ORIGINAL ARTICLE

Protective and curative effects of Livolin forte® on carbon tetrachloride-induced liver damage in Wistar rats

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INTRODUCTION

Man, either by way of lifestyle or job demands, is exposed to many hepatotoxic substances like chemicals, drugs, and pesticides which may lead to liver diseases such as liver cirrhosis, hepatitis and gallbladder dysfunction. The incident of liver diseases is on the increase in recent times. Liver damage inflicted by hepatotoxic agents is of grave consequences [1]. Liver ailments represent a major health problem [2]. Toxic chemicals, xenobiotics, alcohol consumption, malnutrition, anemia, medications, autoimmune disorders [3], viral (hepatitis A, B, C, D, etc.) and microbial infections are harmful and cause damage to the hepatocytes.

Essentiale forte® is a hepatoprotective drug that has been reported to protect and improve liver function in diabetic subjects with nonalcoholic fatty liver [4] and chronic infections. It is also used to treat hepatic cirrhosis, necrosis of the liver cells, liver failure, liver coma, diseases of the cardiovascular system, hyperlipoproteinemia, hypercholesterolemia, atopic dermatitis, eczema and pyelonephritis [5].

Ursodeoxycholic acid is another hepatoprotective drug that has been reported to have antifibrotic activity in bile duct ligation and carbon tetrachloride (CCl₄) induced experimental liver fibrosis in rats [6]. It has also been reported to have antiapoptotic activity on cholangiocytes and hepatocytes [7] and inhibitory effect on generation of reactive oxygen species (ROS).

Livolin forte® (LIV) is a relatively new drug that is being used in hospitals worldwide in the treatment and management of liver diseases. It basically contains phospholipids with vitamins including essential phospholipids: polyunsaturated phosphatidylcholine (300 mg), vitamin B₁ (thiamine mononitrate, 10 mg), vitamin B₂ (riboflavin, 6 mg), vitamin B₆ (pyridoxine HCl, 10 mg), vitamin B₁₂ (cyanocobalamin, 10 µg); nicotinamide (30 mg), and vitamin E (alpha tocopheryl...
acetate, 10 mg). There is a dearth of information on the physiological effects of the drug on the liver in animal models of liver disease. Therefore, this study is aimed at assessing the curative and prophylactic effects of LIV on carbon tetrachloride (CCL₄)-induced liver damage in rats.

MATERIALS AND METHODS
Animal care and management
Twenty-four adult Wistar rats weighing 150-200 g used in this study were obtained from the Animal House of the College of Health Sciences, Obafemi Awolowo University, Ile-Ife, Nigeria. The rats were kept under normal environmental conditions with a 12/12 h light/dark cycle and had free access to standard rodent pellet diet (Caps Feed PLC, Osogbo, Nigeria) and water ad libitum. They were allowed to acclimatize in the laboratory for 2 weeks before the commencement of the study. The experimental procedures adopted in this study were in strict compliance with the guidelines on Experimental Animal Care and Use of Laboratory Animals in Biomedical Research, College of Health Sciences, Obafemi Awolowo University.

Drugs and chemicals
Livolin forte® was from Mega Lifesciences (Pakenham, VIC, Australia; batch number 107050), carbon tetrachloride from Hopkins and Williams (Birmingham, England, UK), and propylene glycol from (Biovision, Milpitas, CA, USA).

Drug preparation
Each capsule contains 366 mg of LIV. One capsule was dissolved in 20 ml of propylene glycol. Therefore, 0.04 ml of the solution equivalent to 0.78 mg of LIV was administered to a rat 150 g orally; this is equivalent to 5.2 mg/kg, which is the therapeutic dose of the drug in humans.

