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Safety Assessment of *Curcuma longa* Root Extract as Anticancer Agent in Colorectal Cancer Indicated by Pharmacological and Pathological Evidence

Abstract

CLASSICAL chemotherapeutic drug 5-fluorouracil has been administered for cancer treatment, although it has awful effects on vital organs. As a result of many therapeutic actions of *Curcuma longa* root extract (CLE), it has been used worldwide in traditional medicine. Recently, many studies suggested it as an anticancer agent. In this study, CLE has been assessed for preclinical safety in a model of dimethylhydrazine-induced colon cancer in albino rats as an alternative drug for 5-fluorouracil. This is achieved through microscopic and chemical examinations to test the protective effect in the liver, kidney, and heart as vital organs. Results: There was a significant ($P < 0.05$) decrease in different function tests of organs for low dose and high dose of CLE groups compared with the 5-fluorouracil-treated group. With the exception of that, the small dose group of it has an insignificant decrease in bilirubin, VLDL, triglycerides, CK-MB, and uric acid results when compared with those of the standard one. Additionally, CLE treatments have been enhancing the pathological pictures of different organs more than the 5-fluorouracil group. In conclusion: Current data supported that 5-fluorouracil has more risky aspects along the life of patients as an anticancer agent. This can be avoided with the usage of CLE instead of 5-fluorouracil.

Keywords: Anticancer agent, *Curcuma longa* root extract (CLE), 5- fluorouracil, Vital organs.

Introduction

Herbal medications are frequently given as liquids, teas, oils, or dry extracts. Numerous medicinal substances, including digoxin, quinidine, morphine, and aspirin, have been derived from plants [1]. Compared to traditional treatment, herbal remedies are considered to be safer, more effective, and less expensive. However, uncontrolled use raises safety issues because it may result in toxicity, allergic reactions, or drug interactions as well. Globally, the use of herbal remedies is expanding quickly [2]. Furthermore, proprietary herbal medicines that are more effective than ordinary extracts are becoming more and more popular. However, the natural source of the proprietary extracts cannot be trusted to ensure their safety. Not every natural remedy is risk-free and without side effects [3-4]. Even though traditional experimental methods are being used to demonstrate the effectiveness of herbal treatments, the formulations' safety data are still insufficient and scant. Toxicological investigations must be conducted more thoroughly in accordance with internationally accepted safety and toxicity study guidelines [5].The first factor in medical therapy that can significantly influence the choice of medication that should be administered to a patient is drug safety. Additionally, taking into account the idea of benefit-risk balance, we discovered that medications with a high risk profile must to be avoided unless absolutely necessary. Drug safety has evolved throughout the past century and into the present, and healthcare systems must analyze past disasters to implement robust patient safety protocols [6]. Preclinical testing in animals and phase I, II, and III studies in humans are used to evaluate the safety and effectiveness of novel medications prior to their approval. Regulatory bodies typically grant marketing authorizations within their specific geographic jurisdiction. Geographic jurisdiction if it finds that the new drug is effective and that there are no significant risks of side effects [7]. About 90% of drug withdrawals are caused by safety issues. Even if some medications work, their toxicity prevents them from being licensed [8].

Curcuma longa is distinguished by their phytochemical profile, Flavonoids: Typical examples of antioxidant and anti-inflammatory compounds include flavonoids, such as derivatives of quercetin and kaempferol [9], Tannins: These polyphenolic compounds provide this plant its astringent, antibacterial, and anti-inflammatory properties [10], Alkaloids: The increased pharmacological activity observed in the leaves is attributed to their mild

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alkaloid content [11]. Essential Oils: Monoterpenes and sesquiterpenes found in the leaves give this plant its scent and therapeutic properties [12]. *Curcuma longa* belongs to the *Zingiberaceae* family of plants. It is brilliant yellow spice, which is made from the rhizome of the plant. It has been used for centuries in folk traditional medicine as a home cure for a variety of illnesses [13]. It has been shown to exert a number of biological actions, including cardio neuroprotective activities, anti-infectious, antioxidant and antithrombotic properties [14].

In a previous work, it has been demonstrated that *Curcuma longa r*oot extract (CLE) exerts an anticancer effect against colorectal cancer (CRC) induced with dimethylhydrazine (DMH) in rats. To suggest CLE as applicable drug in medicinal markets, it is essential to demonstrate its safety in different vital body organs and blood elements.

Material and Methods

Plant part used

The CLE was obtained from local area, where the dry root is available at shops of herbs and spices elsewhere in Egypt. One kilogram of the dry root was bought from a Harraz® company in Shubra, Cairo province, and identified by a botany specialist, Horticulture Department, Faculty of Agriculture, Benha University, Voucher number (178590).

Preparation of CLE

The fundamental methodological techniques for extraction were adjusted in accordance with Harborne recommendations [15]. A fine powder was made from CLE. The extract was obtained by macerating a weighted fraction of the CLE (500 g) in covered Erlenmeyer flasks in a known volume of hydro-ethanolic solvent (bi-distilled water: absolute ethanol, 70:30 v/v). Maceration continued for an additional 72 hours in the refrigerator, with intermittent mixing. Using muslin mesh, the hydro-ethanolic extract was filtered. A semisolid filtrate was prepared by evaporating the extract in wide-mouthed containers using a shaking water bath set at 70 °C. After weighting the semisolid extract, the yield percentage was determined as follows:

Yield% = (wt. of extract/wt. of CLE) ×100

Preparation of suitable extract doses

Depending on the plant part, extract type, and administration method, a variety of CLE types and dosages have been presented in the literature. The dosages used in this study were derived from the 500 mg absolute human dose [5], while taking body weight and surface area into consideration. Animal equivalent dosages of CLE were determined to be 45 mg/kg body weight of rats, using the conversion table developed by Paget and Barnes in relation to body surface area [16]. Additionally, 7 mg/kg was estimated to be the clinical dose of CLE based on body weight. Following that, the averaging of two dosages was utilized to determine the experimental CLE doses with 30 (Low Dose, LD) and 100 (High Dose, HD) mg/kg every day in animals. Rats were given two stock solutions (6 and 20 mg/mL), which corresponded to dosage rates of 30 (LD) and 100 (HD) mg/kg per day. A stomach tube containing roughly 1 ml of the CLE was used for each 200 g weighted rat in treated groups.

Experimental equipment

In the current investigation, Li-heparinized capillary tubes and standard laboratory equipment have been used.

Chemicals and reagents

5-Fluorouracil (5-FU) was bought from Alfa Aesar, Ward Hill, USA while 1,1-DMH was purchased from Sigma Aldrich, USA. Organic solvents and other analytical grade chemicals were obtained from El-Ghomhorya Company for Trading Drugs, Chemicals, and Medical Instruments, Egypt. Isoflurane inhalation anesthesia was obtained from (Sedico®, Egypt). The commercial kits used to estimate safety profiles of CLE which indicated with organs function markers and lipid profile were bought from Diamond ® (Hannover, Germany), Spectrum® (Hannover, Germany), and BIOMED (Egypt).

Preparation of DMH therapy

According to prior investigations of Srimuangwong *et al.* [17], to induce colorectal cancer, so that a 200 g rat was given 8 mg of DMH substance dissolved in a 1 mM solution of EDTA (for 42 days, S.C. twice per week).

Preparation of 5-FU therapy

According to Alazzouni *et al*. [18], a 200 g rat was administrated 10 mg of 5-FU as chemotherapeutic agent dissolved in a 1 in sterilized water (for 42 days, ip once per week).

Experimental Animals

Male Wistar rats (30) weighing 140±20 g and between 10 and 14 weeks of age were employed in this experimental investigation. The animals were housed under typical experimental conditions, which included a 12/12-hour day and night cycle, a temperature of 22 ± 5℃, and a humidity level of 65%.

Experimental design

The animals were randomly divided into five groups, each consisting of six rats, and given the following various medications for six weeks: The first group was kept as a control and given 1 ml of saline via a stomach tube daily. According to *Saini et al.* [19], the second group was administered 40 mg/kg of DMH subcutaneously twice each week,

which dissolved in1mM EDTA, to be the challenge group later. For third one, rats were maintained as a standard group and were given DMH as second one together with 50 mg/kg of 5-FU, ip once weekly according to [20b]. Animals in fourth group received DMH plus CLE (30 mg/kg, per.*os* daily), and named as a CLE -LD treated group. Rats were treated with DMH together with 100 mg/kg, orally, once daily of CLE, and kept as a CLE-HD treated group.

Sampling

According to Saini *et al*. [19], the animal body was weighed in each group and was measured once a week until the experiment was over. After six weeks of exposure to various treatments, rats were fasted overnight with drinking water ad libitum Blood samples were obtained from the retrobulbar venous plexus using a heparinized capillary tube while under mild inhalation anesthesia of isoflurane. Plasma was separated into an Eppendorf tube for biochemical analysis after blood samples were collected into sampling tubes with lithium heparin as an anticoagulant at room temperature and centrifuged at 900xg for five minutes. Until analysis, the plasma samples were stored frozen at -20 °C. Animals were euthanized for organs collection. After removing the tissues around the allocated organs, a 0.9% sodium chloride solution was used to clean the organs. Following filter paper drying, the tissues were immersed in ice-cold phosphate-buffered saline containing 0.1 mM EDTA to eliminate coagulated blood. They were then placed in formalin for subsequent pathological examination.

Clinicochemical assessment:

With a few modest adjustments, the manufacturer's instructions were followed to perform clinicochemical assays spectrophotometrically utilizing particular kits. The methods outlined by Richmond [21] and Vega and Grundy [22] were used to calculate total cholesterol. The method outlined by Fossati and Prencipe [23] was used to determine triacylglycerols (TAGS). Low-density lipoprotein-cholesterol was measured using the methodology outlined by Tietz *et al.* [24] . The method outlined by Friedewald *et al.* [25] was used to determine high-density lipoprotein-cholesterol. Using the formula given by Bauer and Covert [26], the plasma very-low-density lipoprotein-cholesterol value has been determined as follows Cholesterol = (TAGS) / 5 = mg/dl. The Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) enzymes activities in plasma were measured in accordance with Schumann *et al.* [27] and Chen *et al.* [28] . According to *Tietz et al.* [24], total and conjugated bilirubins were measured, and the unconjugated bilirubin was computed numerically by subtraction [29].The method outlined by Horder et al. [30] and *Burtis et al.* [31] was used to determine the plasma CK-MB levels. The method outlined by Adams *et al*. [32] was used to determine the plasma cardiac Troponin-I Assay. Urea, uric acid, and plasma creatinine were measured in accordance with Coulombe and Favreau [33], Tietz [34] and Walker and Shah [35], respectively.

Histopathological examination

Hepatic, renal and cardiac tissue samples were taken after necropsy, washed with sterile normal saline right away, and preserved in 10% neutral buffered formalin. After being dehydrated in progressively higher grades of ethyl alcohol, the fixed specimens were cleaned in xylol, imbedded in paraffin wax, and paraffin blocks were made [36].

Statistical analysis:

For biochemical markers: The mean ±SE of 6 observations is used to represent all data. To find the significant differences between groups, GraphPad Prism® software for Windows (version 8.4.2, San Diego, CA, USA) was used to do one-way analysis of variance (ANOVA) and then Tukey's post-hoc testing. P values less than 0.05 were deemed significant. As a percentage of the 5-FU effect, the potential of CLE was standardized. For histopathological analysis: Three distinct portions from three different rats in each group have been analyzed under a microscope.

Results

Clinicochemical results

A-Hepatic enzymatic markers:

As shown in (Table 1), as expected, there was a marked significant increase in liver enzymatic biomarker activity, mainly AST and ALT, in the DMH-treated group, compared to those of the negative control one. There were insignificant decreases in AST activities and a significant decrease in ALT activities in standard rats (5-FU, 50 mg/kg) to those of the DMH-injected one. On the other hand, LD of CLE (30 mg/kg), HD of CLE (100 mg/kg) and the negative control groups resulted in a significant decrease in hepatic enzymes when compared to the standard group; even that HD of CLE normalized these parameters as control negative group. Data presented in (Table 1) indicate that the percentage of protection against the diseased group, resulting from low and high doses of CLE, exhibited a dose-dependent relationship (32.05 and 76.69 for AST and 39.83 and 57.52% for ALT, respectively), which parallels the effects of 5-FU (24.27 for AST and 19.46% for ALT).

B- Bilirubin markers:

Table 2 demonstrates that the bilirubin biomarkers in the DMH-treated group were significantly higher than those in the negative control group. In comparison to the DMH-injected rats, standard rats (5-FU, 50 mg/kg) showed a significant

decrease in indirect and total bilirubin markers and an insignificant decrease in direct ones. When compared to the 5-FU-treated group, the bilirubin results of LD of CLE (30 mg/kg) showed an insignificant decrease. However, compared to the standard group, HD of CLE (100 mg/kg) group decreased bilirubin parameters significantly, even as those of the negative group. According to the data in (Table 3), the percentage of protection against the diseased group caused by low and high doses of CLE was dose dependent (55.83 and 77.5 for indirect bilirubin and 22.86 and 51.34 for direct bilirubin in addition to 46.67 and 70.67% for total bilirubin, respectively) and parallel to that of 5-FU (45.00 for indirect bilirubin, 14.28 for direct bilirubin, and 36.00% for total bilirubin).

C- Lipids profile

Table 4 illustrates that, as predicted in the DMH-treated group, lipid biomarkers increased significantly while HDL-C biomarker decreased significantly compared to those of the negative control one. There was a significant decrease in LDL-C and total cholesterol activities and an insignificant decrease in VLDL-C and triglycerides activities in standard rats (5-FU, 50 mg/kg) compared to those of the DMH-injected one. Furthermore, compared to the diseased group, the 5-FU-treated group's HDL-C results showed an insignificant rise.

The results of LD of CLE (30 mg/kg) were an insignificant decrease for triglycerides and VLDL-C in addition to a significant decrease for LDL-C and total cholesterol when compared with those of the 5-FU-treated group. In contrast to that, there was an insignificant increase for the HDL-C result in LD of CLE when compared with those of the 5-FU-treated group. On the other hand, HD of CLE (100 mg/kg) and group resulted in a significant decrease in lipid biomarkers except those of HDL-C when compared to the standard group. In HDL-C results of HD of CLE group, there were significant increases when compared to the 5-FU treated group. Lipid parameters levels in HD of CLE group could be similar to those of the negative control.

Data presented in (Table 5) show that the protection% against the diseased group, which was produced by low and high doses of CLE, was dose dependent (31.46 and 47.56 for total cholesterol and 35.35 and 52.14% for triglycerides, respectively) and parallel to that of 5-FU (15.73 for total cholesterol and 18.57% for triglycerides). The percentage of protection against the diseased group, which was generated by both low and high doses of CLE, was dose dependent (44.14 and 65.43 for LDL-C and 35.71 and 51.78% for VLDL-C, respectively) and parallel to that of 5-FU (19.68 for LDL-C and 17.85% for VLDL-C), as demonstrated in (Table 5).

D- Cardiac enzymatic markers

Table 6 illustrates that, as predicted in the DMH-treated group, cardiac enzymatic biomarker results increased significantly, compared to those of the negative control one. Troponin marker activity was significantly lower in standard rats (5-FU, 50 mg/kg) than in the DMH-injected rats. Additionally, the 5-FU-treated group's CK-MB findings revealed an insignificant drop as compared to the diseased group. When compared to the 5-FU-treated group, the findings of LD of CLE (30 mg/kg) showed a significant decrease for troponin and an insignificant decrease for CK-MB. However, when compared to the 5-FU treated group, the HD of CLE (100 mg/kg) group caused a significant decrease in cardiac enzymatic activity as close to that of normal rats. Data presented in (Table 6) show that the protection% against the diseased group, which was produced by low and high doses of CLE, was dose dependent (48.33 and 67.23 for CK-MB and 66.30 and 69.56% for troponin, respectively) and parallel to that of 5-FU (22.22 for CK-MB and 41.31% for troponin).

E- Renal functional markers

The renal functional indicators in the DMH-treated group were substantially greater than those in the negative control group, as shown in (Table 7). The creatinine, uric acid, and urea marker levels of standard rats (5-FU, 50 mg/kg) were insignificantly lower than those of the DMH-injected rats. When compared to the 5-FU-treated group, the uric acid result of LD of CLE (30 mg/kg) revealed an insignificant decrease; in contrast, those of creatinine and urea were significant decreases. However, the HD of CLE (100 mg/kg) group significantly reduced renal function measures in comparison to the standard group, nearly as those of normal rats. According to the data in (Table 8), the percentage of protection against the diseased group caused by low and high doses of CLE was dose dependent (38.57 and 55 for creatinine and 48.23 and 71.76 for uric acid in addition to 36.17 and 48.93% for urea, respectively) and parallel to that of 5-FU (21.43 for creatinine, 30.58 for uric acid, and 12.76% for urea).

Histopathological findings

A-Hepatic tissues

The control group's hepatic tissue underwent histopathological analysis, which revealed normal hepatocytes, sinusoids, central veins, portal regions, and hepatic architecture. Hepatocytes are big, polyhedral cells with spherical nuclei and eosinophilic cytoplasm; (Figure1A). In the meantime, the toxic group's hepatic parenchyma showed a range of pathological alterations after receiving DMH treatment. Significant hepatic sinusoidal and central vein dilatation and congestion were noted. Additionally, Von Kuepfer's cells were activated, resulting in modest peri-vascular leukocytic cellular aggregations; (Figure 1B). Severe

bleeding inside the hepatic parenchyma was occasionally seen; (Figure 1C). The most closely inspected hepatic sections showed the presence of mononuclear leukocytic cellular infiltration together with multifocal regions of coagulative necrosis. The architecture of these necrosed hepatocytes was preserved, exhibiting hypereosinophilic cytoplasm and pyknosis, infrequently karyolysis, or the lack of nuclei; (Figure 1D). The hepatocytes also showed signs of severe degenerative alterations, including hydropic degeneration, which is characterized by enlarged, pale, vacuolated hepatocytes. Cytoplasm rarefaction was found in most zones of the hepatic lobules; (Figure 1E). Mild fibrosis and edema, along with several embedded bile duct profiles of varying sizes (ductular response) and aggregation of mononuclear inflammatory cells, caused the portal triads to diffusely enlarge. The lining epithelium of the bile duct also showed hyperplastic alterations, with desquamated cells in the lumina and eosinophilic debris. The portal veins occasionally included fibrin thrombi; (Figure 1F). When the livers of the treatment groups (DMH+ Fluorouracil, DMH+LD of CLE, and DMH+ HD of CLE) were examined under a microscope, the normal hepatocellular architecture was significantly restored, and there were more regular and less altered hepatocytes than in the DMH-treated rat. Supplementing with CLE reduces the harmful effects of DMH on hepatic tissues more effectively than fluorouracil. Wherein a HD of CLE exhibits a stronger hepatoprotective effect than a LD of CLE. A few hepatic sections under examination showed hepatocyte necrosis and degeneration together with inflammatory responses in the hepatic parenchyma. Additionally, minor vascular alterations were noted in the portal veins, hepatic sinusoids, and central veins. The bile ducts of the portal triads showed minimal leukocytic cellular infiltration and were almost normal; (Figure 2A-F).

