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Hepatoprotective Activity of Livobond A Polyherbal Formulation Against CCl_4 Induced Hepatotoxicity in Rats

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Abstract: In the present study, Livobond was evaluated for its hepatoprotective effects against carbon tetrachloride-induced hepatocellular injury in rats. Hepatotoxicity was induced in male Sprague-Dawley rats by intraperitoneal injection of CCl_4 (1.5 mL kg^{-1}) in olive oil (1:1). Livobond at a dose of 500 and 750 mg/kg/day and silymarin standard 50 mg/kg/day was administered orally for 7 days. The hepatoprotective effect of Livobond and standard was evaluated by the assay of biochemical parameters viz., alanine aminotransaminase (ALT), aspartate aminotransaminase (AST), alkaline phosphatase (ALP), total and direct bilirubin, liver lipid peroxidation, total proteins, catalase and by histopathological studies of the liver. The toxic effects of CCl_4 in Livobond treated group was controlled significantly by restoration of the levels of serum bilirubin, proteins and enzymes as compared to the CCl_4 treated and silymarin treated groups. Histopathological studies further confirmed the hepatoprotective activity of Livobond. The results suggest that Livobond is able to significantly alleviate the hepatotoxicity induced by CCL_4 and may be attributed to the antioxidant property of the formulation.

Key words: Carbon tertrachloride, Livobond, polyherbal formulation, hepatoprotective activity

INTRODUCTION

The liver which occupies the pivotal position in body, plays an essential role in drug and xenobiotic metabolism and in maintaining the biological equilibrium of the organism. The role played by this organ in the removal of substances from the portal circulation makes it susceptible to a persistent attack by offending foreign (xenobiotic) compound culminating in liver dysfunction. Despite the tremendous strides in modern medicine, there are few drugs that stimulate liver function, offer protection to the liver from damage or help to regenerate hepatic cells. However many herbal formulations are available for liver disorders in the Indian market based on Ayurvedic principles (Rajesh and Latha, 2001). Herbal drugs are frequently considered to be less toxic and free from side effects than synthetic drugs. Medicinal plants like *Andrographis paniculata*, *Boerhaavia diffusa* (Rawat *et al*., 1997) *Hibiscus rosasinensis* (Frederick *et al*., 1998), *Phyllanthus amarus* (Sane *et al*., 1995), are well known for their hepatoprotective effects. In the present investigation Livobond, a polyherbal formulation consisting of medicinal plants derived from the traditional system of medicine in India Ayurveda, has been evaluated for its hepatoprotective action. Livobond is a polyherbal formulation consisting of medicinal plants derived from the traditional system of medicine in India, the Ayurveda. Since, there is lack of scientific data regarding pharmacological evaluation of Livobond, it was consequently considered worthwhile to screen Livobond for its hepatoprotective activity. The hepatotoxin used was CCl_4 because CCl_4^- induced liver dysfunction in rats simulates liver cirrhosis in man (Brensilver and Kaplan, 1975). The herbal formulation consists of an aqueous extract of seventeen medicinal plants (Table 1) some of which are known to possess hepatoprotective activities.

Table 1: Composition of Livobond

Botanical name	Sanskrit name	Common name
Salvadora persica	Pilu	Chota pilu
Eclipta alba	Bhringaraja	Bhangra
Tecoma undulata	Rohitak	Rohitak
Boerhaavia diffusa	Punarnava	Beshakapore
Embelia ribes	Vidanga	Vayvidang
Aloe barbadensis	Kumari rasa	Kumari Rasa
Vitis venifera	Draksha	Angur
Coriandrum sativum	Dhanyakama	Dhania
Cassia occidentalis	Kasmarda	Kasondi
Andrographis paniculata	Kalmegh	Kiryata
Cissampelos pereira	Patha	Harjori
Picrorhiza kurrao	Katurohini	Kutki
Terminalia arjuna	Arjuna Tvaka	Arjuna
Berberis aristata	Darvi	Darhaldi
Piperum longum	Pippali	Pipal
Cichorium intybns	Kasni Bij	Kasni
Tefrosia purpurea	Sharapunkha	Sarphankha

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MATERIALS AND METHODS

The research was carried out from April 2008 to May 2008.

Drugs and chemicals: Livobond was provided as a gift sample by Unjha Pharmacy, India. Silymarin was provided Sigma Labs. All chemicals used were of analytical grade.

Animals: Sprague-Dawley albino rats (weighing 170-210 g) were used for the study. Animals were supplied by Haffkines Biopharmaceutical Corporation Ltd., Parel, Mumbai and ACTREC (Advanced Centre for Treatment, Research and Education in Cancer), Kharghar, Navi Mumbai, India and kept under standard laboratory conditions in a 12 h light-dark cycle at 25±2°C. Animals were provided with standard pellet diet and water *ad libitium*. The protocol was approved by Institutional Animal Ethical Committee constituted for the purpose.

