



Hepatotoxicity Induced by the therapeutic dose of Chlorpromazine and Ameliorative for *Saussurea Costus* Roots Extract and Effect of Physiological and Histological Liver of Albino Rats

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Abstract | Chlorpromazine (CPZ) remains a widely used drug in psychiatric practice today. The aim of this study is to investigate the protective role of ethanolic costus root extract in CPZ-induced functional and histological changes in rats' liver. The study was carried out on 30 male albino rats, 12-14 weeks old. The Rats were divided into 5 groups, G1 control negative received distilled water 2 ml. CPZ was administered orally at dose 2 mg/kg of body weight. The rats dosed CPZ were divided as follows: G2 a positive control group, received CPZ alone. G3 was given an ethanolic extract of costus at a dose of 1.5 mg/kg, and G4 was administered an ethanolic extract of costus at a dose of 3 mg/kg. G5 was administered an ethanolic extract of costus plant at a dose of 5 mg/kg. Groups G3, G4, and G5 served as a prophylaxis group dosed 1 hour after CPZ administration. Oral doses are given once daily for 4 weeks. The results of G2 showed increased body weights in rats and liver weights (286.250 ± 13.750 Body Weight) (10.525 ± 0.217 Liver Weight). In contrast a decrease in body and liver Weight is observed among control and Prophylaxis groups (G3, G4 and G5) while no significant difference ($p > 0.05$) in liver and body weights were observed between the groups under study. There was a significant ($p < 0.001$) increase in G2 group ALT, AST and ALP compared a significant decreasing ($p < 0.001$) in prophylactic groups in particular a concentration 5mg/kg or G5 group. Histopathological changes of the liver of G2 including acute cell swelling and necrosis in hepatocyte, inflammatory cells infiltration, blood vessels congestion with perivascular inflammatory cells infiltration, bile ducts epithelial hyperplasia. we noticed that administration of ethanolic extract of *Saussurea costus* root and in resulted in amelioration of the morphological changes in Chlorpromazine treated rats, improved parameters and restored the parameters to near normal compared with group G1. The G5 group that was dosed with the alcoholic extract of the Indian Costus root and in conjunction with the drug Chlorpromazine at a concentration of 5 mg / kg showed higher improvement in the parenchyma of liver tissue's compared to the other groups. The concluded Data revealed that root of costus extract acted as a hepatic protective agent against the side effects or toxicity induced by Chlorpromazine.

Keywords | Chlorpromazine, Liver, Toxicity, Rats, Histology, Costus

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Drug-induced liver damage is a significant medical concern in clinical settings. The liver plays a crucial role in the selective absorption, metabolism, and elimination of drugs, xenobiotics, and environmental pollutants. Chlorpromazine (CPZ), a phenothiazine derivative, has been utilized for over 60 years in psychiatry, particularly for the treatment of schizophrenia and bipolar disorders (Boyd-Kimball *et al.*, 2019). However, its therapeutic use is accompanied by potential hepatotoxicity, which should not be overlooked (Derby *et al.*, 1993). The hepatotoxicity induced by CPZ may be linked to the inflammatory process mediated by the continuous activation of c-Jun N-terminal kinase (JNK). In a study by Gandhi *et al.* (2013) it was shown that inflammation-mediated hepatotoxicity of CPZ depends on Toll-interleukin 1 receptor domain containing adapter protein, and involves prolonged JNK activation *in vivo*. Thus, TIRAP-based pathways may be targeted for prediction of idiosyncratic adverse drug reactions mediated by inflammation and their prevention. Furthermore, CPZ can inhibit bile flow *in vivo*, resulting in cholestasis. Substantial evidence suggests that CPZ serves as an effective model for studying drug-induced liver damage and is commonly used to mimic drug-induced cholestasis (Uzbekova *et al.*, 2002).

Despite proposed mechanisms for CPZ-induced liver injury, the process is not fully understood due to the involvement of numerous factors affecting the liver adversely, also Seeman (2021) indicates the mechanism of CPZ effect by the post-synaptic blockade at the D2 receptors in the mesolimbic pathway. However, blocking D2 receptors in the nigrostriatal pathway is responsible for its extrapyramidal side effects.

CPZ impairs bile secretion in a dose-dependent manner and alters the fluidity of hepatocyte and canalicular membranes, thereby compromising their functional integrity (Samuels and Carey, 1978; Keefe *et al.*, 1980).

