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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

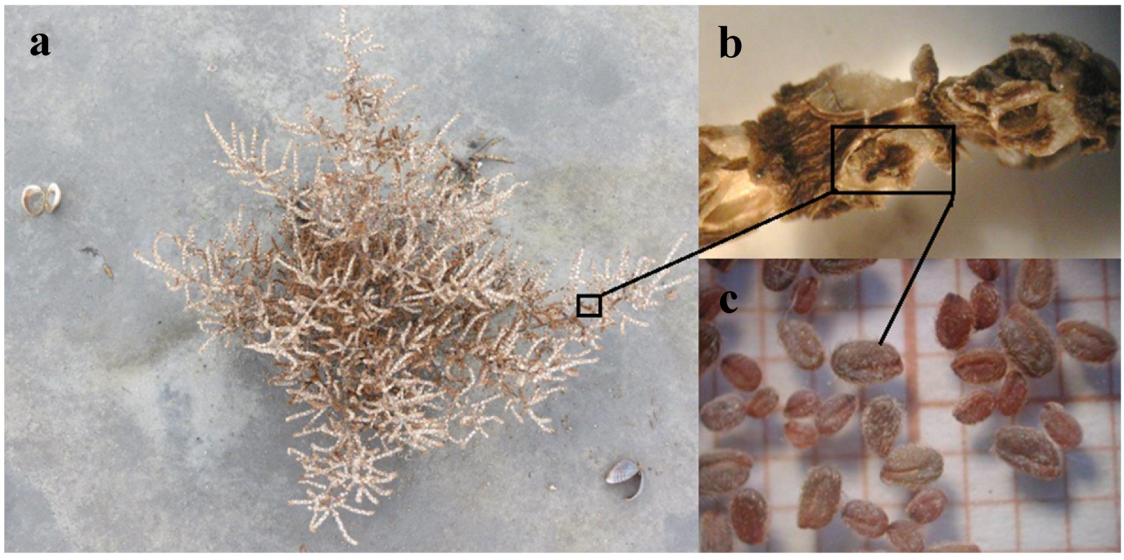

Fig. 1. *Salicornia ramosissima* in natural habitat (a); fructiferous branches (b) and seeds (c).