Experimental design
The rats were randomly divided into four groups of six rats each. The rats were treated as follows: group I (normal control) received 0.3 ml/kg/day of propylene glycol, the vehicle in which LIV was dissolved, orally for one month; group II (toxicant control) was given 0.7 ml/kg/day of carbon tetrachloride (CCL₄) dissolved in olive oil (1:1, v/v) orally for 7 days so as to induce liver damage; group III (LIV+CCL₄) received 5.2 mg/kg/day of LIV orally for one month and subsequently CCL₄ (0.7 ml/kg/day) was administered for one week; group IV (CCL₄+LIV) was given CCL₄ (0.7 ml/kg/day) for one week and subsequently received 5.2 mg/kg/day of LIV orally for one month. One day after the last dose of LIV, CCL₄ and propylene glycol, as the case may be for each group, three of the rats in each group were sacrificed under chloroform anesthesia and blood was obtained by cardiac puncture into separate plain bottles. The blood was centrifuged for 15 min at 4000 rpm using a bench centrifuge. The serum was separated and analyzed for the liver enzymes and other organic constituents that are routinely used in the assessment of liver function. Thereafter, the liver of each rat was carefully excised for weight determination. Portion of the liver was homogenized and centrifuged at 4000 rpm for 30 min to yield a clear supernatant fraction that was used for reduced glutathione (GSH) analysis, while the remaining part was fixed in 10% formo-saline for histopathological studies. The remaining three rats in each group were left for a two week recovery period before they were sacrificed using the same procedure and the same parameters were measured. The entire treatment protocol was summarized in Table 1.

Measurement of body weight
The body weight of the animals were measured once in a week using a weighing balance (Camry; Zhongshan Guangdong, China) during the experiment to access the weight gain or loss in each group.

Measurement of food consumption and water intake
The food consumption and water intake of the animals were determined daily from the onset of the experiment. The volume of water and weight of food given to each group of rats were measured with a measuring cylinder and a weighing balance, respectively. The difference between the previous day volume of water and weight of food and the left-over was taken as the daily food consumption and water intake of the group.

Biochemical analysis
Levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin, total cholesterol and serum glucose were assayed by the use of appropriate biochemical kits purchased from Randox Laboratories (Crumlin, Co. Antrim UK). Total protein (TP) was assayed according to the method of Bradford [8]: to 0.01 ml of serum, 0.01 ml of distilled water and 1 ml of Bradford reagent were added and absorbance was read at 595 nm; this was measured against the blank containing Bradford reagent and distilled water only. Reduced glutathione assay was carried out according to the method of Tietze [9]: 0.1 ml of tissue homogenate was mixed with 0.1 ml of 1 mM of GSH, and 0.1 ml of 1 mM 4-chloro-3,5-dinitrobenzoic acid (CDNB); to the resulting mixture, 2.7 ml of 100 mM phosphate buffer (pH 6.5) was added; the change in absorbance was recorded at a wavelength of 340 nm at 1 min interval for 5 min.

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Histopathologic evaluation
The fixed liver samples were dehydrated in graded alcohol and embedded in paraffin wax. They were then cut into 4-5 μm thick sections and stained with hematoxylin-eosin for photomicroscopic assessment using a Leica DM750 camera microscope at x400 magnification.

Statistical analysis
The results obtained were expressed as mean ± SEM. The data were analyzed using one way ANOVA followed by Tukey’s multiple comparison test using GraphPad 5.03 software. The results were considered significant when P < 0.05.

RESULTS
The toxicant control group showed significant increases in AST, ALT, ALP activities and total bilirubin level when compared to the normal control group in rats that were sacrificed a day after the last dose of CCl₄ (Table 2). An increase in total cholesterol level was seen in the toxicant control group, but not significantly different from the normal control group (Table 3). Also, there were significant reductions in total protein, serum glucose (Table 2) and GSH levels (Table 4) in the toxicant control group compared to the normal control group in rats that were sacrificed a day after the last dose of CCl₄.

There was no significant difference in ALT activity of the toxicant control group compared to the normal control group in rats that were sacrificed after the recovery period. Group III showed reduced ALT and AST activities which were not significantly different from the toxicant control group in rats that were sacrificed a day after the last dose of CCl₄ (Table 2). On the other hand, there were no significant differences in ALT and AST activities of this group compared to the normal control group in those rats that were sacrificed after the recovery period (Table 3). Group IV showed significant reductions in ALT and AST activities compared to the toxicant control group, but no significant difference from the normal control group in rats that were sacrificed a day after the last dose of LIV and even in those sacrificed after the recovery period (Tables 2-3).

Group III showed reduced ALP activity which was not significantly different from the toxicant control group, while group IV showed significant decrease in ALP activity compared to the toxicant control group in rats that were sacrificed a day after the last dose of CCl₄ and LIV as the case may be for each group (Table 2). ALP activities in groups III and IV were significantly higher than the normal control group, but significantly lower than the toxicant control group in those that were sacrificed after the recovery period (Table 3). There was a significant decrease in total cholesterol level of group IV compared to the toxicant control group in rats that were sacrificed a day after the last dose of LIV. However, there were no significant differences in total bilirubin, total cholesterol and total protein levels of the treated groups compared to the normal and toxicant control groups in rats that were sacrificed after the recovery period (Table 3).

