Original Article

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Hepatoprotective properties of *Curcuma longa* L. extract in bleomycin-induced chronic hepatotoxicity

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Summary ***Curcuma longa* L. (CLL) extract has previously been reported to alleviate liver damage. The current study examined the antioxidant activity of CLL by which the extract protects the liver against bleomycin (BLM)-induced hepatotoxicity in mice. The hypothesis was that CLL extract would protect the liver by reducing oxidative stress (induced superoxide dismutase (SOD) and catalase (CAT) activity), inhibiting lipid peroxidation, lowering biochemical parameters, and decreasing ROS production. Hepatic toxicity was induced by intraperitoneal injection of mice once daily with BLM (0.069 U/mL; 0.29 U/kg bw.) for a period of 4 weeks. The CLL was administered once a day for 4 weeks, 2 h prior at dose (40 mg/mL; 0.187 mg/kg/day). CLL extract significantly protected the liver, it decreased plasma bilirubin (BL) and gamma glutamyl transpeptidase (GGT), and it reduced lipid peroxidation levels. BLM intoxication produced oxidative stress, in which the antioxidant system functioned incorrectly and ROS production significantly increased. The CLL extract provided significant hepatic protection against BLM toxicity by improving SOD, CAT ($p < 0.05$), and MDA levels and decreasing ROS in the group receiving BLM ($p < 0.05$), leading to reduced membrane lipid peroxidation. Throughout this study, the CLL extract facilitated recovery from BLM-induced hepatic injury by suppressing oxidative stress. Therefore, the CLL extract has the potential to serve as an antioxidant compound to treat chronic hepatotoxicity.**

Keywords: *Curcuma longa* L., bleomycin, plasmatic protection, MDA, ROS, hepatic protection, oxidative stress

1. Introduction

The basic organ responsible for the biosynthesis, levels, and degradation of circulating biochemical compounds (proteins, enzymes, and hormones) is the liver. Consequently, the liver should be targeted in order to alter and alleviate the risk of different chronic illnesses (*1,2*). Liver injury continues to be among the most common of internal diseases in clinical settings. Hepatoprotectors occur a key place in the treatment of diseases, as well as in the treatment of virtually all other liver diseases. Hepatoprotectors are complex drugs of mainly plant origin intended to increase the resistance of the liver to toxic effects; they help to restore its functions and normalize or enhance the activity of liver cell enzymes.

In traditional medicine, natural plant-based antioxidants have frequently been used to treat or prevent liver disease. Many studies have indicated that the use of plant antioxidants may provide hepatoprotection by restoring the prooxidant-antioxidant balance (*2*), which is completely altered by the induction of oxidative stress. *Curcuma longa* L. (CLL, turmeric, a yellow powder) is a rhizomatous perennial herb that belongs to the Zingiberaceae family; native to India and China, it is also cultivated in places with a tropical climate (*3*). This plant is traditionally used in India for food and medical purposes in Ayurveda Medicine. CLL has various

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chemical, biological and pharmacological properties, including antioxidant and anti-inflammatory activities (*4,5*). Moreover, the anticarcinogenic activity of CLL has been reported in various chemical-induced tumors, including digestive organs (*5,6*), skin cancers (*7*), and cell lines (*8-10*). Although the exact mechanism by which CLL exhibits these effects has yet to be determined, the antioxidant properties of this plant seem to underlie its pleiotropic biological activities (*11*). Most of the biomedical and medical studies of CLL (approximately 80% of the original sources) are devoted to research on curcuminoids, which are considered to be the active principle in rhizomes. These water-insoluble diphenols of yellow-orange color, called curcumin, were isolated in their pure form as early as 1815. Subsequently, curcumin was found to be non-homogenous, and therefore this group of compounds with a similar structure and biological properties began to be called curcuminoids. Curcumin itself (curcumin I) accounts for about 70%, dimethoxycurcumin (curcumin II) accounts for 17%, bis-dimethoxycurcumin (curcumin III) accounts for 3%, and recently added cyclocurcumin (curcumin IV) accounts for about 10% (*12*).