B-Cardiac tissues

Normal cardiomyocytes with their centrally positioned vesicular nuclei were visible in the control group's heart tissue; (Figure 3A). In the meanwhile, DMH caused significant vascular changes, such as vacuolation in the blood vessel walls, muscle hypertrophy, and endothelial damage; (Figure 3B). Additionally, there were large patches of bleeding between the heart's muscles. Disorganized, fragmented muscle fibers caused significant distortion of the cardiac parenchyma, which had severe necrosis with hypereosinophilic, inflated sarcoplasm with pyknosis or no nuclei; (Figure 3C). In the meantime, DMH+ Fluorouracil group the group's cardiac tissue sections revealed considerable myocardial necrosis and myocardial blood vessel congestion; (Figure 3D). When it came to CLE dosage, the high dose treatment demonstrated greater protection than the low dose, with only minor vascular and degenerative alterations in a few heart sections under examination, the histological architecture of the heart in rats in the DMH + HD of CLE was almost identical to that of the control; (Figure 3F). In DMH + LD of CLE group, a few sections under examination showed sporadic focal myocardial hemorrhage, mild congestion of some cardiac blood vessels, and focal areas of cardiomyocyte necrosis; (Figure 3E).

C-Renal tissues

The kidney of the control group showed renal convoluted tubules and glomeruli with normal histological appearance; (Figure 4A). In contrast, the kidneys of the DMH-treated group showed a number of histological changes, including renal blood vessel congestion and dilatation, endothelial cell proliferation, and tunica media vacuolation with perivascular mononuclear leukocytic cellular aggregation. Furthermore, bleeding and perivascular edema were noted. Nuclear pyknosis, loss of cellular detail, blurred cell boundaries, and hypereosinophilic cytoplasm were all signs of extensive necrosis of the epithelial lining in the proximal and distal convoluted tubules. Desquamated and degraded epithelial cells were abundant in the lumens of certain renal tubules; (Figure 4B). Hypersegmentation and vacuolation of the glomerular tuft, hypercellular mesangium, glomerular capillary congestion, hypertrophy of the epithelium lining Bowman's capsule, expansion of the Bowman's space, and mild periglomerular edema were among the abnormalities seen in glomeruli. Some kidney cysts were unintentionally discovered. Together with these lesions, the tubular epithelium displayed massive, pale, vacuolated cytoplasm and widespread degeneration; (Figure 4C). Large-scale tubular degeneration and necrosis were commonly observed in conjunction with partial glomerular tuft shrinkage and atrophy. In the majority of tissue sections, intertubular and glomerular blood capillary congestion was found in (Figure 4D). Mononuclear leukocytic cellular infiltration was common, especially in the spaces between renal tubules, blood vessels, and glomeruli; (Figure 4E).

On the other hand, the glomerular and tubular damage caused by DMH+ Fluorouracil group was lessened than the DMH one. Where there was minor atrophy, mild glomerular lobulation, and mild vacuolation of the endothelial cells lining the glomerular tufts. Rarely, this group showed desquamated epithelial cells in the lumina of afflicted tubules coupled with mild degeneration and necrosis of certain tubular epithelial cells; (Figure 4F). Additionally, compared to the DMH group, the kidneys of the DMH+ LD of CLE group showed an improvement in their histological structure. Both glomerular and intertubular capillary congestion was seen along with a little glomerular growth. In the lumens of certain tubules, eosinophilic debris was occasionally observed along with tubular epithelial

cell degeneration or coagulative necrosis; (Figure 4G). On the other hand, the kidney sections taken from HD of CLE+DMH group showed that most renal tubules had retained normal renal architecture, with sporadic intralumenal casts and modest epithelial cell degeneration in a small number of tubules. Minor glomerular segmentation, proliferation, and congestion were unintentionally seen seldom. Additionally, intertubular blood capillary dilatation and moderate congestion were seen; (Figure 4H).

Discussion

It is established that these days, medicine serves as a natural ally in combating dangerous diseases in increasingly polluted environments.

In order to improve the effectiveness of the drugs that are administered on the one hand and eliminate, or at least lessen, the harmful side effects of the excess drugs that accumulate in the human body on the other, a significant portion of pharmaceutical, medicinal, and biophysical research has been directed toward the exploration and development of new and basic methods in recent years [37].

Around the world, people use herbal remedies for both acute and chronic illnesses without realizing that there are numerous possible side effects that could raise their morbidity, mortality, and hospital admission, length of stay, therapy costs, and quality of life. To control and guarantee the safety and effectiveness of herbal medicines, significant efforts from drug authorities around the world, medical professionals, academic institutions, and researchers are crucial and strongly advised [38].

Curcuma longa is referred to as India's "golden spice." Ayurveda, an Indian ancient medical system, has documented the benefits of it for liver problems, indigestion, nausea, wound healing, inflammation, and skin complexion[39].

Furthermore, CLE is being studied in great detail for its pharmacological properties, including fever reduction and anticancer, antimutagenic, antioxidant, anti-inflammatory, and antidiabetic activities [40]. Due to their varied applications, herbals' toxicity is crucial, and numerous research have been conducted to date on the toxicity of certain plants, including *Crocus sativus* [41] and *Glycyrrhiza glabra* [42]. This study examined the safety and/or toxicity of turmeric in rats because of the overuse of CLE with high efficacy in disease treatment. Our findings revealed that CLE has more protective effect on vital organ as natural antineoplastic than 5FU as chemotherapeutic agent.

For enzymatic liver biomarkers, low-value tests like aspartate aminotransferase (AST), which has lesser specificity as a liver marker than alanine aminotransferase (ALT), have been reduced by recent usage schemes [43]. In the past, ALT and AST have both been employed as indicators of hepatocellular damage. While ALT is mostly found in the cytoplasmic region of hepatocytes, AST is also produced in the kidneys, brain, heart, and skeletal muscle in addition to the liver. Routine chemical testing based on total enzyme kinetics does not distinguish between the immunologically separate forms of AST, cytoplasmic AST, and mitochondrial AST [44]. The use of routine AST testing in conjunction with ALT has been questioned, even though an increase in the overall activity of AST without ALT elevation may indicate cardiac or muscle disease or drug-induced hepatitis, including alcohol and acetaminophen [3].

The present results demonstrated that when compared to the standard group, LD of CLE (30 mg/kg) and HD of CLE (100 mg/kg) groups caused a substantial drop in liver enzymes, being (106 ± 5.0 and 48 ± 9.6 compared to 156 ± 7.6 for AST, 68 ± 3.9 and 44 ± 3.8 vs. 91 ± 6.0 for ALT, respectively), even that HD of CLE normalized these parameters as control negative group, being (48 ± 9.6 compared to 33 ± 5.5 for AST, 44 ± 3.8 vs. 23 ± 4.8 for ALT, respectively). The recorded findings show that the protection % against the diseased group, which was produced by LD and HD of CLE, was dose dependent (32.05 and 76.69 for AST and 39.83 and 57.52% for ALT, respectively) and parallel to that of 5-FU (24.27 for AST and 19.46% for ALT).

These results are consistent with Liu *et al.* [45], who stated that primarily AST and ALT and histopathology could be improved by pretreatment with curcumin and/or α-tocopherol in cisplatin-induced hepatic enzyme damage. Furthermore, a pretreatment with *Curcuma longa* L. extract could prevent doxorubicin-induced hepatotoxicity and significantly raise ALT and AST levels. These findings agree with Eghbali *et al.* [46] , who claimed that patients with β thalassemia who took curcumin had considerably ($p < 0.05$) lower levels of AST and ALT at the end of the study than those in the placebo group. These results support with idea of An *et al.* [47],who found that by reducing the serum levels of AST and ALT in rats and hens, C. longa demonstrated hepatoprotective qualities against liver damage brought on by heavy metals and toxic pesticides.

These results are in contract with Araújo *et al.* [48], who stated that the 5-FU-treated group (intraperitoneally at a dose of 60 mg/kg on the initial day and 40 mg/kg on the following day) and the CLE-treated group (dosage of 50 mg/kg, orally) did not exhibit any significant ($p>0.05$) hepatic changes in hamsters with oral mucositis. This discrepancy was caused by the disease state and the species of the animal difference.

Guerra Ruiz *et al.* [49] and Lala *et al.* [50], who recorded that increase plasma bilirubin level, any of

the following changes in its metabolism can cause it: (a) high formation of bilirubin (i.e., pathologic hemolysis); (b) poor liver tissue uptake, which results in elevated indirect bilirubin; (c) reduced conjugation, which is caused by a defect in UDP-glucuronosyltransferase; and (d) bile clearance disorder, which results in elevated direct bilirubin due to errors in clearance proteins or the inability of the bile to pass through bile ducts and into the small intestine. Ramírez-Mejía *et al.* [51], who found that addition to decreasing the number of hepatocyte cells, a liver lesion of any kind may also affect the uptake of indirect bilirubin from plasma and the transport and clearance of direct bilirubin through the bile ducts.

It had been proved in our study, the bilirubin results of LD of CLE (30 mg/kg) showed an insignificant decrease when compared to the 5-FU-treated group, being (0.53 ± 0.04 vs. 0.66 ± 0.06 for indirect bilirubin, 0.27 ± 0.021 vs. 0.3 ± 0.020 for direct one and 0.80 ± 0.061 vs. 0.96 ± 0.064 for total one, respectively). However, HD of CLE (100 mg/kg) group decreased bilirubin parameters significantly compared to the standard group, being (0.27 ± 0.01 vs. 0.66 ± 0.06 for indirect bilirubin, 0.17 ± 0.015 vs. 0.3 ± 0.020 for direct one and 0.44 ± 0.028 vs. 0.96 ± 0.064 for total one, respectively), even as those of the negative control, being (0.27 ± 0.01 vs. 0.17 ± 0.02 for indirect bilirubin, 0.17 ± 0.015 vs. 0.11 ± 0.01 for direct one and 0.44 ± 0.028 vs. 0.28 ± 0.031 for total one, respectively). The percentage of protection against the diseased group caused by low and high doses of CLE was dose dependent (55.83 and 77.50 for indirect bilirubin and 22.86 and 51.34 for direct bilirubin in addition to 46.6 and 70.67% for total bilirubin, respectively) and parallel to that of 5-FU (45.00 for indirect bilirubin, 14.28 for direct bilirubin, and 36.00% for total bilirubin).

These findings agree with Al-Eisa *et al.* [52], who claimed that total bilirubin levels significantly ($p < 0.05$) dropped in the curcumin group when compared to the $AlCl_3$ group in hepatic intoxication. According to Eghbali *et al.* [46],who suggested that at the conclusion of the trial, the direct and total bilirubin levels of β thalassemia patients who took curcumin were significantly ($p < 0.05$) lower than those in the placebo group. These results agree with An *et al.* [47], who stated that by decreasing the serum levels of total bilirubin in rats and chickens *C. longa* (500 mg/kg daily) demonstrated hepatoprotective qualities against liver damage brought on by heavy metals and toxic pesticides. Even with that, there are no data in the literatures that disagree with our study.

According to Warun Kumar and Harisha [53], who suggested that these lipids and lipoproteins are synthesized, metabolized, and degraded in large part by the liver. Thus, the levels of these lipids and lipoproteins are changed in cirrhosis. The liver and intestines produce nascent HDL, which is the first step in reverse cholesterol transport. Atherosclerosis prevention is significantly aided by the outflow of cholesterol from macrophages to HDL. Ference *et al.* [54] and Feingold [55], who stated that The LDL is created when lipoprotein lipase breaks down the triglycerides found in VLDL in muscle and adipose tissue, releasing free fatty acids. It undergoes additional metabolism to produce LDL, which the LDL receptor absorbs in a variety of organs, with the liver being the primary site of uptake.

In comparison to the standard group, there was an insignificant drop in triglycerides and VLDL-C of CLE (30 mg/kg) group, being (to 228 ± 22 vs. 181 ± 12 for triglycerides, 46 ± 4.4 vs. 36 ± 2.4 for VLDL-C, respectively), in addition to a significant reduce in total cholesterol and LDL-C results, being (225 ± 6.6 vs. 183 ± 7.4 for total cholesterol, 151 ± 6.1 vs. 105 ± 11 for LDL-C, respectively). In contrast to that, the HDL-C result in LD of CLE rats was an insignificant higher than those of the 5-FU-treated group, being (42 ± 6.5 vs. 29 ± 5.3). As compared to the standard group, with the exception of HDL-C results, lipid biomarkers significantly drop in the HD of CLE (100 mg/kg) group when compared to the standard group, being (225 ± 6.6 vs. 140 ± 9.5 for total cholesterol, 228 ± 22 vs. 134 ± 15 for triglycerides, 151 ± 6.1 vs. 65 ± 5.6 for LDL-C, 46 ± 4.4 vs. 27 ± 2.9 for VLDL-C, respectively). In HDL-C result of HD of CLE group was a significant increase more than those of the 5-FU treated group, being (48 ± 6.2 vs. 29 ± 5.3). Lipid parameters levels in HD of CLE group could be similar to those of the negative control. The protection % against the diseased group, which was produced by low and high doses of CLE, was dose dependent (31.46 and 47.56 for total cholesterol and 35.35 and 52.14% for triglycerides, respectively) and parallel to that of 5-FU (15.73 for total cholesterol and 18.57% for triglycerides). The percentage of protection against the diseased group, which was generated by both low and high doses of CLE, was dose dependent (44.14 and 65.43 for LDL-C and 35.71 and 51.78 % for VLDL-C, respectively) and parallel to that of 5-FU (19.68 for LDL-C and 17.85 % for VLDL-C).

The present data may be supported by Udom *et al.* [56], who stated that rats treated with CLE, however, had significantly lower levels of free fatty acids, cholesterol, triglycerides, low density lipoproteins (LDL), very low density lipoproteins (VLDL), and high density lipoproteins (HDL) than the toxic rats (who were given phenobarbital, diethyl nitrosamine, and CCl_4 as hepatic-carcinogenic agents). Rats given CLE prior to treatment did not exhibit any appreciable changes in their lipid profiles as compared to normal control. Current research findings might concur with those of Feingold [55], who proposed that the administration of turmeric powder, curcumin (conventional solvent extract), and

microencapsulated curcumin (supercritical fluid extract) reduced total serum cholesterol in the rat groups' dietary patterns, as indicated by 7.32, 10.54, and 12.81% in the corresponding treated groups, respectively. Adding microencapsulated curcumin to the diets reduces circulating lipids and mitigates the adverse effects of treated rats' hypercholesterolemia. In addition, coincide with those of Mohebbati *et al.* [57] , who proved that male rats' fecundity and serum levels of phospholipids, triglycerides, and cholesterol were decreased when *C. longa* extract (1 mg/kg, p.o.) was administered. These results oppose those of jarhahzadeh *et al.* [58], who claimed that serum levels of VLDL ($p = 0.41$) and total cholesterol ($p = 0.196$) did not significantly alter in obese men. This discrepancy results from variations in technique and research models. Previous researches on the direct effect of turmeric in liver cancer patients with elevated lipid parameters are limited.

The trinity of heart-derived cardiac enzymes, lactate dehydrogenase, aspartate transaminase (serum glutamate oxaloacetate transaminase), and creatinine kinase-MB (CKMB), is traditionally employed in evaluations for the identification of MI, according to [59]. Harahap and Margata [60], who reported that glod standard CK-MB levels, however, have a limited prognostic power when compared to serum troponins (both I and T), which have been validated for post-operative risk stratification for noncardiac surgical procedures and are thought to be more specific and sensitive than CK-MB in the setting of acute coronary syndromes.

The results of LD of CLE (30 mg/kg) revealed a substantial decrease for troponin and an insignificant decrease for CK-MB in comparison to the 5-FU-treated group, being (0.31 ± 0.04 vs. 0.54 ± 0.05 troponin, 0.93 ± 0.09 vs. 1.40 ± 0.2 for CK-MB, respectively). Yet, the HD of CLE (100 mg/kg) group significantly reduced cardiac enzymatic activity as close to that of normal rats as compared to the 5-FU treated group, being (0.28 ± 0.04 vs. 0.54 ± 0.05 for troponin, 0.59 ± 0.05 vs. 1.40 ± 0.2 for CK-MB, respectively). The protection % against the diseased group, which was produced by low and high doses of CLE, was dose dependent (48.33 and 67.23 for CK-MB and 66.30 and 69.56% for troponin, respectively) and parallel to that of 5-FU (22.22 for CK-MB and 41.31% for troponin).

The present findings are consistent with those of Zahed *et al.* [61], who found that 5-fluorouracil administered intraperitoneally resulted in cardiotoxicity, as indicated by higher CK-MB serum level. For ameliorating and prevention of it, CK-MB level significantly ($p < 0.05$) decreased with curcumin administration in 5-FU-treated rats. Also, our data may be agreement with those of A.M. Abdul Azeem *et al.* [62], who proved that treatment of both raw and γ-irradiated turmeric in DOX-administrated group showed a substantial ($p < 0.05$) decrease in kidney function and cardiac marker levels (troponin-I and CK-MB) when compared to the diseased group. No information in earlier literature contradicts our findings.

Toxins and waste materials including urea, creatinine, and uric acid must be eliminated by the kidneys, according to Wasung *et al.* [63]. The byproduct of protein metabolism is urea. Traditionally thought to be biologically inert, urea is a sign of uraemic retention in chronic kidney disease (CKD). Urea itself causes molecular alterations linked to insulin resistance, the generation of free radicals, apoptosis, and disruption of the protective intestinal barrier, according to at least five studies, [64]. According to Ejaz *et al.* [65] reported that research has consistently shown that serum uric acid (SUA) and acute kidney injury (AKI) are strongly correlated across a number of disease models. In two other investigations, lowering SUA also increased GFR and estimated glomerular filtration rate. The mechanisms of action of SUA and diabetic nephropathy, hypertension, cardiovascular disease, chronic renal disease, and metabolic syndrome are similar. The body produces creatinine, a by-product of creatine phosphate in muscle, at a steady rate. Blood creatinine rises as a result of decreased renal clearance. Furthermore, renal function might decline by as much as 50% before a rise in serum creatinine is noticed, indicating renal impairment at a rather late stage, [66]. The uric acid result of LD of CLE (30 mg/kg) showed a negligible drop; nevertheless, the creatinine and urea results showed significant declines, when compared to the 5-FU-treated group, being (4.4 ± 0.51 vs. 5.9 ± 0.78 for uric acid, 0.86 ± 0.08 vs. 1.1 ± 0.08 for creatinine, 30 ± 2.4 vs. 41 ± 1.9 for urea, respectively). In contrast to the standard group, the HD of CLE (100 mg/kg) group considerably decreased renal function measures, which were almost identical to those of normal rats, being (5.9 ± 0.78 vs. 2.4 ± 0.4 for uric acid, 1.1 ± 0.08 vs. 0.63 ± 0.05 for creatinine, 41 ± 1.9 vs. 24 ± 2.1 for urea, respectively). The percentage of protection against the diseased group caused by low and high doses of CLE was dose dependent (38.57 and 55 for creatinine and 48.23 and 71.76 for uric acid in addition to 36.17 and 48.93% for urea, respectively) and parallel to that of 5-FU (21.43 for creatinine, 30.58 for uric acid, and 12.76% for urea).

