SHORT COMMUNICATION

Schizocalyx cuspidatus (A. St.-Hil.) Kainul. & B. Bremer extract improves antioxidant defenses and accelerates the regression of hepatic fibrosis after exposure to carbon tetrachloride in rats

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ABSTRACT

The aim of this study was to investigate the effect of leaves extract of Schizocalyx cuspidatus (A. St.-Hil.) Kainul. & B. Bremer on hepatic morphofunctional dysfunction induced by carbon tetrachloride (CCl4). Liver lesions were induced via intraperitoneal administration of CCl4 every 48 h for 12 days. Forty-nine rats were randomised into seven groups: G1: CCl4; G2: CCl4 (animals euthanised 24 h after last CCl4 application); G3: CCl4 + DMSO; G4: SCE 400 mg/kg; G5: DMSO (700 μl); G6: CCl4 + SCE 200 mg/kg and G7: CCl4 + SCE 400 mg/kg. SCE administration resulted in reduction in hydroperoxide levels, lipidic droplets and necrosis compared to G1, G2 and G3. There was an increase in the amount of collagen fibres in G1, G2 and G3 compared to the groups. These results show that the extract of SCE leaves has great potential for the recovery of liver damage after the application of CCl4.

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1. Introduction

*Schizocalyx cuspidatus* (A.St.-Hil.) Kainul. & B. Bremer, Rubiaceae, is a plant commonly known in Brazil as *quina-do-mato* and its leaves are used in popular medicine for the treatment of stomach and liver disorders, and as a healing agent (Coelho et al. 2012). Its botanical nomenclatures have been recently modified by Kainulainen et al. (2010), who proposed synonymisation of *Bathysa cuspidata* (A. St.-Hil.) Hook. f. ex K. Schum. and *S. cuspidatus* (A. St.-Hil.) Kainul. & B. Bremer According to Gontijo et al. (2012), *S. cuspidatus* is a bitter tonic used in anaemia, cachexia, swamp fever, hookworm and convalescence. These data associated with the relevance of popular knowledge are important for conducting surveys of pharmaceutical bioprospecting, reinforcing the great potential of Brazil’s biodiversity.

The treatment of human diseases using medicinal plants and their derivatives is an ancient practice that is currently gaining popularity worldwide (Das et al. 2015). Nowadays, it is possible to observe a growing interest in plant products, particularly those used in traditional medicine (Joshi et al. 2011).

Carbon tetrachloride (*CCl₄*) is an industrial solvent with known hepatotoxic activity. *CCl₄* induces liver lesions by producing radical species (•CCl₃ and •Cl). The absence of mutagenicity of the *S. cuspidatus* extract and the presence of bioactive phytochemicals such as coumarins, flavonoids, tannins and alkaloids have been demonstrated. The bark extract indicated a potential preventive effect on liver and lung toxic injuries (Gonçalves et al. 2012; Novaes et al. 2012; Blanco-Ayala et al. 2014). However, it is still unknown whether this extract is able to reverse injuries in target organs after exposure to toxic substances and if the leaf extract have a therapeutic potential. The aim of this study was to investigate the effect of a leaf extract of *S. cuspidatus* (SCE) on hepatic dysfunction morphofunctionally induced by *CCl₄* in rats.

![Figure 1. Chromatogram of leaves extract from *S. cuspidatus* recorded at 254 nm and UV spectra of the peaks. Peak numbers correspond to chromatogram above.](image-url)
2. Results and discussion

2.1. Chemical profile of the extract

The HPLC/DAD chemical profile of leaves extract from *S. cuspidatus* at 254 nm is shown in Figure 1. The developed analytical system led to the separation of four major peaks, which were identified as polyphenols. The total phenol and proanthocyanidin content was 45.0 and 27.9 mg/g of dry matter, respectively. Although the molecular mechanism modulated by these compounds remains poorly understood, the upregulation of CAT or glutathione peroxidase gene expression by different flavonoids has been previously reported (Muhammad et al. 2015). Thus, although other factors cannot be ruled out, a similar effect caused by components of the *S. cuspidatus* extract could partially explain the current results (Figure 1).

2.2. Serum analysis and oxidative and antioxidant markers

Serum AST, ALT and GGT levels were significantly higher in the CCl$_4$ group compared to the other groups, indicating a considerable hepatocellular lesion, which was confirmed by histopathological analysis, similar to that found in Al-Waili et al. (2006). The administration of *S. cuspidatus* extract at the doses used in the present study decreased the serum levels of these enzymes, resulting in the subsequent normalisation of these parameters compared to animals in the groups treated with CCl$_4$ alone, confirming the curative effect of SCE (Table 1).

