

Protective effect of Livactine against CCl₄ and paracetamol induced hepatotoxicity in adult Wistar rats

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Abstract

Background: Liver disease has become one of the serious health problems as it is exposed to many kinds of xenobiotics and therapeutic agents. Moreover the rapidly growing morbidity and mortality from liver disease are attributable to the increasing number of chemical compounds and environmental pollution. Unfortunately, so far, in the modern era of medicine there is no specific treatment to counter the menacing impact of these dreaded diseases. Many polyherbal formulations are used widely to treat these disorders. Livactine is a polyherbal formulation and is claimed to be useful in jaundice and biliary dysfunctions. Most of these formulations do not have standard and approved reports stating their pharmacological action or therapeutic efficacy. Therefore, there is a need for experimental confirmation of the pharmacological effects of this formulation. The rationale behind the selection of carbon tetrachloride is due to its free radical mechanism based liver injury, and paracetamol is consumed widely by the human population and it is also a potential liver hazard. **Aim:** To evaluate the anti-hepatotoxic activity of Livactine against carbon tetrachloride & paracetamol induced toxicity in rats. **Material and Methods:** Albino rats of Wistar strain were used to evaluate the hepatoprotective activity of Livactine against carbon tetrachloride & paracetamol induced toxicity. Liver damage was assessed by estimating various biochemical parameters such as serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase, lactate dehydrogenase, alkaline phosphatase, acid phosphatase, total bilirubin, and total protein. The results of the rats treated with Livactine were compared with that of Liv-52. **Results:** Livactine showed significant dose dependent hepatoprotective effect by reducing elevated serum enzyme levels when compared to that of Liv-52. **Conclusion:** Our findings confirm that the formulation was found to be effective pharmacologically at higher dose against carbon tetrachloride and paracetamol induced hepatotoxic models and were comparable to that of Liv-52. The resultant hepatoprotective activity of Livactine could be due to its free radical scavenging property of the ingredients.

Keywords: Livactine, hepatoprotective, carbon tetrachloride, paracetamol.

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Introduction

The liver is a vital organ which regulates many important metabolic functions and is responsible for maintaining homeostasis of the body. A number of chemical agents and drugs which are used on a routine basis produce cellular as well as metabolic liver damage [1]. The traditional systems of medicine like Ayurveda, Siddha, Unani have a major role in the treatment of liver ailments [2]. In the recent years, importance is being given to ayurvedic polyherbal formulations due to their effective therapeutic action and

lack of side effects. Nowadays, there are different marketed formulations available for the treatment of liver disorders such as Liv 42, Liv-52, Livercure, Livol, Livomyn, Livfit, Livogen and Livactine [3-4]. Liv-52 is an indigenous polyherbal formulation that has been used as an anti hepatotoxic agent in various liver disorders. Livactine is a herbal formulation which contains extracts of 9 medicinal plants of which some plants, viz, Boerhavia diffusa, Tinospora cordifolia, Andrographis paniculata and Emblica officianalis have been individually

reported to possess hepatoprotective effect and antioxidant properties. Most of hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and oxidative stress in liver. Carbon tetrachloride (CCl₄) and paracetamol get converted into reactive toxic metabolites by hepatic microsomal cytochrome P450 and cause hepatotoxicity [5-7]. Search for new drugs for limiting hepatic injury has been of interest recently. The present study was conducted to evaluate the hepatoprotective activity of Livactine against CCl₄ and paracetamol induced hepatotoxicity in rats.

Materials and Methods

Chemicals and Drugs

Livactine syrup (Ratnakar's Pharmaceuticals, Andhra Pradesh, India), Liv-52 syrup (The Himalaya Drug Company, Himachal Pradesh, India), CCl₄ (Qualigen's, Mumbai, India), Paracetamol (Yarrow Chem Products, Mumbai, India), Liquid paraffin (Qualigen's, Mumbai, India), Sucrose (Qualigen's, Mumbai, India). Estimation of marker enzymes for liver functions such as SGOT, SGPT, ALP, ACP, Total bilirubin, Total protein were performed by using kits provided by Span Diagnostic Ltd., (Surat, India).

Experimental Animals

Male albino rats of Wistar strain weighing about 250-350 g were used for the experiment. The animals were obtained from the animal house, Bapatla College of Pharmacy, Bapatla and were housed in well ventilated hygienic experimental animal house under constant environmental and nutritional conditions. All the rats were kept in polyacrylic cages and were administered with food and water *ad libitum*. The animals were maintained as per the Committee for the Purpose of Control and Supervision of Experiments on Animals (1032/ac/07/CPCSEA) regulations and the study protocol was approved by the Institutional Animal Ethics Committee (IAEC/II-2/BCOP/2009) of Bapatla College of Pharmacy, Bapatla.