In preparation for the current study, numerous experimental data verified that the main components of curcumin have the ability to modulate a number of cells and body signaling pathways, either directly or indirectly. Experiments involving animal models have established that the polyphenolic components of curcuma are highly active and can be used to treat many human diseases. Due to its activity as a free-radical scavenger, CLL treatment reduces liver pathology and remodulates immune responses in murine models of acute infection (*8*).

Antioxidants act as a radical scavenger, hydrogen/electron donor, peroxide decomposer, singlet oxygen quencher, enzyme inhibitor, synergist, and metal-chelating agent. There are two main mechanisms of antioxidants' action. The first is the chain-breaking mechanism, by which the primary antioxidant donates an electron to the free radical present in the system. The second involves elimination of reactive oxygen species (ROS)/reactive nitrogen species (RNS) by quenching the catalyst that initiates the chain. Antioxidants can influence biological systems through various mechanisms, including electron donation, chelation of metal ions, co-antioxidants, or regulation of gene expression. ROS overproduction beyond the capacity of antioxidants may result in oxidative stress, possibly causing severe metabolic malfunction (*13*). Cellular defense mechanisms have evolved to protect cells from ROS, and these include repair systems and detoxifying enzymes such as superoxide dismutases (SOD), catalase (CAT), and glutathione peroxidase (GPX). Moreover, ROS are involved in both initiation and promotion of multistage carcinogenesis, and tumor cells are more susceptible to oxidative stress than the surrounding normal cells.

Therefore, the aim of the current study was to investigate whether pretreatment with CLL extract against bleomycin-induced chronic hepatotoxicity in IRC mice would decrease liver oxidative stress. To achieve the ultimate goal of this research, this study examined the levels of lipid peroxidation and activity of antioxidant defense enzymes SOD and CAT in plasma samples and liver homogenates of mice treated with the antitumor drug bleomycin. Moreover, the obtained results were compared with reported levels of ROS products.

2. Materials and Methods

2.1. *Preparation of a CLL extract*

CLL rhizomes obtained from ABC Company, New Delhi, India were cleaned, dried, ground, and weighed. After homogenization (100% ethanol, ratio 1:10 plant to ethanol, 2 days/ 27-28.5°C) the mixture was filtered, concentrated (reduced pressure at 45-47°C, 3 days), and lyophilized (Iishin Lab Co., Ltd., USA) to a crude, dark yellow extract. The extract was stored in an airtight glass bottle and kept at 28°C. The extract was then dissolved in d. H_2O before being orally administered to animals in concentrations of 40 mg/kg body weight (0.7 mL/kg body weight).

2.2. *Antioxidant activity of the CLL extract in vitro*

The antioxidant activity (electron donation potential assay, EDP) of the CLL extract was assayed according to the Oyaizu (1986) method (*14*), with slight modifications. A range of concentrations (1-500 µg/mL) was first tested to determine the concentration at which oil exhibited maximal donation potential. The reaction mixture was left for 10 minutes at 25ºC, and the absorbance was measured at 700 nm. Quercetin was used as a positive control. An increased absorbance of the reaction mixture indicates increased reducing power: % Inhibition = $[(OD_{control} - OD_{test}) / OD_{control}] \times 100$.

2.3. *Instruments*

Biochemical analyses were performed with a UV-VIS spectrophotometer-400 (TERMO Sci., RS232C, Stratagene, USA). Electron paramagnetic resonance (EPR) was measured with the X-Band Emxmicro Spectrometer (Bruker, Germany). EPR settings were: a center field of 3,505 g, microwave power of 6.42 mw, a modulated amplitude of 5 g, and 1-5 scans. All experiments were performed in triplicate.

2.4. *Experimental design and diet*

Twenty-six IRC male mice weighing approximately (46

± 1.2 g) were divided into four experimental groups (*n* = 6 of each) and given tap water and a standard pellet diet (12:12 h light-dark cycle at 40-60% humidity) at the Suppliers of Laboratory Animals for the Faculty of Medicine, Trakia University for a period of 4 weeks.

