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Research Article

Antifibrotic Activity of *Saussurea lappa* Root Extract on CCl4-induced Liver Fibrosis in Rats

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Abstract

Natural products emerge as a valuable and affordable source for potential drugs which may be used as lead for treatment of many diseases. Using both *in vitro* and *in vivo* models, Badria and his team were able to find different natural products with superb biological activities.Liver fibrosis represents a major health burden and is one of the most common causes of death worldwide. The possible hepatoprotective effect of methanolic extract of *Saussurea lappa* (Sl) root was investigated against CCl_4-induced liver fibrosis in rats. Intraperitoneal injection of 50% CCl_4 (1ml/kg) twice weekly for 8 weeks, significantly raised the levels of serum ALT, AST, liver MDA and percentage of collagen area and significantly decreased liver GSH as compared to the control group fed on basal diet only. Administration of Sl root extract to the rats in a daily dose of 400 mg/kg, concomitantly with CCl_4, significantly prevented the CCl_4-induced rise in plasma levels of ALT, AST, liver MDA and percentage of collagen area and increased liver GSH as compared to the CCl_4-treated group. Moreover, liver histopathology in Sl-treated group showed relatively preserved architecture, less inflammatory cellular infiltration, less collagen deposition with less positive reaction in immunohistochemical staining for α-SMA, as compared to CCl_4-treated group. These results explained the preventive effect of Sl root extract on liver fibrosis in rats, through antioxidant, anti- inflammatory activities and through prevention of hepatic stellate cell activation.

Keywords: Antifibrotic; *Saussurea lappa*; CCl4; Liver Fibrosis

Introduction

Natural products, their derivatives and traditional remedies have an uprising attention in drug discovery approaches [1-3]. Natural products are characterized by their structural diversity so; they could be utilized as biological function modifiers [4,5]. This open the door for the development of new technologies in discovering new drugs based on natural products' screening, because they are a rich wealth of bioactive compounds [5,6]. Yet, its poor aqueous solubility, inadequate permeability, and suboptimal bioavailability limited their uses. Therefore, several approaches have been developed to improve solubility, permeability and bioavailability of natural products [7,8].

Boswellia carterii is a plant native to India, Arabian, Red Sea region of North-East Africa (Somalia, Eritrea) and rich with oleogum resin with various biological activities. Badria., *et al*. have investigated the various therapeutic applications of *Boswellia* including anti-inflammatory [9-11], hepatoprotective [12], immunomodulatory [13], anti-ulcer [8], antiviral [14], and other biological activities [15].

Saussurea lappa C. B. Clarke (Asteraceae) is well known for its wide therapeutic uses in India. Singh., *et al.* [1] reviewed its chemistry (as shown in Figure 1), traditional uses and pharmacological activities such as its antioxidant, anti-inflammatory, and anti-cancer activities. The hepatoprotective activity of *Saussurea lappa* (Sl) root extract against D-galactosamine and lipopolysaccharide-induced acute liver hepatitis in mice had been reported [2]. However, there is no published work related to the role and mechanism of action of Sl in chronic hepatitis and liver fibrosis. This study was performed to evaluate the ability of Sl methanolic root extract to prevent liver fibrosis which represents a major health burden and is one of the most common cause of death worldwide.

Materials and Methods

Plant material and preparation of methanolic extract

Dried roots of Sl was purchased from a local herbalist and authenticated through a taxonomist, Dr. Nahed Waly at Faculty of Science, Cairo University, Egypt. Plant material was cleaned, ground to powder and extracted with 70% methanol in a container for

24hours. The solvent was then removed and fresh solvent was added to the plant material. The extraction method was repeated twice. It was filtered through filter paper and concentrated into thick semisolid viscous mass under reduced pressure on an evaporator, with a yield of 5% approximately.

Animals and chemicals

Female Sprague-Dawley rats (150-200gm) were obtained from the animal house of Mansoura experimental research center (MERC), Egypt. Experiments performed were approved by the committee on animals' experimentation of Mansoura University, Egypt and met the terms of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council [3]. Carbon tetrachloride, CCl4, was purchased from El-Gomhoria Co., (Egypt). Alanine transaminase (ALT) and aspartate transaminase (AST) estimation kits were purchased from Elitech, (UK). The reduced glutathione (GSH)and malondialdehyde (MDA) estimation kits were purchased from Biodiagnostickits, (Egypt).

Induction of liver fibrosis

Liver fibrosis was induced by intraperitoneal injection of CCl4 at dose of 1ml/kg dissolved in corn oil at 1: 1 ratio twice weekly for 8 weeks [4].

Control animals were fedonbasal diet only. Rats were sacrificed by cervical dislocation 3 days after the last injection of CCl4 administration. Blood samples, collected by direct cardiac puncture, into sterilized and heparinized syringes then centrifuged and plasma obtained was used for spectrophotometric estimation of ALT and AST [5]. Liver cutout and apportion was placed in 10% buffered formal saline. Liver tissue was then dehydrated through ascending concentrations of alcohol, cleared with xylene, impregnated and embedded in paraffin to form block.

