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Croton zehntneri Essential Oil Prevents Acetaminophen-Induced Acute Hepatotoxicity in Mice

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Abstract: Hepatoprotective activity of *Croton zehntneri* Pax & Hoffman (Euphorbiaceae) leaf essential oil (EOCz) was evaluated against single dose of acetaminophen-induced (500 mg/kg, p.o.) acute hepatotoxicity in mice. EOCz significantly protected the hepatotoxicity as evident from the activities of serum glutamate pyruvate transaminase (GPT), serum glutamate oxaloacetate transaminase (GOT) activities, that were significantly ($p<0.01$) elevated in the acetaminophen alone treated animals. Histopathological examinations of liver tissue corroborated well with the biochemical changes. Hepatic steatosis, hydropic degeneration and necrosis were observed in the acetaminophen treated group, while these were completely absent in the standard and EOCz treated groups. In conclusion, these data suggest that the *Croton zehntneri* essential oil can prevent hepatic injuries from acetaminophen-induced hepatotoxicity in mice.

Keywords: *Croton zehntneri;* acetaminophen; hepatoprotective; mice.

1. Introduction

Croton zehntneri, popularly known as "Canela de cunhã", is an aromatic plant native to Northeastern Brazil and used in folk medicine principally as a sedative, as an appetite stimulating, and for the relief of gastrointestinal disturbances [1]. Despite this, little pharmacological investigation has been carried out on the effects of *C. zehntneri* [2]. Phytochemical studies on *Croton zehntneri* leaves

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essential oil (EOCz) revealed the presence of anethole, eugenol, methyl-eugenol and estragole [3]. Pharmacological studies indicated the antihelmintic, larvicidal and antinociceptive [3, 4, 2].

Conventional drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effects. It is, therefore, necessary to search for alternative drugs for the treatment of liver diseases to replace currently used drugs of doubtful efficacy and safety [5]. The acute administration of EOCz up to 3 g kg(-1) by the oral route to mice was devoid of overt toxicity, because of this, EOCz is promising sources for new phytotherapeutic agent [6]. Despite the consumption of Canela de cunhã for the relief of gastrointestinal disturbances, there have been no available reports in literature describing the hepatic effects of *Croton zehntneri* essential oil. In the current study, we therefore analyzed the effets of EOCz on acetaminophen-induced hepatotoxicity in rats.

2. Materials and Methods

2.1. Plant Material

Croton zehntneri was collectecd near Viçosa (Ceará, Brazil) in April, 2004. A voucher specimen (#27477) was deposited at the Herbarium Prisco Bezerra, Federal University of Ceará.

2.2 Essential Oil Extraction

EOCz was extracted from freshly chopped plant leaves by steam distillation and analyzed at PADETEC (UFC). Freshly chopped leaves were placed in a glass flask connected at one end to a glass vessel with water and at the other end to a water-cooled condenser. When the water was boiled, steam percolated through the leaves and was collected in the condenser. After condensation, the essential oil separated from the aqueous phase with its solutes. The composition (w/w) of EOCz, determined by gas chromatography and mass spectrometry, was 57.91% anethole, 27.94% estragole, 5.16% bicyclogermacrene, 1.73% β-caryophyllene, 1.19% myrcene, 1,17% germacrene D, 0.77% 1,8-cineole, 0.62% spathulene, 0.36% β-elemene, 0.30% globulol, 0.27% (E)-β-ocimene, 0.24% alloaromadendrene, 0.23% α-phellandrene and 2.11% unidentified.

2.3 Animals

Male, swiss mice (20-25 g) provided by the Animal Housing, Ceará State University were housed in polyethylene-walled cages (n=8), with food and water *ad libitum*. The animals were kept on a 12 h light: 12 h dark regime (lights on from 7:00 h to 19:00 h) at 23 °C prior to the experiments.

2.4 Induction of acetaminophen-hepatoxicity and EOCz treatment

A total of 48 mice were randomly assigned to six groups of eight in each. The first group received only physiological saline and served as normal control; the second group served as acetaminophen control and received the vehicle used to suspend EOCz (3% DMSO); groups 3 and 4: controls treated with EOCz (30, 100 and 300 mg/kg, respectively in 3% (v/v) DMSO); group 5: N-acetylcisteine (750 mg/kg). All treatments were given orally by gavage at three times points (i.e. 48h. 24h and 2h) before acetaminophen (500 mg/kg, p.o) in a volume of 10 mL/kg. No separate controls using OECz alone were run since in pilot experiments, apparently it had no *per se* effect on the mouse hepatic tissue. Similarly, the vehicle (3% DMSO) used the suspend OECz was also without any effect. 24 h after the induction of hepatotoxixcity, blood was collected by puncturing the retro-orbital plexus

and was allowed to clot at room temperature. Serum was separated by centrifuging at 2500 rpm. The serum obtained was used for the determination of GOT and GPT.