This study complied with Directive 2010/63/EU and it was approved by the Ethical Committee for Animals of BABH and Trakia University, Stara Zagora, Bulgaria (131/6000-0333/09.12.2016). The first group served as controls (CG). In the second group, mice received bleomycin (BLM) at a dose of 0.069 U/mL; 0.29 U/kg body weight in saline (250 μL) injected once intraperitoneally (*i.p.*) on a schedule from day 1 to day 28 of the experiment. In the third group, the mice were fed CLL extract at a concentration of 40 mg/mL; 0.187 mg/kg bw (orally) *via* a feeding cannula for 28 days before meals. In the fourth group, the mice received both CLL extract (0.187 mg/kg bw for 20 days, orally) and BLM (0.29 U/kg body weight in saline for 28 days, *i.p.*). The CLL extract was given orally daily 2 h prior to BLM on a schedule until the end of the experiment. In addition, the toxicological symptoms, physiological status, and behavior (after 24 h) of IRC mice were monitored daily.

2.5. *Blood and liver samples*

Twenty-nine days after the start of the experiment, the mice were anesthetized with nembutal (50 mg/kg, *i.p.*) and sacrificed. Fresh blood (1.3-2 cm^3) was collected directly from the heart in cold EDTA containers (5 cm^3 Monovette, Germany). After centrifugation of blood samples (4,000 rpm at 4°C for 10 min), 200 μL of plasma from each group was stored at – 40°C until further assay. The freshly collected liver (un-extravasation with cold 0.9% saline) of all six animals in each group was stored on ice. After homogenization and addition of solvents, samples were centrifuged at 4,000 rpm at 4°C for 10 min, and 300 μL of the supernatant was stored at – 4°C until further assay.

2.6. *Determination of serum bilirubin and gamma glutamyl transpeptidase levels*

One ml of blood from each group was collected in $Na_3C_6H_5O_7$ tubes for assessment of specific liver biochemical markers. Blood in the cold tubes (gel-activated) was allowed to clot, and the blood was centrifuged at 4,000 rpm for 10 min/4°C. Serum samples were collected to measure the liver markers total bilirubin (TB) and gamma glutamyl transpeptidase (GGT). The markers were assayed at the Central Diagnostic Laboratory of Stara Zagora, Bulgaria and estimated using a commercially available diagnostic kit (AMRT- 2047- KJ, 2017).

2.7. *Evaluation of oxidative stress markers*

2.7.1. *Plasmatic and hepatocellular lipid peroxidation*

The thiobarbituric acid (TBA) method, which measures MDA-reactive products, was used (*15*). In brief, 1 mL of plasma, 1 mL of physiological solution, and 1 mL of 25% trichloroacetic acid were mixed and centrifuged at 7,000 rpm for 20 min. Two mL of protein-free supernatant was mixed with 0.5 mL of 1% TBA and heated at 95°C for 1 h. After cooling, the intensity of pink color in the final fraction was determined at 532 nm. The MDA concentration was calculated according to the following formula: 1 μmol = l MDA = $(OD_{532} \times 1.75)/0.156$, where OD_{532} is the optic density in λ = 532 nm and extinction = 1.56×10^5 $M^{-1}cm^{-1}$.

2.7.2. *Determination of superoxide dismutase activity*

Erythrocyte lysates were assayed for CuZn-SOD activity as described by Sun *et al*. (*16*) with minor modifications. Briefly, the xanthine⁄xanthine oxidase system was used to generate the superoxide anion (O_2^-). This anion reduced nitroblue tetrazolium (NBT) to formazan, which was monitored at 560 nm. SOD in the sample removes the O_2^- and inhibits the reduction. The level of this reduction is used as a measure of SOD activity. One unit of enzymatic activity is defined as the amount of enzyme causing 50% inhibition of the reduction of NBT to formazan. Results are expressed as the units per gram of hemoglobin (U⁄ gHb).

