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Antioxidant and Hepatoprotective activity of lycorine against Carbon tetrachloride-induced oxidative stress in Swiss albino mice

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ABSTRACT

In the present study the ability of lycorine as an antioxidant to protect against CCl_4 induced oxidative stress and hepatotoxicity in Albino mice was investigated. Oral administration of CCl_4, administered twice a week, produced a marked elevation in the serum levels of aspartate transaminase, alanine transaminase, Serum glucose, urea, bilurubin, lipid peroxidation and decreases of antioxidant status such as Catalase and superoxide dismutase. However, intraperitoneal [5 mg/ kg of body weight, IP] administration of lycorine daily for 8 weeks significantly reduced the aspartate transaminase, alanine transaminase, Serum glucose, urea, bilurubin, lipid peroxidation and increased the activities of catalase and superoxide dismutase and it was compared with silymarin. Therefore, the results of this study show that lycorine can be proposed to protect the liver against CCl_4 induced oxidative damage in mice, and the hepatoprotective effect might be correlated with its antioxidant and free radical scavenger effects.

Keywords. Antioxidant, hepatotoxicity, lipid peroxidation, oxidative damage, CCl_4

INTRODUCTION

In these days, a large volume of pharmaceutical is used for the prevention, diagnosis, and treatment of many diseases in humans and animals. Amaryllidaceae family plants and their formulation used for many disorders in ethnomedical practice as well as traditional medicine in all countries, because these plants contain more than twenty important alkaloids. Such an alkaloid has been used as therapeutic agents [1]. Many drugs are alkaloids derived from plants which have been used to treat cancer. For example, Pancratistatin from *Pancratium littorale* Jacq [2] has been selected by the United States Cancer Institutes for preclinical trials. Other biological activities have been established for the amaryllidaceae alkaloid.

Liver is the key organ for metabolism and detoxifications of various components enter into the body. It is involved in wide range of functions and it is continuously exposed to toxic substances and drugs absorbed from the intestine. Actual therapeutic agents have not yet been found. In fact

most of the available remedies support or promote the process of healing or regeneration of the liver.

CCl_4 is a highly toxic organic solvent and has been shown to provide an excellent model for the study of experimental oxidative injury due to its rapid metabolism. CCl_4 is converted into trichloromethyl radical in the liver with a subsequent initiation of lipid peroxidation [3]. It has been shown that CCl_4 induces fatty liver, cell necrosis; induce the triacyl glycerol accumulation, depletion of reduced glutathione, membrane damage, carbonylation of protein and loss of enzyme activity [3]. In addition, CCl_4 also alters the antioxidant profile of the liver including the antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione transferase (GST).

Lycorine is the major leaf and root bulb alkaloid of the amaryllidaceae plants. This alkaloid has been shown to behave as potent therapeutic agent in numerous experimental models. Pharmacological activities such as antiviral [4], anticancer [5], anti-inflammatory [6], antibacterial and analgesics agents [7] have been associated with the ability of lycorine to inhibit the in-vivo growth of a murine ascite tumour and reduced the viability of in vitro grown tumour cells [8]. However, no study has been investigated the hepatocytes ultrastructure protective nature of lycorine in CCl_4 induced mice. Hence, the present study is aimed to investigate the antioxidant potential of lycorine on pathophysiological marker enzyme, enzymic antioxidants, CCl_4 mediated oxidative stress and hepatocytes dysfunction in CCl_4 induced mice and the efficacy of lycorine was compared with silymarin is a standard well known positive hepatoprotective compound.

MATERIAL AND METHODS

Chemicals

Lycorine, Silymarin, NADH, thiobarbituric acid, GSH, and BSA were obtained from Sigma Chemicals [USA] and stored at 2–4°C and protected from sunlight. All other chemicals were analytical grades and were obtained from standard commercial suppliers.

Determination of optimum dosage of lycorine

Initially dose fixation study was conducted with lycorine. Mice administered with lycorine at five different doses 3, 5, 7, 9 and 11 mg/kg body weight] to determine the optimum dosage. At the dose of 5 mg shows the significant [$P < 0.05$] alteration in the activities of pathological marker enzymes such as AST, ALT, ALP and LDH in serum and the histological observations evidenced that lycorine effectively rescues the hepatocytes from CCl_4 induced oxidative damage without disturbing its cellular metabolic function and structural integrity. Hence, the dose of 5 mg/kg BW was chosen in this study.

Experimental design

The experimental animals were divided into four groups with each group comprising of six mice. Group I served as normal and was given olive oil daily for 8 weeks. For inducing hepatotoxicity animals of Groups II–IV were administered with carbon tetrachloride [orally] 1 ml/kg body weight of mice [20% CCl_4 in olive oil] twice a week for 2 months. Group II served as control CCl_4. and group III administered with lycorine [5mg/kg] and Group IV were administered orally with well known hepatoprotective compound silymarin [100 mg/kg] daily for 8 weeks. At the end of the experimental period, the animals were sacrificed by cervical dislocation. All the animal experiments were duly approved by the Institutional Animal Ethics Committee [743/03/abc/ CPCSEA dt 3.3.03] Guidelines.