The serum glucose level in group IV was significantly higher than in the toxicant control group with value being comparable and not significantly different from the control group in rats that were sacrificed a day after the last dose of LIV and those sacrificed after the recovery period (Tables 2-3). Group III showed significant decrease in glucose level compared to the normal control group and group IV in rats that were sacrificed a day after the last dose of CCl₄ and those sacrificed after the recovery period. Serum glucose level was significantly reduced in group III compared to the toxicant control group in rats that were sacrificed after the recovery period.

There was a significant increase in the relative liver weight of the toxicant control group compared to the normal control group in rats that were sacrificed a day after the last dose of CCl₄ and those sacrificed after the recovery period (Table 4). Group III showed no significant difference in the relative liver weight when compared to the normal control group in rats that were sacrificed a day after the last dose of CCl₄. There was a significant decrease in the relative liver weight of this group compared to the toxicant control group in rats that were sacrificed after the recovery period with value also being comparable and not significantly different from the normal control group. Group IV showed a significant decrease in the relative liver weight compared to the toxicant control group, but no significant difference from the control group in rats that were sacrificed a day after the last dose of CCl₄ and those sacrificed after the recovery period.

The GSH activity in the treated groups was significantly higher than in the toxicant control group with values being comparable and not significantly different from the normal control group in rats that were sacrificed a day after the last dose of CCl₄ and LIV as the case may be for each group and those sacrificed after the recovery period (Table 4). However, the GSH level of group III was lower but not significantly different from that of group IV.
Table 1. Treatment protocol is as shown below:

<table>
<thead>
<tr>
<th>Group</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
<th>Week 5</th>
<th>Week 6</th>
<th>Week 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>PG</td>
<td>PG</td>
<td>PG</td>
<td>PG</td>
<td>RP</td>
<td>RP</td>
<td>RP</td>
</tr>
<tr>
<td>Group II</td>
<td>CCl₄*</td>
<td>RP</td>
<td>RP</td>
<td>RP</td>
<td>CCl₄*</td>
<td>RP</td>
<td>RP</td>
</tr>
<tr>
<td>Group III</td>
<td>LIV</td>
<td>LIV</td>
<td>LIV</td>
<td>LIV</td>
<td>CCl₄*</td>
<td>RP</td>
<td>RP</td>
</tr>
<tr>
<td>Group IV</td>
<td>CCl₄*</td>
<td>LIV</td>
<td>LIV</td>
<td>LIV</td>
<td>LIV*</td>
<td>RP</td>
<td>RP</td>
</tr>
</tbody>
</table>

PG, propylene glycol (0.3 ml/kg/day); CCl₄, carbon tetrachloride (0.7 ml/kg/day); LIV, Livolin forte (5.2 mg/kg/day); RP, recovery period; *point of sacrifice of the first half of the group; **point of sacrifice of the second half of the group.

Table 2. Effect of Livolin forte® on serum biochemical parameters in rats that were sacrificed a day after the last dose

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Toxic control</th>
<th>LIV + CCl₄</th>
<th>CCl₄ + LIV</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (U/l)</td>
<td>23.5 ± 4.5</td>
<td>106.33 ± 17.29a</td>
<td>88.5 ± 5.50ac</td>
<td>19 ± 4.73b</td>
</tr>
<tr>
<td>AST (U/l)</td>
<td>25 ± 6</td>
<td>96 ± 6.5a</td>
<td>72.5 ± 13.5ac</td>
<td>29.67 ± 3.28b</td>
</tr>
<tr>
<td>ALP (U/l)</td>
<td>47.5 ± 2.5</td>
<td>657.2 ± 24.26a</td>
<td>570.4 ± 50.18ac</td>
<td>65.03 ± 11.99b</td>
</tr>
<tr>
<td>TB (μmol/l)</td>
<td>3.5 ± 1.5</td>
<td>20 ± 2.89a</td>
<td>5.54 ± 1.66b</td>
<td>4.05 ± 1.05b</td>
</tr>
<tr>
<td>TP (mg/ml)</td>
<td>1.45 ± 0.04</td>
<td>1.27 ± 0.01a</td>
<td>1.51 ± 0.02b</td>
<td>1.46 ± 0.02b</td>
</tr>
<tr>
<td>SG (mg/ml)</td>
<td>119 ± 4</td>
<td>73.8 ± 12.35a</td>
<td>49.80 ± 5.2ac</td>
<td>109.6 ± 16.06b</td>
</tr>
<tr>
<td>TC (mmol/l)</td>
<td>1.17 ± 0.19</td>
<td>1.5 ± 0.15</td>
<td>1.37 ± 0.54a</td>
<td>0.86 ± 0.25b</td>
</tr>
</tbody>
</table>

