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Leiurus quinquestriatus venom ameliorates the hematological and lipid profile alterations in hepatocellular carcinoma-induced rats

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Running title: *Leiurus quinquestriatus* venom mitigates blood and lipid parameters alterations in HCC-induced rats

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Abstract

The health of people is seriously threatened by hepatocellular carcinoma (HCC). Despite its negative effects on lipid and hematological profiles, chemotherapy has continued to be an effective treatment for HCC. The effects of Leiurus quinquestriatus venom (LQV) on the alterations in the lipid profile and hematological state in the rats with HCC were assessed in this study. Diethyl nitrosamine (DEN) at a dose of 100 mg/kg b.wt. was administered intraperitoneally (i.p.) once every week (Wk) for three weeks in a row to cause HCC in rats. A week later, two intraperitoneal injections of carbon tetrachloride (CCl4) (1 ml/kg) were administered. Group 1 (Gp1) was used as a control group, and 50 male Sprague Dawley rats were split up into 5 groups (N = 10). Gp2 had been used as rats with HCC. Gp3 were HCC-rats that received oral sorafinib (SF) treatment at a dose of 30 mg/kg every day for eight weeks. Gp4 were HCC-rats that received intraperitoneal injections of LQV (1/10 of LD50) every day for eight weeks. Gp5 was treated with SF/LQV (daily/8Wks). A variety of hematological parameters were assessed. Low-density lipoproteins (LDL-C), high-density lipoproteins (HDL-C), total cholesterol (TC), and total triglycerides (TG) were also measured as part of the lipid profile. The findings demonstrated that these parameters changed significantly when animals were given HCC. These changes showed improvement after receiving SF or LQV treatment.

Keywords: *Leiurus quinquestriatus*; Venom; hepatocellular carcinoma; Carbon tetrachloride; Diethyl nitrosamine; Sorafenib; Hematology, Lipid profile.

Introduction

The most common kind of liver cancer, hepatocellular carcinoma (HCC), accounts for between 75 and 85 percent of all hepatic cancers. The frequency of HCC was predicted to rise by about 55% by 2040 (1,2). Numerous risk factors, including exposure to aflatoxins, food additives, alcohol intake, and viral hepatitis, are associated with HCC

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(3,4). Chemotherapies used to treat HCC cause harm to developing drugs and essential organs (5). HCC in patients was linked to a number of negative impacts on the biochemical, histological, and hematological markers. There have been reports of notable hematological changes in animals with DEN-induced HCC (6). They found that rats given DEN/CCl4 injections had significantly lower mean values for hemoglobin (Hb) level, hematocrit percentages (Hct %), total platelets count, and total red blood cells (R.B.Cs) count (7,8). In light of this, injury to the hepatic cells affects liver function and may result in changes to lipid metabolism (9).

Chemotherapy works well for treating a variety of malignancies (10). Consequently, it is not advised to administer them frequently, and it is crucial to take their risk-benefit ratio into account (11). An oral multi-kinase inhibitor called Sorafenib (SF) is used to treat kidney cancer and HCC. However, because of its negative consequences, SF's effectiveness is limited (12). Hematologic effects following SF therapy were documented in a prior study. Chemotherapy is still not enough to effectively treat HCC (13,14). Consequently, there is an urgent need for novel and improved therapies (15).

Natural products have been shown to improve against side effects caused by SF during the treatment of liver cancer (16). The anti-inflammatory and antioxidant qualities may be the origin of this action, as they lessen inflammation and shield cells from harm brought on by reactive oxygen species (ROS) (17). Numerous toxins, proteins, and peptides found in animal venoms function as ionic channel inhibitors and interfere with essential physiological functions. Their target selectivity against cancer cells increased as a result (18). The venom of *Leiurus quinquestratus* (LQV) has been documented as a medicinal agent in the past (19). Oligopeptides, nucleotides, amino acids, enzymes, and other organic materials make up LQV. Since these chemicals may be promising leads for the development of new anti-cancer drugs, LQV is being studied more and more (20). This study assessed the impact of LQV treatment on the alterations in lipid profiles and hematological abnormalities in rats with HCC following SF or/LQV treatment.

Materials and methods

Chemicals

Sigma Aldrich (USA) supplied the diethyl nitrosamine and CCl4 (anhydrous ≥ 99.50%, Product number 289116). The Bio-diagnostic Company (Egypt) provided the phosphate buffer saline (PBS) and the biochemical kits for the lipid profile, which included the levels of high-density lipoprotein cholesterol (HDL-C), triglycerides (TGs), total lipids, and total cholesterol (TC).