Biochemical analysis

Serum Aspartate transaminase [AST], Alanine transaminase, Serum bilurubin, Urea, glucose were estimated by using commercially available kits according to the manufacturer's instruction.[AGGAPPE Diagnostic, Kerala and ENSURE BIOTECH, Hyderabad, India].

Measurement of CCl_4 mediated oxidative stress

The activities of enzymatic antioxidants such as SOD [8], Catalase [9], were assayed in control and experimental group of mice. Further, the levels of lipid peroxides [10] were determined in control and experimental groups of mice.

Statistical Analysis

Data were evaluated with SPSS/10 software hypothesis testing methods included one way analysis of variance [ANOVA] followed by least significant difference [LSD] test P values of less than 0.05 were considered to show statistical significance. All these results were expressed as mean ± SD for six animals in each group

RESULTS

The experimental results showed that the body weight of mice were reduced by the CCl_4 administered group, Nevertheless, the relative organs weight such as liver, kidney and spleen weight of the mice were significantly increased (Table 1). Conversely, the body weight and relative organ weight were reverted ($P < 0.05$) to near normalcy in CCl_4 induced group of mice by the lycorine and silymarin treatment. These findings suggested that lycorine can prevent the free radical induced oxidative damage caused by CCl_4

Table- 1 Effect of lycorine on body weight and organ weight (Relative weight (g/g of the body weight, %)

Groups	Body weight	Liver	Kidney	Spleen
Control	32.66±0.40	5.54±0.23	1.43±0.18	0.35±0.02
CCl_4 alone	27.29±0.23 [a]	6.46±0.20 [a]	2.18±0.19 [a]	0.64±.0.05 [a]
CCl_4 + lycorine	28.61 ±1.43 [b]	5.98±0.20 [b]	1.77±0.19 [b]	0.52±0.02 [b]
CCl_4 +Silymarin	31.49±0.32 [b]	5.62±0.20 [b]	1.41±0.17 [b]	0.42±0.02 [b]

Results were expressed as Mean± SD (nos= 6) ; a P <0.05 compare with Control groups mice; b P<0.05 compare with CCL4 induced groups mice

Table—2 Effect of lycorine on serum glucose, urea, bilurubin

Groups	Glucose mg/dl	Urea mg/dl	Total bilurubin mg/dl	Direct bilurubin mg/dl
Control	99±1.3	18±0.5	0.33±0.01	2.1±0.01
CCl_4 alone	150±2.5 [a]	35±0.1 [a]	0.66±0.04 [a]	2.4±0.05 [a]
CCl_4 + lycorine	125±6.2 [b]	19±0.4 [b]	0.43±0.03 [b]	2.2±0.01 [b]
CCl_4 +Silymarin	105±7.2 [b]	18±0.6 [b]	0.33±0.04 [b]	2.0±0.04 [b]

Results were expressed as Mean± SD (nos= 6) ; a P <0.05 compare with Control groups mice
b P<0.05 compare with CCL4 induced groups mice

Table 2 shows the effect of lycorine on serum glucose, urea, total bilurubin and direct bilurubin in control and experimental group of mice. The level of serum glucose, urea, total bilurubin and direct bilurubin were elevated in CCl_4 induced group of mice. Moreover, mice administered with lycorine the level of glucose, urea, total bilurubin and direct bilurubin were reduced when

compared with CCl_4 induced group of mice. Similarly, silymarin also reduced the level of glucose, urea, total bilurubin and direct bilurubin as compared to CCl_4 induced group of mice.
Table 3 shows the activities of biochemical enzymes such as ALT, and AST in control and experimental mice. The activities of ALT and AST were significantly [$p < 0.05$] increased when compared with control group of mice. However, the increased levels of the above enzymes were significantly reverted to normal levels by the treatment with lycorine at dosage level of 5mg / body weight of mice. The activities of the lycorine are comparable with reference drug Silymarin.

Table—3 Effect of lycorine on serum pathophysiological enzymes

Groups	AST(U/L)	ALT(U/L)
Control	92.90±5.0	59.00±2.6
CCl_4 alone	255.66±14.7 [a]	235.50±2.0 [a]
CCl_4+lycorine	137.16±13 [b]	64.8±2.4 [b]
Silymarin+ CCl_4	119.00±3.2 [b]	94.66±11 [b]

Results were expressed as Mean± SD (nos= 6) ; a P <0.05 compare with Control groups mice
b P<0.05 compare with CCL4 induced groups mice

MDA level is generally used as a marker of free radical mediated lipid peroxidation injury. We measured MDA levels in the serum and the results are shown in Table 4. MDA levels in the CCl_4 treated group were significantly higher than that in the control group [$p < 0.05$]. However, MDA levels in lycorine and silymarin treated group of mice at dose of 5 mg/kg and 100 mg/ kg were significantly lower than that in the CCl_4 administered group of mice [$p < 0.05$].