2.7.3. *Determination of CAT activity*

CAT activity was estimated in erythrocyte lysate using the method of Beers and Sizer (*17*). Hydrogen peroxide (30 mM) was used as a substrate, and a decrease in its concentration at 22°C in phosphate buffer (50 mM, pH = 7.0) was followed at 240 nm for 1 min. One unit of CAT activity was determined to be the quantity of enzyme that removes 1 μmol H_2O_2 for 1 min. The results are expressed as U/gHb (units per gram of hemoglobin). The hemoglobin concentration of the lysate was determined by the cyanmethemoglobin method of Mahoney *et al*. (*17*).

2.7.4. *Plasma and hepatocellular ex vivo evaluation of ROS production*

One hundred mg of liver tissue homogenate and 100 μL of plasma were homogenized with 900 μL of 50 mM spin-trap *N*-tert-butyl-alpha-phenylnitrone (PBN) dissolved in dimethyl sulfoxide (DMSO) using sonication at one cycle for 2 min. After 5 min on ice, the suspension was centrifuged at 4,000 rpm for 10 min at 4ºC. Supernatants were transferred to cold Eppendorf tubes and immediately analyzed. The real-time formation of ROS products in the supernatant was estimated using the methods described previously (*19*)

with some modifications (*20*).

2.8. *Statistical analysis*

EPR spectral processing was performed using the software Bruker Win-EPR and Sim-fonia. Statistical analysis was performed with Statistica 8.0, StatSoft, Inc. One-way ANOVA and the Student *t*-test were used to determine significant differences among groups. The results are expressed as the mean ± standard error (SE). A value of $p < 0.05$ was considered significant.

3. Results

In the current study, the CLL extract was examined as a promising protector from liver injury, and its antioxidant activity was evaluated *in vitro* (Figure 1) along with other biochemical values. The maximal electron donation potential of CLL (40 µg/mL) towards Fe^{3+} complex was observed at maximal concentration (500 µg/mL, 0.0977 ± 0.016 *vs*. 0.0485 ± 0.022, $p < 0.05$, *t*-test), which is higher than quercetin, which was the standard. However, this suggested that CLL and its constituents had sufficient antioxidant activity/defense to overcome hepatic oxidative disorders caused by BLM *in vivo*.

To examine the role of CLL in hepatic toxicity, the extract was administered to animals with BLM-induced chronic toxicity. The hepatoprotective effects of the CLL extract on the development of liver toxicity, induced by prolonged (4 weeks) exposure to BLM intoxication, were determined in this study.

Serum BL (39.05 ± 0.91 µmol/L *vs*. 13.57 ± 1.06 µmol/L; $p < 0.05$, *t*-test) and CG (50.34 ± 2.23 µmol/L *vs*. 19.417 ± 1.06 µmol/L; $p < 0.05$, *t*-test) levels increased significantly in the BLM group compared to those in the CG (Figure 2). Serum BL and GGT levels increased by BLM decreased significantly after 2 h of oral pretreatment with the CLL extract (BL: 18.08 ± 0.99 µmol/L; $p < 0.05$ and GGT: 15.55 ± 0.089 µmol/L; $p < 0.05$, *t*-test) as an antioxidant.

An examination of plasmatic and hepatic homogenate lipid peroxidation (MDA) is shown in Figure 3. In generally, mice with BLM intoxication had significantly higher levels of oxidative stress biomarkers than the CG, in both plasma (26.75 ± 1.56 µmol/ngPr *vs*. 11.588 ± 0.516 µmol/ngPr; $p < 0.05$, *t*-test) and liver homogenate (49.934 ± 2.11 µmol/ngPr *vs*. 21.417 ± 0.99 µmol/ngPr; $p < 0.05$, *t*-test). Notably, the CLL extract group and CLL + BLM group had significantly lower levels of hepatic and plasmatic MDA compared to levels in the BLM group ($p < 0.05$).