LIV, Livolin forte®; CCl₄, carbon tetrachloride; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; TB, total bilirubin; TP, total protein; SG, serum glucose; TC, total cholesterol. Significantly different from control, toxic control, and from CCl₄ + LIV group.

Table 3. Effect of Livolin forte® on serum biochemical parameters in rats that were sacrificed after the recovery period

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Toxic control</th>
<th>LIV + CCl₄</th>
<th>CCl₄ + LIV</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (U/l)</td>
<td>26 ± 3</td>
<td>32 ± 15.39</td>
<td>20 ± 1</td>
<td>24 ± 1.5</td>
</tr>
<tr>
<td>AST (U/l)</td>
<td>28 ± 9</td>
<td>60 ± 4.93a</td>
<td>25 ± 2b</td>
<td>23 ± 4b</td>
</tr>
<tr>
<td>ALP (U/l)</td>
<td>99.85 ± 1.15</td>
<td>715.6 ± 80.83a</td>
<td>330.5 ± 10.5ab</td>
<td>258 ± 116ab</td>
</tr>
<tr>
<td>TB (μmol/l)</td>
<td>4.35 ± 0.85</td>
<td>5.67 ± 0.88</td>
<td>5.3 ± 0.1</td>
<td>3.8 ± 0.6</td>
</tr>
<tr>
<td>TP (mg/ml)</td>
<td>1.45 ± 0.02</td>
<td>1.48 ± 0.01</td>
<td>1.5 ± 0.01</td>
<td>1.37 ± 0.05</td>
</tr>
<tr>
<td>SG (mg/ml)</td>
<td>118.6 ± 9.64</td>
<td>120.51 ± 6.2</td>
<td>24.13 ± 0.63abc</td>
<td>87.4 ± 10.5</td>
</tr>
<tr>
<td>TC (mmol/l)</td>
<td>1.18 ± 0.15</td>
<td>1.5 ± 1.6</td>
<td>1.37 ± 0.03</td>
<td>1.03 ± 0.06</td>
</tr>
</tbody>
</table>

LIV, Livolin forte®; CCl₄, carbon tetrachloride; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; TB, total bilirubin; TP, total protein; SG, serum glucose; TC, total cholesterol. Significantly different from control, toxic control, and from CCl₄ + LIV group.

Table 4. Effect of Livolin forte® on the liver weight and reduced glutathione

<table>
<thead>
<tr>
<th></th>
<th>Relative liver weight (g)</th>
<th>Reduced glutathione (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>One day</td>
<td>Recovery period</td>
</tr>
<tr>
<td>Control</td>
<td>3.53 ± 0.06</td>
<td>3.42 ± 1.38</td>
</tr>
<tr>
<td>Toxic control</td>
<td>4.53 ± 0.19a</td>
<td>4.21 ± 0.25a</td>
</tr>
<tr>
<td>LIV + CCl₄</td>
<td>3.72 ± 0.34</td>
<td>3.35 ± 0.1b</td>
</tr>
<tr>
<td>CCl₄ + LIV</td>
<td>3.57 ± 0.09b</td>
<td>3.43 ± 0.03b</td>
</tr>
</tbody>
</table>

LIV, Livolin forte®; CCl₄, carbon tetrachloride. Significantly different from control, toxic control group.