Scorpion venom preparation

Professional hunters gathered one hundred *L. quinquestriatus* from Aswan, Egypt, and moved them to the Zoology Department of Tanta University's Faculty of Science in Egypt. A specialist in animal taxonomy then verified the authenticity of the specimens. LQV was lyophilized after scorpions were milked with electrical stimulation (12–17V). After 24 hours of injection, the median fatal dose (LD50) was calculated using sublethal doses of various LQV concentrations (21).

Determination of the median lethal dose of LQV

36 male Sprague Dawley rats weighing 120 ± 5 grams each were split up into six groups (N = 6). To determine the LD_{50}, these groups received a single intraperitoneal injection of LQV (0.1–5 mg/kg) and were observed for a whole day. Probit analysis was used to determine this value.

Induction of HCC in rats

A dose of 100 mg/kg b.wt. per week for three weeks was administered intraperitoneally (i.p.) to male rats after the DEN was dissolved in PPB, filtered, and administered. Following a week of recuperation, CCl4, the promoting reagent, was administered twice weekly for eight weeks in a row at a rate of 1 ml/kg b.wt. Following four weeks, the treatment plans were administered every day for eight weeks in a row (22).

Design of experiments

Male adult rats weighing 120 ± 8 g on average were acquired from the National Research Center in Cairo, Egypt. The temperature was approximately 22 ± 1 ºC, and the relative humidity was 55 ± 5%. Animals were treated following Tanta University's Faculty of Science-approved ethical norms (Protocol number: IACUC-SCI-TU-0228). Group 1 had been used as a negative control (Gp1). Gp2: Rats with HCC. Gp3: For eight weeks, rats with HCC were given oral injections of SF (30 mg/kg b.wt) (23, 24). Gp4: HCC-induced rats were injected with LQV (1/10 LD50) i.p. daily for 8 Wks. Gp5: HCC-induced rats were injected with SF and LQV (1/10 LD50) daily for 8 Wks. Sera samples, liver tissues from all groups were collected for assessment of biochemical parameters.

Determination of the total body weight changes

At the start of the experiment (I.b.wt) and the end (F.b.wt), each group was weighted. The following formula was used to determine the percentage change (% b.wt) in the total body weight: (F.b.wt – I.b.wt / I.b.wt) × 100.

Determination of the hematological parameters

The electronic blood counter (Mendary, China) was used to measure the R.B.Cs count, Hb g/dl, Hct %, total W.B.Cs, and total platelets count from fresh blood samples.

Determination of lipid profile

Kits provided by Bio-diagnostic Company were used to measure the levels of total lipids, TC, TG, and HDL-C. The following formulas were used to determine the amounts of low-density lipoprotein cholesterol (LDL-C) and very low-density lipoprotein cholesterol (VLDL-C): LDL-C = TC - HDL-C - TG/5 and VLDL-C = TG/5 (25, 26).

Statistical analysis

The outcomes of the study were presented as mean ± standard error means (SEM). One-way analysis of variance (ANOVA) was employed to evaluate the data, followed by the Tukey test for multiple comparisons. Values with a $P < 0.05$ were statistically significant.

Results

The LD_{50} value of LQV

To determine the LD_{50} that killed 50% of rats, six groups of animals (6 rats/each) were injected i.p with different doses of the LQV for 24 hrs. The LD_{50} value of LQV that killed 50% of rats was 0.3 mg/kg b.wt (Figure 1).

The kinetic changes in the body weight of rats in different groups

The results demonstrated that there were no significant differences found in the I.b.wt of the rats in all the studied groups ($p > 0.05$). However, a significant increase in the F.b.wt was observed in the Gp1 ($p < 0.05$). In contrast, no such increase was noted in the Gp2 from Wk-0 to Wk-16. The study's kinetic change in b. wt was calculated for every group. After 16 Wks of post-DEN/CCl4 induction, the ratio of T.b.wt increase was 103.3 % in the Gp1 and increased to 68.2 % in the Gp2. Therefore, DEN/CCl_4-induced rats showed a significant ($p < 0.05$) decrease in the T.b.wt as compared to the Gp1 (Table 1A and Figure 2A).