For millennia, medicinal plants have been a primary source of bioactive substances, used either as pure compounds or raw materials to treat various illnesses. Of the estimated 250,000–500,000 plant species on Earth, humans utilize only 1–10%. Many contemporary medications have been derived from traditional medicinal plants, following ethnobotanical pathways from indigenous remedies used by traditional medical systems, thereby playing a pivotal role in the development and advancement of modern studies (Hayta *et al.*, 2014).

Natural products are abundant in active antioxidant compounds, including flavonoids and polyphenolic chemicals

(Enseleit *et al.*, 2012). *Saussurea costus*, commonly known as Indian costus, is renowned for its medicinal properties (Singh *et al.*, 2017). It has been used in modern medicine to treat various conditions, including ulcers, gastrointestinal issues, inflammatory diseases, and asthma (Amara *et al.*, 2017). *Saussurea costus* is noted for its high antioxidant content, particularly sesquiterpene lactones such as dihydrocostose lactone, cinnarobricin, and costunolide, which contribute to its pharmacological effects, including anti-inflammatory, immunomodulatory, hypoglycemic, anti-hepatotoxic, hypolipidemic, anti-parasitic, antiviral, and anti-cancer properties (Saleem *et al.*, 2013; Zahara *et al.*, 2014).

This study aims to investigate the effects of CPZ administration on liver function in rats and to evaluate the protective effects of Indian costus root extract on liver enzymes and the histological structure of the liver.

MATERIALS AND METHODS

ANIMALS AND TREATMENT

Thirty adult male albino rats (weighing 150–220 g, aged 12–14 week) were obtained from animal house of College of Agriculture at Baghdad University. In the College of Education for Women /Anbar University's animal home, animals were placed in plastic animal cages. The study designs and protocols confirm the requirements of the ethics considered by our institute in accordance with the Standard Guide for the Care and Use of Laboratory Animals. The cages were covered with sawdust, with attention to the cleanliness of the cages, and the sawdust was changed three times a week. Six rats were placed in each cage when rats were distributed. The rats were acclimatized at suitable conditions in laboratories for ventilation, temperature (23±2 °C) with controlled humidity conditions (50–55 %) at 12 h light and dark cycle, and a constant supply of water and a standard dietary requirement. The drug CPZ (trade name "Lagactil" in the dose 100 mg) used is manufactured by "Aventis" (Oubari pharma). The experimental rats were divided into 5 groups (treated orally with one daily dose for 30 days) as follows:

G1 group, A negative control group was given water only. G2 group A positive control group was given oral chlorpromazine doses of 2 mg/kg of body weight as a single dose once a day (Al-Mushhadani *et al.*, 2020) While the groups of Costus root extract (prophylactic groups) were distributed according to the concentration of the extract into three groups G3, G4 and G5: each one was given a concentration of 1.5, 3 and 5 mg/kg respectively, In conjunction with chlorpromazine (2mg/kg). In the study, groups G3, G4, and G5 served as a prophylaxis group dosed 1 hour after CPZ administration.

Botanists scientists recognized and verified the costus (*Saussurea costus*) roots that were collected from Al-Ramadi city local markets. An electric grinder is used to dry and crush, then stored in tightly sealed bottles for use in the extraction process later on. Regarding 100 g of the produced powder, it was steeped for 72 hours at room temperature in 1000 mL of 70% ethanol. This solution passed through Whatman grade⁻¹ filter paper in a funnel under vacuum. The filtrate was next dried and evaporated under lower pressure in a rotary evaporator. *S. Costus* root crude ethanolic extract was gathered and then lyophilized to a dry powder (Abd El-Rahman *et al.*, 2020). Until needed (the yield of the extract was 2gm), the samples were finally kept at 4 °C.

