ weights of experimental groups.

Organ index (g/100 g b.w.)	SRS *per se*		Protection studies		Regeneration studies	
	Control (I)	SRS (II)	W + CCl_4 (III)	SRS + CCl_4 (IV)	CCl_4 + W (V)	CCl_4 + SRS (VI)**
Liver	5.990 ± 0.133	4.951 ± 0.227[a]	5.644 ± 0.386	4.585 ± 0.127[b]	5.050 ± 0.143	5.132 ± 0.0
Right kidney	0.722 ± 0.031	0.702 ± 0.032	0.777 ± 0.034	0.727 ± 0.018	0.720 ± 0.036	0.808 ± 0.0
Left kidney	0.700 ± 0.044	0.675 ± 0.034	0.730 ± 0.027	0.719 ± 0.037	0.706 ± 0.019	0.795 ± 0.0

Data are presented as mean ± SEM.
** Not considered in statistical analysis (regeneration studies excluded from 2-way ANOVA).
[a] Differs significantly from group I ($p < 0.05$).
[b] Differs significantly from group III ($p < 0.05$).

Table 3
Malondialdehyde (MDA) levels in mouse tissues.

MDA (ng/g wet tissue)	SRS *per se*		Protection studies		Regeneration studies	
	Control (I)	SRS (II)	W + CCl_4 (III)	SRS + CCl_4 (IV)	CCl_4 + W (V)	CCl_4 + SRS (VI)**
Liver	250.9 ± 33.63	301.1 ± 63.53	211.4 ± 52.99	152.6 ± 72.63	195.7 ± 39.16	199.4 ± 0.0
Kidneys	522.0 ± 45.53	481.5 ± 52.59	375.4 ± 59.72	623.5 ± 58.40	801.3 ± 124.60	113.4 ± 0.0

Data are presented as mean ± SEM.
** Not considered in statistical analysis (regeneration studies excluded from 2-way ANOVA).

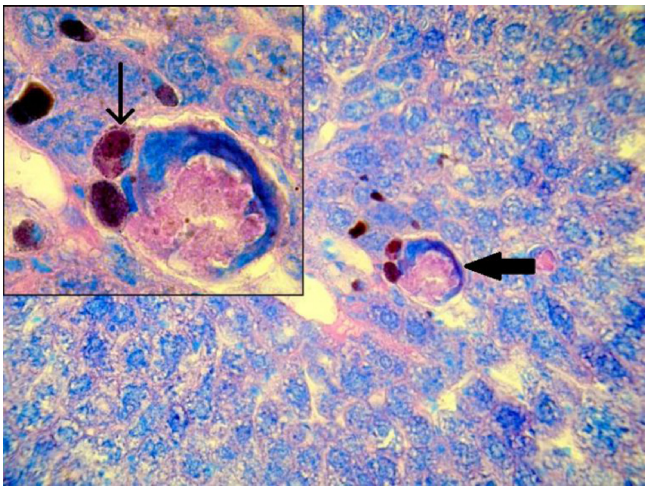

Fig. 6. Microphotograph of hepatic section from CCl_4 + SRS group (Long Ziehl-Neelsen stain, 400× original magnification; the inset shows 1000× magnification of same visual field), where pigmented cells (thin arrow) and microgranuloma (thick arrow) are shown.

the current histopathological profile obtained without significant levels of MDA.

Our results in the renal tissue showed that the glomerular function and tubular absorption capacity may have been altered with exposure to CCl_4. The kidneys are susceptible to vasoactive substances and CCl_4-induced vasoconstriction produces a local ischemic environment, which leads to cellular injury by deteriorating of membrane integrity [34]. The resulting tubular necrosis leads to the detachment of tubular epithelial cells from the basement membrane, forming clusters that can obstruct the filtrate flow and increase intraluminal pressure and decrease the glomerular filtration rate (GFR). As a result of this, a return leakage of the filtrate to the interstice occurs, which further decreases GFR [23]. All these events can lead to functional overload of the nephrons with subsequent renal failure [35]. The findings of group IV show the inability of SRS to prevent CCl_4-induced hepato- and nephrotoxicity. Contrary to these results, a previous study on a seed extract of *Spinacia oleracea* (Chenopodiaceae) revealed a hepatoprotective effect against CCl_4-induced injury and was shown to be safe up to 2000 mg/kg [36]. However, the present results may be supported by a study in which the consumption of unprocessed vegetables of *Corchorus olitorius* aggravated the damage caused by CCl_4, possibly induced by antinutritional content [37]. As discussed above, the presence of antinutrients or contaminants may antagonize the presumed bioactive properties of the SRS, leading to an unfavorable histological outcome.