Experimental Protocol

Carbon tetrachloride induced hepatotoxicity Adult male albino rats (250-350 g) were used for the study. Animals were divided into five groups of six animals each. Group I served as normal control and received normal saline 1ml/kg, p.o. Group II was administered with CCl₄ in liquid paraffin (30% v/v) 1ml/kg, i.p. Group III was treated with the standard drug Liv-52 at a dose of 1ml/kg, p.o. Group IV and V was treated with 1, 2 ml/kg dose levels of Livactine per orally. Group III, IV and V received drug treatment along with CCl₄ in liquid paraffin (30% v/v) 1ml/kg. Carbon tetrachloride in liquid paraffin was administered every 72 hours [8]. The treatment was carried out for a period of 10 days.

Paracetamol induced hepatotoxicity Group I served as normal control and received normal saline 1ml/kg, p.o, Group II was administered with a dose of paracetamol (3g/kg, p.o) in 50% sucrose solution, Group III was

administered with Liv-52 (1ml/kg/p.o) & paracetamol (3g/kg p.o) in 50% sucrose solution, Group IV and V was administered Livactine (1, 2 ml/kg p.o) & paracetamol (3g/kg p.o) in 50% sucrose solution. The duration of treatment was 10 days. Paracetamol (single dose) was administered on the tenth day of the treatment. The blood samples were withdrawn on the 11th day through the retro-orbital puncture for the estimation of biochemical parameters [9].

Biochemical Estimation The blood samples were collected without any anticoagulant and were allowed to clot for 10 minutes at room temperature. The blood was centrifuged at 2500 rpm for 20 minutes at 30^oC. The obtained serum was stored at 4^oC for the estimation of SGOT [10], SGPT [10], ALP [11], ACP [11], LDH [12], Total bilirubin [13], Total protein [14]. These estimations were done according to the standard procedures given along with the kits purchased.

Statistical Analysis

The data of biochemical estimations were reported as mean ± SEM. The statistical significance was determined by using one way analysis of variance (ANOVA) followed by Dunnet's multiple comparison tests. P < 0.05 was used to determine statistical significance.

Results

The results of Livactine against CCl₄ and paracetamol induced hepatotoxicity are shown in Table 1 and 2. Administration of CCl₄ & paracetamol to rats caused severe liver damage as there was a significant increase in the levels of SGPT, SGOT, ALP, ACP, LDH, total bilirubin whereas a significant decrease in the level of total proteins was observed which may be due to acute hepatocellular damage and biliary obstruction. Rats treated with Livactine exhibited a significant reduction in the CCl₄ & paracetamol induced increase in the levels of SGPT, SGOT, ALP, ACP, LDH, total bilirubin and increased the levels of total proteins. The protective effect was comparable with Liv-52.

Discussion

It is well established that CCl₄ induces hepatotoxicity by metabolic activation; therefore it selectively causes toxicity in liver cells maintaining semi normal metabolic function. CCl₄ is biotransformed by cytochrome P450 system in the endoplasmic reticulum to produce trichloromethyl free radical (CCl₃). Trichloromethyl free radical when combined with cellular lipids and proteins in the presence of oxygen form trichloromethylperoxyl radical, which may attack lipids on the membrane of endoplasmic reticulum faster than trichloromethyl free radical. Thus, trichloromethylperoxyl free radical leads to elicit lipid peroxidation. The destruction of Ca²⁺ homeostasis, finally results in cell death. Hepatoprotective activity of any drug is the ability of its constituents to inhibit the aromatase activity of cytochrome P450 thereby favoring liver regeneration [15-17].

Table 1 Effect of Livactine on CCl₄ induced hepatotoxicity in rats.