Animal treatment started from Wk-17. The results showed that the F.b.wt of Gp2 significantly decreased compared to the Gp1 (Table 1B). The treatment of HCC-induced rats with SF, LQV, or SF/LQV led to a significant decrease in the F.b.wt compared to that of the Gp1 ($p < 0.05$) Table (1B). Of note, the results showed that the treatment with SF led to a significant decrease in the F.b.wt compared to Gp2 ($p < 0.05$). A significant increase in the F.b.wt was observed in HCC-treated rats with LQV or SF/LQV from Wk-18 to Wk-26 when compared to Gp3 (Table 1B and Figure 2B).

Effect of the treatment with SF or/and LQV on the hematological parameters

The results showed that the total R.B.Cs count, Hb level, Hct%, and total platelets count were significantly ($P < 0.05$) decreased in Gp2 as compared to Gp1 (Table 2). The total number of W.B.Cs count increased significantly in the Gp2 compared to the Gp1 (Table 2). The total number of W.B.Cs count was significantly ($P < 0.05$) decreased

in the Gp3, Gp4, and Gp5 when compared to that of the Gp2. Interestingly, the treatment with SF and LQV showed a pronounced improvement in the hematological parameters when compared to Gp3 and Gp4 (Table 2).

Effect of the treatment with SF or/and LQV on the lipid profile

The results showed that there was a significant increase ($P < 0.05$) in the total lipids, TC, TGs, LDL-C, and VLDL-C with a significant decrease in HDL-C levels in the Gp2 as compared to the Gp1 (Table 3). The treatment of HCC-induced rats with SF, LQV, or SF/LQV led to significant change ($P < 0.05$) in the previously mentioned lipid parameters compared to those of the Gp2 (Table 3 and Figure 3).

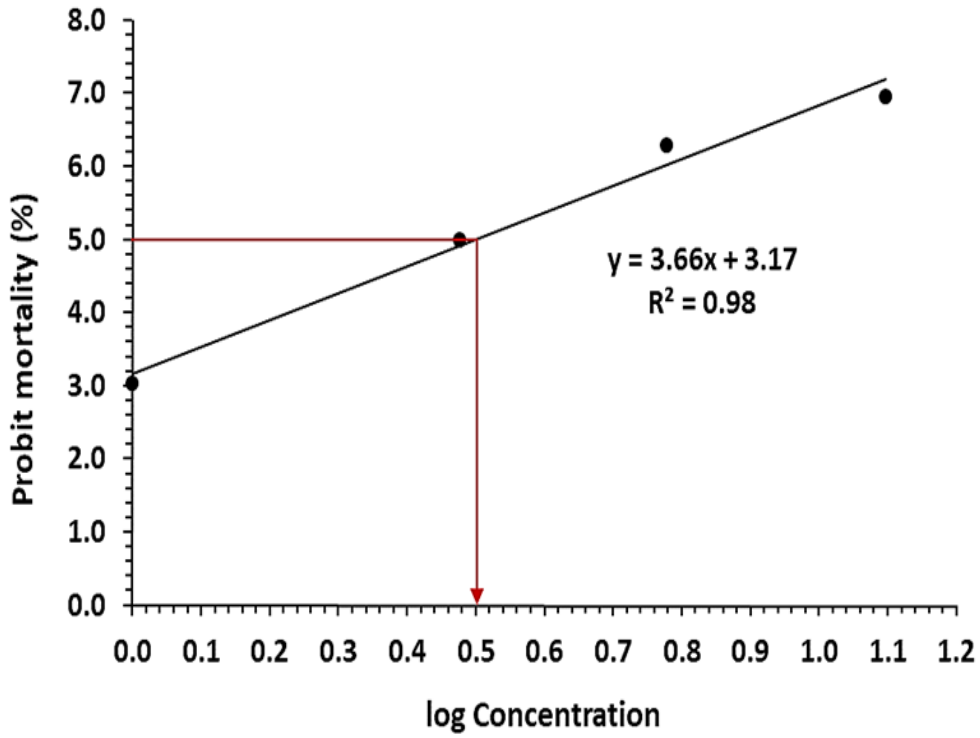

Figure 1. The LD_{50} of LQV on rats after 24 hrs using probit analysis.

Table 1A. Initial, final body weight and their relative weights during HCC induction from Wk-0 to Wk-16

Groups	I.b.wt (g)	F.b.wt (g)	% b.wt change	Paired Samples Test	
				t- value	p-value
Control	138.13 ± 5.30	280.0 ± 5.98	103.3 ± 11.8	36.48	< 0.001
HCC-induced rats	143.18 ± 6.80	240.3 ± 7.88	68.2 ± 10.5	50.45	< 0.001

The values represented mean ± SD. **HCC:** Hepatocellular carcinoma; **Wk:** Weeks; **I.b.wt:** Initial body weight; **F.b.wt:** Final body weight. *P*-value < 0.05 was statistically significant. The means that do not share the same letter are significantly different (Tukey's test).