The current results are similar to those of Ejaz *et al.* [65] , who suggested that the 5-FU group had significantly ($p<0.05$) higher serum urea levels than the CLE-treated and control groups. There was no difference in urea between the CLE-treated group and control groups. The 5-FU group had considerably ($p<0.05$) higher serum creatinine level than the other groups. The group treated with CLE showed a substantial ($p<0.05$) drop in its serum level when compared to the 5-FU group. Also these agree with those of Zeng *et al.* [67], who stated that

when compared to a placebo, they discovered that curcumin intervention tended to lower serum uric acid, although the effect was not of statistical significance in gout disease.

In comparison to the DMH-treated rat, the treatment groups (DMH+ Fluorouracil, DMH+LD of CLE, and DMH+ HD of CLE) displayed more regular and less altered tissue structure in histological analysis of the various studied organs, and the normal architecture was significantly restored. Supplementation of CLE reduces the harmful effects of DMH on critical organs more than fluorouracil does. Dosing with HD of CLE has a stronger cell-protective effect in liver, heart, and kidney sections compared to this of LD of CLE. The microscopic results of our study may be in agreement with Youssef *et al.* [68], who reported that following four weeks of therapy with compounds 1 (curcumin) and 2 (ethyl curcumin), liver, kidney and heart sections displayed normal structure, which were identical to that observed control-negative group with ameliorating and protection from toxic-inducible DMH action. Additionally, the present data are consistent with those of Ejaz *et al.* [65], who proved that pathological picture of renal tubular epithelium and interstitial blood vessels in CLE treated-groups were improved when compared with those of 5-FU treated group, as well as those of Zahed *et al.* [61], who stated that in curcumin administrated animals, the results were the same as those shown in the control group, and the cardiac histopathology lesions were not as severe as those seen in the 5-FU group.

Clinical-chemical parameter data corroborated pathological findings.

Conclusion

The current work, histopathological findings and statistical data analysis of vital organs indicate that CLE improves toxicological changes brought on by 5-FU as chemotherapy. As a result, it is suggested as a superior safer treatment for cancer than 5-FU.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

The Scientific Research Ethics Committee (Animal Care and Use), Faculty of Veterinary Medicine, Benha University, Benha, Egypt, gave ethical approval for the study's experiments (Approval no. BUFVTM 07-02-25).

Figures and Tables

TABLE 1. Effects of CLE and Standard on the plasma hepatic enzymatic markers in 6 rats administrated DMH in contrasted with normal ones.

Groups	Marker			
	AST (U/l)		ALT (U/L)	
	Means ± SE	Protection % against diseased	Means ± SE	Protection % against diseased
Control	33.0 ± 5.50 ^{c}	-	23.0 ± 4.80 ^{d}	-
Diseased (DMH treated)	206 ± 21.0 ^{a}	100.0	113 ± 5.20 ^{a}	100.0
Standard (5-FU treated)	156 ± 7.60 ^{a}	24.27	91.0 ± 6.00 ^{b}	19.46
LD of CLE	106 ± 5.00 ^{b}	32.05	68.0 ± 3.90 ^{c}	39.83
HD of CLE	48.0 ± 9.60 ^{c}	76.69	44.0 ± 3.80 ^{d}	57.52

The values are shown as the means ± SE of six rats in each group. The means in a single column with distinct letter superscripts show significant differences over time ($P < 0.05$; ANOVA with Tukey's post-hoc test); the HD of CLE indicates a high dose of *Curcuma longa* root extract, and the LD of CLE indicates a low dose of *Curcuma longa* root extract with protection% against diseased.

TABLE 2. Effects of CLE and Standard on bilirubin markers in 6 rats administrated DMH in contrasted with normal ones.

Groups	Bilirubin Marker (mg/dl)		
	Indirect	Direct	Total
	Means ± SE		
Control	0.17 ± 0.02 ^{c}	0.11 ± 0.010 ^{b}	0.28 ± 0.031 ^{c}
Diseased (DMH treated)	1.20 ± 0.10 ^{a}	0.35 ± 0.018 ^{a}	1.50 ± 0.084 ^{a}

Standard (5-FU treated)	0.66 ± 0.06 [b]	0.30 ± 0.020 [a]	0.96 ± 0.064 [b]
LD of CLE	0.53 ± 0.04 [b]	0.27 ± 0.021 [a]	0.80 ± 0.061 [b]
HD of CLE	0.27 ± 0.01 [c]	0.17 ± 0.015 [b]	0.44 ± 0.028 [c]

The values are shown as the means ± SE of six rats in each group. The means in single column with distinct letter superscripts show significant differences over time ($P < 0.05$; ANOVA with Tukey's post-hoc test); the HD of CLE indicates a high dose of *Curcuma longa* root extract, and the LD of CLE indicates a low dose of *Curcuma longa* root extract.

TABLE 3. Protection% of CLE and Standard on the renal functional markers against diseased in rats.

Groups	Bilirubin Marker (mg/dl)		
	Indirect	Direct	Total
	Protection diseased	%	against
Diseased (DMH treated)		100	
Standard (5-FU treated)	45.00	14.28	36.00
LD of CLE	55.83	22.86	46.67
HD of CLE	77.50	51.34	70.67

Values present as Protection% against diseased group for the HD of CLE indicates a high dosage of *Curcuma longa* root extract, the LD of CLE indicates a low dose of *Curcuma longa* root extract and Standard group.

TABLE 4. Effects of CLE and Standard on lipid markers in 6 rats administrated DMH in contrasted with normal ones.

Groups	Marker (mg/dl)				
	Total cholesterol	Triglycerides	LDL-C	HDL-C	VLDL-C
	Means ± SE				
Control	126 ± 6.70 [d]	81.0 ± 8.50 [bc]	52.0 ± 8.70 [c]	58 ± 6.6 [bc]	16 ± 1.7 [bc]
Diseased (DMH treated)	267 ± 6.80 [a]	280 ± 18.0 [a]	188 ± 13.0 [a]	23 ± 3.7 [a]	56 ± 3.6 [a]
Standard (5-FU treated)	225 ± 6.60 [b]	228 ± 22.0 [ac]	151 ± 6.10 [b]	29 ± 5.3 [a]	46 ± 4.4 [ac]
LD of CLE	183 ± 7.40 [c]	181 ± 12.0 [bc]	105 ± 11.0 [c]	42 ± 6.5 [ac]	36 ± 2.4 [bc]
HD of CLE	140 ± 9.50 [d]	134 ± 15.0 [bc]	65.0 ± 5.60 [c]	48 ± 6.2 [bc]	27 ± 2.9 [bc]

The values are shown as the means ± SE of six rats in each group. The means in single column with distinct letter superscripts show significant differences over time ($P < 0.05$; ANOVA with Tukey's post-hoc test); the HD of CLE indicates a high dose of *Curcuma longa* root extract, and the LD of CLE indicates a low dose of *Curcuma longa* root extract.

TABLE 5. Protection% of CLE and Standard on lipid markers against diseased in rats.

Groups	Marker (mg/dl)			
	Total cholesterol	Triglycerides	LDL-C	VLDL-C
	Protection % against diseased			
Diseased (DMH treated)		100		
Standard (5-FUtreated)	15.73	18.57	19.68	17.85
LD of CLE	31.46	35.35	44.14	35.71
HD of CLE	47.56	52.14	65.43	51.78

Values present as Protection% against diseased group for the HD of CLE indicates a high dosage of *Curcuma longa* root extract, the LD of CLE indicates a low dose of *Curcuma longa* root extract and Standard group.

TABLE 6.Effects of CLE and Standard on the CK-MB and troponin markers in 6 rats administrated DMH in contrasted with normal ones.

Groups	Marker (ng/ml)			
	CK-MB		Troponin	
	Means ± SE	Protection % against diseased	Means ± SE	Protection % against diseased
Control	0.35 ± 0.03 [b]	-	0.17 ± 0.03 [c]	-

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Diseased (DMH treated)	1.80 ± 0.22 [a]	100.0	0.92 ± 0.08 [a]	100.0
Standard (5-FU treated)	1.40 ± 0.20 [ac]	22.22	0.54 ± 0.05 [b]	41.31
LD of CLE	0.93 ± 0.09 [bc]	48.33	0.31± 0.04 [c]	66.30
HD of CLE	0.59 ± 0.05 [b]	67.23	0.28 ± 0.04 [c]	69.56

The values are shown as the means ± SE of six rats in each group. The means in single column with distinct letter superscripts show significant differences over time ($P < 0.05$; ANOVA with Tukey's post-hoc test); the HD of CLE indicates a high dosage of *Curcuma longa* root extract, and the LD of CLE indicates a low dose of *Curcuma longa* root extract with protection% against diseased.

TABLE 7. Effects of CLE and Standard on renal functional markers in 6 rats administrated DMH in contrasted with normal ones.

Groups	**Marker (mg/dl)**		
	Creatinine	**Uric acid**	**Urea**
	Means ± SE		
Control	0.54 ± 0.04[b]	2.3 ± 0.46 [bc]	17 ± 0.9 [b]
Diseased (DMH treated)	1.40 ± 0.20 [a]	8.5 ± 1.30 [a]	47 ± 3.4 [a]
Standard (5-FU treated)	1.10± 0.08 [ac]	5.9 ± 0.78 [a]	41 ± 1.9 [a]
LD of CLE	0.86 ± 0.08 [bc]	4.4 ± 0.51 [ac]	30 ± 2.4 [b]
HD of CLE	0.63± 0.05 [b]	2.4 ± 0.40 [bc]	24 ± 2.1 [b]

The values are shown as the means ± SE of six rats in each group. The means in single columns with distinct letter superscripts show significant differences over time ($P < 0.05$; ANOVA with Tukey's post-hoc test); the HD of CLE indicates a high dose of *Curcuma longa* root extract, and the LD of CLE indicates a low dose of *Curcuma longa* root extract.

TABLE 8. Protection% of CLE and Standard on renal functional markers against diseased in rats.

Groups	**Marker (mg/dl)**		
	Creatinine	**Uric acid**	**Urea**
	Protection % against diseased		
Diseased (DMH treated)		100	
Standard (5-FU treated)	21.43	30.58	12.76
LD of CLE	38.57	48.23	36.17
HD of CLE	55.00	71.76	48.93

Values present as Protection% against diseased group for the HD of CLE indicates a high dosage of *Curcuma longa* root extract, the LD of CLE indicates a low dose of *Curcuma longa* root extract and Standard group.

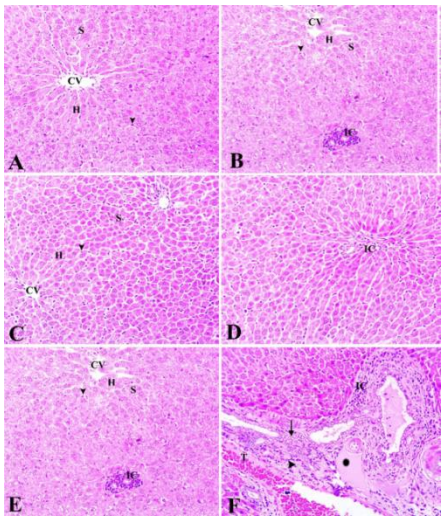

Fig.1. Liver sections of control (A), and toxic (B- F) groups, H&E (x200). Control group (A), showing normal histological appearance of central veins (CV), sinusoids (S), and hepatocytes (H). DMH group (B), mild inflammatory cellular (IC) aggregation (C) congestion of central vein (C) with severe hemorrhage between hepatocytes (arrow), (D) coagulative necrosis (N) of hepatocytes infiltrated with mononuclear inflammatory cells (arrowhead), (E) focal area of lymphocytic aggregation (asterisk) with hydropic degeneration of hepatocytes (arrow), (F) fibrin thrombi (T) in portal vein; portal area expanded by fibrosis (arrowhead) and

edema (asterisk); bile duct hyperplasia (arrow), mixed with aggregates of mononuclear inflammatory cells (IC).

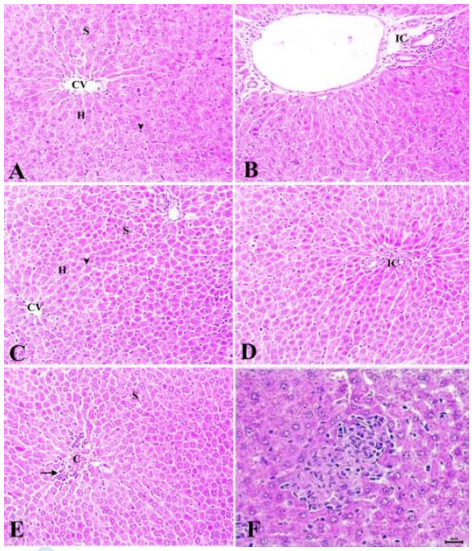

Fig. 2. Liver sections of treated groups with HD of CLE (A, B), LD of CLE (C, D), and 5-flu (E, F), H&E (x200). (A, C) Nearly normal appearance of central vein (CV) hepatocytes (H), with mild congestion and dilatation of hepatic sinusoid (S) in association with enlargement and activation of Von Kuepfer's cells (arrowhead). Note, (E) showing marked congestion (C) of the central vein and hepatic sinusoids (S) with peri-vascular leukocytic cellular aggregations (arrow), (F) Focal area of necrosis associated with mononuclear cellular infiltration in 5-flu. (B, D) Portal area showing mild dilatation of portal vein along with mild periductal edema and inflammatory cellular infiltration (IC).

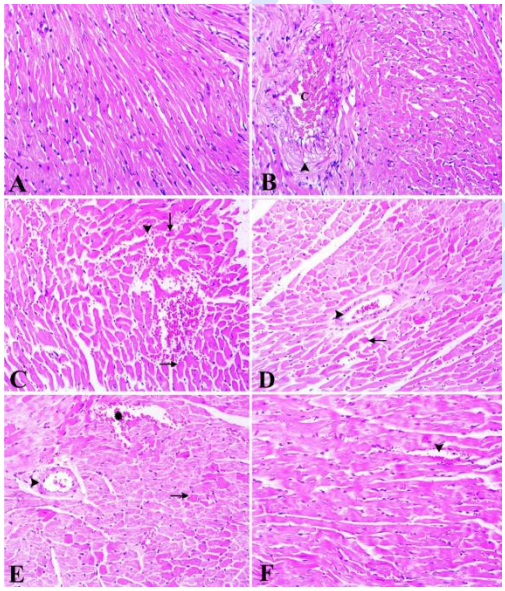

Fig. 3. Heart sections of control (A), toxic (B, C) and treatment (D- F) groups, H&E (x200). Control (A) group, normal histological structure of cardiac muscles. DMH (B, C) group, (B) Endothelial damage, congestion (C) with marked vesiculation of the cardiac blood vessels wall (arrowhead), (C) Extensive intermuscular hemorrhage (arrowhead) and myocardial necrosis (arrow). DMH+ Fluorouracil (D) group, myocardial necrosis (arrow) with mild congestion (arrowhead) the myocardial blood vessels. DMH+ LD of CLE (E) group, myocardial necrosis (arrow), mild congestion (arrowhead) and focal hemorrhage between cardiac muscles (asterisk). DMH+ HD of CLE (F) group, nearly normal cardiac striation with slight congestion of intermuscular capillaries (arrowhead).

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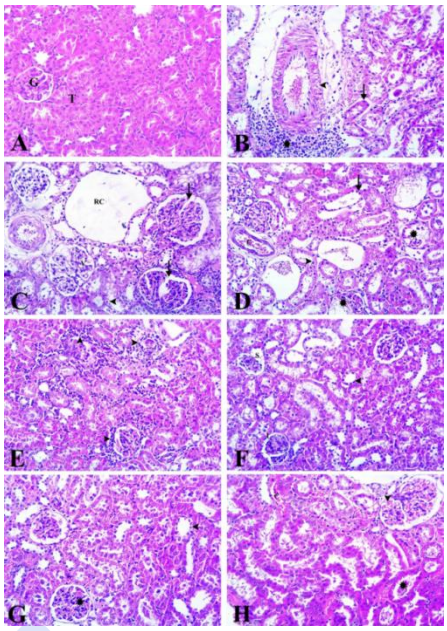

Fig. 4. Kidney sections of control (A), toxic (B- E) and treatment (F- H) groups, H&E (x200). Control (A) group, normal histological structure of glomeruli (G) and convoluted tubules (T). DMH (B- E) group, (B) proliferation of endothelial cells with vacuolation of the tunica media of the renal blood vessels (arrowhead), perivascular mononuclear leukocytic cellular aggregation (asterisk). Also noted severe necrosis of the tubular epithelium with hypereosinophilic cytoplasm and nuclear pyknosis (arrow), (C) hypersegmentation (arrow) and vacuolation (V) of the glomerular tuft, congestion of glomerular capillaries, widening of the Bowman's space and mild periglomerular edema with presence of renal cysts (RC). Note also, hydropic degeneration of tubular epithelium characterized by cytoplasmic vacuolation (arrowhead), (D) glomerular tuft shrinkage and atrophy (asterisk) with extensive tubular degeneration (arrowhead) and necrosis (arrow). Congestion (C) of intertubular and glomerular blood capillaries, (E) Mononuclear leukocytic cellular infiltration (arrowhead) around glomeruli, blood vessels, and in between renal tubules. DMH+ Fluorouracil (F) group, slight glomerular lobulation (L) and mild glomerular shrinkage (S). Note also, mild degeneration and necrosis of some tubular epithelial cells (arrowhead) along with desquamated epithelial cells in the lumina of affected tubules. DMH+ LD of CLE (G) group, improvement in the histological structure of kidneys with slight glomerular proliferation (asterisk) with mild degeneration (arrowhead) of tubular epithelial cells and eosinophilic debris in lumens of some tubules. DMH+ HD of CLE (H) group, nearly normal renal architecture in most renal tubules with mild degeneration of epithelial cells and intralumenal casts in few tubules (asterisk). Also find, minor glomerular proliferation, segmentation and congestion (arrowhead) with mild congestion (C) of intertubular blood capillaries.