BLOOD AND TISSUE SAMPLING (HISTOPATHOLOGY)

Rats were weighed after the treatment and then starved for the night. The animals received ether anesthetic so that capillary tubes could be used to extract 5 ml of blood from their eye sockets. The blood was then split into laboratory tubes without the use of anticoagulant. After whole blood was put into a standard disposable tube and let to coagulate in a 37°C water bath for 15 to 30 minutes, the serum was separated by centrifugation at 3000 rpm. The biochemical parameters involved—AST, ALT, and ALP—were measured with sera. Animals were given up for autopsy and liver removal after blood was drawn under anesthesia by intramuscular injection of 50 mg/kg ketamine hydrochloride. After dissecting and cleaning the liver with tap water, the liver of each specimen was taken out and immediately fixed in 10% buffered formalin solution for 72 hours. Fixed tissues were dehydrated in aggraded series of alcohols, cleared in xylene, embedded in paraffin wax and cut with microtome at 6 to 7 µm. Sections were mounted on glass slides, deparaffinized and stained by Hematoxylin and Eosin (H and E) (Drury and Wallington, 1980) and then examined under olumpis light microscopy in Japan. Digital camera (sony-japa 14 migapixill) is used for the photos.

BIOCHEMICAL DETERMINATIONS

Alanine amino transferase (ALT) activities were measured in serum using the modified kinetic method of (Hafkenscheid, 1979). Aspartate amino transferase (AST) activities were measured in serum using the modified kinetic method of Henary (1974), serum alkaline phosphatase (ALP) was carried out according to the method of (Belfied and Goldberg, 1971).

STATISTICAL ANALYSIS

The statistical analysis of the data in this study was conducted by following the steps of the linear model of the statistical program (CoStat), and the significant differences (ANOVA) between the means were compared by SPSS programming difference were deemed significant at $p \leq 0.05$ after the data were presented as mean $\pm$ standard error

RESULTS AND DISCUSSION

The liver is the main target of poisoning for a number of compounds, as most substances pass through metabolism. As a result, it becomes an organ of great importance for studying the effects of different chemicals excreted in the body, so the present work aims to study the potential modifying effects of Costus extract against hepatotoxicity. Drug-induced hepatotoxicity is a major concern in clinical practice. Although relatively uncommon, drug-induced liver injury is the leading cause of acute liver failure in the world and a major reason for liver transplantation (Reuben *et al.*, 2010). In addition, drugs used in psychiatry and neurology are the second most important group of drugs causing hepatotoxicity, after anti-infective drugs. After discontinuing feeding for 30 days of experiment for all animals, the G2 (CPZ group) gained weight at faster rate (286.250 ± 13.750 g Body Weight) (10.525 ± 0.217 g Liver Weight), while the G1 or control group gained weight at slower rate (231.250 ± 21.250 g Body Weight) (8.160 ± 0.628 g Liver Weight)., the prophylactic group (G3, G4 and G5) gradually began to lose weight (260 ± 10.607 , 242.500 ± 16.008 g and 241.250 ± 6.575 g respectively of Body Weight) (10.163 ± 0.545 g , 9.050 ± 0.674 g and 8.725 ± 0.837 g respectively of Liver Weight). The results observed in Table 1, There was no significant change ($p > 0.05$) in liver and body weight of animals among CPZ group, control group and prophylactic group.

Table 1: Comparison of Body and liver Weight of rats in control and experimental groups after administration of *Saussurea costus*.

Groups	Body Weight	Liver Weight
	Mean $\pm$ St. Error	Mean $\pm$ St. Error
(G1) Control negative group	231.250 ± 21.250	8.160 ± 0.628
(G2) Chlorpromazine mg/ kg	286.250 ± 13.750	10.525 ± 0.217
(G3) Antioxidant 1.5 mg/kg	260.000 ± 10.607	10.163 ± 0.545
(G4) Antioxidant 3 mg/kg	242.500 ± 16.008	9.050 ± 0.674
(G5) Antioxidant 5 mg/kg	241.250 ± 6.575	8.725 ± 0.837
LSD	43.74	1.855
P-Value	0.11533	0.078
C.V.	11.504	13.201

There are several theories put out to explain why antipsychotics tend to make people gain weight. Treatment of psychosis patients is plagued by antipsychotic-induced

weight gain (AIWG); antipsychotics are also known to lower glucose metabolism, increase cholesterol and triglyceride levels, and cause arterial hypertension which leads to metabolic syndrome (De Hert *et al.*, 2011). Interest in antipsychotics resulting in weight gain was aroused after the landmark study by Allison *et al.* (1999), the first meta-analysis on the subject. Over ten weeks, the study evaluated the weight gain caused by first- and second-generation antipsychotics (FGAs and SGAs, respectively). Molindone has been associated to a mean weight drop of 0.39 kg, clozapine, olanzapine, thioridazine, sertinazole, chlorpromidine, and risperidone have all been proven to cause significant weight increase between 4.45 and 2.19 kg. These medications' effects at serotonin 5-HT_{2A} and 5-HT_{2C}, dopamine D₂ and D₃, histamine H₁, and muscarinic M₃ receptors have been connected to the high likelihood of weight gain (Fleischhacker *et al.*, 2010).

