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EXPERIMENTAL STUDY

Antioxidant and hepatoprotective activities of Dicoma anomala Sond. aqueous root extract against carbon tetrachloride-induced liver damage in Wistar rats

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Abstract

OBJECTIVE: To evaluates the antioxidant and hepatoprotective potentials of Dicoma anomala Sond. (Asteraceae) on body weight, feed and water intake, biochemical parameters and organ histology.

METHODS: Various concentrations (1.56-25 μg/mL) were used in the *in vitro* assays 1,1-diphenyl-2-picryl hydrazyl (DPPH, superoxide anion, hydroxyl radicals, etc.). The effects of treatment with 125, 250 and 250 mg/mL Dicoma anomala aqueous roots extract (DARE) was investigated *in vivo* in the CCl_4-induced hepatotoxic rats during the 15 days study.

RESULTS: Water extract exhibited the best activity (IC_{50}: 15.20 ± 0.03, 11.70 ± 0.10, and 0.84 ± 0.05 μg/mL) *in vitro* in DPPH, hydroxyl and superoxide anion radicals, respectively, when compared with standards. Pre-treatment and treatment with different concentrations of DARE significantly ($P < 0.05$) attenuated the elevated serum activities of aspartate transaminase, alanine transaminase levels while increasing the activities of superoxide dismutase, catalase and glutathione peroxidase. The histopathological evaluations revealed extensive liver damage characterized by severe vacuolar and cytoplasmic degeneration, hepatic necrosis, and cellular infilteration in pre-treated groups while in the treated groups; such liver damages were not observed most especially at 500 mg/kg dose.

CONCLUSION: The results proved the hepatoprotective potential of DARE against CCl_4-induced oxidative stress. Moreover, histopathological examinations revealed better therapeutic advantage of DARE than prophylactic use.

Key words: Dicoma anomala Sond. (Asteraceae); Antioxidant enzymes; Carbon tetrachloride; Hepatoprotection; Lipid peroxidation

INTRODUCTION

Free radicals are implicated in the pathogenesis of several degenerative diseases including liver damage. Their deleterious influence causes oxidative stress which is a reflection of disequilibrium between them and the body's antioxidant defence system. This result in significant damage to important cellular macromolecules (proteins, lipids and DNA) and ultimately cell necrosis.[1] When this happens, swiftly intervention with exogenous antioxidants[2] (which could be easily and readily achieved through consumption of vegetables and fruits) augments the cellular defence system thereby preventing cell death. Foods of plant origin usually contain natural antioxidants that can scavenge free radi-

cals[2] and there is a great deal of interest in edible plants with excellent phytonutrients primarily due to their health benefits.

Dicoma anomala Sond. (Asteraceae) commonly called fever or stomach bush (Eng.) or hloenya (South Sotho) is a prostrate. Decumbent or erect perennial herb with underground tuber. D. anomala is widely distributed in sub-Sahara Africa including most Provinces within South Africa.[3] The plant is ethno-botanically indicated in the treatment of cold and coughs, fever, ulcers and dermatosis. Its pharmacological potentials as antiplasmodial, antibacterial, anthelmintic, antiviral and anti-inflammatory have also been documented.[4] Till date, there is paucity of information in scientific literatures on the hepatoprotective potentials of D. anomala. Therefore, the present study reports on this with focus on its curative and prophylactic models.

MATERIALS AND METHODS

Chemicals

Carbon tetrachloride, 2, 2- azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) and assay kits were purchased from Sigma-Aldrich (Johannesburg, South Africa). All other chemicals and reagents used were of analytical grade.