References

1. Vitalone, A., Menniti-Ippolito, F., Moro, P. A., Firenzuoli, F., Raschetti, R. & Mazzanti, G. Suspected adverse reactions associated with herbal products used for weight loss: a case series reported to the Italian National Institute of Health. *European journal of clinical pharmacology*, **67**, 215-224 (2011).
2. Amrita Chatterjee & Sarkar, B. Polyphenols and terpenoids derived from Ocimum species as prospective hepatoprotective drug leads: a comprehensive mechanistic review. *Phytochemistry Reviews,* **24**(2), 2087-2129 (2025).
3. Mohammed-Ali, Z., Brinc, D., Kulasingam, V. & Selvaratnam, R. Defining appropriate utilization of AST testing. *Clinical Biochemistry*, **79**, 75-77 (2020).
4. Joshi, J., Ghaisas, S., Vaidya, A., Vaidya, R., Kamat, D., Bhagwat, A. & Bhide, S. Early human safety study of turmeric oil (Curcuma longa oil) administered orally in healthy volunteers. *Journal-Association of Physicians of India*, **51**, 1055-1060 (2003).
5. Soleimani, V., Sahebkar, A. & Hosseinzadeh, H. Turmeric (Curcuma longa) and its major constituent (curcumin) as nontoxic and safe substances. *Phytotherapy Research*, **32**(6), 985-995 (2018).
6. Alshammari, T. M. Drug safety: The concept, inception and its importance in patients' health. *Saudi Pharmaceutical Journal*, **24**(4), 405-412 (2016).
7. Onakpoya, I. J., Heneghan, C. J. & Aronson, J. K. Worldwide withdrawal of medicinal products because of adverse drug reactions: a systematic

review and analysis. *Critical reviews in toxicology*, **46**(6), 477-489 (2016)

8. Craveiro, N. S., Lopes, B. S., Tomás, L. & Almeida, S. F. Drug withdrawal due to safety: a review of the data supporting withdrawal decision. *Current drug safety*, **15**(1), 4-12 (2020).
9. Al-Khayri, J. M., Sahana, G. R., Nagella, P., Joseph, B. V., Alessa, F. M. & Al-Mssallem, M. Q. Flavonoids as potential anti-inflammatory molecules: A review. *Molecules*, **27**(9), 2901 (2022).
10. Mal, S. & Pal, D. Tannins and polyphenols extracted from natural plants and their versatile application. *Bioactive natural products for pharmaceutical applications*, 715-757 (2020).
11. Niranjan, R. K., Thimmaraju, M., Malik, J. K., Gupta, V. & Singh, G. PHARMACOGNOSTIC INSIGHTS AND THERAPEUTIC POTENTIAL OF CURCUMA LONGA LEAVES WITH A FOCUS ON ANTI-INFLAMMATORY PROPERTIES. *International Journal of Pharmaceutical Science and Medicine*, **3**(1), 10-19 (2025).
12. Butnariu, M. Plants as source of essential oils and perfumery applications. *Bioprospecting of plant biodiversity for industrial molecules*, 261-292 (2021).
13. Sharma, S. Phytochemical and Pharmacological Review and Importance of Curcuma longa. *International Journal of Recent Research and Review*, **14** (4), ISSN 2277 – 8322 (2021).
14. Micucci, M., Aldini, R., Cevenini, M., Colliva, C., Spinozzi, S., Roda, G., Montagnani, M., Camborata, C., Camarda, L. & Chiarini, A. Curcuma longa L. as a therapeutic agent in intestinal motility disorders. 2: Safety profile in mouse. *PloS one*, **8**(11), e80925 (2013).
15. Harborne, A. *Phytochemical methods a guide to modern techniques of plant analysis*, springer science & business media, (1998).
16. Paget, G. & Barnes, J. *Toxicity tests. Evaluation of drug activities, Pharmacometrics*, Academic Press, Massachusetts, **1**, 135-166 (1964).
17. Srimuangwong, K., Tocharus, C., Tocharus, J., Suksamrarn, A. & Chintana, P. Y. Effects of hexahydrocurcumin in combination with 5-fluorouracil on dimethylhydrazine-induced colon cancer in rats. *World journal of Gastroenterology*, **18**(47), 6951 (2012 b).
18. Alazzouni, A. S., Dkhil, M. A., Gadelmawla, M. H., Gabri, M. S., Farag, A. H. & Hassan, B. N. Ferulic acid as anticarcinogenic agent against 1, 2-dimethylhydrazine induced colon cancer in rats. *Journal of King Saud University-Science*, **33**(2), 101354 (2021).
19. Saini, M. K., Vaiphei, K. & Sanyal, S. N. Chemoprevention of DMH-induced rat colon carcinoma initiation by combination administration of piroxicam and C-phycocyanin. *Molecular and cellular biochemistry*, **361**(1), 217-228 (2012).
20. Srimuangwong, K., Tocharus, C., Chintana, P. Y., Suksamrarn, A. & Tocharus, J. Hexahydrocurcumin enhances inhibitory effect of 5-fluorouracil on HT-29 human colon cancer cells. *World Journal of Gastroenterology*, **18**(19), 2383 (2012 a).
21. Richmond, W. Analytical reviews in clinical biochemistry: the quantitative analysis of cholesterol. *Annals of clinical biochemistry*, **29**(6), 577-597 (1992).
22. Vega, G. L. & Grundy, S. M. Lipoprotein responses to treatment with lovastatin, gemfibrozil, and nicotinic acid in normolipidemic patients with hypoalphalipoproteinemia. *Archives of internal medicine*, **154**(1), 73-82 (1994).
23. Fossati, P. & Prencipe, L. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *Clinical chemistry*, **28**(10), 2077-2080 (1982).
24. Tietz , Finley PR & E, P. *Clinical guide to laboratory tests*, WB Saunders company Philadelphia, pp.1096 (1995).
25. Friedewald, W. T., Levy, R. I. & Fredrickson, D. S. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical chemistry*, **18**(6), 499-502 (1972).
26. Bauer, J. E. & Covert, S. J. The influence of protein and carbohydrate type on serum and liver lipids and lipoprotein cholesterol in rabbits. *Lipids*, **19** (11), 844-850 (1984).
27. Schumann, G., Canalias, F., Joergensen, P. J., Kang, D., Lessinger, J.-M. & Klauke, R. IFCC reference procedures for measurement of the catalytic concentrations of enzymes: corrigendum, notes and useful advice: International Federation of Clinical Chemistry and Laboratory Medicine (IFCC)–IFCC Scientific Division. *Clinical chemistry and laboratory medicine*, **48**(5), 615-621 (2010).
28. Chen, Y., Krishan, M., Nebert, D. W. & Shertzer, H. G. Glutathione-deficient mice are susceptible to TCDD-Induced hepatocellular toxicity but resistant to steatosis. *Chemical research in toxicology*, **25**(1), 94-100 (2012).

29. Liman, M. L. & Atawodi, S. E. Hepatoprotective and nephroprotective effects of methanolic extract of different parts of Tamarindus indica Linn in rats following acute and chronic carbon tetrachloride intoxication. *Annual Research & Review in Biology,* **5**(2), 109 (2015).

30. Horder, M., Elser, R., Gerhardt, W., Mathieu, M. & Sampson, E. IFCC methods for the measurements of catalytic concentration of enzymes. *Journal of the International Federation of Clinical Chemistry*, **2**(2), 80-83 (1990).

31. Burtis, C. A., Ashwood, E. R. & Tietz, N. W. *Tietz textbook of clinical chemistry*. Saunders, (1999).

32. Adams, J., Abendschein, D. R. & Jaffe, A. S. Biochemical markers of myocardial injury. Is MB creatine kinase the choice for the 1990s?. *Circulation*, **88**(2), 750-763 (1993).

33. Coulombe, J. & Favreau, L. A new simple semimicro method for colorimetric determination of urea. *Clinical chemistry*, **9**(1), 102-108 (1963).

34. Tietz, N. W. *Fundamentals of Clinical Chemistry,* Saunders, Philadelphia (1976).

35. Walker, P. D. & Shah, S. V. Reactive oxygen metabolites in endotoxin-induced acute renal failure in rats. *Kidney international*, **38**(6), 1125-1132 (1990).

36. Bancroft, D, J. & Layton, *C. Connective and mesenchymal 11 tissues with their stains. Bancroft's Theory and Practice of Histological Techniques E-Book,* 187 (2012).

37. Kundu, P., Das, S. & Chattopadhyay, N. Managing efficacy and toxicity of drugs: targeted delivery and excretion. *International Journal of Pharmaceutics*, **565,** 378-390 (2019).

38. Jităreanu, A., Trifan, A., Vieriu, M., Caba, I.-C., Mârțu, I. & Agoroaei, L. Current trends in toxicity assessment of herbal medicines: A narrative review. *Processes*, **11**(1), 83 (2022).

39. Slika, L. & Patra, D. Traditional uses, therapeutic effects and recent advances of curcumin: a mini-review. *Mini Reviews in Medicinal Chemistry,* **20**(12), 1072-1082 (2020).

40. Krup, V., Prakash, L. H. & Harini, A. Pharmacological activities of turmeric (Curcuma longa Linn): a review. *Journal of Homeopathy & Ayurvedic Medicine*, **2**(133), 2167-1206 (2013).

41. Bostan, H. B., Mehri, S. & Hosseinzadeh, H. Toxicology effects of saffron and its constituents: a review. *Iranian journal of basic medical sciences*, **20**(2), 110 (2017).

42. Nazari, S., Rameshrad, M. & Hosseinzadeh, H. Toxicological effects of Glycyrrhiza glabra (licorice): a review. *Phytotherapy research*, **31**(11), 1635-1650 (2017).

43. Xu, S., Hom, J., Balasubramanian, S., Schroeder, L. F., Najafi, N., Roy, S. & Chen, J. H. Prevalence and predictability of low-yield inpatient laboratory diagnostic tests. *JAMA network open*, **2**(9), e1910967 (2019).

44. Kwo, P. Y., Cohen, S. M. & Lim, J. K. ACG clinical guideline: evaluation of abnormal liver chemistries. *Official journal of the American College of Gastroenterology*, **112**(1), 18-35 (2017).

45. Liu, Z., Huang, P., Law, S., Tian, H., Leung, W. & Xu, C. Preventive effect of curcumin against chemotherapy-induced side-effects. *Frontiers in pharmacology*, **9**, 1374 (2018).

46. Eghbali, A., Nourigheimasi, S., Ghasemi, A., Afzal, R. R., Ashayeri, N., Eghbali, A., Khanzadeh, S. & Ghaffari, K. The effects of curcumin on hepatic T2* MRI and liver enzymes in patients with β-thalassemia major: a double- blind randomized controlled clinical trial. *Frontiers in Pharmacology,* **14**, 1284326 (2023).

47. An, S., Jang, E. & Lee, J.-H. Preclinical evidence of curcuma longa and its noncurcuminoid constituents against hepatobiliary diseases: a review. *Evidence-Based Complementary and Alternative Medicine*, **2020(**1), 8761435 (2020).

48. Araújo, A. A. d., Silva, E. M., Mafra, C. A. d. C. C., Costa, Í. d. C. C., Barbalho, A. L. A., Matos, I. A. F. d., Santos, M. A. d., Lopes, M. L. D. d. S., Medeiros, C. A. C. X. d. & Soares, L. A. L. Curcuma longa extract protects against 5-fluorouracil-induced oral mucositis in hamsters. *Brazilian Journal of Pharmaceutical Sciences,* **58,** e20114 (2022).

49. Guerra Ruiz, A. R., Crespo, J., López Martínez, R. M., Iruzubieta, P., Casals Mercadal, G., Lalana Garcés, M., Lavin, B. & Morales Ruiz, M. Measurement and clinical usefulness of bilirubin in liver disease. *Advances in Laboratory Medicine/Avances en Medicina de Laboratorio*, **2**(3), 352-361 (2021).

50. Lala, V., Zubair, M. & Minter, D*. Liver function tests,* StatPearls Publishing, [Internet] (2023).

51. Ramírez-Mejía, M. M., Castillo-Castañeda, S. M., Pal, S. C., Qi, X. & Méndez-Sánchez, N. The multifaceted role of bilirubin in liver disease: a literature review. *Journal of Clinical and Translational Hepatology*, **12**(11), 939 (2024).

52. Al-Eisa, R., Khouja, H. & Al-Nahari, H. Turmeric (Curcuma Longa) protection against the Liver Toxicity Caused by Aluminum Chloride (AlCl3) in Adult Male Rats. *International Journal of Pharmaceutical Research & Allied Sciences*, **6**(2), 110-127 (2017).

53. Warun Kumar, M. & Harisha, E. Assessment of lipid profile changes with respect to severity of liver dysfunction in cirrhosis of liver. *Indian Journal of Basic and Applied Medical Research,* **4**, 56-63 (2015).

54. Ference, B. A., Kastelein, J. J. & Catapano, A. L. Lipids and lipoproteins in 2020. *Jama*, **324**(6), 595-596 (2020).

55. Feingold, K. R. *Introduction to lipids and lipoproteins,* Endotext [internet]. South Dartmouth (MA), PP1-2 (2024).

56. Udom, G. J., Ogbuagu, E. O., Udobangd, J. A., Ndifon, B. T., Jovita, N. O., Ise, U. P., Udo, N. M. & Yemitan, O. K. Antidotal assessment of hydroethanol root extract of Curcuma longa L.(Turmeric) against chemically-induced hepatic neoplasm in wistar rats. *Global scientific journal*, **7**(5), ISSN 2320-9186 (2019).

57. Mohebbati, R., Anaeigoudari, A. & Khazdair, M. The effects of Curcuma longa and curcumin on reproductive systems. *Endocrine regulations*, **51**(4), 220-228 (2017).

58. jarhahzadeh, M., Alavinejad, P., Farsi, F., Husain, D. & Rezazadeh, A. The effect of turmeric on lipid profile, malondialdehyde, liver echogenicity and enzymes among patients with nonalcoholic fatty liver disease: a randomized double blind clinical trial. *Diabetology & Metabolic Syndrome*, **13**(1), 112 (2021).

59. Malhotra, M. & Ahmed, F. Correlation between CK-MB, TSH, LDL, HDL, Troponin T, Troponin I and myocardial infarction. International Journal of Health Sciences, 6(S4), 5645-5650 (2022).

60. Harahap, U. & Margata, L. The Significance of Troponin and Ck-Mb in Association with Q-Wave Myocardial Infarction. *Indonesian Journal of Pharmaceutical and Clinical Research*, **1**(1), 11-17 (2018).

61. Zahed, T., Ahmadi, N., Noorbakhsh, M. F., Saeed, N. & Ahmad, O. Comparative Evaluation of the Preventive Effects of Citral, Silymarin, Thymoquinone, and Curcumin on 5-Fluorouracil-Induced Cardiac and Pulmonary Toxicity in Rats. *Journal of Pharmacopuncture*, **27**(4), 277 (2024).

62. A.M. Abdul Azeem, A.N. El shahat, Megid, M. H. M. A. E. & Mansour, a. A. A. Effects of Gamma-Irradiated Aqueous Extract of Turmeric on Doxorubicin Induced Cardio-Nephrotoxicity in Male Rat. *Pakistan Journal Zoology*, **57**(1), 343-349 (2025).

63. Wasung, M. E., Chawla, L. S. & Madero, M. Biomarkers of renal function, which and when?. *Clinica chimica acta*, **438,** 350-357 (2015).

64. Vanholder, R., Gryp, T. & Glorieux, G. Urea and chronic kidney disease: the comeback of the century?(in uraemia research). *Nephrology Dialysis Transplantation*, **33**(1), 4-12 (2018).

65. Ejaz, A. A., Johnson, R. J., Shimada, M., Mohandas, R., Alquadan, K. F., Beaver, T. M., Lapsia, V. & Dass, B. The role of uric acid in acute kidney injury. *Nephron*, **142**(4), 275-283 (2019).

66. Gounden, Verena, Harshil Bhatt & Jialal, a. I. *Renal function tests*, StatPearls Publishing, [Internet], (2024).

67. Zeng, L., Yang, T., Yang, K., Yu, G., Li, J., Xiang, W. & Chen, H. Efficacy and safety of curcumin and curcuma longa extract in the treatment of arthritis: a systematic review and meta-analysis of randomized controlled trial. *Frontiers in immunology*, **13,** 891822 (2022).

68. Youssef, K. M., Ezzo, A. M., El-Sayed, M. I., Hazzaa, A. A., EL-Medany, A. H. & Arafa, M. Chemopreventive effects of curcumin analogs in DMH-Induced colon cancer in albino rats mode. *Future Journal of Pharmaceutical Sciences*, **1**(2), 57-72 (2015).

تقييم سلامة مستخلص جذر الكركم كعامل مضاد للسرطان في سرطان القولون والمستقيم، استنادًا إلى الأدلة الدوائية والمرضية

الملخص

تم استخدام عقار العلاج الكيماوى التقليدي 5-فلوروبوراسيل لعلاج السرطان، على الرغم من أن له آثاراً مهلكه على الأعضاء الحيوية . ونتيجة للعديد من التأثيرات العلاجية لمستخلص جذور الكركم (*Curcuma longa* root) والمشار اليها ب اختصار (CLE) ، فقد تم استخدامه في جميع أنحاء العالم في الطب التقليدي. وفي الآونة الأخيرة، أشارت العديد من الدراسات إلى أنه يمتلك تأثيرمضاد للسرطان. وقد تم تقييم سلامته قبل السريرية في نموذج لسرطان القولون المستحدث بمادة (DMH) في الفئران البيضاء كدواء بديل لـ 5-

فلوروبوراسيل. ويتحقق ذلك من خلال الفحوص المجهريه والكيميائية لاختبار التأثير الوقائي في الكبد والكلى والقلب كأعضاء حيوية. النتائج: كان هناك انخفاض كبير في نتائج اختبارات الوظائف المختلفة للأعضاء بالنسبة للمجموعات التي تلقت جرعات مختلفه للنبات مقارنة ($p < 0.05$) بالمجموعة التي عولجت بـ 5-فلوروبوراسيل. وباستثناء ذلك، فإن مجموعة الجرعة الصغيرة منه أظهرت انخفاضاً غير كبير إحصائياً ($p < 0.05$) في نتائج bilirubin ، triglycerides ،(VLDL)،(CK-MB)،و uric acid عند مقارنتها بنتائج المجموعة القياسية. بالإضافة إلى ذلك، حسنت علاجات (CLE) الصور المرضية للأعضاء المختلفة بشكل أفضل من مجموعة 5-فلوروبوراسيل. الخلاصة: دعمت النتائج الحالية أن 5-فلوروبوراسيل ينطوي على جوانب أكثر خطورة على حياة المرضى كدواء مضاد للسرطان. ويمكن تجنب ذلك باستخدام (CLE) بدلاً من 5-فلوروبوراسيل.

الكلمات الدالة: عامل مضاد للسرطان، مستخلص جذور الكركم CLE، 5-فلوروبوراسيل، الأعضاء الحيوية.

