Hepatoprotective activity of Livshis, a polyherbal formulation in CCl₄-induced hepatotoxic male Wistar rats: A toxicity screening approach

Tushar Kanti Bera, Kausik Chatterjee, Debasis De, Kazi Monjur Ali, Kishalay Jana, Soumyajit Maiti, Debidas Ghosh

Department of Bio-Medical Laboratory Science & Management (UGC Innovative Funded Department), Vidyasagar University, West Bengal, India
Pharmaceutical Division, Southern Health Improvement Samity (SHIS), Bhangar, South 24 - Paraganas, West Bengal, India

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Abstract: This study investigated the hepatoprotective activity of the polyherbal formulation Livshis in CCl₄-induced hepatotoxic male albino rats, and included an assessment of the toxicity of the formulation. Male Wistar albino rats (140 ± 10 g) were divided into five groups (n = 6). Liver necrosis was induced by intraperitoneal injection of CCl₄ (1 mL/kg body weight, 50% v/v with olive oil). Antioxidant enzyme activities, such as lipid peroxidation, and liver function test biosensors were assessed. Hematological and renotoxicity markers were evaluated to assess the general toxicity of the formulation. For acute toxicity evaluation of Livshis, the formulation was administered orally at doses ranging from 25 to 3200 mg/kg body weight. Hepatic antioxidant enzyme activities diminished significantly, and hepatic lipid peroxidation rates were elevated in CCl₄-treated animals that were pretreated with distilled water (Group II). The activities of serum toxicity marker enzymes and serum liver function test biosensors increased significantly in Group II animals, whereas these biosensors were significantly protected in Livshis pretreated, CCl₄-treated animals. Group V animals, treated with Livshis alone, did not exhibit any significant variation in levels of hematological and renotoxicity markers compared to controls. In an acute toxicity study, there were no toxic symptoms up to the dose level of 3200 mg/kg body weight. We conclude that Livshis is safe for long-term treatment for hepatic protection at doses of 50 mg/kg body weight.

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* Corresponding author. Department of Bio-Medical Laboratory Science & Management (UGC Innovative Funded Department), Vidyasagar University, West Bengal, India.
E-mail address: debidas.ghosh@yahoo.co.in (D. Ghosh).
Introduction

Exposure to various organic compounds, including a number of drugs and environmental pollutants, can cause cellular damage by metabolic activation of those compounds to form highly reactive substances such as reactive oxygen species (ROS). The industrial solvent CCl₄ is a well-established hepatotoxin. The importance of medicinal plants in traditional healthcare practices, providing clues to new areas of research and in biodiversity conservation, is now well recognized. However, information on the medicinal uses of plants is not always available in isolated areas of India. In India, >43% of flowering plants are reported to be of medicinal importance. Herbal medicines are popular remedies for diseases, and are used by the vast majority of the population worldwide. The pharmacological effects of many plants have received much attention by researchers, and in India, herbal formulations that have attained widespread acceptability as therapeutic agents include antidiabetics, hepatoprotective agents, and lipid-reducing agents. However, there are many limitations such as collection, storage, doses, and duration regarding the safety and efficacy of these preparations. Livshis is a hepatoprotective polyherbal formulation containing seven medicinal plant parts; it is used in traditional medicine to treat hepatic disorders. In the state of West Bengal, India, the Livshis polyherbal formulation is used in Ayurvedic medicine for the treatment of hepatic disorders. Its constituents are Aloe barbadensis Mill., Andrographis paniculata Wall. ex Nees., Asteraeflanta longifolia Nees., Berberis chitria Lindl., Fumaria parviflora Lam., Phyllanthus fraternus Webs. and Picrorhiza kurroa Royle ex Benth. (Table 1). A. barbadensis Mill. has been linked with improved blood glucose levels in diabetes and improvements in acute hepatitis. A. paniculata Wall. ex Nees. is used by traditional Sidha and Ayurvedic systems of medicine, and in tribal medicine in India and other countries, for multiple clinical applications. The leaves and roots of A. longifolia Nees. are used for the treatment of hepatic obstruction, jaundice, and diseases of the urinogenital tract. The stem, roots and fruits of B. chitria Lindl. are used in many Ayurvedic preparations. The stem bark of B. chitria is used for the treatment of malaria, diarrhea, and jaundice. Fresh and dried leaves, and fresh juice from the whole F. parviflora Lam. plant have been widely used in traditional remedies and folklore medicines for liver disorders. P. fraternus Webs. has a long history as an herbal medicine; it exhibits antioxidant properties and is used in the management of kidney stones, gallbladder stones, tuberculosis, and hepatitis. P. kurroa Royle ex Benth. is used to treat diabetes, jaundice, hepatomegaly, spleen disorders, and skin disorders. Inspite of the past reputation of Livshis as a medicine at the local level, the scientific basis of its mode of action on hepatoprotective activity and toxicity profile have not been investigated. We investigated the toxicity and hepatoprotective effects of Livshis to assess its safety and tolerability profile for long-term treatment.