Experimental design: Rats were randomly divided into six groups with six animals each. Group 1 served as a normal control and received distilled water for 7 days. Group 2 served as toxin control and received distilled water for 6 days and on the 7th day received a single dose of CCl_4 (1.5 mL kg^{-1}, 60 in olive oil, 1:1) intraperitoneally. Group 3 served as a drug control and received Livobond, 750 mg/kg/day, orally for 7 days. Group 4 and 5 were treated daily with 500 and 750 mg/kg/day Livobond orally for 7 days and on the 7th day received a single dose of CCl_4 (1.5 mL kg^{-1}, in olive oil, 1:1, 30 min after administration of Livobond). Group 5 served as a standard and received silymarin (50 mg/kg/day) daily for 7 days and on the 7th day received a single dose of CCl_4 (1.5 mL kg^{-1}, in olive oil, 1:1, 30 min after administration of silymarin). At the end of the experimental period, rats were deprived of food overnight and sacrificed by decapitation. Blood samples were collected and allowed to clot for 30-40 min. Serum was separated by centrifugation at 37°C and was used for estimation of various biochemical parameters. Liver was removed rapidly, and cut into separate portions for hepatic catalase, lipid peroxidation estimation, total proteins and histopathological studies.

Biochemical evaluation: The activities of aspartate aminotransaminase (AST) (Bradley *et al*., 1972; Wolf *et al*., 1972), alanine aminotransaminase (ALT) (Bradley *et al*., 1972; Wolf *et al*., 1972), alkaline phosphatase (ALP) (Bessey *et al*., 1946) and total and direct bilirubin (Pearlman and Lee, 1974) in serum and total proteins (Flack and Woollen, 1984) in the tissue homogenate were determined using standard diagnostics kits of Erba in ERBA auto analyzer (Erba Manheim, Germany). The contents of malondialdehyde (MDA) and catalase (CAT) were determined by the methods of Buege and Aust (1978) and Aebi (1984), respectively.

Statistical analysis: Results are expressed as Mean±SEM. Total variation present in a set of data was estimated by one-way analysis of variance (ANOVA) followed by Dunnet's test. $p<0.05$ was considered significant.

RESULTS AND DISCUSSION

Assessment of serum marker enzymes and hepatic antioxidants: Hepatic damage induced by CCl_4 causes significant rise in marker enzymes AST, ALT, ALP and also serum total and direct bilirubin (Table 2). Enhanced liver lipid peroxidation (LP) expressed in terms of thiobarbituric acid reacting substances and reduced activities of catalase (CAT) and Total Proteins (TP) observed in CCl_4 treated rats also confirms hepatic damage (Table 3). Pretreatment of Livobond at doses of 500 and 750 mg kg^{-1} showed a significant decrease ($p\leq0.05$) in AST, ALT, ALP, total and direct bilirubin and liver lipid peroxidation levels and a significant increase ($p\leq0.05$) in liver tissue catalase and total proteins. The degree of protection was observed maximally with the highest dose of the herbal formulation (750 mg kg^{-1} body weight). Standard control drug, silymarin at a dose of 50 mg kg^{-1} also prevented elevation serum enzymes and liver lipid peroxidation, and lowering of tissue catalase and total proteins. However protection offered by silymarin was relatively lower.

Histopathological evaluation: The histopathological observations supported the results obtained from

Table 2: Effect of Livobond and Silymarin on biochemical serum parameters in rats subjected to CCl_4 induced toxicity

Groups	AST (IU L^{-1})	ALT (IU L^{-1})	ALP (IU L^{-1})	Total bilirubin (mg dL^{-1})	Direct bilirubin (mg dL^{-1})
1	227.2±11.99	78.42±4.741	130.1±6.752	0.559±0.099	0.663±0.098
2	318.8±10.15	148.50±11.14	220.8±11.03	1.467±0.055	1.449±0.256
3	230.5±9.106*	70.66±4.315*	133.5±9.981*	0.633±0.142*	0.520±0.054*
4	266.3±7.025*	115.50±5.868*	181.9±9.324*	0.924±0.121*	0.934±0.094*
5	231.5±8.657*	84.99±5.642*	136.8±8.729*	0.644±0.114*	0.699±0.106*
6	267.3±8.745*	98.55±7.048*	162.6±12.92*	0.766±0.082*	0.747±0.110*

Values are expressed as mean±SEM; n = 6, *$p\leq0.05$, one way ANOVA followed by Dunnet's test when compared with CCl_4 control