Table 5. Effect of Livolin forte® on food consumption and water intake

<table>
<thead>
<tr>
<th></th>
<th>Food consumption (g)</th>
<th>Water intake (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>One day</td>
<td>Recovery period</td>
</tr>
<tr>
<td>Control</td>
<td>15.08 ± 0.44</td>
<td>17.86 ± 1.38</td>
</tr>
<tr>
<td>Toxic control</td>
<td>14.05 ± 1.92</td>
<td>16.07 ± 0.77</td>
</tr>
<tr>
<td>LIV + CCl₄</td>
<td>16.18 ± 0.39</td>
<td>11.67 ± 0.74ac</td>
</tr>
<tr>
<td>CCl₄ + LIV</td>
<td>15.9 ± 0.56</td>
<td>17.98 ± 0.7</td>
</tr>
</tbody>
</table>

LIV, Livolin forte®; CCl₄, carbon tetrachloride. Significantly different from control, toxic control, and from CCl₄ + LIV group.
There was no significant difference in food consumption of the toxicant control group compared to the normal control group in rats that were sacrificed a day after the last dose of CCl₄ and those sacrificed after the recovery period (Table 5). However, there was a significant decrease in water intake of the toxicant control group compared to the normal control group in rats that were sacrificed a day after the last dose of CCl₄ and even in those sacrificed after the recovery period. Water intake, as shown in Table 5, was significantly higher in the treated groups compared to the toxicant control group in rats that were sacrificed a day after the last dose of CCl₄. Conversely, there was a significant decrease in food and water consumption in this group compared to the normal control in those that were sacrificed after the recovery period. The food and water intake were significantly reduced in group III compared to group IV in rats that were sacrificed after the recovery period.

There was no significant difference in body weight of the toxicant control group compared to the normal control group in rats that were sacrificed a day after the last dose of CCl₄ and those sacrificed after the recovery period (Fig.1). Group III showed no significant difference in body weight compared to the normal control group in rats that were sacrificed a day after the last dose of CCl₄, but there was a significant decrease in body weight in this group compared to the normal control group after the recovery period. However, no significant difference in body weight was seen in group IV when compared to the normal control group in rats that were sacrificed a day after the last dose of LIV and even in those sacrificed after the recovery period.

The photomicrograph of the liver section of toxicant control rats shows distortion of hepatic cells with slightly ballooned sinusoid (Fig.6) when compared to the normal control group which shows normal hepatic cells with distinct and intact central vein (Fig.7). Group III shows restoration of the normal architecture of the liver as a result of tissue regeneration (Fig.8) when compared to the toxicant control group in rats that were sacrificed after the recovery period. Similarly, group IV shows appreciable tissue regeneration of the hepatic cells (Fig.9) when compared to the toxicant control group.

Figure 1. Course of the mean body weights of rats. Significantly different from *control, or *CCl₄ + LIV groups.

Figure 2. Photomicrograph of the liver section of toxicant control rats sacrificed a day after the last dose of CCl₄ showing distortion in the arrangement of the hepatic cells with few perivascular vacuoles (arrow).

Figure 3. Photomicrograph of the liver section of normal control rats sacrificed a day after the last dose of propylene glycol showing normal hepatocytes with well-preserved cytoplasm and prominent nucleus. The portal vein, hepatic artery, interlobular bile duct are well-defined.
Figure 4. Photomicrograph of the liver section of CCl\textsubscript{4} + LIV rats sacrificed a day after the last dose of LIV showing well preserved hepatocytes which are arranged normally. Few Kupffer cells appear around the sinusoids.

Figure 5. Photomicrograph of the liver section of LIV + CCl\textsubscript{4} rats sacrificed a day after the last dose of CCl\textsubscript{4} showing a general distortion of liver architecture with degenerated hepatic cells (broken arrow). Vascular wall appears thickened.

Figure 6. Photomicrograph of the liver section of toxicant control rats sacrificed after the recovery period showing slightly ballooned sinusoids with distortion of hepatic cells. Perivascular vacuole (arrow) are present but not prominent.

Figure 7. Photomicrograph of the liver section of normal control rats sacrificed after the recovery period showing the central vein which is distinct and intact. Hepatocytes appear normal with intact cytoplasm. The sinusoids are normal in size, shape and number and well demarcated.

Figure 8. Photomicrograph of the liver section of LIV + CCl\textsubscript{4} rats sacrificed after the recovery period showing normal and well preserved hepatocytes and sinusoids.

Figure 9. Photomicrograph of the liver section of CCl\textsubscript{4} + LIV rats sacrificed after the recovery period showing normal and well preserved hepatocytes. The sinusoids appear slightly dilated.
DISCUSSION

The toxicity of CCl₄ is initiated by formation of a reactive metabolite, trichloromethyl radical, which binds covalently to the macromolecules and induces peroxidative degradation of membrane lipids of endoplasmic reticulum rich in polyunsaturated fatty acids. This peroxidative degradation of membrane lipids leads to the formation of lipid peroxides which probably causes damage to cell membrane [10, 11].