Materials and methods

Preparation of the Livshis polyherbal formulation

The Pharmaceutical Division of Southern Health Improvement Samity (SHIS; West Bengal, India) provided the seven medicinal plants used in the preparation of Livshis. Prof. R. K. Bhakat, Department of Botany and Forestry, Vidyasagar University, West Bengal, India, taxonomically identified the plants. Herbal specimens were kept as dried samples by the Departmental Herbarium Museum. Table 1 lists the herbarium specimen numbers of these plants. Selected parts of seven medicinal plants (Table 1) were dried in an incubator for 24 hours at 37 °C, then crushed separately with an electrical grinder and pulverized. Powdered forms of the plant parts were combined in fixed ratios as per Table 1, and labeled Livshis. This preparation of the Livshis polyherbal formulation was carried out according to a proposed Ayurvedic hepatoprotective formulation.

Selection of animals and animal care

The hepatoprotective study was conducted using 30 mature male Wistar albino rats weighing 140 ± 10 g. Forty-eight rats (140 ± 10 g) were selected for the acute toxicity study. Animals were acclimated to laboratory conditions for 15 days prior to beginning the experiment. Rats were housed in Tarsons cages at an ambient temperature of 25 ± 2 °C with a 12-hour light:dark cycle. Rats were fed food and water ad libitum. The Principles of Laboratory Animal Care were followed throughout the duration of the experiment, and instructions regarding injection and other treatment procedures as provided by our Institutional Ethical Committee.

Table 1 Composition of the Livshis polyherbal formulation.

<table>
<thead>
<tr>
<th>Botanical name</th>
<th>Family</th>
<th>Voucher specimen number</th>
<th>Common name</th>
<th>Parts used</th>
<th>Amount present in 450 mg Livshis sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aloe barbadensis Mill.</td>
<td>Liliaceae</td>
<td>RKB-BMLSM-11/10</td>
<td>Ghritkumari</td>
<td>Leaves</td>
<td>50 mg</td>
</tr>
<tr>
<td>Andrographis paniculata Wall. ex Nees.</td>
<td>Acanthaceae</td>
<td>RKB-BMLSM-12/10</td>
<td>Kalmegh</td>
<td>Leaves</td>
<td>60 mg</td>
</tr>
<tr>
<td>Asteraeflanta longifolia Nees.</td>
<td>Acanthaceae</td>
<td>RKB-BMLSM-13/10</td>
<td>Kulekhar</td>
<td>Leaves</td>
<td>90 mg</td>
</tr>
<tr>
<td>Berberis chitria Lindl.</td>
<td>Berberidaceae</td>
<td>RKB-BMLSM-14/10</td>
<td>Darhaldi</td>
<td>Bark, stem</td>
<td>80 mg</td>
</tr>
<tr>
<td>Fumaria parviflora Lam.</td>
<td>Fumariaceae</td>
<td>RKB-BMLSM-15/10</td>
<td>Khetapatra</td>
<td>Leaves</td>
<td>50 mg</td>
</tr>
<tr>
<td>Phyllanthus fraternus Webs.</td>
<td>Euphorbiaceae</td>
<td>RKB-BMLSM-16/10</td>
<td>Bhuiamla</td>
<td>Leaves</td>
<td>70 mg</td>
</tr>
<tr>
<td>Picrorhiza kurroa Royle ex Benth.</td>
<td>Scrophulariaceae</td>
<td>RKB-BMLSM-17/10</td>
<td>Picrorhiza</td>
<td>Rhizomes</td>
<td>50 mg</td>
</tr>
</tbody>
</table>
Administration of Livshis

Rats in a fasting state were treated orally by forceful gavage with 50 mg Livshis powder suspended in 5 mL distilled water per kilogram body weight. This dose was selected from our pilot study using doses from 20 mg/kg to 200 mg/kg, in which the present dose of 50 mg/kg was noted as the threshold dose. In traditional medicine, Livshis is given to humans at a dose of 20–30 mg/kg body weight (2–3 mg/100 g body weight).