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Safety Assessment of *Curcuma longa* Root Extract as Anticancer Agent in Colorectal Cancer Indicated by Pharmacological and Pathological Evidence

Abstract

CLASSICAL chemotherapeutic drug 5-fluorouracil has been administered for cancer treatment, although it has awful effects on vital organs. As a result of many therapeutic actions of *Curcuma longa* root extract (CLE), it has been used worldwide in traditional medicine. Recently, many studies suggested it as an anticancer agent. In this study, CLE has been assessed for preclinical safety in a model of dimethylhydrazine-induced colon cancer in albino rats as an alternative drug for 5-fluorouracil. This is achieved through microscopic and chemical examinations to test the protective effect in the liver, kidney, and heart as vital organs. Results: There was a significant ($P < 0.05$) decrease in different function tests of organs for low dose and high dose of CLE groups compared with the 5-fluorouracil-treated group. With the exception of that, the small dose group of it has an insignificant decrease in bilirubin, VLDL, triglycerides, CK-MB, and uric acid results when compared with those of the standard one. Additionally, CLE treatments have been enhancing the pathological pictures of different organs more than the 5-fluorouracil group. In conclusion: Current data supported that 5-fluorouracil has more risky aspects along the life of patients as an anticancer agent. This can be avoided with the usage of CLE instead of 5-fluorouracil.

Keywords: Anticancer agent, *Curcuma longa* root extract (CLE), 5- fluorouracil, Vital organs.

Introduction

Herbal medications are frequently given as liquids, teas, oils, or dry extracts. Numerous medicinal substances, including digoxin, quinidine, morphine, and aspirin, have been derived from plants [1]. Compared to traditional treatment, herbal remedies are considered to be safer, more effective, and less expensive. However, uncontrolled use raises safety issues because it may result in toxicity, allergic reactions, or drug interactions as well. Globally, the use of herbal remedies is expanding quickly [2]. Furthermore, proprietary herbal medicines that are more effective than ordinary extracts are becoming more and more popular. However, the natural source of the proprietary extracts cannot be trusted to ensure their safety. Not every natural remedy is risk-free and without side effects [3-4]. Even though traditional experimental methods are being used to demonstrate the effectiveness of herbal treatments, the formulations' safety data are still insufficient and scant. Toxicological investigations must be conducted more thoroughly in accordance with internationally accepted safety and toxicity study guidelines [5].The first factor in medical therapy that can significantly influence the choice of medication that should be administered to a patient is drug safety. Additionally, taking into account the idea of benefit-risk balance, we discovered that medications with a high risk profile must to be avoided unless absolutely necessary. Drug safety has evolved throughout the past century and into the present, and healthcare systems must analyze past disasters to implement robust patient safety protocols [6]. Preclinical testing in animals and phase I, II, and III studies in humans are used to evaluate the safety and effectiveness of novel medications prior to their approval. Regulatory bodies typically grant marketing authorizations within their specific geographic jurisdiction. Geographic jurisdiction if it finds that the new drug is effective and that there are no significant risks of side effects [7]. About 90% of drug withdrawals are caused by safety issues. Even if some medications work, their toxicity prevents them from being licensed [8].

Curcuma longa is distinguished by their phytochemical profile, Flavonoids: Typical examples of antioxidant and anti-inflammatory compounds include flavonoids, such as derivatives of quercetin and kaempferol [9], Tannins: These polyphenolic compounds provide this plant its astringent, antibacterial, and anti-inflammatory properties [10], Alkaloids: The increased pharmacological activity observed in the leaves is attributed to their mild

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alkaloid content [11]. Essential Oils: Monoterpenes and sesquiterpenes found in the leaves give this plant its scent and therapeutic properties [12]. *Curcuma longa* belongs to the *Zingiberaceae* family of plants. It is brilliant yellow spice, which is made from the rhizome of the plant. It has been used for centuries in folk traditional medicine as a home cure for a variety of illnesses [13]. It has been shown to exert a number of biological actions, including cardio neuroprotective activities, anti-infectious, antioxidant and antithrombotic properties [14].

In a previous work, it has been demonstrated that *Curcuma longa r*oot extract (CLE) exerts an anticancer effect against colorectal cancer (CRC) induced with dimethylhydrazine (DMH) in rats. To suggest CLE as applicable drug in medicinal markets, it is essential to demonstrate its safety in different vital body organs and blood elements.

Material and Methods

Plant part used

The CLE was obtained from local area, where the dry root is available at shops of herbs and spices elsewhere in Egypt. One kilogram of the dry root was bought from a Harraz® company in Shubra, Cairo province, and identified by a botany specialist, Horticulture Department, Faculty of Agriculture, Benha University, Voucher number (178590).

Preparation of CLE

The fundamental methodological techniques for extraction were adjusted in accordance with Harborne recommendations [15]. A fine powder was made from CLE. The extract was obtained by macerating a weighted fraction of the CLE (500 g) in covered Erlenmeyer flasks in a known volume of hydro-ethanolic solvent (bi-distilled water: absolute ethanol, 70:30 v/v). Maceration continued for an additional 72 hours in the refrigerator, with intermittent mixing. Using muslin mesh, the hydro-ethanolic extract was filtered. A semisolid filtrate was prepared by evaporating the extract in wide-mouthed containers using a shaking water bath set at 70 °C. After weighting the semisolid extract, the yield percentage was determined as follows:

$$\text{Yield\%} = (\text{wt. of extract/wt. of CLE}) \times 100$$

Preparation of suitable extract doses

Depending on the plant part, extract type, and administration method, a variety of CLE types and dosages have been presented in the literature. The dosages used in this study were derived from the 500 mg absolute human dose [5], while taking body weight and surface area into consideration. Animal equivalent dosages of CLE were determined to be 45 mg/kg body weight of rats, using the conversion table developed by Paget and Barnes in relation to body surface area [16]. Additionally, 7 mg/kg was estimated to be the clinical dose of CLE based on body weight. Following that, the averaging of two dosages was utilized to determine the experimental CLE doses with 30 (Low Dose, LD) and 100 (High Dose, HD) mg/kg every day in animals. Rats were given two stock solutions (6 and 20 mg/mL), which corresponded to dosage rates of 30 (LD) and 100 (HD) mg/kg per day. A stomach tube containing roughly 1 ml of the CLE was used for each 200 g weighted rat in treated groups.

Experimental equipment

In the current investigation, Li-heparinized capillary tubes and standard laboratory equipment have been used.

Chemicals and reagents

5-Fluorouracil (5-FU) was bought from Alfa Aesar, Ward Hill, USA while 1,1-DMH was purchased from Sigma Aldrich, USA. Organic solvents and other analytical grade chemicals were obtained from El-Ghomhorya Company for Trading Drugs, Chemicals, and Medical Instruments, Egypt. Isoflurane inhalation anesthesia was obtained from (Sedico®, Egypt). The commercial kits used to estimate safety profiles of CLE which indicated with organs function markers and lipid profile were bought from Diamond ® (Hannover, Germany), Spectrum® (Hannover, Germany), and BIOMED (Egypt).

Preparation of DMH therapy

According to prior investigations of Srimuangwong *et al.* [17], to induce colorectal cancer, so that a 200 g rat was given 8 mg of DMH substance dissolved in a 1 mM solution of EDTA (for 42 days, S.C. twice per week).

Preparation of 5-FU therapy

According to Alazzouni *et al.* [18], a 200 g rat was administrated 10 mg of 5-FU as chemotherapeutic agent dissolved in a 1 in sterilized water (for 42 days, ip once per week).

Experimental Animals

Male Wistar rats (30) weighing 140±20 g and between 10 and 14 weeks of age were employed in this experimental investigation. The animals were housed under typical experimental conditions, which included a 12/12-hour day and night cycle, a temperature of 22 ± 5℃, and a humidity level of 65%.

Experimental design

The animals were randomly divided into five groups, each consisting of six rats, and given the following various medications for six weeks: The first group was kept as a control and given 1 ml of saline via a stomach tube daily. According to *Saini et al.* [19], the second group was administered 40 mg/kg of DMH subcutaneously twice each week,

which dissolved in1mM EDTA, to be the challenge group later. For third one, rats were maintained as a standard group and were given DMH as second one together with 50 mg/kg of 5-FU, ip once weekly according to [20b]. Animals in fourth group received DMH plus CLE (30 mg/kg, per.*os* daily), and named as a CLE -LD treated group. Rats were treated with DMH together with 100 mg/kg, orally, once daily of CLE, and kept as a CLE-HD treated group.

Sampling

According to Saini *et al*. [19], the animal body was weighed in each group and was measured once a week until the experiment was over. After six weeks of exposure to various treatments, rats were fasted overnight with drinking water ad libitum Blood samples were obtained from the retrobulbar venous plexus using a heparinized capillary tube while under mild inhalation anesthesia of isoflurane. Plasma was separated into an Eppendorf tube for biochemical analysis after blood samples were collected into sampling tubes with lithium heparin as an anticoagulant at room temperature and centrifuged at 900xg for five minutes. Until analysis, the plasma samples were stored frozen at -20 °C. Animals were euthanized for organs collection. After removing the tissues around the allocated organs, a 0.9% sodium chloride solution was used to clean the organs. Following filter paper drying, the tissues were immersed in ice-cold phosphate-buffered saline containing 0.1 mM EDTA to eliminate coagulated blood. They were then placed in formalin for subsequent pathological examination.

Clinicochemical assessment:

With a few modest adjustments, the manufacturer's instructions were followed to perform clinicochemical assays spectrophotometrically utilizing particular kits. The methods outlined by Richmond [21] and Vega and Grundy [22] were used to calculate total cholesterol. The method outlined by Fossati and Prencipe [23] was used to determine triacylglycerols (TAGS). Low-density lipoprotein-cholesterol was measured using the methodology outlined by Tietz *et al.* [24] . The method outlined by Friedewald *et al.* [25] was used to determine high-density lipoprotein-cholesterol. Using the formula given by Bauer and Covert [26], the plasma very-low-density lipoprotein-cholesterol value has been determined as follows Cholesterol = (TAGS) / 5 = mg/dl. The Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) enzymes activities in plasma were measured in accordance with Schumann *et al.* [27] and Chen *et al.* [28] . According to *Tietz et al.* [24], total and conjugated bilirubins were measured, and the unconjugated bilirubin was computed numerically by subtraction [29].The method outlined by Horder et al. [30] and *Burtis et al.* [31] was used to determine the plasma CK-MB levels. The method outlined by Adams *et al*. [32] was used to determine the plasma cardiac Troponin-I Assay. Urea, uric acid, and plasma creatinine were measured in accordance with Coulombe and Favreau [33], Tietz [34] and Walker and Shah [35], respectively.

Histopathological examination

Hepatic, renal and cardiac tissue samples were taken after necropsy, washed with sterile normal saline right away, and preserved in 10% neutral buffered formalin. After being dehydrated in progressively higher grades of ethyl alcohol, the fixed specimens were cleaned in xylol, imbedded in paraffin wax, and paraffin blocks were made [36].

Statistical analysis:

For biochemical markers: The mean ±SE of 6 observations is used to represent all data. To find the significant differences between groups, GraphPad Prism® software for Windows (version 8.4.2, San Diego, CA, USA) was used to do one-way analysis of variance (ANOVA) and then Tukey's post-hoc testing. P values less than 0.05 were deemed significant. As a percentage of the 5-FU effect, the potential of CLE was standardized. For histopathological analysis: Three distinct portions from three different rats in each group have been analyzed under a microscope.

Results

Clinicochemical results

A-Hepatic enzymatic markers:

As shown in (Table 1), as expected, there was a marked significant increase in liver enzymatic biomarker activity, mainly AST and ALT, in the DMH-treated group, compared to those of the negative control one. There were insignificant decreases in AST activities and a significant decrease in ALT activities in standard rats (5-FU, 50 mg/kg) to those of the DMH-injected one. On the other hand, LD of CLE (30 mg/kg), HD of CLE (100 mg/kg) and the negative control groups resulted in a significant decrease in hepatic enzymes when compared to the standard group; even that HD of CLE normalized these parameters as control negative group. Data presented in (Table 1) indicate that the percentage of protection against the diseased group, resulting from low and high doses of CLE, exhibited a dose-dependent relationship (32.05 and 76.69 for AST and 39.83 and 57.52% for ALT, respectively), which parallels the effects of 5-FU (24.27 for AST and 19.46% for ALT).

B- Bilirubin markers:

Table 2 demonstrates that the bilirubin biomarkers in the DMH-treated group were significantly higher than those in the negative control group. In comparison to the DMH-injected rats, standard rats (5-FU, 50 mg/kg) showed a significant

decrease in indirect and total bilirubin markers and an insignificant decrease in direct ones. When compared to the 5-FU-treated group, the bilirubin results of LD of CLE (30 mg/kg) showed an insignificant decrease. However, compared to the standard group, HD of CLE (100 mg/kg) group decreased bilirubin parameters significantly, even as those of the negative group. According to the data in (Table 3), the percentage of protection against the diseased group caused by low and high doses of CLE was dose dependent (55.83 and 77.5 for indirect bilirubin and 22.86 and 51.34 for direct bilirubin in addition to 46.67 and 70.67% for total bilirubin, respectively) and parallel to that of 5-FU (45.00 for indirect bilirubin, 14.28 for direct bilirubin, and 36.00% for total bilirubin).

C- Lipids profile

Table 4 illustrates that, as predicted in the DMH-treated group, lipid biomarkers increased significantly while HDL-C biomarker decreased significantly compared to those of the negative control one. There was a significant decrease in LDL-C and total cholesterol activities and an insignificant decrease in VLDL-C and triglycerides activities in standard rats (5-FU, 50 mg/kg) compared to those of the DMH-injected one. Furthermore, compared to the diseased group, the 5-FU-treated group's HDL-C results showed an insignificant rise.

The results of LD of CLE (30 mg/kg) were an insignificant decrease for triglycerides and VLDL-C in addition to a significant decrease for LDL-C and total cholesterol when compared with those of the 5-FU-treated group. In contrast to that, there was an insignificant increase for the HDL-C result in LD of CLE when compared with those of the 5-FU-treated group. On the other hand, HD of CLE (100 mg/kg) and group resulted in a significant decrease in lipid biomarkers except those of HDL-C when compared to the standard group. In HDL-C results of HD of CLE group, there were significant increases when compared to the 5-FU treated group. Lipid parameters levels in HD of CLE group could be similar to those of the negative control.

Data presented in (Table 5) show that the protection% against the diseased group, which was produced by low and high doses of CLE, was dose dependent (31.46 and 47.56 for total cholesterol and 35.35 and 52.14% for triglycerides, respectively) and parallel to that of 5-FU (15.73 for total cholesterol and 18.57% for triglycerides). The percentage of protection against the diseased group, which was generated by both low and high doses of CLE, was dose dependent (44.14 and 65.43 for LDL-C and 35.71 and 51.78% for VLDL-C, respectively) and parallel to that of 5-FU (19.68 for LDL-C and 17.85% for VLDL-C), as demonstrated in (Table 5).

D- Cardiac enzymatic markers

Table 6 illustrates that, as predicted in the DMH-treated group, cardiac enzymatic biomarker results increased significantly, compared to those of the negative control one. Troponin marker activity was significantly lower in standard rats (5-FU, 50 mg/kg) than in the DMH-injected rats. Additionally, the 5-FU-treated group's CK-MB findings revealed an insignificant drop as compared to the diseased group. When compared to the 5-FU-treated group, the findings of LD of CLE (30 mg/kg) showed a significant decrease for troponin and an insignificant decrease for CK-MB. However, when compared to the 5-FU treated group, the HD of CLE (100 mg/kg) group caused a significant decrease in cardiac enzymatic activity as close to that of normal rats. Data presented in (Table 6) show that the protection% against the diseased group, which was produced by low and high doses of CLE, was dose dependent (48.33 and 67.23 for CK-MB and 66.30 and 69.56% for troponin, respectively) and parallel to that of 5-FU (22.22 for CK-MB and 41.31% for troponin).

E- Renal functional markers

The renal functional indicators in the DMH-treated group were substantially greater than those in the negative control group, as shown in (Table 7). The creatinine, uric acid, and urea marker levels of standard rats (5-FU, 50 mg/kg) were insignificantly lower than those of the DMH-injected rats. When compared to the 5-FU-treated group, the uric acid result of LD of CLE (30 mg/kg) revealed an insignificant decrease; in contrast, those of creatinine and urea were significant decreases. However, the HD of CLE (100 mg/kg) group significantly reduced renal function measures in comparison to the standard group, nearly as those of normal rats. According to the data in (Table 8), the percentage of protection against the diseased group caused by low and high doses of CLE was dose dependent (38.57 and 55 for creatinine and 48.23 and 71.76 for uric acid in addition to 36.17 and 48.93% for urea, respectively) and parallel to that of 5-FU (21.43 for creatinine, 30.58 for uric acid, and 12.76% for urea).

Histopathological findings

A-Hepatic tissues

The control group's hepatic tissue underwent histopathological analysis, which revealed normal hepatocytes, sinusoids, central veins, portal regions, and hepatic architecture. Hepatocytes are big, polyhedral cells with spherical nuclei and eosinophilic cytoplasm; (Figure1A). In the meantime, the toxic group's hepatic parenchyma showed a range of pathological alterations after receiving DMH treatment. Significant hepatic sinusoidal and central vein dilatation and congestion were noted. Additionally, Von Kuepfer's cells were activated, resulting in modest peri-vascular leukocytic cellular aggregations; (Figure 1B). Severe

bleeding inside the hepatic parenchyma was occasionally seen; (Figure 1C). The most closely inspected hepatic sections showed the presence of mononuclear leukocytic cellular infiltration together with multifocal regions of coagulative necrosis. The architecture of these necrosed hepatocytes was preserved, exhibiting hypereosinophilic cytoplasm and pyknosis, infrequently karyolysis, or the lack of nuclei; (Figure 1D). The hepatocytes also showed signs of severe degenerative alterations, including hydropic degeneration, which is characterized by enlarged, pale, vacuolated hepatocytes. Cytoplasm rarefaction was found in most zones of the hepatic lobules; (Figure 1E). Mild fibrosis and edema, along with several embedded bile duct profiles of varying sizes (ductular response) and aggregation of mononuclear inflammatory cells, caused the portal triads to diffusely enlarge. The lining epithelium of the bile duct also showed hyperplastic alterations, with desquamated cells in the lumina and eosinophilic debris. The portal veins occasionally included fibrin thrombi; (Figure 1F). When the livers of the treatment groups (DMH+ Fluorouracil, DMH+LD of CLE, and DMH+ HD of CLE) were examined under a microscope, the normal hepatocellular architecture was significantly restored, and there were more regular and less altered hepatocytes than in the DMH-treated rat. Supplementing with CLE reduces the harmful effects of DMH on hepatic tissues more effectively than fluorouracil. Wherein a HD of CLE exhibits a stronger hepatoprotective effect than a LD of CLE. A few hepatic sections under examination showed hepatocyte necrosis and degeneration together with inflammatory responses in the hepatic parenchyma. Additionally, minor vascular alterations were noted in the portal veins, hepatic sinusoids, and central veins. The bile ducts of the portal triads showed minimal leukocytic cellular infiltration and were almost normal; (Figure 2A-F).