Another research pointed out that steatosis is a different kind of lesion. This response is often chronic and results from the liver gradually and increasingly accumulating lipids, particularly triglycerides (Goncalves *et al.*, 2015), which can be brought on by a number of illnesses, including the use of specific drugs. Sometimes benign macrovesicular steatosis can progress to steatohepatitis and cirrhosis in drug-induced steatosis, which is nearly always reversible (Zhang *et al.*, 2013).

Adipokines made in white adipose tissue, leptin and adiponectin, have been linked to weight gain; elevated leptin levels and decreased adiponectin levels (Ak *et al.*, 2013). Antipsychotics also alter ghrelin, which stimulates food intake and deposits fat tissue by acting on the arcuate nucleus of the hypothalamus (Lu *et al.*, 2015).

The ethanolic extract of *S. Costus* was found to decrease the body and liver weights of rats. According to several studies, the extracts of *S. costus* are the best inhibitors of pancreatic lipase and α glucosidase. Interestingly, the ethanolic extract of *S. Costus* showed notable levels of antioxidant activity in DPPH free radical scavenging assay. Moreover, flavonoids lessen the absorption of triacylglycerol by preventing α -glucosidase activity and the formation of micelle in the small intestine. Therefore, these studies clarified that reducing the gastrointestinal adverse effects linked to undigested starch reaching the colon would be possible by maintaining the balance between α -amylase and α -glucosidase inhibitors. Strongly positive correlations were observed between the antioxidant, antidiabetic, and anti-obesity potential of phenolic and flavonoid compounds with the inhibition activity of enzymes (alpha-amylase, alpha-glucosidase, and pancreatic lipase) and free radical scavenging/antioxidant activity (Naseer *et al.*, 2022; Kumar *et al.*, 2023).

BIOCHEMICAL RESULTS

In the current study details of findings observed in Table 2 revealed the treatment of rats that treated with CPZ or G2 group (2mg / day orally) for 30 days resulted in a high significant rise ($p < 0.001$) in serum of liver enzymes (AST, ALT and ALP) compared with G1 (control group) and showed a gradually significant decreasing in the level of enzymes in the prophylactic group (G3, G4 and G5) respectively.

Table 2: Effect of ethanolic extract of *Saussurea costus* on AST, ALT and ALP in rat treated with Chlorpromazine.

Groups	AST (U/L)	ALT (U/L)	ALP (IU/L)
	Mean $\pm$ St. Error	Mean $\pm$ St. Error	Mean $\pm$ St. Error
(G1) Control negative group	147.74 $\pm$ 7.75 C	70.77 $\pm$ 4.23 D	78.83 $\pm$ 3.47 C
(G2) Chlorpromazine mg/ kg	251.28 $\pm$ 10.58 A	169.27 $\pm$ 9.04 A	116.42 $\pm$ 3.82 A
(G3) Antioxidant 1.5 mg/kg	200.09 $\pm$ 10.09 B	127.39 $\pm$ 7.39 B	94.72 $\pm$ 2.66 B
(G4) Antioxidant 3 mg/kg	170.23 $\pm$ 6.90 C	104.12 $\pm$ 8.61 C	86.47 $\pm$ 4.01 BC
(G5) Antioxidant 5 mg/kg	149.49 $\pm$ 7.04 C	85.31 $\pm$ 3.50 CD	74.86 $\pm$ 3.83 C
Total	183.77 $\pm$ 10.84	111.37 $\pm$ 9.61	90.26 $\pm$ 4.17
LSD	27.142	21.850	11.311
P-Value	<.001	<.001	<.001

Thus, Chlorpromazine causes a dose-related decrease in bile secretion and changes the fluidity of the hepatocyte and canalicular membranes, which in turn affects the function integrity of these sites and is well known to cause hepatotoxicity in humans (Derby *et al.*, 1993).