Biochemical analysis of the serum of the toxicant control group revealed a significant increase in AST and ALT activities compared to the normal control group. Kew [12] reported that administration of CCl₄ to rats induced liver damage resulting in raised activities of the liver transaminases. The results of the present study indicated that the liver cells of the toxicant control group had been damaged by CCl₄ as evidenced by the photomicrograph in Fig.2. The curative group showed a significant reduction in the activities of the transaminases compared to the toxicant control group, while the prophylactic group had high activities of the enzymes which were not significantly different from the toxicant control group in rats that were sacrificed a day after the last dose of CCl₄. Using the drug prophylactically, cause a decrease in the activities of the enzymes that were not significantly different from that of the toxicant control group. It could be that phosphatidylcholine contained in the drug does not make the hepatocytes resilient to toxic agents as much as they facilitate regeneration resulting from hepatotoxic substances. The photomicrograph of this group is an attestation to this assertion as it appeared more necrotic than that of the curative group (Fig.5). However, this can be further investigated by tissue culture experiments. The significant decrease in enzyme activities of the liver transaminases in the curative group relative to the toxicant control group could be attributed to phosphatidylcholine present in LIV. Phosphatidylcholine is a class of phospholipids that is known to protect the liver through the regeneration of liver cells [13]. This assertion is also corroborated by the representative liver photomicrograph of animals in this group (Fig.7). There were no significant differences in the activities of transaminases in the treated rats compared to the normal control group in rats that were sacrificed after the recovery period, due to restoration of the normal histology of their liver tissue.

The activity of alkaline phosphatase (ALP) was significantly higher in the toxicant control group than in the normal control rats as a result of CCl₄-induced liver damage. In the prophylactic group, the activity of this enzyme was not significantly different from the toxicant control group in rats that were sacrificed a day after the last dose of CCl₄, indicating that the drug was not able to prevent the cells of the liver from being damaged by CCl₄. However in the curative group, the activity of ALP was significantly lower than the toxicant control group but not significantly different from the normal control group. This could be attributed to the regenerative action of phosphatidylcholine in the drug.

ALP activity in the treated groups was significantly higher than the normal control group but significantly lower than the toxicant control group in rats that were sacrificed after the recovery period. This later increase in ALP activity in the treated groups could be due to the release of the enzyme from other sources apart from the liver. This needs to be investigated, more so as the photomicrographs of the livers of the treated rats sacrificed after the recovery period actually revealed appreciable healing or tissue regeneration (Figs.8&9).

Equally, total bilirubin level was significantly increased in the toxicant control group compared to the normal control group in rats that were sacrificed a day after the last dose of CCl₄. The elevation in bilirubin level could have resulted from decreased uptake and conjugation of bilirubin by the liver or blockage of bile ducts [14] indicating a possible hepatic injury. The bilirubin level in both the curative and prophylactic groups were not significantly different from the normal control group in rats that were sacrificed a day after the last dose of LIV and CCl₄, respectively. The significant decrease in bilirubin level in the treated groups compared to the toxicant control group may have been as a result of enhanced bilirubin uptake and conjugation by the regenerated liver and its subsequent secretion into the bile ducts.

The significant decrease in bilirubin level in the treated groups could also be attributed to the presence of vitamin B₁₂ in this drug. Vitamin B₁₂ helps to maintain the integrity of red blood cells, thereby decreasing hemolysis of red blood cells. Bilirubin is a by-product of red blood cells destruction [15]; hence decreases in hemolysis of red blood cells will subsequently lead to a decrease in bilirubin formation. The treated groups and the toxicant control group showed no significant differences in bilirubin level compared to the normal control group in rats that were sacrificed after the recovery period due to the restoration of the normal architecture of the liver as a result of tissue regeneration.