Table 1B. The percentage of the body weight changes from Wk-18 to Wk-26

Groups	Wk-18	Wk-26
Control	301.25 ± 7.44 [a]	351.88 ± 11.32 [a]
HCC-induced rats	252.5 ± 11.29 [b]	279.83 ± 7.08 [b]
HCC-induced rats/SF	252 ± 7.58 [b]	226 ± 8.22 [d]
HCC-induced rats/LQV	249 ± 6.52 [b]	277.6 ± 7.99 [b, c]
HCC-induced rats/SF/LQV	250 ± 5.48 [b]	262.5 ± 9.35 [c]

The values represented mean ± SD. **HCC:** Hepatocellular carcinoma; **SF:** Sorafenib; **LQV:** *L. quinquestriatus* venom; **Wk:** Weeks. *P*-value < 0.05 was statistically significant. The means that do not share the same letter are significantly different (Tukey's test).

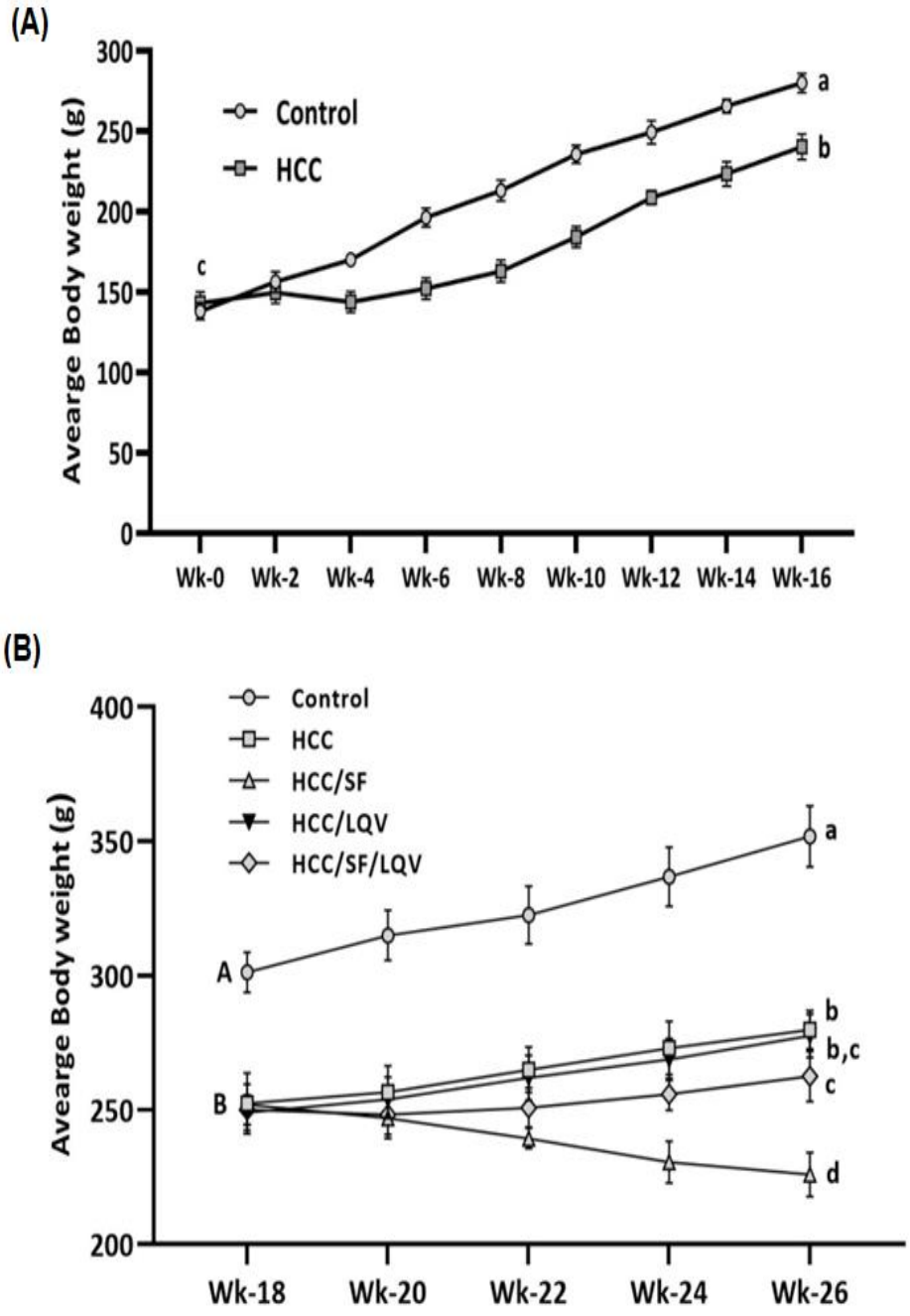

Figure 2 (A and B): Kinetics of body weight changes during the induction of HCC in different groups from Wk-0 to Wk-16 **(A),** the percentage of the b.wt changes from Wk-18 to Wk-26 in different treated groups **(B).** The data represented mean ± SD. **HCC:** Hepatocellular carcinoma; **SF:** Sorafenib; **LQV:** *L. quinquestriatus* venom; **Wk:** Weeks; **b.wt:** Body weight. *P*-value < 0.05 was statistically significant. The means that do not share the same letter are significantly different (Tukey's test).

Table 2. Total W.B.Cs, R.B.Cs counts, Hb level, Hct %, and the total platelets count in different treated groups

Groups	W.B.Cs ($\times 10^3$/ul)	R.B.Cs ($\times 10^6$/ul)	Hb (g/dl)	Hct (%)	Platelets ($\times 10^3$/ul)
Control	8.48 ± 0.35^{e}	9.28 ± 0.56^{a}	13.4 ± 0.57^{a}	41.78 ± 1.95^{a}	596 ± 33.6^{a}
HCC	14.15 ± 0.41^{a}	5.18 ± 0.31^{d}	8.9 ± 0.7^{d}	26.25 ± 1.21^{d}	307.5 ± 27.5^{d}
HCC/SF	11.98 ± 0.47^{c}	7.28 ± 0.37^{b}	$11.32 \pm 0.48^{b,c}$	$33.16 \pm 0.89^{b,c}$	426 ± 31.1^{c}