Livshis hepatoprotective study and chronic toxicity assessment

Male Wistar albino rats weighing 140 ± 10 g were divided into five groups (n = 6) for assessment of the Livshis hepatoprotective effect and its general toxicity. Group I (placebo control): rats were fed by per oral (p.o.) with 5 mL distilled water/ kg body weight each day for 28 days under fasting conditions. Group II [pretreatment (distilled water) and CCl4 treatment]: rats were fed by per oral (p.o.) with 5 mL distilled water/ kg body weight for 27 days and on day 28, each received a single intraperitoneal injection of 0.5 mL/kg CCl4, 50% v/v with olive oil (1 mL total). Group III (pretreatment with Livshis and CCl4 treatment): rats were treated with 50 mg Livshis in 5 mL distilled water per kilogram body weight each day under fasting conditions for 27 days. On day 28, each rat received a single intraperitoneal injection of 0.5 mL/kg CCl4, 50% v/v with olive oil (1 mL total). Group IV [pretreatment (Silymarin) and CCl4 treatment]: rats were treated with 20 mg Silymarin in 5 mL distilled water per kilogram body weight each day under fasting conditions for 27 days by gavage. On day 28, each rat received a single intraperitoneal injection of 0.5 mL/kg CCl4, 50% v/v with olive oil (1 mL total). Group V (distilled water + Livshis): rats were fed 50 mg Livshis in 5 mL distilled water per kilogram body weight by gavage each day for 28 days under fasting conditions.

All animals were rested, with no treatment on day 29 of the experiment. Animals were observed daily for toxic effects, mortality, body weight, food intake, and water intake. On day 30 of the experiment, the final body weights were recorded, and all the animals were sacrificed by light ether anesthesia followed by decapitation. Approximately 6 mL blood was collected from the dorsal aorta of each rat. Two milliliters of 6-mL sample were dispensed into EDTA for hematological study, and the remaining 4 mL into heparin for biochemical analysis. The liver and kidneys were excised and weighed. Liver tissue samples were stored in Bouin’s fixative for histological study. Remaining hepatic excised and weighed. Liver tissue samples were stored in for biochemical analysis. The liver and kidneys were hematological study, and the remaining 4 mL into heparin (2–3 mg/100 g body weight).

Statistical analysis

Analysis of variance followed by multiple comparison two-tail t test was used for statistical analysis of collected data. Differences were considered significant for p < 0.05 and p < 0.001. All the values shown in tables are mean ± standard error of the mean.

Results

Body weight and organo-somatic indices

Group II rats exhibited significantly increased organo-somatic indices following treatment with CCl4; specifically, hepato-somatic and reno-somatic indices (p < 0.001), glutamic pyruvate transaminase (SGPT), and alkaline phosphatase (ALP) using standard kits. Histopathological study of liver

Liver tissues were dissected out from all the groups and fixed in Bouin’s fixative. After 24 hours, small pieces of liver tissue were dehydrated in an ascending series of ethanol solutions. The tissues were subjected to paraffin embedding and 5-μm sections were cut with a Leica microtome. Deparaffinized sections were stained with hematoxylin and eosin. Histological examinations were carried out on stained sections with a computer-aided microphotography system (Caliper pro, Dewinter Software Inc., Delhi, India). Renotoxicity biosensors

Levels of serum urea, uric acid, creatinine, and blood urea nitrogen (BUN) were determined using commercially available standard kits (Span Diagnostic Ltd., Surat, India) for assessment of renotoxicity biosensors. Hematotoxicity markers

Hemoglobin (Hb), red blood cell (RBC) count, total leucocyte count (TLC), platelet index (PI), packed cell volume (PCV), and erythrocyte sedimentation rate (ESR) were determined using standard techniques. Study of acute toxicity of Livshis

An acute toxicity study (LD50 determination) was carried out by the method described by Mythilpriya et al., in which 48 healthy male Wistar albino rats weighing roughly 140 ± 10 g were randomly distributed to eight different groups of six animals each. The animals were fasted overnight and Livshis was administered orally at a dose of 25, 50, 100, 200, 400, 800, 1600 or 3200 mg/kg body weight each day. During the Livshis treatment, all animals were observed for awareness, interactivity, posture, tremors, salivation, diarrhea, lethargy, sleep, coma, and death in addition to continuous observation from the first 4 hours, up until 14 days to determine mortality rate.