There was a significant decrease in total protein in the toxicant control group compared to the normal control group and treated groups in rats that were sacrificed a day after the last dose of CCl₄.
observed reduction in total protein in the toxicant control group is a sign of liver damage, arising from the administration of the CCl₄. This may be an indication of diminished synthetic function of the liver which may consequently lead to enhanced retention of fluid in the tissues spaces [16] causing generalized edema and ascites. There were no significant differences in the serum level of total protein of the treated groups compared to the control group in rats that were sacrificed a day after the last dose of LIV and CCl₄ as the case may be for each group and those sacrificed after the recovery period. The increase in total protein level in the treated groups relative to the toxicant control group could have been due to the presence of phosphatidylcholine in LIV because phosphatidylcholine has been reported to enhance cell membrane integrity [17], thereby increasing protein synthesis by the liver.

The toxicant control group showed an insignificant increase in cholesterol level compared to the normal control and treated groups indicating a possible attack on the liver cells by CCl₄. The observed increase in cholesterol level indicates the inhibition of bile acids synthesis from cholesterol or blockage of bile outflow either inside the liver or outside the liver, as a result of which the blood cholesterol level rises. The curative group showed a significant reduction in the serum cholesterol level compared to the toxicant control group, while the prophylactic group showed a lower but not significant difference in the serum cholesterol level compared to the toxicant control group in rats that were sacrificed a day after the last dose of LIV and CCl₄ as the case may be for each group. The decrease in cholesterol level in the treated groups relative to the toxicant control group could have been due to phosphatidylcholine [18] and vitamin B₆ [19] present in LIV, because both constituents have been reported to lower blood cholesterol level.

The serum glucose level also dropped significantly in the toxicant control group and prophylactic group compared to the normal control group in rats that were sacrificed a day after the last dose of CCl₄. The observed reduction in the serum glucose might reflect the impairment of glucose buffer function of the liver because the liver is important for maintaining a normal blood glucose concentration [15]. The significant decrease in the serum glucose level observed in the prophylactic group in rats that were sacrificed after the recovery period may have resulted from decreased food consumption observed in this group, more so as the representative photomicrograph of the liver of this group showed appreciable tissue regeneration of the liver cells (Fig.8).

The significant increase in the relative liver weight observed in the toxicant control group when compared to the normal control group may have resulted from inflammation in response to injury caused by CCl₄ or reduction in protein causing rapid mitosis of the hepatic cells and growth of the liver to a larger size [15].

Similarly, there was a significant decrease in GSH level in the toxicant control group compared to the normal control group and treated groups. Li et al [20] reported that the administration of CCl₄ to rats in order to induce liver damage resulted in decreased level of GSH. The observed decrease in GSH level in the toxicant control group could be attributed to increased use of GSH in scavenging of reactive substances that were produced as a result of the necrosis of the hepatocytes or possible decreased hepatic production of GSH [20]. The significant increase in GSH level, both in the curative and prophylactic groups, could be ascribed to the presence of vitamin E in LIV. Vitamin E has been reported to protect the polyunsaturated fatty acids of cellular membranes and low-density lipoproteins (LDL) from oxidative damage caused by free radicals. In this manner, vitamin E serves to protect and stabilize the cellular membrane through its role as a powerful antioxidant [21].

The significant decrease in water intake observed in the toxicant control group and group prophylactically treated with the drug maybe as a result of depression of thirst center located in the lateral hypothalamus [15] by CCl₄. This needs to be further investigated. Also, there was a significant decrease in food consumption in the prophylactic group compared to the normal control group in rats that were sacrificed after the recovery period. This may be as a result of lethargy observed in this group during the period of CCl₄ administration. The significant decrease in body weight seen in the prophylactic group when compared to the normal control rats could be attributed to the decrease in food consumption observed in this group.

The group that was treated curatively with the drug showed significant reversal of the changes induced by CCl₄ compared to the toxicant control group both in rats that were sacrificed a day after the last dose of LIV and those sacrificed after the recovery period. In contrast, the group treated prophylactically with LIV showed reductions in almost all the parameters measured but not significantly different from the toxicant control group in rats that were sacrificed a day after the last dose of CCl₄, but rats that were sacrificed after the recovery period showed no significant differences in nearly all the parameters compared to the normal control group. This could be
that the drug does not have much membrane stabilizing properties as they facilitate regeneration of the liver cells when used as remedies against hepatotoxic substances.

In conclusion, the results of this study showed that Livolin forte was better as a curative agent rather than a prophylactic agent in rats. This supports the use of the drug for treating hepatic damage in humans.

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COMPETING INTERESTS

The authors declare that there are no conflicts of interest for this study.

REFERENCES