Figure 4 shows that the erythrocyte SOD activity in BLM mice was significantly lower than that in the healthy controls (1,232 ± 124 U/gHb *vs*. 2,489 ± 345 U/gHb, $p < 0.01$, *t*-test). The same decrease was observer in liver samples from mice treated with BLM (1,627 ± 151 U/gHb *vs*. 2,813 ± 415 U/gHb, $p < 0.01$, *t*-test). The levels of erythrocyte SOD activity were close to those in the controls in the group treated with CCL (2,712 ± 307 U/gHb *vs*. 2,813 ± 415 U/gHb, $p < 0.07$,

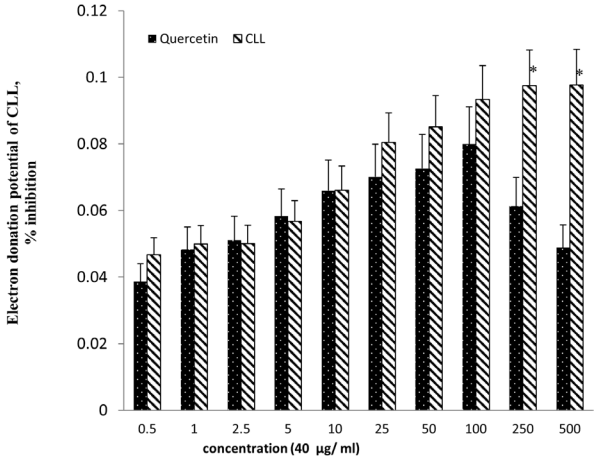

Figure 1. Antioxidant activity of CLL extract in the concentration range of 0.5-500 µg/mL compared to quercetin as the positive standard. Experiments were performed in triplicate. Values are expressed as the mean ± SE. *$p < 0.05$ *vs*. standard.

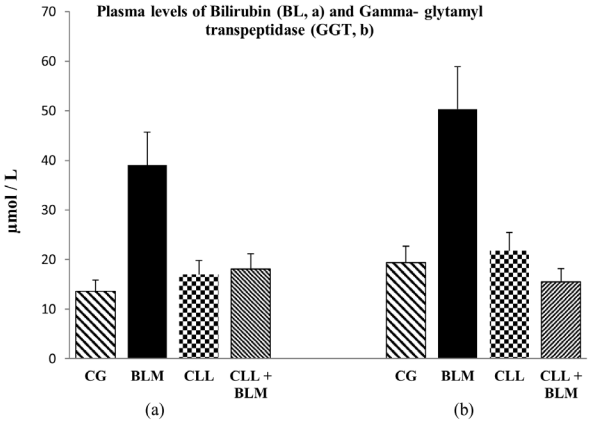

Figure 2. Effect of CLL extract on serum BL (a) and GGT (b) level in BLM-intoxicated IRC mice. Experiments were performed in triplicate. Values are expressed as the mean ± SE. *$p < 0.05$ *vs*. CG group; **$p < 0.05$ *vs*. BLM group.

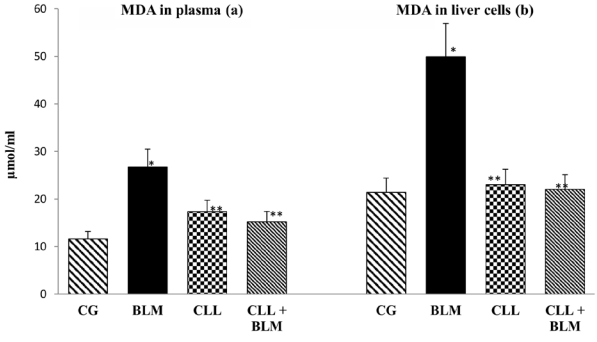

Figure 3. The levels of MDA measured in plasma (a) and MDA in liver cells (b). *$p < 0.05$ *vs*. CG; **$p < 0.05$ *vs*. BLM group.