Groups	Treatment	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	ACP (IU/L)	LDH (IU/L)	Total Bilirubin (mg/dL)	Total Protein (g/dL)
1	Normal Saline	88.00±	44.33±	133.80±	4.31±	247.50±	0.50±	7.65±
	1 ml/kg	2.53 ^{b#} c [#]	2.44 ^{b#} c [#]	1.60 ^{b#} c [#]	0.19 ^{b#} c [#]	2.81 ^{b#} c [#]	0.02 ^{b#} c ^{ns}	0.08 ^{b#} c ^{ns}
2	CCl ₄ /Liquidparaffin (30%v/v)	516.20±	475.30±	772.20±	15.03±	453.20±	0.83±	6.40±
	1 ml/kg	3.55 ^{a#} c [#]	1.74 ^{a#} c [#]	2.08 ^{a#} c [#]	0.15 ^{a#} c [#]	5.10 ^{a#} c [#]	0.03 ^{a#} c [#]	0.13 ^{a#} c [#]
3	Liv-52	141.21±	74.17±	280.00±	7.10±	280.30±	0.51±	7.55±
	1 ml/kg	2.38 ^{a#} b [#]	2.38 ^{a#} b [#]	2.88 ^{a#} b [#]	0.14 ^{a#} b [#]	2.76 ^{a#} b [#]	0.03 ^{a#} b [#]	0.15 ^{a#} b [#]
4	Livactine	335.80±	267.70±	454.50±	8.05±	343.00±	0.63±	7.08±
	1 ml/kg	2.40 ^{a#} b [#] c [#]	2.39 ^{a#} b [#] c [#]	5.37 ^{a#} b [#] c [#]	0.14 ^{a#} b [#] c [#]	2.94 ^{a#} b [#] c [#]	0.06 ^{a#} b [#] c ^{ns}	0.34 ^{a#} b [#] c ^{ns}
5	Livactine	248.80±	195.00±	318.7±	7.71±	300.80±	0.60±	7.46±
	2 ml/kg	3.27 ^{a#} b [#] c [#]	4.02 ^{a#} b [#] c [#]	2.04 ^{a#} b [#] c [#]	0.09 ^{a#} b [#] c [#]	5.06 ^{a#} b [#] c [#]	0.02 ^{a#} b [#] c ^{ns}	0.08 ^{a#} b [#] c ^{ns}

The values are Mean ± S.E.M of 6 observations, a- represents the probability of significance when compared to Group 1, b- represents the probability of significance when compared to Group 2, c- represents the probability of significance when compared to Group 3, * - p<0.05; ** - p<0.01; # - p<0.001; ns-non significant. (ANOVA followed by Dunnett's multiple comparison test).

Table 2 Effect of Livactine on paracetamol induced hepatotoxicity in rats.

Groups	Treatment	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	ACP (IU/L)	LDH (IU/L)	Total Bilirubin (mg/dL)	Total Protein (g/dL)
1	Normal Saline	86.67±	42.00±	132.7±	4.06±	250.8±	0.50±	7.63±
	1 ml/kg	1.76 ^{b#} c [#]	1.46 ^{b#} c [#]	1.52 ^{b#} c [#]	0.12 ^{b#} c [#]	3.00 ^{b#} c [#]	0.01 ^{b#} c ^{ns}	0.12 ^{b#} c ^{ns}
2	Paracetamol	150.00±	112.00±	340.00±	6.71±	362.7±	0.71±	6.83±
	3 g/kg	2.30 ^{a#} c [#]	1.46 ^{a#} c [#]	2.30 ^{a#} c [#]	0.16 ^{a#} c [#]	1.76 ^{a#} c [#]	0.02 ^{a#} c [#]	0.17 ^{a#} c [#]
3	Liv-52	112.00±	53.50±	152.30±	5.15±	260±	0.53±	7.70±
	1 ml/kg	1.36 ^{a#} b [#]	1.31 ^{a#} b [#]	1.74 ^{a#} b [#]	0.14 ^{a#} b [#]	2.92 ^{a#} b [#]	0.02 ^{a#} b [#]	0.08 ^{a#} b [#]
4	Livactine	146.30±	98.33±	300.70±	6.70±	343.30±	0.61±	7.10±
	1 ml/kg	1.40 ^{a#} b ^{ns} c [#]	1.40 ^{a#} b ^{ns} c [#]	1.33 ^{a#} b ^{ns} c [#]	0.11 ^{a#} b ^{ns} c [#]	0.95 ^{a#} b ^{ns} c [#]	0.01 ^{a#} b ^{ns} c [#]	0.08 ^{a#} b ^{ns} c [#]
5	Livactine	128.30±	59.00±	278.70±	6.43±	295.30±	0.56±	7.73±
	2 ml/kg	0.95 ^{a#} b ^{ns} c [#]	1.41 ^{a#} b ^{ns} c [#]	0.98 ^{a#} b ^{ns} c [#]	0.18 ^{a#} b ^{ns} c [#]	2.82 ^{a#} b ^{ns} c [#]	0.01 ^{a#} b ^{ns} c [#]	0.12 ^{a#} b ^{ns} c [#]

The values are Mean ± S.E.M of 6 observations, a- represents the probability of significance when compared to Group 1, b- represents the probability of significance when compared to Group 2, c- represents the probability of significance when compared to Group 3, * - p<0.05; ** - p<0.01; # - p<0.001; ns-non significant. (ANOVA followed by Dunnett's multiple comparison test).