B-Cardiac tissues

Normal cardiomyocytes with their centrally positioned vesicular nuclei were visible in the control group's heart tissue; (Figure 3A). In the meanwhile, DMH caused significant vascular changes, such as vacuolation in the blood vessel walls, muscle hypertrophy, and endothelial damage; (Figure 3B). Additionally, there were large patches of bleeding between the heart's muscles. Disorganized, fragmented muscle fibers caused significant distortion of the cardiac parenchyma, which had severe necrosis with hypereosinophilic, inflated sarcoplasm with pyknosis or no nuclei; (Figure 3C). In the meantime, DMH+ Fluorouracil group the group's cardiac tissue sections revealed considerable myocardial necrosis and myocardial blood vessel congestion; (Figure 3D). When it came to CLE dosage, the high dose treatment demonstrated greater protection than the low dose, with only minor vascular and degenerative alterations in a few heart sections under examination, the histological architecture of the heart in rats in the DMH + HD of CLE was almost identical to that of the control; (Figure 3F). In DMH + LD of CLE group, a few sections under examination showed sporadic focal myocardial hemorrhage, mild congestion of some cardiac blood vessels, and focal areas of cardiomyocyte necrosis; (Figure 3E).

C-Renal tissues

The kidney of the control group showed renal convoluted tubules and glomeruli with normal histological appearance; (Figure 4A). In contrast, the kidneys of the DMH-treated group showed a number of histological changes, including renal blood vessel congestion and dilatation, endothelial cell proliferation, and tunica media vacuolation with perivascular mononuclear leukocytic cellular aggregation. Furthermore, bleeding and perivascular edema were noted. Nuclear pyknosis, loss of cellular detail, blurred cell boundaries, and hypereosinophilic cytoplasm were all signs of extensive necrosis of the epithelial lining in the proximal and distal convoluted tubules. Desquamated and degraded epithelial cells were abundant in the lumens of certain renal tubules; (Figure 4B). Hypersegmentation and vacuolation of the glomerular tuft, hypercellular mesangium, glomerular capillary congestion, hypertrophy of the epithelium lining Bowman's capsule, expansion of the Bowman's space, and mild periglomerular edema were among the abnormalities seen in glomeruli. Some kidney cysts were unintentionally discovered. Together with these lesions, the tubular epithelium displayed massive, pale, vacuolated cytoplasm and widespread degeneration; (Figure 4C). Large-scale tubular degeneration and necrosis were commonly observed in conjunction with partial glomerular tuft shrinkage and atrophy. In the majority of tissue sections, intertubular and glomerular blood capillary congestion was found in (Figure 4D). Mononuclear leukocytic cellular infiltration was common, especially in the spaces between renal tubules, blood vessels, and glomeruli; (Figure 4E).

On the other hand, the glomerular and tubular damage caused by DMH+ Fluorouracil group was lessened than the DMH one. Where there was minor atrophy, mild glomerular lobulation, and mild vacuolation of the endothelial cells lining the glomerular tufts. Rarely, this group showed desquamated epithelial cells in the lumina of afflicted tubules coupled with mild degeneration and necrosis of certain tubular epithelial cells; (Figure 4F). Additionally, compared to the DMH group, the kidneys of the DMH+ LD of CLE group showed an improvement in their histological structure. Both glomerular and intertubular capillary congestion was seen along with a little glomerular growth. In the lumens of certain tubules, eosinophilic debris was occasionally observed along with tubular epithelial

cell degeneration or coagulative necrosis; (Figure 4G). On the other hand, the kidney sections taken from HD of CLE+DMH group showed that most renal tubules had retained normal renal architecture, with sporadic intralumenal casts and modest epithelial cell degeneration in a small number of tubules. Minor glomerular segmentation, proliferation, and congestion were unintentionally seen seldom. Additionally, intertubular blood capillary dilatation and moderate congestion were seen; (Figure 4H).

Discussion

It is established that these days, medicine serves as a natural ally in combating dangerous diseases in increasingly polluted environments.

In order to improve the effectiveness of the drugs that are administered on the one hand and eliminate, or at least lessen, the harmful side effects of the excess drugs that accumulate in the human body on the other, a significant portion of pharmaceutical, medicinal, and biophysical research has been directed toward the exploration and development of new and basic methods in recent years [37].

Around the world, people use herbal remedies for both acute and chronic illnesses without realizing that there are numerous possible side effects that could raise their morbidity, mortality, and hospital admission, length of stay, therapy costs, and quality of life. To control and guarantee the safety and effectiveness of herbal medicines, significant efforts from drug authorities around the world, medical professionals, academic institutions, and researchers are crucial and strongly advised [38].

Curcuma longa is referred to as India's "golden spice." Ayurveda, an Indian ancient medical system, has documented the benefits of it for liver problems, indigestion, nausea, wound healing, inflammation, and skin complexion[39].

Furthermore, CLE is being studied in great detail for its pharmacological properties, including fever reduction and anticancer, antimutagenic, antioxidant, anti-inflammatory, and antidiabetic activities [40]. Due to their varied applications, herbals' toxicity is crucial, and numerous research have been conducted to date on the toxicity of certain plants, including *Crocus sativus* [41] and *Glycyrrhiza glabra* [42]. This study examined the safety and/or toxicity of turmeric in rats because of the overuse of CLE with high efficacy in disease treatment. Our findings revealed that CLE has more protective effect on vital organ as natural antineoplastic than 5FU as chemotherapeutic agent.

For enzymatic liver biomarkers, low-value tests like aspartate aminotransferase (AST), which has lesser specificity as a liver marker than alanine aminotransferase (ALT), have been reduced by recent usage schemes [43]. In the past, ALT and AST have both been employed as indicators of hepatocellular damage. While ALT is mostly found in the cytoplasmic region of hepatocytes, AST is also produced in the kidneys, brain, heart, and skeletal muscle in addition to the liver. Routine chemical testing based on total enzyme kinetics does not distinguish between the immunologically separate forms of AST, cytoplasmic AST, and mitochondrial AST [44]. The use of routine AST testing in conjunction with ALT has been questioned, even though an increase in the overall activity of AST without ALT elevation may indicate cardiac or muscle disease or drug-induced hepatitis, including alcohol and acetaminophen [3].

The present results demonstrated that when compared to the standard group, LD of CLE (30 mg/kg) and HD of CLE (100 mg/kg) groups caused a substantial drop in liver enzymes, being (106 ± 5.0 and 48 ± 9.6 compared to 156 ± 7.6 for AST, 68 ± 3.9 and 44 ± 3.8 vs. 91 ± 6.0 for ALT, respectively), even that HD of CLE normalized these parameters as control negative group, being (48 ± 9.6 compared to 33 ± 5.5 for AST, 44 ± 3.8 vs. 23 ± 4.8 for ALT, respectively). The recorded findings show that the protection % against the diseased group, which was produced by LD and HD of CLE, was dose dependent (32.05 and 76.69 for AST and 39.83 and 57.52% for ALT, respectively) and parallel to that of 5-FU (24.27 for AST and 19.46% for ALT).

These results are consistent with Liu *et al.* [45], who stated that primarily AST and ALT and histopathology could be improved by pretreatment with curcumin and/or α-tocopherol in cisplatin-induced hepatic enzyme damage. Furthermore, a pretreatment with *Curcuma longa* L. extract could prevent doxorubicin-induced hepatotoxicity and significantly raise ALT and AST levels. These findings agree with Eghbali *et al.* [46] , who claimed that patients with β thalassemia who took curcumin had considerably ($p < 0.05$) lower levels of AST and ALT at the end of the study than those in the placebo group. These results support with idea of An *et al.* [47],who found that by reducing the serum levels of AST and ALT in rats and hens, C. longa demonstrated hepatoprotective qualities against liver damage brought on by heavy metals and toxic pesticides.

These results are in contract with Araújo *et al.* [48], who stated that the 5-FU-treated group (intraperitoneally at a dose of 60 mg/kg on the initial day and 40 mg/kg on the following day) and the CLE-treated group (dosage of 50 mg/kg, orally) did not exhibit any significant ($p>0.05$) hepatic changes in hamsters with oral mucositis. This discrepancy was caused by the disease state and the species of the animal difference.

Guerra Ruiz *et al.* [49] and Lala *et al.* [50], who recorded that increase plasma bilirubin level, any of

the following changes in its metabolism can cause it: (a) high formation of bilirubin (i.e., pathologic hemolysis); (b) poor liver tissue uptake, which results in elevated indirect bilirubin; (c) reduced conjugation, which is caused by a defect in UDP-glucuronosyltransferase; and (d) bile clearance disorder, which results in elevated direct bilirubin due to errors in clearance proteins or the inability of the bile to pass through bile ducts and into the small intestine. Ramírez-Mejía *et al.* [51], who found that addition to decreasing the number of hepatocyte cells, a liver lesion of any kind may also affect the uptake of indirect bilirubin from plasma and the transport and clearance of direct bilirubin through the bile ducts.

It had been proved in our study, the bilirubin results of LD of CLE (30 mg/kg) showed an insignificant decrease when compared to the 5-FU-treated group, being (0.53 ± 0.04 vs. 0.66 ± 0.06 for indirect bilirubin, 0.27 ± 0.021 vs. 0.3 ± 0.020 for direct one and 0.80 ± 0.061 vs. 0.96 ± 0.064 for total one, respectively). However, HD of CLE (100 mg/kg) group decreased bilirubin parameters significantly compared to the standard group, being (0.27 ± 0.01 vs. 0.66 ± 0.06 for indirect bilirubin, 0.17 ± 0.015 vs. 0.3 ± 0.020 for direct one and 0.44 ± 0.028 vs. 0.96 ± 0.064 for total one, respectively), even as those of the negative control, being (0.27 ± 0.01 vs. 0.17 ± 0.02 for indirect bilirubin, 0.17 ± 0.015 vs. 0.11 ± 0.01 for direct one and 0.44 ± 0.028 vs. 0.28 ± 0.031 for total one, respectively). The percentage of protection against the diseased group caused by low and high doses of CLE was dose dependent (55.83 and 77.50 for indirect bilirubin and 22.86 and 51.34 for direct bilirubin in addition to 46.6 and 70.67% for total bilirubin, respectively) and parallel to that of 5-FU (45.00 for indirect bilirubin, 14.28 for direct bilirubin, and 36.00% for total bilirubin).

These findings agree with Al-Eisa *et al.* [52], who claimed that total bilirubin levels significantly ($p < 0.05$) dropped in the curcumin group when compared to the $AlCl_3$ group in hepatic intoxication. According to Eghbali *et al.* [46],who suggested that at the conclusion of the trial, the direct and total bilirubin levels of β thalassemia patients who took curcumin were significantly ($p < 0.05$) lower than those in the placebo group. These results agree with An *et al.* [47], who stated that by decreasing the serum levels of total bilirubin in rats and chickens *C. longa* (500 mg/kg daily) demonstrated hepatoprotective qualities against liver damage brought on by heavy metals and toxic pesticides. Even with that, there are no data in the literatures that disagree with our study.

According to Warun Kumar and Harisha [53], who suggested that these lipids and lipoproteins are synthesized, metabolized, and degraded in large part by the liver. Thus, the levels of these lipids and lipoproteins are changed in cirrhosis. The liver and intestines produce nascent HDL, which is the first step in reverse cholesterol transport. Atherosclerosis prevention is significantly aided by the outflow of cholesterol from macrophages to HDL. Ference *et al.* [54] and Feingold [55], who stated that The LDL is created when lipoprotein lipase breaks down the triglycerides found in VLDL in muscle and adipose tissue, releasing free fatty acids. It undergoes additional metabolism to produce LDL, which the LDL receptor absorbs in a variety of organs, with the liver being the primary site of uptake.

In comparison to the standard group, there was an insignificant drop in triglycerides and VLDL-C of CLE (30 mg/kg) group, being (to 228 ± 22 vs. 181 ± 12 for triglycerides, 46 ± 4.4 vs. 36 ± 2.4 for VLDL-C, respectively), in addition to a significant reduce in total cholesterol and LDL-C results, being (225 ± 6.6 vs. 183 ± 7.4 for total cholesterol, 151 ± 6.1 vs. 105 ± 11 for LDL-C, respectively). In contrast to that, the HDL-C result in LD of CLE rats was an insignificant higher than those of the 5-FU-treated group, being (42 ± 6.5 vs. 29 ± 5.3). As compared to the standard group, with the exception of HDL-C results, lipid biomarkers significantly drop in the HD of CLE (100 mg/kg) group when compared to the standard group, being (225 ± 6.6 vs. 140 ± 9.5 for total cholesterol, 228 ± 22 vs. 134 ± 15 for triglycerides, 151 ± 6.1 vs. 65 ± 5.6 for LDL-C, 46 ± 4.4 vs. 27 ± 2.9 for VLDL-C, respectively). In HDL-C result of HD of CLE group was a significant increase more than those of the 5-FU treated group, being (48 ± 6.2 vs. 29 ± 5.3). Lipid parameters levels in HD of CLE group could be similar to those of the negative control. The protection % against the diseased group, which was produced by low and high doses of CLE, was dose dependent (31.46 and 47.56 for total cholesterol and 35.35 and 52.14% for triglycerides, respectively) and parallel to that of 5-FU (15.73 for total cholesterol and 18.57% for triglycerides). The percentage of protection against the diseased group, which was generated by both low and high doses of CLE, was dose dependent (44.14 and 65.43 for LDL-C and 35.71 and 51.78 % for VLDL-C, respectively) and parallel to that of 5-FU (19.68 for LDL-C and 17.85 % for VLDL-C).

The present data may be supported by Udom *et al.* [56], who stated that rats treated with CLE, however, had significantly lower levels of free fatty acids, cholesterol, triglycerides, low density lipoproteins (LDL), very low density lipoproteins (VLDL), and high density lipoproteins (HDL) than the toxic rats (who were given phenobarbital, diethyl nitrosamine, and CCl_4 as hepatic-carcinogenic agents). Rats given CLE prior to treatment did not exhibit any appreciable changes in their lipid profiles as compared to normal control. Current research findings might concur with those of Feingold [55], who proposed that the administration of turmeric powder, curcumin (conventional solvent extract), and

microencapsulated curcumin (supercritical fluid extract) reduced total serum cholesterol in the rat groups' dietary patterns, as indicated by 7.32, 10.54, and 12.81% in the corresponding treated groups, respectively. Adding microencapsulated curcumin to the diets reduces circulating lipids and mitigates the adverse effects of treated rats' hypercholesterolemia. In addition, coincide with those of Mohebbati *et al.* [57] , who proved that male rats' fecundity and serum levels of phospholipids, triglycerides, and cholesterol were decreased when *C. longa* extract (1 mg/kg, p.o.) was administered. These results oppose those of jarhahzadeh *et al.* [58], who claimed that serum levels of VLDL ($p = 0.41$) and total cholesterol ($p = 0.196$) did not significantly alter in obese men. This discrepancy results from variations in technique and research models. Previous researches on the direct effect of turmeric in liver cancer patients with elevated lipid parameters are limited.

The trinity of heart-derived cardiac enzymes, lactate dehydrogenase, aspartate transaminase (serum glutamate oxaloacetate transaminase), and creatinine kinase-MB (CKMB), is traditionally employed in evaluations for the identification of MI, according to [59]. Harahap and Margata [60], who reported that glod standard CK-MB levels, however, have a limited prognostic power when compared to serum troponins (both I and T), which have been validated for post-operative risk stratification for noncardiac surgical procedures and are thought to be more specific and sensitive than CK-MB in the setting of acute coronary syndromes.

The results of LD of CLE (30 mg/kg) revealed a substantial decrease for troponin and an insignificant decrease for CK-MB in comparison to the 5-FU-treated group, being (0.31 ± 0.04 vs. 0.54 ± 0.05 troponin, 0.93 ± 0.09 vs. 1.40 ± 0.2 for CK-MB, respectively). Yet, the HD of CLE (100 mg/kg) group significantly reduced cardiac enzymatic activity as close to that of normal rats as compared to the 5-FU treated group, being (0.28 ± 0.04 vs. 0.54 ± 0.05 for troponin, 0.59 ± 0.05 vs. 1.40 ± 0.2 for CK-MB, respectively). The protection % against the diseased group, which was produced by low and high doses of CLE, was dose dependent (48.33 and 67.23 for CK-MB and 66.30 and 69.56% for troponin, respectively) and parallel to that of 5-FU (22.22 for CK-MB and 41.31% for troponin).

The present findings are consistent with those of Zahed *et al.* [61], who found that 5-fluorouracil administered intraperitoneally resulted in cardiotoxicity, as indicated by higher CK-MB serum level. For ameliorating and prevention of it, CK-MB level significantly ($p < 0.05$) decreased with curcumin administration in 5-FU-treated rats. Also, our data may be agreement with those of A.M. Abdul Azeem *et al.* [62], who proved that treatment of both raw and γ-irradiated turmeric in DOX-administrated group showed a substantial ($p < 0.05$) decrease in kidney function and cardiac marker levels (troponin-I and CK-MB) when compared to the diseased group. No information in earlier literature contradicts our findings.

Toxins and waste materials including urea, creatinine, and uric acid must be eliminated by the kidneys, according to Wasung *et al.* [63]. The byproduct of protein metabolism is urea. Traditionally thought to be biologically inert, urea is a sign of uraemic retention in chronic kidney disease (CKD). Urea itself causes molecular alterations linked to insulin resistance, the generation of free radicals, apoptosis, and disruption of the protective intestinal barrier, according to at least five studies, [64]. According to Ejaz *et al.* [65] reported that research has consistently shown that serum uric acid (SUA) and acute kidney injury (AKI) are strongly correlated across a number of disease models. In two other investigations, lowering SUA also increased GFR and estimated glomerular filtration rate. The mechanisms of action of SUA and diabetic nephropathy, hypertension, cardiovascular disease, chronic renal disease, and metabolic syndrome are similar. The body produces creatinine, a by-product of creatine phosphate in muscle, at a steady rate. Blood creatinine rises as a result of decreased renal clearance. Furthermore, renal function might decline by as much as 50% before a rise in serum creatinine is noticed, indicating renal impairment at a rather late stage, [66]. The uric acid result of LD of CLE (30 mg/kg) showed a negligible drop; nevertheless, the creatinine and urea results showed significant declines, when compared to the 5-FU-treated group, being (4.4 ± 0.51 vs. 5.9 ± 0.78 for uric acid, 0.86 ± 0.08 vs. 1.1 ± 0.08 for creatinine, 30 ± 2.4 vs. 41 ± 1.9 for urea, respectively). In contrast to the standard group, the HD of CLE (100 mg/kg) group considerably decreased renal function measures, which were almost identical to those of normal rats, being (5.9 ± 0.78 vs. 2.4 ± 0.4 for uric acid, 1.1 ± 0.08 vs. 0.63 ± 0.05 for creatinine, 41 ± 1.9 vs. 24 ± 2.1 for urea, respectively). The percentage of protection against the diseased group caused by low and high doses of CLE was dose dependent (38.57 and 55 for creatinine and 48.23 and 71.76 for uric acid in addition to 36.17 and 48.93% for urea, respectively) and parallel to that of 5-FU (21.43 for creatinine, 30.58 for uric acid, and 12.76% for urea).