Table 3: Effect of Livobond on liver catalase, lipid peroxidation and total proteins

Groups	Catalase	Lipid peroxidation	Total proteins (g dL^{-1})
1	116.5±8.478	18.11±2.176	1.466±0.119
2	67.7±9.044	33.98±3.004	0.841±0.092
3	111.7±5.527*	19.57±1.774*	1.270±0.138*
4	95.2±10.11*	25.47±2.405*	1.031±0.140*
5	114.1±7.556*	19.70±1.844*	1.319±0.087*
6	119.9±12.70*	22.88±3.107*	1.176±0.078*

Catalase (CAT) - µmoles of H_2O_2 decomposed/mg protein/min. Lipid Peroxidation (LP) - moles of MDA g^{-1} of tissue. Values are expressed as Mean±SEM; n = 6, *p ≤ 0.05 one way ANOVA followed by Dunnet's test when compared with CCl_4 control

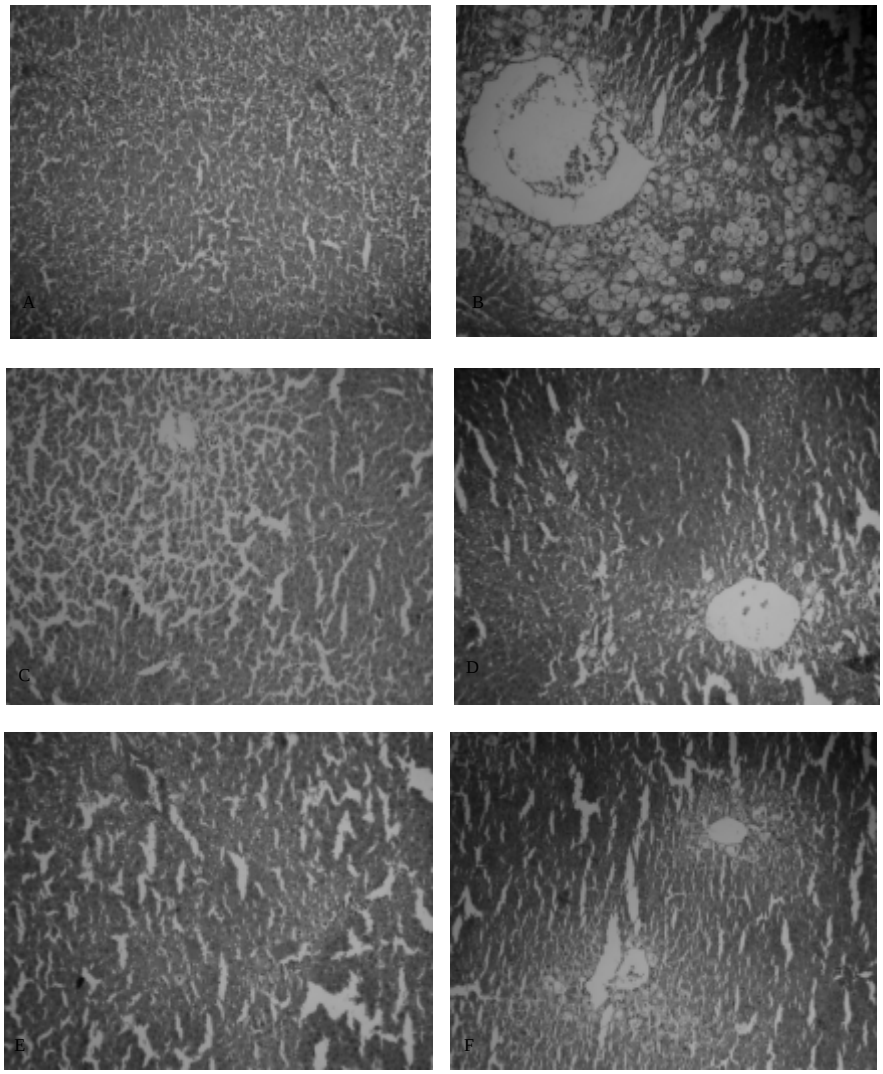

Fig. 1: Effect of LIVOBOND on histopathological changes that occurred in rats during carbon tetrachloride intoxication. (A) Normal control, (B) CCl_4 control, (C) Drug control (750 mg kg^{-1}), (D) Livobond (500 mg kg^{-1}), (E) Livobond (750 mg kg^{-1}) and (F) Silymarin (50 mg kg^{-1}). Scale bar: 100 µ (0.1 mm)

biochemical estimations. The histological profile of the control animals showed normal hepatic architecture (Fig. 1A). In rats treated with CCl_4 (Fig. 1B), the normal architecture of the liver was completely lost with the appearance of centrilobular necrosis with tiny vacuoles, lymphocytes infiltration of the periportal area and fatty changes were observed. The animals administered with Livobond at 500 and 750 mg kg^{-1} (Fig. 1D, E) and silymarin 50 mg kg^{-1} (Fig. 1F) showed a significant recovery from CCl_4 induced liver damage as evident from normal hepatic architectural pattern.