2.5 Histopathological Studies

The animals were sacrificed, and the abdomen was cut open to remove the liver. Then, 5 mm thick pieces of the liver were fixed in Bouin's solution (mixture of 75 ml of saturated picric acid, 25 mL of 40% formaldehyde and 5 ml of glacial acetic acid) for 12 h and then embedded in paraffin, using conventional methods, and cut into 5 mm thick sections and stained, using haematoxylin-eosin dye, and finally mounted in diphenylxylene. Then the sections were observed under microscope for histopathological changes in liver architecture, and their photomicrographs were taken.

2.6 Statistical Analysis

All data are expressed as the mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) followed by Student-Newman-Keuls test. Differences between experimental groups were considered significant if $p < 0.05$.

3. Results and Discussion

No mortality was observed in rats subjected to acetaminophen-induced hepatotoxicity study. Significant elevations of serum GOT and GPT levels were observed at 24 hours after the oral administration of 500 mg/kg acetaminophen. Assessment of liver damage by acetaminophen is usually made by determination of serum enzyme levels of GOT and GPT. Necrosis results in the release of these enzymes into circulation; therefore, it can be measured in serum. High levels of GOT indicate liver damage, GPT catalyses the conversion of alanine to pyruvate and glutamate and is released in similar manner. Therefore, GPT is more specific to liver, and is thus a better parameter for detecting liver damage [8]. Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver. These results were further confirmed by histopathological studies. Liver cell necrosis was observed in control group at 24 hours after acetaminophen administration. However, the number of necrotic cells was visibly decreased after *Croton zehntneri* administration (Fig. 3).

"Cenela-de-cunhã" is a traditional Brazilian medicine that has been for the relief of gastrointestinal and nervous disturbances [1]. *Croton zehntneri* is commonly used as a kind of green vegetable in Brazil Northeastern and no toxicity has been reported. Since *C. zehntneri* is very popular in Ceará (Brazil), in this study the hepatoprotective effect of *C. zehntneri* leafs essential oil on acetaminophen-induced hepatotoxicity in rats was investigated.

Acetaminophen is a common antipyretic agent, which is safe in therapeutic doses but can produce fatal hepatic necrosis in man, rats and mice with toxic doses. Protection against acetaminophen-induced toxicity has been used as a test for potential hepatoprotective activity by several investigations [7]. The efficacy of any hepatoprotective drug is dependent on its capacity of either reducing the harmful effect or restoring the normal hepatic physiology that has been distributed by a hepatotoxin.

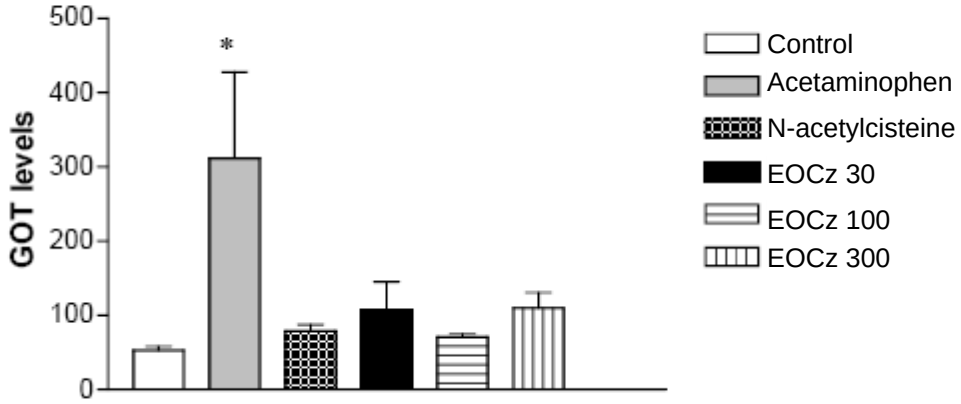

Figure 1. Effect of *Croton zehntneri* essential oil on GOT levels on acute acetaminophen-induced hepatotoxicity. $p < 0.05$ *vs* control (ANOVA, Student-Newman-Keul´s test.).