HCC/LQV	13.08 ± 0.44^{b}	6.3 ± 0.37^{c}	10.3 ± 0.55^{c}	30.95 ± 1.09^{c}	388.8 ± 24.6^{c}
HCC/SF/LQV	10.8 ± 0.39^{d}	8.13 ± 0.56^{b}	12.18 ± 0.59^{b}	35.75 ± 1.5^{b}	487.5 ± 29.9^{b}

The values represented mean ± SD. **HCC:** Hepatocellular carcinoma; **SF:** Sorafenib; **LQV:** *L. quinquestriatus* venom. Total **R.B.Cs:** Red blood cells count; Total **W.B.Cs:** White blood cells; **Hb:** Hemoglobin; **Hct:** Hematocrit. P-value < 0.05 was statistically significant. The means that do not share the same letter are significantly different (Tukey's test).

Table 3. The TC, TG, HDL-C, LDL-C, and VLDL-C levels in different treated groups

Groups	TC (mg/dL)	TGs (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	VLDL-C (mg/dL)
Control	121.80 ± 1.63^{a}	121.25 ± 3.88^{a}	48.50 ± 0.3^{a}	71.05 ± 1.63^{a}	24.25 ± 0.08^{a}
HCC	203.50 ± 3.01^{c}	214.30 ± 5.49^{d}	23.70 ± 1.86^{c}	136.94 ± 3.01^{c}	42.86 ± 0.49^{b}
HCC/SF	157.20 ± 2.55^{d}	148.42 ± 4.35^{e}	29.90 ± 0.93^{d}	97.62 ± 2.55^{b}	29.68 ± 0.35^{c}
HCC/LQV	168.50 ± 4.18^{d}	180.21 ± 1.98^{c}	27.90 ± 0.74^{d}	104.56 ± 4.18^{b}	$36.04 \pm 0.98^{b,c}$
HCC/SF/LQV	140.10 ± 1.74^{b}	130.33 ± 2.66^{d}	39.50 ± 0.36^{e}	85.54 ± 1.74^{e}	26.06 ± 0.16^{c}

The values represented mean ± SD. **HCC:** Hepatocellular carcinoma; **SF:** Sorafenib; **LQV:** *L. quinquestriatus* venom. **TC:** total cholesterol; **TGs:** triglycerides; **HDL-C:** high-density lipoprotein cholesterol; **LDL-C:** low-density lipoprotein cholesterol; **VLDL-C:** very low-density lipoprotein cholesterol. P-value < 0.05 was statistically significant. The means that do not share the same letter are significantly different (Tukey's test).