DISCUSSION

Since the changes associated with the CCl_4 induced liver damage are similar to that of acute viral hepatitis (Suja *et al*., 2004), CCl_4^- mediated hepatotoxicity was chosen as the experimental model. The ability of a hepatoprotective drug to reduce the injurious effects or to preserve the normal hepatic physiological mechanism, that have been disturbed by a hepatotoxin, is the index of its protective effect (Yadav and Dixit, 2003).

CCl_4 is biotransformed by cytochrome P450 system to produce trichloromethyl free radicals. These free radicals may again react with oxygen to form trichloromethyl peroxyl radical, which may attack lipids on the membrane of endoplasmic reticulum to elicit lipid peroxidation, finally resulting in cell necrosis and consequently cell death (Shyamal *et al*., 2006).

Hepatocellular necrosis leads to elevation of the serum marker enzymes, which are released from the liver into blood (Shenoy *et al*., 2001). Hepatic cells participate in a variety of metabolic activities and contain a host of enzymes. In tissues, AST and ALT are found in higher concentrations in cytoplasm and AST in particular also exists in 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. The elevated levels of AST and ALT in serum are indicative of cellular leakage and loss of the functional integrity of cell membranes in liver. Administration of CCl_4 significantly raises the serum levels of enzymes like AST and ALT (Rajesh and Latha, 2004) in rats as observed in present results. Oral administration of Livobond at a dose of 750 mg kg^{-1} body weight to rats caused a decrease in the activity of the above enzymes, which may be a consequence of the stabilization of plasma membrane as well as repair of hepatic tissue damage caused by CCl_4. The activity of serum alkaline phosphatase was also elevated during CCl_4 administration. Alkaline phosphatase is excreted normally via bile by the liver. In liver injury due to hepatotoxin, there is a defective excretion of bile by the liver, which is reflected in their increased levels in serum. Hyperbilirubinaemia is a very sensitive test to substantiate the functional integrity of the liver and severity of necrosis which increases the binding, conjugating and excretory capacity of hepatocytes that is proportional to the erythrocytes degeneration rate (Rajesh and Latha, 2004).

Depletion of elevated bilirubin level together with the suppression of the activity of ALP in the serum of rats treated with Livobond, suggests the possibility of the herbal product being able to stabilize biliary dysfunction of rat liver during chronic injury with CCl_4. Malondialdehyde (MDA) is a major reactive aldehyde resulting from the peroxidation of biological membrane polyunsaturated fatty acid. MDA, a secondary product of lipid peroxidation, is used as an indicator of tissue damage involving a series of chain reactions. It reacts with thiobarbituric acid, producing red-coloured products. Lipid peroxidation has been implicated in the pathogenesis of increased membrane rigidity, osmotic fragility, reduced erythrocyte survival and perturbation in lipid fluidity. It has been hypothesized that one of the principal cause of CCl_4 induced hepatotoxicity is lipid peroxidation of hepatocyte membranes by free radical derivatives of CCl_4 (Jayakumar *et al*., 2006). The observation of elevated levels of hepatic MDA in Group 2 rats (administered CCl_4 alone) in the present study is consistent with this hypothesis. Thus the maintenance of near normal levels of hepatic MDA in Group 5 rats (administered CCl_4 and Livobond, 750 mg kg^{-1} body weight) is of great interest since it provides additional evidence to suggest a hepatoprotective role for Livobond.

Catalase is an enzyme which is an important component of the innate defence mechanism of living tissues. A reduction in the activity of this enzyme is associated with the accumulation of highly- reactive free radicals, leading to deleterious effects such as loss of integrity and function of cell membranes. Administration of CCl_4 leads to generation of peroxy radical, O_2^- which is associated with inactivation of CAT. This probably explains the significantly reduced activities of CAT observed in rats challenged with CCl_4 (Group 2). In rats receiving CCl_4 and Livobond (Group 5), the activity of CAT were significantly higher than in Group 2 rats and very similar to the values noted in normal.

The protective effect of Livobond was further substantiated by histopathological assessment, where necrotic and infiltrative changes were observed.

In a dose response study, it was found that Livobond at a dose of 750 mg kg^{-1} could almost protect the liver from toxic injury, which was better, when compared with the standard drug, silymarin. The results of the present study shows that Livobond confer protection against liver damage induced by CCl_4, which strongly suggests that the polyherbal formulation have a potential clinical application in therapy for liver diseases.

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