| Groups/treatment               | ALT (IU/L) | AST (IU/L) | GGT (IU/L) | HPX (nmol/mg protein) | SOD (U/mg protein) |
|-------------------------------|------------|------------|------------|----------------------|-------------------|
| G1/CCl$_4$ + 12 days          | 121.71 ± 31.14a | 166.57 ± 42.34a | 5.28 ± 1.89a | 166.53 ± 42.35b | 1.27 ± 1.14a |
| G2/CCl$_4$                    | 217.42 ± 57.68b | 718.71 ± 318.02b | 5.14 ± 1.06a | 642.08 ± 209.01a | 1.15 ± 0.73a |
| G3/CCl$_4$ + DMSO             | 127.57 ± 50.73a | 166.14 ± 44.99a | 3.71 ± 0.75b | 166.1 ± 44.98b | 4.32 ± 1.51b |
| G4/SCE 400                    | 70.71 ± 9.65c | 114.71 ± 16.88c | 2.28 ± 0.75b | 85.83 ± 5.09c | 8.53 ± 1.55c |
| G5/DMSO                       | 76.71 ± 15.11c | 111.85 ± 14.33c | 3.80 ± 1.39b | 149.96 ± 35.21b | 8.23 ± 1.50c |
| G6/CCl$_4$ + SCE 200          | 48.14 ± 2.03d | 85.57 ± 5.28d | 3.42 ± 1.51b | 84.61 ± 10.30c | 8.07 ± 0.84c |
| G7/CCl$_4$ + SCE 400          | 52.11 ± 6.09d | 90.27 ± 5.18d | 2.85 ± 1.06b | 82.28 ± 12.91c | 7.42 ± 0.92c |

ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: α-glutamyl transferase; HPX: hydroperoxides; SOD: superoxide dismutase. Data are expressed as mean ± SD. Different letters in the columns indicate statistical difference between groups (p < 0.05), and groups that have some common letter do not differ statistically, one-way ANOVA.
showed the highest number of SOD. Similar effects were evidenced on nicotine-induced oxidative stress in rat liver using a dietary supplementation with vitamin E capable of protecting the liver (Taysi et al. 2010).

### 2.3. Liver morphometry and histopathology

Histopathological alterations such as the accumulation of lipid droplets, necrosis and fibrosis were found in the CCl₄ and DMSO groups (Table 2). The liver of rats treated with SCE did not reveal any degenerative signs and was comparable with control groups of the plant and DMSO. The treatment with CCl₄ promotes a profound change in the histological architecture.

**Table 2.** Histological area of cell necrosis, lipid droplets and fibrosis in the liver of rats exposed to CCl₄ and treated with *S. cuspidatus* extract (SCE 200 and 400 mg/kg).

| Groups/treatment | Lipidic droplets (%) | Necrosis (%) | Fibrosis (%) |
|------------------|----------------------|--------------|--------------|
| G1/CCl₄ + 12 days| 2.71 ± 1.09<sup>a</sup> | 33.31 ± 2.85<sup>b</sup> | 8.54 ± 1.30<sup>c</sup> |
| G2/CCl₄         | 15.30 ± 5.77<sup>d</sup> | 58.57 ± 10.55<sup>a</sup> | 12.61 ± 3.92<sup>b</sup> |
| G3/CCl₄ + DMSO  | 1.89 ± 0.54<sup>c</sup> | 4.11 ± 1.10<sup>b</sup> | 10.86 ± 2.66<sup>c</sup> |
| G4/ SCE 400     | 0.35 ± 0.16<sup>d</sup> | 0.88 ± 0.16<sup>d</sup> | 2.35 ± 1.7<sup>b</sup> |
| G5/DMSO         | 0.30 ± 0.12<sup>c</sup> | 0.75 ± 0.15<sup>d</sup> | 3.34 ± 1.15<sup>b</sup> |
| G6/CCl₄ + SCE 200| 0.27 ± 0.10<sup>c</sup> | 2.36 ± 0.68<sup>d</sup> | 3.37 ± 1.06<sup>c</sup> |
| G7/CCl₄ + SCE 400| 0.59 ± 0.29<sup>c</sup> | 0.84 ± 0.15<sup>c</sup> | 2.05 ± 1.02<sup>c</sup> |

Data are expressed as mean ± SD. Different letters in the columns indicate statistical difference between groups (p < 0.05), and groups that have some common letter do not differ statistically, one-way ANOVA.

**Figure 2.** Photomicrographs of the liver of rats exposed to CCl₄ and treated with *S. cuspidatus* extract (SCE 200 and 400 mg/kg) showing fibrosis areas (A–F, Sirius red staining under polarising microscopy; bars = 60 μm). A = G1 (CCl₄ + 12 days); B = G2 (CCl₄); C = G3 (CCl₄ + DMSO); D = G4 and G5 (SCE and DMSO, respectively); E = G6 (CCl₄ and SCE 200); F = G7 (CCl₄ and SCE 400).
of the liver, provoking haemorrhage, oedema, fibrosis, steatosis and necrosis (Srivastava & Shivanandappa 2011). Data found in this study show that \textit{S. cuspidatus} effectively restored lesions and avoided the accumulation of fat caused by CCl$_4$ in the liver of these animals. The proportion of cell necrosis was lower in SCE groups when compared to CCl$_4$ groups (Figures 2 and S1), where a higher density of collagen fibres can be observed in A = G1 (CCl$_4$ + 12 days) and B = G2 (CCl$_4$) compared to the other groups.

3. Conclusions

The present study has shown that the extract of \textit{S. cuspidatus} leaves has a therapeutic effect, since it has great potential for the recovery of liver damage previously established after the application of CCl$_4$, stimulates the antioxidant defense system and reduces morphological and functional liver damage.

Disclosure statement

No potential conflict of interest was reported by the authors.

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