In the regeneration study, the results from CCl_4 + W group showed that liver recovery can be achieved in 5–7 days after a single administration of CCl_4 as previously demonstrated [38]. In fact, the liver has a strong regenerative capacity and the loss of hepatic tissue from the action of toxic compounds initiates a mechanism by which the surviving hepatocytes undergo promitogenic activity [39]. These newly divided cells are resilient, allowing the original mass of tissue and its functions to be restored, while restraining the progression of toxic injury [40]. The proximal tubular epithelium of the kidneys can also be recovered within 5 to 7 days through the sequential process of dedifferentiation, migration, proliferation and re-differentiation of surviving cells, which restores the tubular cell layer and function of tubular cells. After 21 to 28 days, the tubules showed a completely mature, functionally normal epithelium [41]. Our findings are consistent with this outcome, and by this time, CCl_4 may be already completely excreted from the body. However, inflammatory processes remain and obviously more time would be required for a full recovery. Due to the reduced sample size of the CCl_4 + SRS group, it was only pertinent to discuss the histopathological evaluation of the other animals. The observed granulomatous liver changes were comparable to those previously found in Balb/c mice, in the first 7 days following a single injection of CCl_4 with similar dosages of the present experiment [42,43]. The reported granulomas, composed by perisinusoidal inflammatory and activated stellate cells, were formed *de novo* in the surrounding regenerating parenchymal tissue rather than by the collapse of degenerated tissue [42], being almost solved on the 14th day [43]. In contrast, the presence of microgranulomas 23 days after CCl_4 intoxication could have been triggered or prolonged by SRS treatment in the surviving mice of our regeneration study. The pigmentation may result from cellular and erythrocyte degradation products, lipid peroxidation of cell membranes or altered heme metabolism. Circulatory disorders and treatment with xenobiotics may induce the accumulation of pigments (lipofuscin, hemosiderin or porphyrin) in hepatic tissue [44]. In non-lysosomal hepatic steatosis, for example, Küpffer cells often form resorbent microgranulomas around hepatocytes that die, with or without ceroid pigmentation [45]. Moreover, when swollen with lipofuscin and ceroid pigments, these macrophages may be useful markers of recent necrosis due to the engulfed debris of the adjacent necrotic hepatocytes [46]. A previous study demonstrated that single CCl_4 administration can cause lipofuscin accumulation in the rat liver [47]. Similarly, the exposure to SRS may have deprived hepatocytes of their normal recovery and enhanced liver impairment that was already under CCl_4 intoxication. Basophilia observed in the renal tubules may suggest an earlier sign of tubular regeneration. These basophilic cells are characterized by high mitotic activity and normally appear in the reconstructive tissue after 5 to 7 days of tubular cell necrosis, where the basement membrane remained intact [41]. However, in this experimental group, these cells persisted in the renal tissue after 22 days, indicating that, although the epithelial lining is complete, epithelial cell renewal persisted for a considerable time, as also seen in renal tubulonecrosis induced by mercuric chloride [48]. In general, evidence shows that treatment with SRS after administration of CCl_4 may delay the rate of normal renal turnover and cause accumulation of liver breakdown products. The observed result was possibly caused by interactions and synergisms among different antinutrients or contaminants of SRS and CCl_4, which makes it difficult to assign liability to any particular substance. This fact may be one of the reasons behind the low survival rate associated with this group. As deaths occurred within the first 8 days, and understanding that vital mechanisms of regenerating of hepatic and renal tissues end during this period, we speculate that SRS disrupted these CCl_4-induced mechanisms and after

that period the surviving animals may have a longer recovery.

5. Conclusion

Based on the study of the potential of *S. ramosissima* as a therapeutic agent, data from the present study contrasts the results of the available literature. Seeds of *S. ramosissima*, under experimental conditions considered, were unable to act as a therapeutic agent against hepatorenal toxicity and may have affected the normal regenerative function. For this reason, further research is under way to elucidate the composition and effects of seed extracts and compounds in a dose and time dependent manner.

Conflicts of interest

Authors declare that there is no conflict of interest regarding the publication of this article.

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