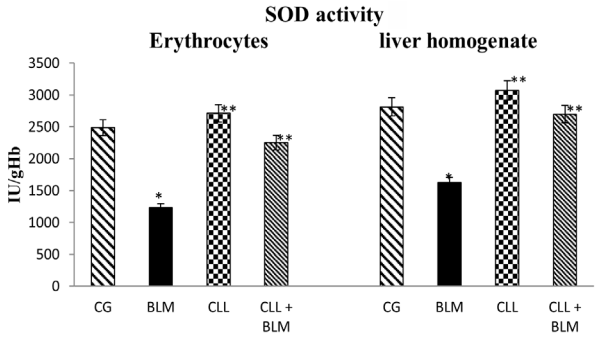

Figure 4. SOD activity in plasma and liver homogenate. *$p < 0.05$ *vs*. CG group; **$p < 0.05$ *vs*. BLM group.

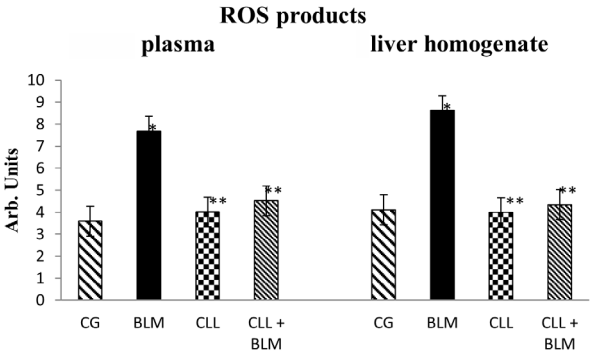

Figure 6. Formation of ROS products in plasma (a) and liver homogenate (b) samples expressed in Arb units. Experiments were repeated three times; *$p < 0.05$ *vs*. CG group; **$p < 0.05$ *vs*. BLM group.

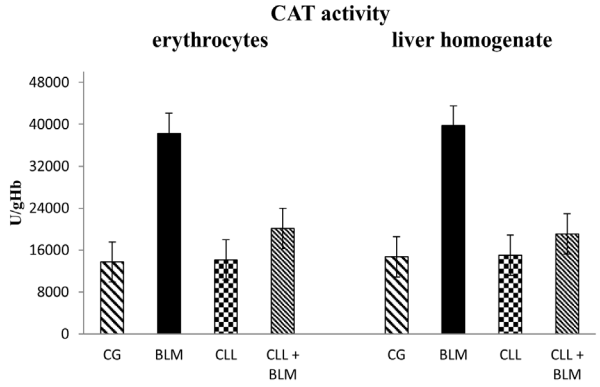

Figure 5. CAT activity in plasma and liver homogenate. *$p < 0.05$ *vs*. CG group; **$p < 0.05$ *vs*. BLM group.

t-test) or with a combination of CLL + BLM (2,253 ± 4,271 U/gHb *vs*. 2,813 ± 415 U/gHb, $p < 0.05$, *t*-test). Liver SOD activity in the group treated with CCL alone was higher than that in controls, but not significantly so (3,071 ± 397 U/gHb *vs*. 2,813 ± 415 U/gHb), and activity in the group treated with a combination of CLL + BLM was close to that in controls (2,698 ± 229 U/gHb *vs*. 2,813 ± 415 U/gHb, *t*-test).

Figure 5 shows that the CAT activity in both erythrocyte samples from mice treated with BLM was significantly higher than that in controls (mean: 38,274 ± 245 U/gHb *vs*. 13,717 ± 289 U/gHb, $p < 0.01$, *t*-test). The examined plasma indicated that activity in mice treated with CCL alone (mean: 14,121 ± 374 U/gHb *vs*. 13,717 ± 289 U/gHb, *t*-test) or a combination of BLM and CCL (20,112 ± 378U/gHb *vs*. 13,717 ± 289 U/gHb, *t*-test) was close to that in controls and significantly lower than that in the group treated with BLM alone ($p < 0.01$).