The current results are similar to those of Ejaz *et al.* [65] , who suggested that the 5-FU group had significantly ($p<0.05$) higher serum urea levels than the CLE-treated and control groups. There was no difference in urea between the CLE-treated group and control groups. The 5-FU group had considerably ($p<0.05$) higher serum creatinine level than the other groups. The group treated with CLE showed a substantial ($p<0.05$) drop in its serum level when compared to the 5-FU group. Also these agree with those of Zeng *et al.* [67], who stated that

when compared to a placebo, they discovered that curcumin intervention tended to lower serum uric acid, although the effect was not of statistical significance in gout disease.

In comparison to the DMH-treated rat, the treatment groups (DMH+ Fluorouracil, DMH+LD of CLE, and DMH+ HD of CLE) displayed more regular and less altered tissue structure in histological analysis of the various studied organs, and the normal architecture was significantly restored. Supplementation of CLE reduces the harmful effects of DMH on critical organs more than fluorouracil does. Dosing with HD of CLE has a stronger cell-protective effect in liver, heart, and kidney sections compared to this of LD of CLE. The microscopic results of our study may be in agreement with Youssef *et al.* [68], who reported that following four weeks of therapy with compounds 1 (curcumin) and 2 (ethyl curcumin), liver, kidney and heart sections displayed normal structure, which were identical to that observed control-negative group with ameliorating and protection from toxic-inducible DMH action. Additionally, the present data are consistent with those of Ejaz *et al.* [65], who proved that pathological picture of renal tubular epithelium and interstitial blood vessels in CLE treated-groups were improved when compared with those of 5-FU treated group, as well as those of Zahed *et al.* [61], who stated that in curcumin administrated animals, the results were the same as those shown in the control group, and the cardiac histopathology lesions were not as severe as those seen in the 5-FU group.

Clinical-chemical parameter data corroborated pathological findings.

Conclusion

The current work, histopathological findings and statistical data analysis of vital organs indicate that CLE improves toxicological changes brought on by 5-FU as chemotherapy. As a result, it is suggested as a superior safer treatment for cancer than 5-FU.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

The Scientific Research Ethics Committee (Animal Care and Use), Faculty of Veterinary Medicine, Benha University, Benha, Egypt, gave ethical approval for the study's experiments (Approval no. BUFVTM 07-02-25).

Figures and Tables

TABLE 1. Effects of CLE and Standard on the plasma hepatic enzymatic markers in 6 rats administrated DMH in contrasted with normal ones.

Groups	Marker			
	AST (U/l)		ALT (U/L)	
	Means ± SE	Protection % against diseased	Means ± SE	Protection % against diseased
Control	33.0 ± 5.50 ^{c}	-	23.0 ± 4.80 ^{d}	-
Diseased (DMH treated)	206 ± 21.0 ^{a}	100.0	113 ± 5.20 ^{a}	100.0
Standard (5-FU treated)	156 ± 7.60 ^{a}	24.27	91.0 ± 6.00 ^{b}	19.46
LD of CLE	106 ± 5.00 ^{b}	32.05	68.0 ± 3.90 ^{c}	39.83
HD of CLE	48.0 ± 9.60 ^{c}	76.69	44.0 ± 3.80 ^{d}	57.52

The values are shown as the means ± SE of six rats in each group. The means in a single column with distinct letter superscripts show significant differences over time ($P < 0.05$; ANOVA with Tukey's post-hoc test); the HD of CLE indicates a high dose of *Curcuma longa* root extract, and the LD of CLE indicates a low dose of *Curcuma longa* root extract with protection% against diseased.

TABLE 2. Effects of CLE and Standard on bilirubin markers in 6 rats administrated DMH in contrasted with normal ones.

Groups	Bilirubin Marker (mg/dl)		
	Indirect	Direct	Total
	Means ± SE		
Control	0.17 ± 0.02 ^{c}	0.11 ± 0.010 ^{b}	0.28 ± 0.031 ^{c}
Diseased (DMH treated)	1.20 ± 0.10 ^{a}	0.35 ± 0.018 ^{a}	1.50 ± 0.084 ^{a}

Standard (5-FU treated)	0.66 ± 0.06 [b]	0.30 ± 0.020 [a]	0.96 ± 0.064 [b]
LD of CLE	0.53 ± 0.04 [b]	0.27 ± 0.021 [a]	0.80 ± 0.061 [b]
HD of CLE	0.27 ± 0.01 [c]	0.17 ± 0.015 [b]	0.44 ± 0.028 [c]

The values are shown as the means ± SE of six rats in each group. The means in single column with distinct letter superscripts show significant differences over time ($P < 0.05$; ANOVA with Tukey's post-hoc test); the HD of CLE indicates a high dose of *Curcuma longa* root extract, and the LD of CLE indicates a low dose of *Curcuma longa* root extract.

TABLE 3. Protection% of CLE and Standard on the renal functional markers against diseased in rats.

Groups	Bilirubin Marker (mg/dl)		
	Indirect	Direct	Total
	Protection % against diseased		
Diseased (DMH treated)		100	
Standard (5-FU treated)	45.00	14.28	36.00
LD of CLE	55.83	22.86	46.67
HD of CLE	77.50	51.34	70.67

Values present as Protection% against diseased group for the HD of CLE indicates a high dosage of *Curcuma longa* root extract, the LD of CLE indicates a low dose of *Curcuma longa* root extract and Standard group.

TABLE 4. Effects of CLE and Standard on lipid markers in 6 rats administrated DMH in contrasted with normal ones.

Groups	Marker (mg/dl)				
	Total cholesterol	Triglycerides	LDL-C	HDL-C	VLDL-C
	Means ± SE				
Control	126 ± 6.70 [d]	81.0 ± 8.50 [bc]	52.0 ± 8.70 [c]	58 ± 6.6 [bc]	16 ± 1.7 [bc]
Diseased (DMH treated)	267 ± 6.80 [a]	280 ± 18.0 [a]	188 ± 13.0 [a]	23 ± 3.7 [a]	56 ± 3.6 [a]
Standard (5-FU treated)	225 ± 6.60 [b]	228 ± 22.0 [ac]	151 ± 6.10 [b]	29 ± 5.3 [a]	46 ± 4.4 [ac]
LD of CLE	183 ± 7.40 [c]	181 ± 12.0 [bc]	105 ± 11.0 [c]	42 ± 6.5 [ac]	36 ± 2.4 [bc]
HD of CLE	140 ± 9.50 [d]	134 ± 15.0 [bc]	65.0 ± 5.60 [c]	48 ± 6.2 [bc]	27 ± 2.9 [bc]

The values are shown as the means ± SE of six rats in each group. The means in single column with distinct letter superscripts show significant differences over time ($P < 0.05$; ANOVA with Tukey's post-hoc test); the HD of CLE indicates a high dose of *Curcuma longa* root extract, and the LD of CLE indicates a low dose of *Curcuma longa* root extract.

TABLE 5. Protection% of CLE and Standard on lipid markers against diseased in rats.

Groups	Marker (mg/dl)			
	Total cholesterol	Triglycerides	LDL-C	VLDL-C
	Protection % against diseased			
Diseased (DMH treated)		100		
Standard (5-FUtreated)	15.73	18.57	19.68	17.85
LD of CLE	31.46	35.35	44.14	35.71
HD of CLE	47.56	52.14	65.43	51.78

Values present as Protection% against diseased group for the HD of CLE indicates a high dosage of *Curcuma longa* root extract, the LD of CLE indicates a low dose of *Curcuma longa* root extract and Standard group.

TABLE 6.Effects of CLE and Standard on the CK-MB and troponin markers in 6 rats administrated DMH in contrasted with normal ones.

Groups	Marker (ng/ml)			
	CK-MB		Troponin	
	Means ± SE	Protection % against diseased	Means ± SE	Protection % against diseased
Control	0.35 ± 0.03 [b]	-	0.17 ± 0.03 [c]	-

Diseased (DMH treated)	1.80 ± 0.22 [a]	100.0	0.92 ± 0.08 [a]	100.0
Standard (5-FU treated)	1.40 ± 0.20 [ac]	22.22	0.54 ± 0.05 [b]	41.31
LD of CLE	0.93 ± 0.09 [bc]	48.33	0.31± 0.04 [c]	66.30
HD of CLE	0.59 ± 0.05 [b]	67.23	0.28 ± 0.04 [c]	69.56

The values are shown as the means ± SE of six rats in each group. The means in single column with distinct letter superscripts show significant differences over time ($P < 0.05$; ANOVA with Tukey's post-hoc test); the HD of CLE indicates a high dosage of *Curcuma longa* root extract, and the LD of CLE indicates a low dose of *Curcuma longa* root extract with protection% against diseased.

TABLE 7. Effects of CLE and Standard on renal functional markers in 6 rats administrated DMH in contrasted with normal ones.

Groups	**Marker (mg/dl)**		
	Creatinine	**Uric acid**	**Urea**
	Means ± SE		
Control	0.54 ± 0.04[b]	2.3 ± 0.46 [bc]	17 ± 0.9 [b]
Diseased (DMH treated)	1.40 ± 0.20 [a]	8.5 ± 1.30 [a]	47 ± 3.4 [a]
Standard (5-FU treated)	1.10± 0.08 [ac]	5.9 ± 0.78 [a]	41 ± 1.9 [a]
LD of CLE	0.86 ± 0.08 [bc]	4.4 ± 0.51 [ac]	30 ± 2.4 [b]
HD of CLE	0.63± 0.05 [b]	2.4 ± 0.40 [bc]	24 ± 2.1 [b]

The values are shown as the means ± SE of six rats in each group. The means in single columns with distinct letter superscripts show significant differences over time ($P < 0.05$; ANOVA with Tukey's post-hoc test); the HD of CLE indicates a high dose of *Curcuma longa* root extract, and the LD of CLE indicates a low dose of *Curcuma longa* root extract.

TABLE 8. Protection% of CLE and Standard on renal functional markers against diseased in rats.

Groups	**Marker (mg/dl)**		
	Creatinine	**Uric acid**	**Urea**
	Protection % against diseased		
Diseased (DMH treated)	100		
Standard (5-FU treated)	21.43	30.58	12.76
LD of CLE	38.57	48.23	36.17
HD of CLE	55.00	71.76	48.93

Values present as Protection% against diseased group for the HD of CLE indicates a high dosage of *Curcuma longa* root extract, the LD of CLE indicates a low dose of *Curcuma longa* root extract and Standard group.

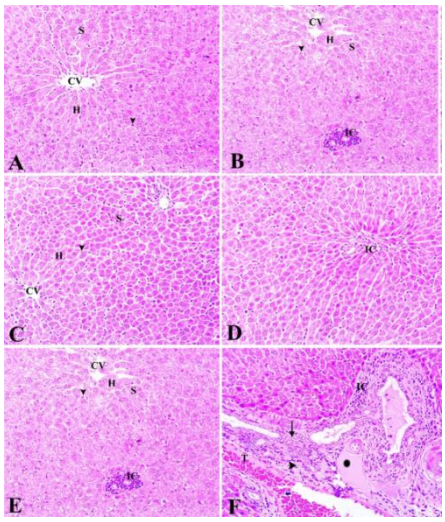

Fig.1. Liver sections of control (A), and toxic (B- F) groups, H&E (x200). Control group (A), showing normal histological appearance of central veins (CV), sinusoids (S), and hepatocytes (H). DMH group (B), mild inflammatory cellular (IC) aggregation (C) congestion of central vein (C) with severe hemorrhage between hepatocytes (arrow), (D) coagulative necrosis (N) of hepatocytes infiltrated with mononuclear inflammatory cells (arrowhead), (E) focal area of lymphocytic aggregation (asterisk) with hydropic degeneration of hepatocytes (arrow), (F) fibrin thrombi (T) in portal vein; portal area expanded by fibrosis (arrowhead) and

edema (asterisk); bile duct hyperplasia (arrow), mixed with aggregates of mononuclear inflammatory cells (IC).

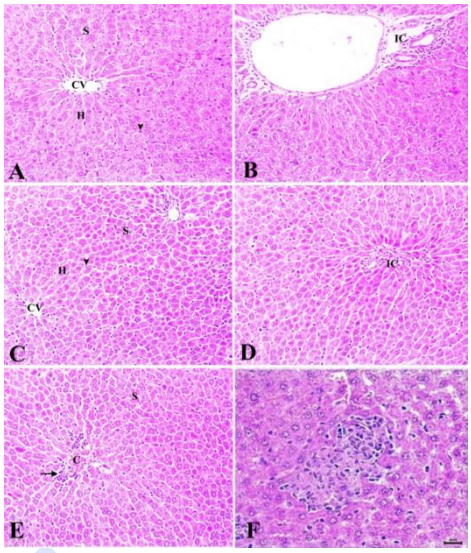

Fig. 2. Liver sections of treated groups with HD of CLE (A, B), LD of CLE (C, D), and 5-flu (E, F), H&E (x200). (A, C) Nearly normal appearance of central vein (CV) hepatocytes (H), with mild congestion and dilatation of hepatic sinusoid (S) in association with enlargement and activation of Von Kuepfer's cells (arrowhead). Note, (E) showing marked congestion (C) of the central vein and hepatic sinusoids (S) with peri-vascular leukocytic cellular aggregations (arrow), (F) Focal area of necrosis associated with mononuclear cellular infiltration in 5-flu. (B, D) Portal area showing mild dilatation of portal vein along with mild periductal edema and inflammatory cellular infiltration (IC).

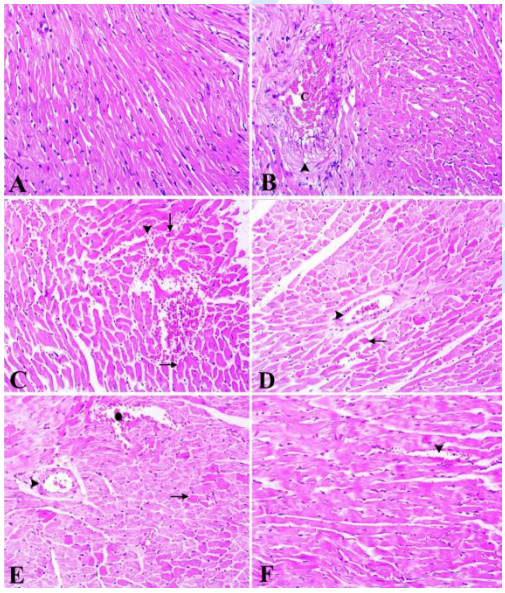

Fig. 3. Heart sections of control (A), toxic (B, C) and treatment (D- F) groups, H&E (x200). Control (A) group, normal histological structure of cardiac muscles. DMH (B, C) group, (B) Endothelial damage, congestion (C) with marked vesiculation of the cardiac blood vessels wall (arrowhead), (C) Extensive intermuscular hemorrhage (arrowhead) and myocardial necrosis (arrow). DMH+ Fluorouracil (D) group, myocardial necrosis (arrow) with mild congestion (arrowhead) the myocardial blood vessels. DMH+ LD of CLE (E) group, myocardial necrosis (arrow), mild congestion (arrowhead) and focal hemorrhage between cardiac muscles (asterisk). DMH+ HD of CLE (F) group, nearly normal cardiac striation with slight congestion of intermuscular capillaries (arrowhead).

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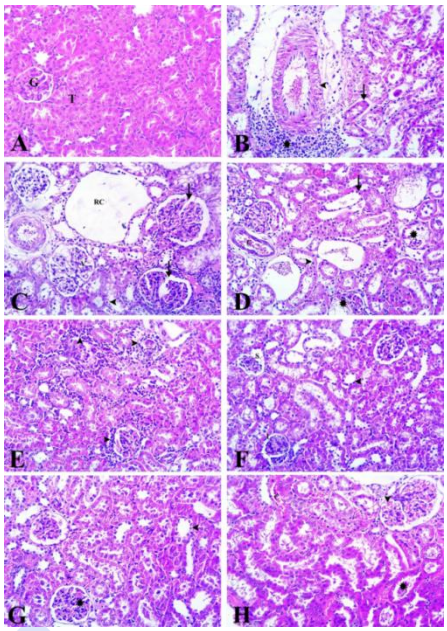

Fig. 4. Kidney sections of control (A), toxic (B- E) and treatment (F- H) groups, H&E (x200). Control (A) group, normal histological structure of glomeruli (G) and convoluted tubules (T). DMH (B- E) group, (B) proliferation of endothelial cells with vacuolation of the tunica media of the renal blood vessels (arrowhead), perivascular mononuclear leukocytic cellular aggregation (asterisk). Also noted severe necrosis of the tubular epithelium with hypereosinophilic cytoplasm and nuclear pyknosis (arrow), (C) hypersegmentation (arrow) and vacuolation (V) of the glomerular tuft, congestion of glomerular capillaries, widening of the Bowman's space and mild periglomerular edema with presence of renal cysts (RC). Note also, hydropic degeneration of tubular epithelium characterized by cytoplasmic vacuolation (arrowhead), (D) glomerular tuft shrinkage and atrophy (asterisk) with extensive tubular degeneration (arrowhead) and necrosis (arrow). Congestion (C) of intertubular and glomerular blood capillaries, (E) Mononuclear leukocytic cellular infiltration (arrowhead) around glomeruli, blood vessels, and in between renal tubules. DMH+ Fluorouracil (F) group, slight glomerular lobulation (L) and mild glomerular shrinkage (S). Note also, mild degeneration and necrosis of some tubular epithelial cells (arrowhead) along with desquamated epithelial cells in the lumina of affected tubules. DMH+ LD of CLE (G) group, improvement in the histological structure of kidneys with slight glomerular proliferation (asterisk) with mild degeneration (arrowhead) of tubular epithelial cells and eosinophilic debris in lumens of some tubules. DMH+ HD of CLE (H) group, nearly normal renal architecture in most renal tubules with mild degeneration of epithelial cells and intralumenal casts in few tubules (asterisk). Also find, minor glomerular proliferation, segmentation and congestion (arrowhead) with mild congestion (C) of intertubular blood capillaries.