Liver function test parameters

For liver function tests (LFTs), we determined levels of serum total bilirubin (TB), total protein (TP), and activities of serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvate transaminase (SGPT), and alkaline phosphatase (ALP) using standard protocols.
and a decreased final body weight compared to the placebo control animals. Livshis (Group III) and Silymarin (Group IV) pretreated animals showed significantly reduced effects in response to CCl₄ exposure. Group V Livshis-treated control animals showed insignificant changes in final body weight, and hepato-somatic and reno-somatic indices with respect to controls (Table 2).

Hepatic antioxidant enzyme activities and lipids peroxidation level

Vitamin C levels and activities of hepatic antioxidant enzymes CAT, GST and SOD biosensors were significantly decreased (p < 0.001). There was an accompanying elevation of lipids with oxidation level, that is, TBARS biosensor, in Group II rats compared to the placebo controls. Group III animals pretreated with Livshis exhibited less dramatic changes in response to treatment with CCl₄, and so received significant protection from the pretreatment regime (Table 3). There were insignificant differences in biosensor data between Livshis (Group III) and Silymarin (Group IV) pretreated rats. Livshis treatment alone, as provided to control Group V rats, had no noticeable effect on the activities of the enzyme and lipid peroxidation biosensors (Table 3).

LFT biosensors

Group II rats, exposed to CCl₄ with only distilled water pretreatment, exhibited significant increases (p < 0.001) in LFT parameters: serum TB, SGOT, SGPT and ALP, along with a reduction in the level of serum TP. Group III animals, with Livshis pretreatment, showed significant protection of LFT biosensor activities. There were insignificant variations in LFT biosensor data between Livshis and Silymarin pretreated Groups III and IV. Livshis treatment of the Group V control animals produced no significant differences in the LFT biosensors compared to the placebo control animals (Table 4).

Histopathological evaluation

The histological profile of the hepatic tissue of the placebo control animals showed a normal lobular architecture. Normal hepatocytes were arranged in single cell cords radiating away from a central vein (A). Group II rats, treated with distilled water and CCl₄, showed disturbed liver architecture, exhibiting central lobular necrosis with tiny vacuoles, and fatty infiltrations (B). Group III and IV animals, pretreated with Livshis and Silymarin respectively, and subsequently treated with CCl₄ retained normal hepatic tissue architecture, so received significant protection from CCl₄-induced hepatic damage (C and D). Group V animals, treated with Livshis alone, did not show any significant hepatic tissue architectural changes (E) (Fig. 1).

Renotoxicity biosensors

Group II animals showed significant elevation in renotoxicity biosensors urea, uric acid and creatinine levels in blood, and BUN. Group III rats, receiving Livshis pretreatment, acquired significant protection from the effects of CCl₄, as shown by the renotoxicity biosensor results. There were no significant differences in renotoxicity biosensor measurements for Group III and IV rats receiving Livshis and Silymarin pretreatment, respectively. Livshis treatment of the Group V control animals did not produce any significant differences in renotoxicity biosensor data compared to the Group I placebo control group (Table 5).

Hematotoxicity markers

Group II rats exhibited decreases in Hb levels, RBC count, TLC, PI, and PCV (p < 0.001). Additionally, ESR for this test group was elevated. However, Group III and IV rats did not exhibit any noticeable changes in these indicators. The Livshis control group (Group V) showed no changes in these hematotoxicity markers (Table 6).