The same increase was seen in liver homogenate samples treated with BLM (39,716 ± 377 U/gHb *vs*. 14,721 ± 451 U/gHb, $p < 0.01$, *t*-test). Moreover, CAT activity in liver homogenate samples was close to that in controls after treatment with CCL (15,021 ± 376 U/gHb *vs*. 14,721 ± 451 U/gHb, *t*-test) or a combination of CCL+ BLM (19,072 ± 395 U/gHb *vs*. 14,721 ± 451 U/gHb, *t*-test).

Figure 6 shows the ROS products in plasma and liver homogenate measured in arbitrary units. The ROS products in mice treated with BLM increased significantly compared to levels in controls (7.68 ± 0.89 *vs*. 3.59 ± 0.25, $p < 0.01$, *t*-test). However, the level of ROS products was roughly close to that in controls in the group treated with CCL (4.01 ± 0.14 *vs*. 3.59 ± 0.25, *t*-test), or with a combination of CCL + BLM (4.52 ± 0.14 *vs*. 3.59 ± 0.25, *t*-test). A significant decrease was observed in both groups compared to the group treated with BLM alone (CCL: 4.01 ± 0.14 *vs*. 7.68 ± 0.89, $p < 0.01$, *t*-test; and CCL + BLM: 4.52 ± 0.14 *vs*. 7.68 ± 0.89, $p < 0.01$, *t*-test).

A significant difference was observed in the liver homogenate (Figure 5) obtained from the group treated with BLM alone compared to controls (8.62 ± 0.71 *vs*. 4.11 ± 0.53, $p < 0.01$, *t*-test). Moreover, insignificant differences were observed in the group treated with CCL (3.99 ± 0.22 *vs*. 4.11 ± 0.53, *t*-test) or a combination of CCL + BLM (4.34 ± 0.27 *vs*. 4.11 ± 0.53, *t*-test) *vs*. controls.

4. Discussion

Antitumor drugs (like bleomycin, which has cytotoxic activity resulting in the generation of toxic ROS products and oxidative organ changes) cause significant adverse reactions and, as a result, lead to irreversible oxidative disorders of liver function (*10,19-21*). As a consequence, this study focused on finding new therapeutic alternatives (natural combinations or plant antioxidants) to reduce, remodel, or protect from hepatic injury (*2,10,22*). Experimentally, BLM (dose of 0.069 U/mL; 0.29 U/kg bw. in saline) has been used to induce chronic toxicity in IRC mice to produce various grades of oxidative hepatocellular damage and damage to other tissues (*23,24*).

Results revealed that the plasma levels of TB include

free and conjugated bilirubin. In the context of induced hepatotoxicity, TB in the blood increased, and this was due to decreased bilirubin intake in liver cells, impaired protein conjugation, or biliary duct obstruction (*2,25*). GGT is also used as a marker in preneoplastic lesions in the liver during chemical induction, carcinogenesis, and as a key enzyme in glutathione (GSH) and cysteine metabolism (*26*).

Based on these facts, serum levels of BL and GGT can be used as a biochemical marker for early stages of chronic hepatotoxicity. In line with the current results, Sengupta *et al*. (*2*) reported that administration of the CLL extract to mice administered CCl_4 restored the serum level of BL, and they attributed this to the hepatoprotective effect of the CLL extract and its compounds. A serum reduction in GGT is probably due to the antioxidant activity of CLL in the early inflammatory response *via* regulation of GSH and cysteine levels (*27*) after BLM-induced liver injury. BLM intoxication has been reported to stimulate additional oxidative stress mechanisms and the exhaustion of the endogenous antioxidant system, exacerbating organ/tissue damage (*28*).

The CLL extract significantly ($p < 0.05$) improved the deficiency in endogenous antioxidant levels due to BLM, and it induced the survival of hepatocytes. These findings simultaneously support the assertion that treatment with 40 mg/mL of CLL provided protection against the effects of BLM-induced stress on SOD and CAT by enhancing intrahepatic activity. In addition, administration of CLL optimized the level of hepatocellular antioxidant enzymes by scavenging/clearing free-radical formations (*10*) caused by BLM intoxication. Progressive CLL antioxidant action can be attributed to the antioxidant characteristics of the chemical constituents in the extract (*29*).