Table—4 Effect of lycorine serum antioxidant and lipid peroxidation status

Groups	Catalase U/mg	SOD U/Mg	MDA (nm/mg of Protein)
Normal	13.56±0.3	43.37±2.1	2.37±0.42
CCl_4 alone	8.64±0.5 [a]	24.71±1.3 [a]	2.95±0.54 [a]
CCl_4 +lycorine	11.41±0.2 [b]	41.13±2.53 [b]	7.26±0.76
CCl_4 +silymarin	16.49±0.2 [b]	67.23±1.9 [b*]	4.67±0.46

Results were expressed as Mean± SD (nos= 6) ; a P <0.05 compare with Control groups mice
b P<0.05 compare with CCL4 induced groups mice

Table 4 represents the effect of lycorine on the activities of enzymatic antioxidants such as catalase, SOD in serum of control and experimental groups of mice. The activities were significantly ($p < 0.05$) diminished in the serum of CCl_4 induced group of mice. However, treatment of lycorine and silymarin, significantly ($p < 0.05$) attenuated the altered activities of these enzymic antioxidants to near normalcy in serum of CCl_4 induced mice.

DISCUSSION

Carbon tetrachloride is xenobiotics that produce hepatotoxicity in human as well as in various experimental animals and it was biotransformed by Cytochrome P450 (CYP) into trichloromethyl radical $CCl^{\cdot}_3$ and trichloromethyl peroxyl radical. Which is initiated the lipid peroxidation [11] and involved in the pathogenesis of liver [12] both radicals are capable of binding to proteins or lipids, leading to membrane lipid peroxidation and finally cell necrosis [13]. Many studies have shown that a crucial mechanism of the hepatoprotective effects may be

related to the antioxidant capacities to scavenge reactive oxygen species [14, 15],. In fact, a considerable body of literature has reported that numerous antioxidant agent such as vitamin E [16] vitamin C and A [17] reduce CCl_4 induced hepatotoxicity effects by prevention of lipid peroxidation. Hepatic cells participate in a variety of metabolic activities and contain host of enzymes. In tissues Aspartate transaminase (AST) and Alanine transaminase (ALT) were found in higher concentrations in cytoplasm and mitochondria. In liver injury, the transport function of the hepatocytes is disturbed; resulting in the leakage of plasma membrane, thereby causing an increased enzyme level in serum and soluble enzymes like that AST will also be released. The elevated levels of AST and ALT in serum are indicating of cellular leakage and loss of functional integrity of cell membranes in liver [18]. Similarly our present investigation result shows that level of AST and ALT was increased after CCl_4 administration. However, treatment with lycorine to CCl_4 induced group of mice decline the activity of AST, ALT, glucose and urea which may be the stabilization of plasma membrane as well as repair of hepatic tissue damage caused by CCl_4.

Bilirubin is one of the most useful clinical parameters to know the severity of hepatic necrosis. It is an important degradation product of hemoglobin and is normally excreted into the bile. If hepatic parenchymal damage is severe, less bilirubin will be excreted and hyperbilirubinemia is observed that reflects pathophysiology of liver damage [19] A noticeable observation with lycorine was decreased the elevated level of serum bilirubin, which suggests that it can be used in the acute condition of jaundice. In the present investigation there was a significant rise in serum bilirubin and urea concentration after toxicant administration. It may be due to dysfunctional and dystrophic changes in the liver and kidney.

Oxidative stress is the state of imbalance between the level of antioxidant defence system and production of oxygen-derived species. Increased O_2concentration and production of oxygen-derived species such as superoxide radical ($*O_2\_$), hydroxyl radical (OH*) and hydrogen peroxide cause oxidative stress [20] Antioxidant activity has been hypothesized that one of the principal causes of CCl_4 induced liver injury is formation of lipid peroxides by free radical derivatives of CCl_4 (CCl^{3-}). Thus, the antioxidant activity or the inhibition of the generation of free radicals is important in the protection against CCl_4induced hepatopathy [21]. The body has an effective defense mechanism to prevent and neutralize the free radical-induced damage. This is accomplished by a set of endogenous antioxidant enzymes such as SOD, CAT and GPx. In CCl_4 induced group of mice shows the reduced activities of Catalase and Superoxide dismutase. However, after treatment with lycorine significantly increased the activities of Catalase and Super oxides dismutase.

Our results demonstrate a very good protective effect of lycorine against CCl_4 induced liver injury, which is probably due at least partly to its antioxidant activities, scavenging CCl_4 associated free radicals

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