Livshis acute toxicity study

In the acute toxicity study, treatment with Livshis alone did not produce any lethal or toxic symptoms up to a dosage of 3200 mg/kg body weight. No mortality or morbidity occurred in animals during the 14-day period following oral administration for all Livshis doses. We observed no tremors, salivation, diarrhea, sleep, coma, death, or unusual behaviors such as walking backwards, and the animals had a normal reaction to handling. The LD₅₀ for oral administration of Livshis was >3200 mg/kg body weight; treatment with 50 mg/kg Livshis was well tolerated by the

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight (g)</th>
<th>Hepato-somatic index (g %)</th>
<th>Reno-somatic index (g %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(I) Placebo</td>
<td>143.42 ± 4.73</td>
<td>3.90 ± 0.36</td>
<td>1.31 ± 0.03</td>
</tr>
<tr>
<td>(II) Distilled water + CCl₄</td>
<td>146.64 ± 4.77</td>
<td>6.07 ± 0.31*</td>
<td>1.92 ± 0.05*</td>
</tr>
<tr>
<td>(III) Livshis + CCl₄</td>
<td>147.55 ± 5.26</td>
<td>3.94 ± 0.28</td>
<td>1.33 ± 0.04</td>
</tr>
<tr>
<td>(IV) Silymarin + CCl₄</td>
<td>145.37 ± 4.87</td>
<td>3.92 ± 0.33</td>
<td>1.29 ± 0.03</td>
</tr>
<tr>
<td>(V) Distilled water + Livshis</td>
<td>141.34 ± 5.58</td>
<td>3.88 ± 0.27</td>
<td>1.32 ± 0.04</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± standard error of the mean, n = 6. Analysis of variance followed by multiple comparison two-tail t test.
* p < 0.001 compared with placebo control Group I.
Hepatoprotective activity of Livshis

animals, and did not produce any behavioral changes during long-term treatment (Table 7).

Discussion

Toxicological evaluation is carried out in experimental animals to predict the toxicity and safety of drugs and plant products for human use, and to produce guidelines for a dose that is safe for humans. A World Health Organization survey has indicated that worldwide 70–80% of the population relies on alternative medicines, mainly from herbal sources, for their primary health care.33 We investigated the hepatoprotective effects of Livshis against CCl₄-induced hepatotoxicity, and screened the general toxicity of Livshis to assess its safety and tolerability for long-term treatment. CCl₄ is a well-known hepatotoxin that is widely used to induce hepatotoxicity in laboratory animals.34 The cytochrome P450 system forms the reactive metabolite trichloromethyl radical (CCl₃·) from CCl₄. As oxygen concentration increases, any CCl₃· radicals present in the system react very rapidly with the oxygen to produce highly reactive ROS, which in turn causes cell necrosis and subsequent organ death.35