Plant collection and extraction

Fresh root stocks of Dicoma anomala were procured in April 2014 from Setsing market, Phuthaditjhaba, Free State Province, South Africa. The sample was confirmed by Dr. AOT Ashafa of Plant Sciences department, University of the Free State, South Africa. A voucher specimen was prepared and deposited at the herbarium. The rootstocks were cut into smaller pieces; oven dried (40 ℃), and pulverized using a Waring commercial blender (Waring Instrument, Torrington, CT, USA) into fine powder. Approximately 10 g each from the powdered materials was exhaustively extracted with 40 mL each of water, ethanol, aqueous ethanol and methanol. They were filtered and organic extracts were concentrated using a rotary evaporator (Cole-Palmer, model SB-1100 Shanghai, China) to obtain dry brown crude extracts. The water extract was dried on a water bath (Memmert W600, Schwabash, Germany) at 45 ℃. The crude extracts were reconstituted in respective solvents to prepare various concentrations used for the *in vitro* antioxidant assays.

Similarly, 200 g of the powdered material was extracted with 2 L of water, filtered and concentrated to dryness on water bath at 40 ℃. The extraction yielded 48.87 g of brown gum (24.435% w/w of dry plant material). The crude extract was reconstituted in water to give various concentrations used for the *in vivo* assays.

In vitro antioxidant assays

The 1,1-diphenyl-2-picryl hydrazyl DPPH radical scavenging activity of the extracts determined using Braca *et al*.[5] method, nitric oxide,[6] reducing power,[7] metal chelating,[8] 2, 2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid ABTS,[9] superoxide anion,[10] hydroxyl radical,[11] total antioxidant capacity,[12] total phenolic content[13] and total flavonoids according to Chang *et al*.[14]

In vivo hepatoprotective study

Experimental animals: male and female Wistar rats (Rattus norvegicus, 10 weeks old) weighing 145.00 ± 10.00 g were used in this study. They were procured from the animal house of the University of the Free State, Bloemfontein. They were acclimatized for one week, fed with standard rat feed (Epol mice cubes, Westville, South Africa) and water ad libitum. Ethical clearance (number NR 02/13) to undertake the study was obtained from the Interfaculty Animal Ethical Committee of the University of the Free State prior to the commencement of the study.

Experimental design: the hepatoprotective study was conducted according to Chandan *et al*.[15] method with slight modifications. A total of 66 rats were randomly divided into eleven groups of six animals each (n = 6). Group A animals were orally administered 1 mL normal saline for 15 days. Groups B and C rats received 1 mL/kg CCl_4 (in olive oil; ratio 1∶1 v/v) i.p on days 1 and 15 and represented curative and prophylactic hepatotoxic rats, respectively. Groups D-G rats were curative hepatotoxic rats treated orally with silymarin (100 mg/kg body weight BW), 125, 250 and 500 mg/kg BW Dicoma anomala aqueous roots extract DARE respectively. Groups H-K represented prophylactic hepatotoxic rats given respective similar treatments as in the curative study and CCl_4 i.p on the 15th day of the experiment.

Serum preparation and organ isolation: at the end of experimental period, all the rats were anaesthetized with halothane and blood was collected by cardiac puncture. An aliquot (2 mL) of the blood collected into ethylenediamine tetraacetic acid EDTA bottle was used for the analysis of haematological parameters, while another 5 mL of the blood collected in non-heparinized bottle was centrifuged at 1285 × g for 10 min and the resulting serum was aspirated and used for other serum bioassays.

The animals were quickly dissected and the liver, kidney, heart and lungs were excised, freed of fat and weighed for evaluation of organ -body weight ratios. The liver was further divided into two portions and a portion immediately fixed in 10% formalin for histopathological examination, while the other was homogenized in ice cold 0.1 mol/L Tris-HCl buffer (pH 7.2), at 10 000 rpm for 15 min and the supernatant obtained was kept at − 80 ℃ freezer prior further bioassays.

Determination of haematological and biochemical parameters: the automated haematologic analyzer (Sysmex, KX-21, Chuo-ku, Kobe, Japan) was used to analyse total protein, bilirubin levels and haematological parameters. The serum levels of total cholesterol, high