References

1. Vitalone, A., Menniti-Ippolito, F., Moro, P. A., Firenzuoli, F., Raschetti, R. & Mazzanti, G. Suspected adverse reactions associated with herbal products used for weight loss: a case series reported to the Italian National Institute of Health. *European journal of clinical pharmacology*, **67**, 215-224 (2011).
2. Amrita Chatterjee & Sarkar, B. Polyphenols and terpenoids derived from Ocimum species as prospective hepatoprotective drug leads: a comprehensive mechanistic review. *Phytochemistry Reviews,* **24**(2), 2087-2129 (2025).
3. Mohammed-Ali, Z., Brinc, D., Kulasingam, V. & Selvaratnam, R. Defining appropriate utilization of AST testing. *Clinical Biochemistry*, **79**, 75-77 (2020).
4. Joshi, J., Ghaisas, S., Vaidya, A., Vaidya, R., Kamat, D., Bhagwat, A. & Bhide, S. Early human safety study of turmeric oil (Curcuma longa oil) administered orally in healthy volunteers. *Journal-Association of Physicians of India*, **51**, 1055-1060 (2003).
5. Soleimani, V., Sahebkar, A. & Hosseinzadeh, H. Turmeric (Curcuma longa) and its major constituent (curcumin) as nontoxic and safe substances. *Phytotherapy Research*, **32**(6), 985-995 (2018).
6. Alshammari, T. M. Drug safety: The concept, inception and its importance in patients' health. *Saudi Pharmaceutical Journal*, **24**(4), 405-412 (2016).
7. Onakpoya, I. J., Heneghan, C. J. & Aronson, J. K. Worldwide withdrawal of medicinal products because of adverse drug reactions: a systematic

review and analysis. *Critical reviews in toxicology*, **46**(6), 477-489 (2016)

8. Craveiro, N. S., Lopes, B. S., Tomás, L. & Almeida, S. F. Drug withdrawal due to safety: a review of the data supporting withdrawal decision. *Current drug safety*, **15**(1), 4-12 (2020).

9. Al-Khayri, J. M., Sahana, G. R., Nagella, P., Joseph, B. V., Alessa, F. M. & Al-Mssallem, M. Q. Flavonoids as potential anti-inflammatory molecules: A review. *Molecules*, **27**(9), 2901 (2022).

10. Mal, S. & Pal, D. Tannins and polyphenols extracted from natural plants and their versatile application. *Bioactive natural products for pharmaceutical applications*, 715-757 (2020).

11. Niranjan, R. K., Thimmaraju, M., Malik, J. K., Gupta, V. & Singh, G. PHARMACOGNOSTIC INSIGHTS AND THERAPEUTIC POTENTIAL OF CURCUMA LONGA LEAVES WITH A FOCUS ON ANTI-INFLAMMATORY PROPERTIES. *International Journal of Pharmaceutical Science and Medicine*, **3**(1), 10-19 (2025).

12. Butnariu, M. Plants as source of essential oils and perfumery applications. *Bioprospecting of plant biodiversity for industrial molecules,* 261-292 (2021).

13. Sharma, S. Phytochemical and Pharmacological Review and Importance of Curcuma longa. *International Journal of Recent Research and Review,* **14** (4), ISSN 2277 – 8322 (2021).

14. Micucci, M., Aldini, R., Cevenini, M., Colliva, C., Spinozzi, S., Roda, G., Montagnani, M., Camborata, C., Camarda, L. & Chiarini, A. Curcuma longa L. as a therapeutic agent in intestinal motility disorders. 2: Safety profile in mouse. *PloS one*, **8**(11), e80925 (2013).

15. Harborne, A. *Phytochemical methods a guide to modern techniques of plant analysis,* springer science & business media, (1998).

16. Paget, G. & Barnes, J. *Toxicity tests. Evaluation of drug activities, Pharmacometrics,* Academic Press, Massachusetts, **1**, 135-166 (1964).

17. Srimuangwong, K., Tocharus, C., Tocharus, J., Suksamrarn, A. & Chintana, P. Y. Effects of hexahydrocurcumin in combination with 5-fluorouracil on dimethylhydrazine-induced colon cancer in rats. *World journal of Gastroenterology*, **18**(47), 6951 (2012 b).

18. Alazzouni, A. S., Dkhil, M. A., Gadelmawla, M. H., Gabri, M. S., Farag, A. H. & Hassan, B. N. Ferulic acid as anticarcinogenic agent against 1, 2-dimethylhydrazine induced colon cancer in rats. *Journal of King Saud University-Science*, **33**(2), 101354 (2021).

19. Saini, M. K., Vaiphei, K. & Sanyal, S. N. Chemoprevention of DMH-induced rat colon carcinoma initiation by combination administration of piroxicam and C-phycocyanin. *Molecular and cellular biochemistry*, **361**(1), 217-228 (2012).

20. Srimuangwong, K., Tocharus, C., Chintana, P. Y., Suksamrarn, A. & Tocharus, J. Hexahydrocurcumin enhances inhibitory effect of 5-fluorouracil on HT-29 human colon cancer cells. *World Journal of Gastroenterology,* **18**(19), 2383 (2012 a).

21. Richmond, W. Analytical reviews in clinical biochemistry: the quantitative analysis of cholesterol. *Annals of clinical biochemistry*, **29**(6), 577-597 (1992).

22. Vega, G. L. & Grundy, S. M. Lipoprotein responses to treatment with lovastatin, gemfibrozil, and nicotinic acid in normolipidemic patients with hypoalphalipoproteinemia. *Archives of internal medicine*, **154**(1), 73-82 (1994).

23. Fossati, P. & Prencipe, L. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *Clinical chemistry*, **28**(10), 2077-2080 (1982).

24. Tietz , Finley PR & E, P. *Clinical guide to laboratory tests*, WB Saunders company Philadelphia, pp.1096 (1995).

25. Friedewald, W. T., Levy, R. I. & Fredrickson, D. S. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical chemistry*, **18**(6), 499-502 (1972).

26. Bauer, J. E. & Covert, S. J. The influence of protein and carbohydrate type on serum and liver lipids and lipoprotein cholesterol in rabbits. *Lipids*, **19** (11), 844-850 (1984).

27. Schumann, G., Canalias, F., Joergensen, P. J., Kang, D., Lessinger, J.-M. & Klauke, R. IFCC reference procedures for measurement of the catalytic concentrations of enzymes: corrigendum, notes and useful advice: International Federation of Clinical Chemistry and Laboratory Medicine (IFCC)–IFCC Scientific Division. *Clinical chemistry and laboratory medicine,* **48**(5), 615-621 (2010).

28. Chen, Y., Krishan, M., Nebert, D. W. & Shertzer, H. G. Glutathione-deficient mice are susceptible to TCDD-Induced hepatocellular toxicity but resistant to steatosis. *Chemical research in toxicology*, **25**(1), 94-100 (2012).

29. Liman, M. L. & Atawodi, S. E. Hepatoprotective and nephroprotective effects of methanolic extract of different parts of Tamarindus indica Linn in rats following acute and chronic carbon tetrachloride intoxication. *Annual Research & Review in Biology*, **5**(2), 109 (2015).

30. Horder, M., Elser, R., Gerhardt, W., Mathieu, M. & Sampson, E. IFCC methods for the measurements of catalytic concentration of enzymes. *Journal of the International Federation of Clinical Chemistry*, **2**(2), 80-83 (1990).

31. Burtis, C. A., Ashwood, E. R. & Tietz, N. W. *Tietz textbook of clinical chemistry*. Saunders, (1999).

32. Adams, J., Abendschein, D. R. & Jaffe, A. S. Biochemical markers of myocardial injury. Is MB creatine kinase the choice for the 1990s?. *Circulation*, **88**(2), 750-763 (1993).

33. Coulombe, J. & Favreau, L. A new simple semimicro method for colorimetric determination of urea. *Clinical chemistry*, **9**(1), 102-108 (1963).

34. Tietz, N. W. *Fundamentals of Clinical Chemistry*, Saunders, Philadelphia (1976).

35. Walker, P. D. & Shah, S. V. Reactive oxygen metabolites in endotoxin-induced acute renal failure in rats. *Kidney international*, **38**(6), 1125-1132 (1990).

36. Bancroft, D, J. & Layton, *C. Connective and mesenchymal 11 tissues with their stains. Bancroft's Theory and Practice of Histological Techniques E-Book*, 187 (2012).

37. Kundu, P., Das, S. & Chattopadhyay, N. Managing efficacy and toxicity of drugs: targeted delivery and excretion. *International Journal of Pharmaceutics*, **565,** 378-390 (2019).

38. Jităreanu, A., Trifan, A., Vieriu, M., Caba, I.-C., Mârțu, I. & Agoroaei, L. Current trends in toxicity assessment of herbal medicines: A narrative review. *Processes*, **11**(1), 83 (2022).

39. Slika, L. & Patra, D. Traditional uses, therapeutic effects and recent advances of curcumin: a mini-review. *Mini Reviews in Medicinal Chemistry*, **20**(12), 1072-1082 (2020).

40. Krup, V., Prakash, L. H. & Harini, A. Pharmacological activities of turmeric (Curcuma longa Linn): a review. *Journal of Homeopathy & Ayurvedic Medicine*, **2**(133), 2167-1206 (2013).

41. Bostan, H. B., Mehri, S. & Hosseinzadeh, H. Toxicology effects of saffron and its constituents: a review. *Iranian journal of basic medical sciences*, **20**(2), 110 (2017).

42. Nazari, S., Rameshrad, M. & Hosseinzadeh, H. Toxicological effects of Glycyrrhiza glabra (licorice): a review. *Phytotherapy research*, **31**(11), 1635-1650 (2017).

43. Xu, S., Hom, J., Balasubramanian, S., Schroeder, L. F., Najafi, N., Roy, S. & Chen, J. H. Prevalence and predictability of low-yield inpatient laboratory diagnostic tests. *JAMA network open*, **2**(9), e1910967 (2019).

44. Kwo, P. Y., Cohen, S. M. & Lim, J. K. ACG clinical guideline: evaluation of abnormal liver chemistries. *Official journal of the American College of Gastroenterology*, **112**(1), 18-35 (2017).

45. Liu, Z., Huang, P., Law, S., Tian, H., Leung, W. & Xu, C. Preventive effect of curcumin against chemotherapy-induced side-effects. *Frontiers in pharmacology*, **9**, 1374 (2018).

46. Eghbali, A., Nourigheimasi, S., Ghasemi, A., Afzal, R. R., Ashayeri, N., Eghbali, A., Khanzadeh, S. & Ghaffari, K. The effects of curcumin on hepatic T2* MRI and liver enzymes in patients with β-thalassemia major: a double- blind randomized controlled clinical trial. *Frontiers in Pharmacology*, **14**, 1284326 (2023).

47. An, S., Jang, E. & Lee, J.-H. Preclinical evidence of curcuma longa and its noncurcuminoid constituents against hepatobiliary diseases: a review. *Evidence-Based Complementary and Alternative Medicine*, **2020(**1), 8761435 (2020).

48. Araújo, A. A. d., Silva, E. M., Mafra, C. A. d. C. C., Costa, Í. d. C. C., Barbalho, A. L. A., Matos, I. A. F. d., Santos, M. A. d., Lopes, M. L. D. d. S., Medeiros, C. A. C. X. d. & Soares, L. A. L. Curcuma longa extract protects against 5-fluorouracil-induced oral mucositis in hamsters. *Brazilian Journal of Pharmaceutical Sciences*, **58,** e20114 (2022).

49. Guerra Ruiz, A. R., Crespo, J., López Martínez, R. M., Iruzubieta, P., Casals Mercadal, G., Lalana Garcés, M., Lavin, B. & Morales Ruiz, M. Measurement and clinical usefulness of bilirubin in liver disease. *Advances in Laboratory Medicine/Avances en Medicina de Laboratorio*, **2**(3), 352-361 (2021).

50. Lala, V., Zubair, M. & Minter, D. *Liver function tests*, StatPearls Publishing, [Internet] (2023).

51. Ramírez-Mejía, M. M., Castillo-Castañeda, S. M., Pal, S. C., Qi, X. & Méndez-Sánchez, N. The multifaceted role of bilirubin in liver disease: a literature review. *Journal of Clinical and Translational Hepatology*, **12**(11), 939 (2024).

52. Al-Eisa, R., Khouja, H. & Al-Nahari, H. Turmeric (Curcuma Longa) protection against the Liver Toxicity Caused by Aluminum Chloride (AlCl3) in Adult Male Rats. *International Journal of Pharmaceutical Research & Allied Sciences*, **6**(2), 110-127 (2017).

53. Warun Kumar, M. & Harisha, E. Assessment of lipid profile changes with respect to severity of liver dysfunction in cirrhosis of liver. *Indian Journal of Basic and Applied Medical Research,* **4**, 56-63 (2015).

54. Ference, B. A., Kastelein, J. J. & Catapano, A. L. Lipids and lipoproteins in 2020. *Jama*, **324**(6), 595-596 (2020).

55. Feingold, K. R. *Introduction to lipids and lipoproteins,* Endotext [internet]. South Dartmouth (MA), PP1-2 (2024).

56. Udom, G. J., Ogbuagu, E. O., Udobangd, J. A., Ndifon, B. T., Jovita, N. O., Ise, U. P., Udo, N. M. & Yemitan, O. K. Antidotal assessment of hydroethanol root extract of Curcuma longa L.(Turmeric) against chemically-induced hepatic neoplasm in wistar rats. *Global scientific journal*, **7**(5), ISSN 2320-9186 (2019).

57. Mohebbati, R., Anaeigoudari, A. & Khazdair, M. The effects of Curcuma longa and curcumin on reproductive systems. *Endocrine regulations*, **51**(4), 220-228 (2017).

58. jarhahzadeh, M., Alavinejad, P., Farsi, F., Husain, D. & Rezazadeh, A. The effect of turmeric on lipid profile, malondialdehyde, liver echogenicity and enzymes among patients with nonalcoholic fatty liver disease: a randomized double blind clinical trial. *Diabetology & Metabolic Syndrome*, **13**(1), 112 (2021).

59. Malhotra, M. & Ahmed, F. Correlation between CK-MB, TSH, LDL, HDL, Troponin T, Troponin I and myocardial infarction. International Journal of Health Sciences, 6(S4), 5645-5650 (2022).

60. Harahap, U. & Margata, L. The Significance of Troponin and Ck-Mb in Association with Q-Wave Myocardial Infarction. *Indonesian Journal of Pharmaceutical and Clinical Research*, **1**(1), 11-17 (2018).

61. Zahed, T., Ahmadi, N., Noorbakhsh, M. F., Saeed, N. & Ahmad, O. Comparative Evaluation of the Preventive Effects of Citral, Silymarin, Thymoquinone, and Curcumin on 5-Fluorouracil-Induced Cardiac and Pulmonary Toxicity in Rats. *Journal of Pharmacopuncture*, **27**(4), 277 (2024).

62. A.M. Abdul Azeem, A.N. El shahat, Megid, M. H. M. A. E. & Mansour, a. A. A. Effects of Gamma-Irradiated Aqueous Extract of Turmeric on Doxorubicin Induced Cardio-Nephrotoxicity in Male Rat. *Pakistan Journal Zoology*, **57**(1), 343-349 (2025).

63. Wasung, M. E., Chawla, L. S. & Madero, M. Biomarkers of renal function, which and when?. *Clinica chimica acta*, **438,** 350-357 (2015).

64. Vanholder, R., Gryp, T. & Glorieux, G. Urea and chronic kidney disease: the comeback of the century?(in uraemia research). *Nephrology Dialysis Transplantation*, **33**(1), 4-12 (2018).

65. Ejaz, A. A., Johnson, R. J., Shimada, M., Mohandas, R., Alquadan, K. F., Beaver, T. M., Lapsia, V. & Dass, B. The role of uric acid in acute kidney injury. *Nephron*, **142**(4), 275-283 (2019).

66. Gounden, Verena, Harshil Bhatt & Jialal, a. I. *Renal function tests*, StatPearls Publishing, [Internet], (2024).

67. Zeng, L., Yang, T., Yang, K., Yu, G., Li, J., Xiang, W. & Chen, H. Efficacy and safety of curcumin and curcuma longa extract in the treatment of arthritis: a systematic review and meta-analysis of randomized controlled trial. *Frontiers in immunology*, **13,** 891822 (2022).

68. Youssef, K. M., Ezzo, A. M., El-Sayed, M. I., Hazzaa, A. A., EL-Medany, A. H. & Arafa, M. Chemopreventive effects of curcumin analogs in DMH-Induced colon cancer in albino rats mode. *Future Journal of Pharmaceutical Sciences*, **1**(2), 57-72 (2015).

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تقييم سلامة مستخلص جذر الكركم كعامل مضاد للسرطان في سرطان القولون والمستقيم، استنادًا إلى الأدلة الدوائية والمرضية

الملخص

تم استخدام عقار العلاج الكيماوى التقليدي 5-فلوروبوراسيل لعلاج السرطان، على الرغم من أن له آثاراً مهلكه على الأعضاء الحيوية . ونتيجة للعديد من التأثيرات العلاجية لمستخلص جذور الكركم (*Curcuma longa* root) والمشار اليها ب اختصار (CLE) ، فقد تم استخدامه في جميع أنحاء العالم في الطب التقليدي. وفي الآونة الأخيرة، أشارت العديد من الدراسات إلى أنه يمتلك تأثيرمضاد للسرطان. وقد تم تقييم سلامته قبل السريرية في نموذج لسرطان القولون المستحدث بمادة (DMH) في الفئران البيضاء كدواء بديل لـ 5-فلوروبوراسيل. ويتحقق ذلك من خلال الفحوص المجهريه والكيميائية لاختبار التأثير الوقائي في الكبد والكلى والقلب كأعضاء حيوية. النتائج: كان هناك انخفاض كبير في نتائج اختبارات الوظائف المختلفة للأعضاء بالنسبة للمجموعات التي تلقت جرعات مختلفه للنبات مقارنة ($p < 0.05$) بالمجموعة التي عولجت بـ 5-فلوروبوراسيل. وباستثناء ذلك، فإن مجموعة الجرعة الصغيرة منه أظهرت انخفاضاً غير كبير إحصائياً ($p < 0.05$) في نتائج bilirubin ، (VLDL) ، triglycerides ، (CK-MB)،و uric acid عند مقارنتها بنتائج المجموعة القياسية. بالإضافة إلى ذلك، حسنت علاجات (CLE) الصور المرضية للأعضاء المختلفة بشكل أفضل من مجموعة 5-فلوروبوراسيل. الخلاصة: دعمت النتائج الحالية أن 5-فلوروبوراسيل ينطوي على جوانب أكثر خطورة على حياة المرضى كدواء مضاد للسرطان. ويمكن تجنب ذلك باستخدام (CLE) بدلاً من 5-فلوروبوراسيل.

الكلمات الدالة: عامل مضاد للسرطان، مستخلص جذور الكركم CLE، 5-فلوروبوراسيل، الأعضاء الحيوية.