Approximately 5μm sections were cut and fixed on glass slides, stained with hematoxylin and eosin, Sirius red stain and immune histochemical staining for alpha smooth muscle actin (α-SMA) for microscopic examination. Percentage of area occupied by collagen was measured by image analysis of Sirius red stained sections (40*magnification) using Image J program. Another portion of liver was homogenized for quantitative analysis of (MDA and GSH) in tissue homogenate.

Toxicity issue

According to the OECD-423 guidelines for acute oral toxicity, the LD50 dose of 2000 mg/kg and above for Sl root extract is categorized a sun-classified. 1/5of the LD50, 400mg/kg was taken as dose for the evaluation of antifibrotic activity.

Hepatoprotective study

Animals were divided into three groups of six rats each. Group 1 (negative control) received basal diet. Group2 (CCl_4-treated) received intraperitoneal injection of CCl_4 at dose of 1ml/kg dissolved in corn oil at 1:1 ratio twice weekly for 8 weeks. Group 3 (Sl-treated group) received both CCl_4, as described in group 2, and Sl methanolic root extract via gastric tube in a dose of 400mg/ kg daily for 8 weeks.

Statistical analysis

Serum ALT and AST, liver MDA and GSH, percentage of area occupied by collagen tissue were all presented as mean and standard deviation and the mean of two groups were compared by the independent samplet-test. P-value of <0.05 was considered as significant.

Results and Discussion

CCl_4-induced liver fibrosis in rat is a commonly used model. CCl_3, the toxic metabolite of CCl_4, binds to lipoprotein causing lipid peroxidation, Kupffer cell activation and initiation of an inflammatory response [6]. Yaeesh., *et al.* [2] were the first one to report the hepatoprotective activity of Sl root extract against acute hepatitis induced by injection of d-galactosamine (D-GalN) and lipopolysaccharide in mice. However, the present study differs from that of Yaeesh., *et al.* [2] in that, the present study evaluated the efficacy of Sl root extract in prevention of chronic liver toxicity induced by CCl_4 in rats, applying different techniques: histopathological, immunohistochemistry and measuring serum liver enzymes and hepatic oxidative stress marker levels.

In our study, Sl-treated group showed significant decrease in serum ALT and AST, Liver MDA and in the area occupied by collagen with significant increase in liver GSH as compared to the CCl_4-treated group (Table 1). Liver histopathology of Sl-treated group showed a decrease in; liver distortion, inflammatory cell infiltration and collagen deposition, with fewer thinner septa and less positive reaction for α-SMAascomparedtotheCCl_4-treatedgroup (Table 1, Figure 1). The hepatoprotective effect of Sl root, in our results, may be due to its antioxidant activity against CCl_4 induced oxidative stress, a major contributor to the liver fibrosis cascade. This antioxidant effect was confirmed by the significant decrease in liver MDA with significant increase in liver GSH in Sl-treated group as compared to CCl_4-treated group. In addition, an anti-inflammatory activity of Sl was proved by the decrease in the inflammatory cell infiltration seen in H and E stained sections, parallel to the results of Matsuda., *et al.* [7] who reported the inhibitory effect of Sl root on expression of macro phages, TNF-α and NO which drive the hepatic inflammation. Moreover, the diminished α-SMA immune histochemical positive reaction means a reduction in the number of hepatic myofibroblasts.

	Serum ALT (U/L)	Serum AST (U/L)	Liver MDA (nmol/gm)	Liver GSH (umol/gm)	Percentageof collagen area
Group1:negative control	24 ± 5.29	118.17 ± 13.73	8.47 ± 1.09	1.51 ± 0.09	2.98 ± 0.53
Group2:CCl_4 fibrosismodel	76.5 ± 6.47*	283.33 ± 20.06*	18.16 ± 0.52*	0.57 ± 0.05*	9.35 ± 0.72*
Group3:CCl_4+Sl root extract	39.17 ± 5.12#	187.83 ± 19.69#	11.8 ± 0.81#	1.11 ± 0.17#	4.22 ± 0.4#

Table 1: Effect of *Saussurea lappa* root extract on CCl4-induced change in liver enzymes, oxidative stress markers and percent age of liver collagen area.

*: p< 0.05; group1 versus2

#: p< 0.05; group2 versus3

Values represent mean ± standard deviation

Figure 1: Major chemical constituents of Saussurea Lappa Root Extract [16].

Conclusion

This may result from prevention of HSCs activation by the antioxidant and anti-inflammatory effects of Slor loss of those cells after activation. This loss is probably through apoptosis correlated to the results of Kim., *et al.* [8] who reported an apoptotic effect of dehydrocostus lactone, extracted from Sl root, against cancer cells.

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