Oxidative stress is as an imbalance between production and purification of ROS products (*30*). ROS production and oxidative damage caused by BLM contribute to liver injury, and those phenomena cause increased damage to cellular macromolecules (especially lipids) and they induce membrane malformation and hepatic cell changes (*31-34*). The effects of the CLL extract on BLM-induced oxidative toxicity in the liver and blood are shown in Figure 5. In these experiments, EPR indicated an increased ROS concentration in liver ($p < 0.05$) and blood ($p < 0.05$) samples. Chronic BLM exposure resulted in ROS production and lower levels of lipid peroxidation in the liver and blood of mice treated with the CLL extract alone. Greater effects were observed in mice treated with CLL + BLM ($p < 0.05$). However, oral administration of the CLL extract completely ameliorated BLM-induced toxicity by attenuating ROS accumulation ($p < 0.05$). Several studies have suggested that the plant extract has a protective effect against disturbances in hepatic function due to direct antioxidant and free-radical scavenging mechanisms and regulation of ROS production (*10,33,35*). Moreover, Lee *et al*. reported that the CLL extract regulates ROS levels by countering chronic stress and by controlling enhanced ROS signaling and subsequent ER stress in hepatotoxicity (*36*).

The surge in interest in curcuminoids over the last decade is not accidental: these diphenols contained in the rhizomes of the plant *Curcuma Longa*, due to the pronounced pleiotropic nature of their biological effects, have long been considered to be promising compounds that can serve as "multi-purpose" adjuvant drugs (*37*). According to its chemical structure, the main component in the curcuminoid preparation, curcumin, is a bis-α, β-unsaturated β-diketone resulting from the conjugation of two molecules of ferulic acid, connected *via* a methylene bridge. In solution, the molecules of curcuminoids are in the keto-enol form stabilized by hydrogen bonds (*38*). The direction of the equilibrium shift in keto-enol tautomerism depends on the polarity of the solvent and the pH of the solution. In non-polar solvents, curcumin is predominantly present in the enol form, which is maintained through the formation of an intramolecular hydrogen bond, and in polar solvents curcumin takes the diketo form. At low and neutral pH levels, the keto-enol equilibrium shifts towards the keto form, and curcumin acts as a proton donor. At pH levels above 8, the enol form prevails in the solution, and it also acts as a proton donor (*39,40*). As enolates, the molecules of curcuminoids exhibit pronounced antiradical properties. In the enol form, curcumin molecules can act both as a donor and an acceptor in the formation of hydrogen bonds, and keto-enol tautomerism determines whether curcumin exhibits the properties of a Michael acceptor in nucleophilic addition reactions. The presence of two hydrophobic phenoxyl sites, the conjugated keto and alcohol groups, and the presence of hydroxy and methoxy groups determine whether curcuminoids interact *via* Van der Waals bonds with aromatic amino acid residues and whether they form covalent bonds with sulfhydryl groups of cysteine residues that are part of the protein molecules (*41*).

CLL is reported to affect hepatic enzymes by suppressing transcription factor NF-kB (*9*). Sengupta *et al*. suggested that CLL would be an effective immunotherapeutic natural agent preventing disease and the ability to ameliorate hepatotoxicity in mice with carbon tetra chloride intoxication (*2*). Salama *et al*. found that the CLL extract has hepatoprotective action in terms of CLL's properties, functions, and structure against toxins and that CLL has pharmacologic potential in the treatment of liver cirrhosis (*10*).

5. Conclusion

The current study contends that CLL extract is an

antioxidant that is effective in preventing chronic BLM intoxication in the liver and blood, and this study provides new findings into the pharmacologic potential of the CLL extract as protection from hepatocellular disease.

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