Oxidative damage to a cell induces lipid peroxidation of cell membrane lipids. Studies have shown that acute or chronic exposure of animals to CCl₄ increases the rate of formation of lipid peroxidation products such as TBARS or malondialdehyde.36 Some authors have postulated that damage that occurs in hepatic tissue injury following exposure to CCl₄ is a result of lipid peroxidation.37 In the present study, the significant increases in TBARS levels seen in Group II rats exposed to CCl₄ indicate increased lipid peroxidation, tissue damage, and subsequent failure of antioxidant defense mechanisms that would otherwise prevent the formation of excessive free radicals. Moreover, histopathological changes such as central lobular necrosis, seen as the formation of tiny vacuoles, and FL were observed in hepatic tissue in Group II rats. Liver injury disturbs the hepatocyte transport function, resulting in the leakage of plasma membrane, thereby causing increased TB and increased enzyme levels in serum. TB, SGOT, SGPT and ALP enzymes are normally excreted in bile by the liver. In the presence of hepatotoxicity, the liver bile excretion process becomes defective, resulting in increased enzyme levels in the serum.38 Similarly, CAT, GST, SOD and vitamin C antioxidants constitute a mutually supportive line of defense against ROS.37 Decreased activities of these enzymatic and nonenzymatic antioxidant species seen in the hepatic tissue of Group II rats also supports the suggestion of a failure of antioxidant defense mechanisms. Such a failure could cause accumulation of highly reactive free radicals, leading to deleterious effects such as loss of integrity and function of cell membranes.39 This is supported by the observation that CCl₄ induces the generation of hydroxyl radicals, peroxyl radicals, and superoxide radicals, and increases the leakage of plasma membrane, thereby causing increased TB and increased enzyme levels in serum. TB, SGOT, SGPT and ALP enzymes are normally excreted in bile by the liver. In the presence of hepatotoxicity, the liver bile excretion process becomes defective, resulting in increased enzyme levels in the serum.38 Similarly, CAT, GST, SOD and vitamin C antioxidants constitute a mutually supportive line of defense against ROS.37 Decreased activities of these enzymatic and nonenzymatic antioxidant species seen in the hepatic tissue of Group II rats also supports the suggestion of a failure of antioxidant defense mechanisms. 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Such a failure could cause accumulation of highly reactive free radicals, leading to deleterious effects such as loss of integrity and function of cell membranes.39 This is supported by the observation that CCl₄ induces the generation of hydroxyl radicals, peroxyl radicals, and superoxide cities of living organisms, and is used to induce hepatotoxicity in experimental animals. However, its toxic effects are not limited to the liver, as it can also cause damage to other organs, including the kidneys and the cardiovascular system. The toxic effects of CCl₄ are primarily due to the formation of reactive oxygen species (ROS), which are produced when the molecule reacts with biological molecules. These ROS can damage cellular structures and cause cellular death. In this study, we investigated the protective effects of Livshis, a plant extract, against CCl₄-induced hepatotoxicity in male albino rats. The extract was found to be effective in reducing the levels of liver function test biomarkers, such as aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), and total bilirubin (TB), which are commonly used to assess liver function. The extract also showed significant protective effects on the activities of antioxidant enzymes, such as catalase (CAT), superoxide dismutase (SOD), and glutathione-S-transferase (GST), which play a crucial role in defending against oxidative stress. The results of this study suggest that Livshis has the potential to be used as a hepatoprotective agent in the treatment of liver diseases induced by CCl₄. However, further studies are needed to investigate the mechanism of action of Livshis and to determine its optimal dose for effective protection against CCl₄-induced hepatotoxicity.

Table 3: Protective effect of Livshis on hepatic antioxidant enzyme activities and lipid peroxidation levels against CCl₄-induced hepatotoxicity in male albino rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>CAT (mM H₂O₂ consumption/mg tissue/min)</th>
<th>SOD (U/mg tissue)</th>
<th>GST (µM/min/mg tissue)</th>
<th>Vitamin C (mg/dL)</th>
<th>TBARS (nM/mg tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(I) Placebo</td>
<td>9.43 ± 0.54</td>
<td>2.74 ± 0.17</td>
<td>1.85 ± 0.04</td>
<td>16.21 ± 1.77</td>
<td>42.63 ± 3.16</td>
</tr>
<tr>
<td>(II) Distilled water + CCl₄</td>
<td>3.58 ± 0.31*</td>
<td>1.03 ± 0.06*</td>
<td>0.57 ± 0.02*</td>
<td>7.63 ± 1.31*</td>
<td>73.35 ± 4.72*</td>
</tr>
<tr>
<td>(III) Livshis + CCl₄</td>
<td>9.37 ± 0.36</td>
<td>2.31 ± 0.14**</td>
<td>1.81 ± 0.03</td>
<td>14.51 ± 1.96**</td>
<td>51.28 ± 4.25*</td>
</tr>
<tr>
<td>(IV) Silymarin + CCl₄</td>
<td>9.34 ± 0.48</td>
<td>2.68 ± 0.15</td>
<td>1.83 ± 0.04</td>
<td>14.66 ± 1.68**</td>
<td>49.81 ± 4.38*</td>
</tr>
<tr>
<td>(V) Distilled water + Livshis</td>
<td>9.45 ± 0.52</td>
<td>2.73 ± 0.18</td>
<td>1.82 ± 0.05</td>
<td>16.19 ± 2.04</td>
<td>43.22 ± 3.46</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± standard error of the mean, n = 6. Analysis of variance followed by multiple comparison two-tail t test.

* p < 0.001.
** p < 0.05 compared with placebo control Group I.

CAT: Catalase; SOD: Superoxide dismutase; TBARS: Thiobarbituric acid reactive substance.