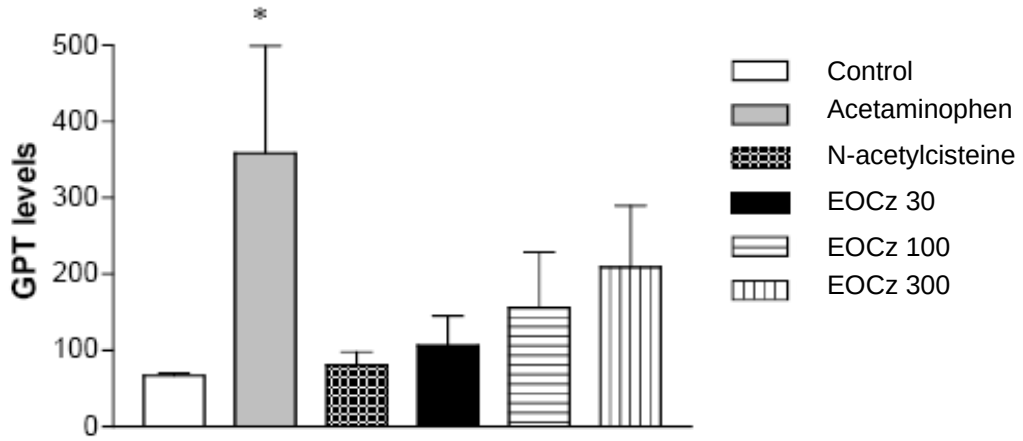

Figure 2. Effect of *Croton Croton zehntneri* essential oil on GPT levels on acute acetaminophen-induced hepatotoxicity. $p < 0.05$ *vs* control (ANOVA, Student-Newman- Keul´s test).

The results of this study demonstrate that *C. zehntneri* treatment can decrease acetaminophen-induced liver injuries in rats. The hepatoprotective effect was shown by the decreases in the activities

of GOT and GPT,), that is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by acetaminophen, as well as histopathological observations in liver tissue. *C. zehntneri* was administered 48, 24 and 2h before acetaminophen administration. The recovery effects were evident at 24 hours after acetaminophen administration.

The histopathological profile of the rat treated with EOCz (Fig. 3) showed no visible changes confirming the safety of the essential oil at selected dose regimen. Examination of liver sections of control group showed normal cellular architecture. In the liver sections of the rats intoxicated with acetaminophen, there is disarrangement and degeneration of normal hepatic cells with intense centrilobular necrosis. According to microscopic examinations, severe hepatic lesions induced by acetaminophen were remarkably reduced by the chronic administration of the EOCz, which were in good agreement with the results of the biochemical tests.

These findings suggest that *C. zehntneri* can interact directly with acetaminophen metabolites or induce a protective event to reduce the hepatotoxicity of acetaminophen. Thus, *C. zehntneri* appears to be a suitable treatment herb for overdose intoxication of acetaminophen. Possible mechanism that may be responsible for the protection of acetaminophen induced the following EOCz by if self-act as a free radical scavenger intercepting those radicals involved in acetaminophen metabolism by microsomal enzymes. Its ability is to inhibit rat hepatic microsomal membrane lipid peroxidation and to scavenge on radicals, as well as to interact with 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) [3]. Thus, by trapping oxygen related free radicals EOCz could hinder their interaction with polyester fatty acids and would abolish the enhancement of lipids peroxidative processes [7].

Which component(s) of the extract is responsible for this effect, however, was not investigated. The main constituent of EOCz is anethole that has appreciable free radical scavenging properties [9]. Generation of free radicals may be, at least partially, the basis of many human diseases and conditions. Therefore, the antioxidant action of *C. zehntneri* may explain its claimed usefulness in folk medicine. In order to elucidate the mechanism(s) by which EOCz components exhibit the hepatoprotective effect, which we demonstrated in this study, further studies with the isolated components will follow.

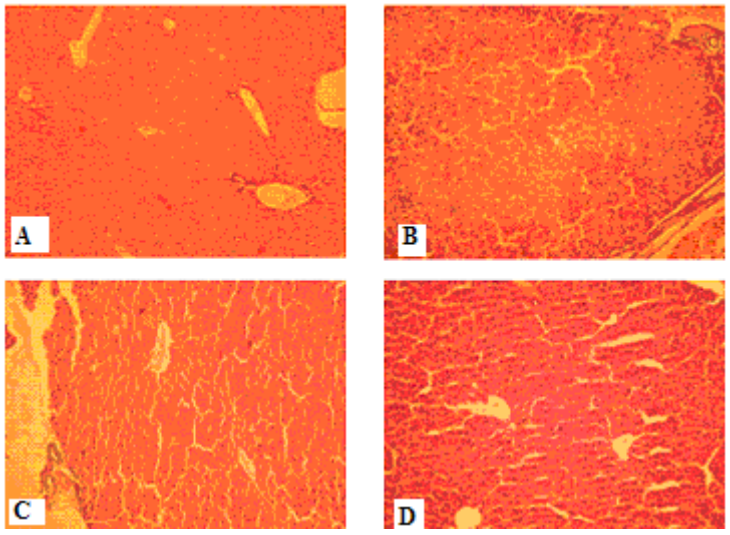

Figure 3. Photomicrographs of mice liver obtained from different treatment groups A: Control, B: Acetaminophen control, C: EOCz 30 mg/kg, D: EOCz 100 mg/kg. H and E (10x).

Acknowledgments

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