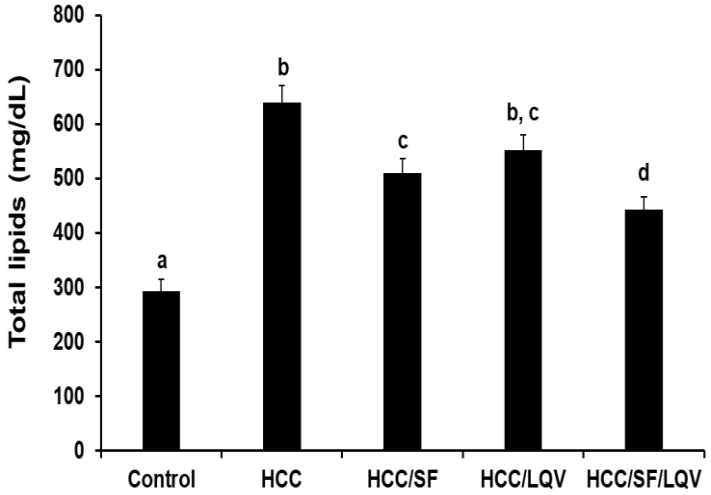

Figure 3: The total lipids levels in the different treated groups. The data represented mean ± SD. **HCC:** Hepatocellular carcinoma; **SF:** Sorafenib; **LQV:** *L. quinquestriatus* venom; **Wk:** Weeks; **b.wt:** Body weight. *P*-value < 0.05 was statistically significant. The means that do not share the same letter are significantly different (Tukey's test).

Discussion

It is known that diethyl nitrosamine is the initiating for rat liver carcinogenesis (27). Hepatic cirrhosis, carcinogenesis, and mimicking HCC in humans are all consequences of CCl4 promotion (28). The existence of bioactive chemicals extracted from a range of venomous species has led to the development of new medicinal medicines from natural components. Potential pharmacological applications were demonstrated by venomous creatures such as frogs, spiders, bees, snakes, and scorpions (29, 30). The venom of scorpions, in particular, has a number of pharmacological and biological uses, such as antibacterial and anti-cancer properties (20, 31). The LQV LD50 value in this investigation was determined to be 0.3 mg/kg b.wt. In mice models, sub-lethal dosages of LQV do not result in liver or renal failure, tissue damage, or allergies (22). The venom of the *Hottentotta saulcyi* scorpion has an LD_{50} of 0.73 mg/kg in mice, according to a prior study (32).

Comparing the DEN/CCl4 group to the normal rats, these results showed a significant decrease in body weight, which may have been caused by food consumption (33). The percentage b.wt change was significantly higher in HCC-treated rats with LQV or SF/LQV than in the SF-treated group, according to the data. The ability of LQV to maintain and regain body weight more quickly may be the reason why treating rats with HCC with LQV increased the rats' body weight (33).

The findings demonstrated that the mean values of R.B.Cs, Hb level, Hct%, and the total platelets count were significantly lower than their corresponding levels in the Gp1 and Gp2, while the W.B.Cs count was significantly higher. This result was consistent with earlier research showing a decline in Hb concentration and overall R.B.Cs counts in individuals with primary HCC (7, 34). LQV therapy lessened the negative impact of DEN/CCl4 on the hematological parameters in rats with HCC.

The mean values of R.B.Cs, W.B.Cs, Hb level, Hct%, and total platelets count are all reduced by LQV treatment. This result implied that LQV might offer some defense against hematological changes brought on by DEN/CCl4. This suggests that LQV can promote the production of erythropoietin, which in turn promotes the production of blood cells by bone marrow stem cells (35, 36).

The development of liver cancer can be prevented and fought by addressing metabolic dysregulation, which has important ramifications for developing new cancer management and therapy approaches (37). In rats with HCC, elevated TC, TGs, and LDL-C levels accompanied by decreased HDL-C levels could indicate hepatic dysfunction (38, 39). Rats with HCC were treated with SF, which reduced the lipid changes. These results were consistent with earlier research showing that mice with HCC who received SF alone or in combination with natural products had improved lipid profiles (40). Rats with HCC showed a marked improvement in their lipid profile after receiving SF or LQV. This might be a result of LQV's role in controlling hepatic lipid metabolism. These results were consistent with earlier research that documented the impact of natural ingredient treatment on improving the cholesterol profile in test animals (41–43). All things considered, LQV therapy ameliorated the lipid profile and hematological changes in the rats with HCC.

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