Table 4: Protective role of Livshis on liver function test biosensors in CCl₄-induced hepatotoxicity in male albino rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>TB (mg/dL)</th>
<th>TP (mg/dL)</th>
<th>SGOT (IU/L)</th>
<th>SGPT (IU/L)</th>
<th>ALP (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(I) Placebo</td>
<td>0.54 ± 0.03</td>
<td>9.47 ± 0.83</td>
<td>62.72 ± 4.87</td>
<td>31.47 ± 3.78</td>
<td>90.64 ± 4.21</td>
</tr>
<tr>
<td>(II) Distilled water + CCl₄</td>
<td>1.42 ± 0.06*</td>
<td>5.33 ± 0.62*</td>
<td>134.7 ± 6.48*</td>
<td>81.72 ± 5.24*</td>
<td>154.72 ± 5.3*</td>
</tr>
<tr>
<td>(III) Livshis + CCl₄</td>
<td>0.55 ± 0.04</td>
<td>9.38 ± 0.67</td>
<td>68.03 ± 5.21**</td>
<td>35.24 ± 4.21**</td>
<td>94.85 ± 4.57**</td>
</tr>
<tr>
<td>(IV) Silymarin + CCl₄</td>
<td>0.56 ± 0.03</td>
<td>9.40 ± 0.91</td>
<td>67.53 ± 5.46**</td>
<td>37.03 ± 4.63**</td>
<td>96.25 ± 5.13**</td>
</tr>
<tr>
<td>(V) Distilled water + Livshis</td>
<td>0.52 ± 0.03</td>
<td>9.50 ± 0.88</td>
<td>64.21 ± 5.21</td>
<td>33.16 ± 4.11</td>
<td>91.61 ± 4.33</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± standard error of the mean, n = 6. Analysis of variance followed by multiple comparison two-tail t test.

* p < 0.001.
** p < 0.05 compared with placebo control Group I.

ALP: alkaline phosphatase. SGOT: serum glutamate oxaloacetate transaminase; SGPT: serum glutamate pyruvate transaminase; TB: total bilirubin; TP: total protein.
radicals, associated with inactivation of both enzymatic and nonenzymatic antioxidants. Group III rats, pretreated with Livshis, retained significant liver function following treatment with CCl₄. Thus, Livshis plays a vital role in the maintenance of normal liver cell membrane function, protecting hepatic tissue against CCl₄ toxicity by means of suppressing the generation of free radicals in hepatic tissue. It is possible that Livshis stabilizes the plasma membrane of hepatocytes against CCl₄, and helps to maintain hepatocyte transport function by reducing the formation of CCl₃O• and trichloromethyl peroxo radical (CCl₃OO•) from CCl₄. As a result, formation of ROS is reduced (e.g., superoxide anion, H₂O₂, and hydroxyl radicals). Moreover, there is inhibition of hepatocyte plasma membrane lipids by these free radicals.

The elevation in concentrations of renotoxicity markers urea, uric acid, creatinine and BUN, together with the reduction in serum TP concentration in Group II rats are indicative of cellular leakage and loss of the functional integrity of cell membranes in renal tissue.⁴⁰,⁴¹ Livshis treatment alone resulted in no significant changes in any of the renotoxicity biosensors. The normal renotoxicity biosensors indicate that Livshis did not interfere with renal function or renal integrity. Analysis of blood parameters is important for risk evaluation because changes in the hematological system are predictive of toxicity. We attributed the observed reductions in Hb, RBC, TLC, PI and PCV, and elevation of ESR in Group II rats to destruction of erythrocytes, reduction in the rate of erythrocyte formation, and disturbed hematopoiesis, and/or to enhanced

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<table>
<thead>
<tr>
<th>Groups</th>
<th>Urea (mg/dL)</th>
<th>UA (mg/dL)</th>
<th>BUN (mg/dL)</th>
<th>Creatinine (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(I) Placebo control</td>
<td>40.82 ± 3.65</td>
<td>3.44 ± 0.03</td>
<td>19.06 ± 2.31</td>
<td>0.65 ± 0.006</td>
</tr>
<tr>
<td>(II) Distilled water + CCl₄</td>
<td>67.52 ± 3.88*</td>
<td>8.78 ± 0.05*</td>
<td>31.53 ± 3.26*</td>
<td>0.82 ± 0.007*</td>
</tr>
<tr>
<td>(III) Livshis + CCl₄</td>
<td>45.3 ± 4.17**</td>
<td>3.53 ± 0.04</td>
<td>21.45 ± 3.02</td>
<td>0.68 ± 0.005**</td>
</tr>
<tr>
<td>(IV) Silymarin + CCl₄</td>
<td>43.95 ± 4.02</td>
<td>3.51 ± 0.04</td>
<td>20.52 ± 2.21</td>
<td>0.67 ± 0.006</td>
</tr>
<tr>
<td>(V) Distilled water + Livshis</td>
<td>41.06 ± 3.74</td>
<td>3.47 ± 0.04</td>
<td>19.17 ± 1.97</td>
<td>0.67 ± 0.005</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± standard error of the mean, n = 6. Analysis of variance followed by multiple comparison two-tail t test.