In the present study it was observed that the administration of CCl₄ decreased the levels of proteins and increased the levels of serum marker enzymes significantly (P<0.001) which is an evidence of existence of liver toxicity when compared to normal animals (Table 1). These elevated marker enzymes were brought back and the total protein levels were elevated in case of Liv-52 treated animals and was found to be highly significant (P<0.001). Livactine at a dose of 1 ml/kg produced highly significant (P<0.001) reduction in the elevated marker enzymes like SGOT, SGPT, ALP, ACP and LDH whereas the restoration of bilirubin level was found to be less significant (P<0.01). The protein levels were not significantly altered when compared to that of carbon tetrachloride intoxicated group. Significant variation (P<0.001) were observed when compared with Liv-52 treated rats in case of SGOT, SGPT, ALP, ACP, LDH etc whereas the difference were found to be insignificant in case of total bilirubin and total protein. Treatment with Livactine at a dose of 2 ml/kg has shown a significant (P<0.001) protective effect against CCl₄ induced toxicity which is clearly evident from the restoration of elevated marker enzymes and increase of protein parameter. The activity of Livactine at 2 ml/kg dose level was comparatively similar to that of the standard treatment and the difference was found to be insignificant. The marked elevation of bilirubin in the

serum of CCl₄ intoxicated rats were significantly decreased in groups treated with the Livactine.

Bilirubin is the conventional indicator of liver diseases and these biochemical restorations may be due the promotion of its glucuronidation [16]. The ingredients of Livactine were reported to possess antioxidant and hepatoprotective property. The possible mechanism by which Livactine exhibited significant protection against CCl₄ induced hepatotoxicity may be due its free radical scavenging mediated restoration of cellular methylation and inhibition of loss of calcium sequestration [17]. Attainment of normal levels of proteins and other serum enzyme levels in Livactine treated rats confirms the hepatoprotective effect of the polyherbal formulation. The activity was found to be dose dependant and higher doses of the formulation may result in better protection against liver disorders.

Paracetamol is a commonly and widely used analgesic antipyretic agent. Hepatotoxic doses of paracetamol deplete the normal levels of hepatic glutathione (see at <http://en.wikipedia.org/wiki/Paracetamol> - cite _note -Goldfrank-42). The hepatic cytochrome P450 enzyme system metabolizes paracetamol, forming a minor yet significant alkylating metabolite known as NAPQI (N-acetyl-p-benzo-quinone imine). NAPQI is then

irreversibly conjugated with the sulfhydryl groups of glutathione [18]. NAPQI is primarily responsible for the toxic effects of paracetamol.

Production of NAPQI is primarily due to, two isoenzymes of cytochrome P450: CYP2E1 and CYP1A2. The P450 gene is highly polymorphic, however, and individual differences in paracetamol toxicity were believed to be due to a third isoenzyme, CYP2D6. CYP2D6 metabolises paracetamol into NAPQI to a lesser extent than other P450 enzymes, its activity may contribute to paracetamol toxicity in extensive and ultrarapid metabolisers, and when paracetamol is taken at very large doses. In the liver, the cytochrome P450 enzymes CYP2E1 and CYP3A4 were primarily responsible for the conversion of paracetamol to NAPQI which undergoes conjugation with glutathione. Conjugation depletes glutathione, a natural antioxidant. This in combination with direct cellular injury by NAPQI, leads to cell damage and death [19-21]. Excess production of paracetamol metabolite causes the initial hepatic damage and subsequent activation of inflammatory mediator TNF- α which in turn contribute to tissue necrosis.

In the present investigation it was observed that the administration of paracetamol decreased the levels of proteins and increased the levels of serum marker enzymes significantly ($P < 0.001$) which is an evidence of existence of liver toxicity, (Table 2). Livactine at a dose of 1 ml/kg produced highly significant ($P < 0.001$) reduction in the elevated marker enzymes like SGPT, ALP and LDH only. Whereas the restoration of bilirubin level was found to be less significant ($P < 0.01$). The protein levels were not significantly altered when compared to that of paracetamol intoxicated control group animals. Treatment with Livactine at a dose of 2 ml/kg showed significant ($P < 0.001$) protective effect which is clearly evident from the restoration of elevated marker enzymes and increase of protein parameter similar to that of Liv-52 treated group. The possible mechanism by which Livactine exhibited significant protection against paracetamol induced hepatotoxicity may be due to the active constituents present in various ingredients like flavonoids, alkaloids, sterols etc and its free radical scavenging activity. It is well reported that these active constituents are responsible for the hepatoprotective activity and attainment of normal levels of proteins and other serum enzyme levels in Livactine treated rats confirms the hepatoprotective effect of the polyherbal formulation.

Based on the obtained results it is well evident that Livactine is pharmacologically effective for the treatment of liver disorders at a higher dose levels when compared to Liv-52.

Conclusion

The present results provide strong evidence that the formulation Livactine inhibits hepatotoxicity induced by carbon tetrachloride and paracetamol. The hepatoprotective action was much more significant at the

dose of 2ml/kg when compared to 1ml/kg. So, the physicians are advised to prescribe higher doses of Livactine for treating liver disorders in case of humans.

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