* p < 0.001.
** p < 0.05 compared with placebo control Group I.
BUN: blood urea nitrogen; UA: uric acid.
removal of erythrocytes from the circulation. Treatment of Group III rats with Livshis resulted in no significant changes in Hb, RBC, TC, PI, PCV or ESR. Thus, Livshis is non-toxic to circulating red cells, and does not interfere with their production. Hematopoiesis and leukopoiesis remain unaffected, even though the hematopoietic system is one of the most sensitive targets for toxic compounds and is an important index for physiological and pathological status assessment in humans and animals. Therefore, we conclude that Livshis is not hematotoxic.

During the acute toxicity study, rats in all groups did not exhibit any adverse effects at a Livshis dose of 3200 mg/kg. The Organization for Economic Cooperation and Development (OECD) guidelines for acute oral toxicity categorizes an LD50 dose of ≥2000 mg/kg as unclassified, and hence the Livshis formulation is considered to be safe.

The active phyto-ingredients present in Livshis stabilize the plasma membranes of hepatocytes and help to maintain their transport function. The formulation also facilitates the repair of hepatic tissue by protecting serum marker enzyme activities. Catalase, superoxide dismutase and glutathione peroxidase activities in various rat tissues after carbon tetrachloride intoxication.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Hb (%)</th>
<th>RBC (10^6/mm^3)</th>
<th>TLC (10^3/mm^3)</th>
<th>PI (10^5/mm^3)</th>
<th>PCV (%)</th>
<th>ESR (mm/1st h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(I) Placebo control</td>
<td>13.2 ± 0.46</td>
<td>7.28 ± 0.31</td>
<td>5.21 ± 0.08</td>
<td>2.86 ± 0.04</td>
<td>38.46 ± 3.4</td>
<td>7.86 ± 0.25</td>
</tr>
<tr>
<td>(II) Distilled water + CCl4</td>
<td>7.8 ± 0.32*</td>
<td>5.17 ± 0.24*</td>
<td>3.46 ± 0.07*</td>
<td>1.53 ± 0.02*</td>
<td>27.62 ± 3.2*</td>
<td>13.83 ± 0.4*</td>
</tr>
<tr>
<td>(III) Livshis + CCl4</td>
<td>12.8 ± 0.38</td>
<td>7.22 ± 0.27</td>
<td>5.18 ± 0.06</td>
<td>2.82 ± 0.03</td>
<td>32.8 ± 4.1**</td>
<td>8.02 ± 0.31</td>
</tr>
<tr>
<td>(IV) Silymarin + CCl4</td>
<td>13.0 ± 0.41</td>
<td>7.25 ± 0.30</td>
<td>5.20 ± 0.08</td>
<td>2.84 ± 0.04</td>
<td>36.62 ± 3.6</td>
<td>7.98 ± 0.27</td>
</tr>
<tr>
<td>(V) Distilled water + Livshis</td>
<td>13.4 ± 0.52</td>
<td>7.31 ± 0.31</td>
<td>5.22 ± 0.06</td>
<td>2.87 ± 0.03</td>
<td>38.51 ± 3.8</td>
<td>7.85 ± 0.26</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± standard error of the mean, n = 6. Analysis of variance followed by multiple comparison two-tail t test.

* p < 0.001.
** p < 0.05 compared with placebo control Group I.

ESR: erythrocyte sedimentation rate; Hb: hemoglobin; PCV: packed cell volume; PI: platelet index; RBC: red blood cell; TLC: total leukocyte count.

References


15. CPCSEA guidelines for laboratory animal facility. Committee for the purpose of control and supervision on experiments on animals. Ind J Pharmacol. 2003;35:257–274.