Ninety percent of drug-induced hepatotoxicity is caused by hepatocellular damage, which is linked to abnormally high serum alanine aminotransferase (ALT) and little or no rise in alkaline phosphatase (ALP); a high serum bilirubin level that is present in cases of severe hepatocellular damage which is a sign of a bad prognosis (Telles-Correia *et al.*, 2017). It can be prevented by regular monitoring with liver function tests and in case of the early detection of liver injury, the drug should be stopped, and symptomatic management should start (Velayudham and Farrell, 2003; Morgan *et al.*, 2019).

The current investigation revealed an increase in serum as AST, ALT, and ALP activities in rats treated with CPZ alone compared to control group. This results in leakage of specific intracellular enzymes such as AST, ALT, and ALP to the plasm boosting their activities there. Similar findings have been published in a number of researches (Ab-

del-Wahhab *et al.*, 2017; Al-Fatlawi *et al.*, 2019; Dejanovic *et al.*, 2014). Additionally noted was an increase in ALT, AST, and ALP levels.

Table 2 shows that the ethanol extract of *S. costus* is reducing the toxic effect of chlorpromazine since the plasma levels of AST, ALT, and ALP in prophylactic groups of rats by *S. costus* were much lower than those of the CPZ group. Previous investigations have revealed that an extract of Indian costus decreased the activity of liver enzymes (Hegazy *et al.*, 2020; Toussoni *et al.*, 2020) and this may be attributed to phytochemical compounds such as flavonoids and chlorogenic acid that serve as antioxidant material that help to inhibit free radicals that caused lipid peroxidation and prevents chlorpromazine poisoning. These results correspond with (Ansari *et al.*, 2018; Kadhemi, 2019).

Whereas reactive oxygen species (ROS) are produced at a higher rate in the organ, which is well known to cause oxidative stress (Abdel-Wahhab *et al.*, 2016). and this contributes in inhibiting the defensive system. Concerning this same issue, Jorgačević *et al.* (2014) found that although neutrophils and Kupffer cells are significant sources of ROS, the main sources of ROS within hepatocytes are iron overload, cyclooxygenase, lipoxygenase, cytochrome P450 2E1, mitochondria, and nicotinamide adenine dinucleotide phosphate oxidase. Consequently, the reduction of hepatocyte antioxidant ability adds to the ROS-induced liver damage.

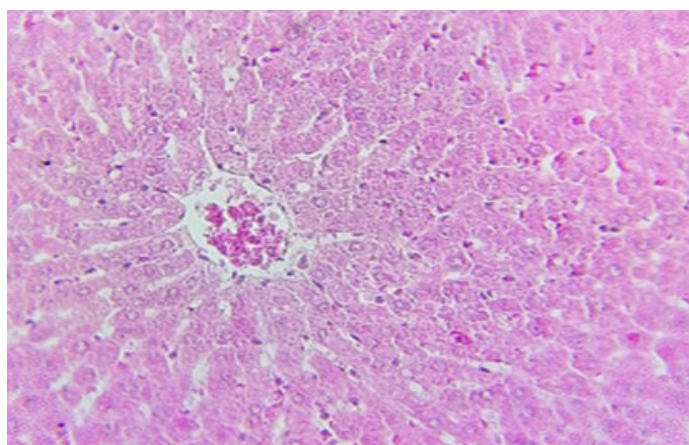


Figure 1: Histophotograph of the liver of control group screening hepatic architecture, central vein hepatocyte and blood sinusoids H and E400X.

HISTOLOGICAL EXAMINATION

The histopathological changes of the rat's liver tissue revealed the normal architecture in the G1 or control group (normal structure of the cords of the hepatocytes, the central vein and the blood sinusoids) (Figure 1) with many disorders lesions in the G2 or CPZ group (in a dose of 2 mg/kg body weight/day of chlorpromazine only). This is histologically represented by hepatocyte suffering from

acute cell swelling, where most hepatocyte were in necrosis and inflammatory cells infiltration was observed. Also, blood vessels congestion and perivascular inflammatory cells infiltration are manifested in the field of view X400, (Figures 2, 3, and 4). In the portal tract, dilation was in the portal vein with perivascular edema, bile ducts epithelial hyperplasia, and hemosiderin deposition also peri central area of Hydropic Degeneration were observed in the field of X100 (Figures 5, 6, and 7).

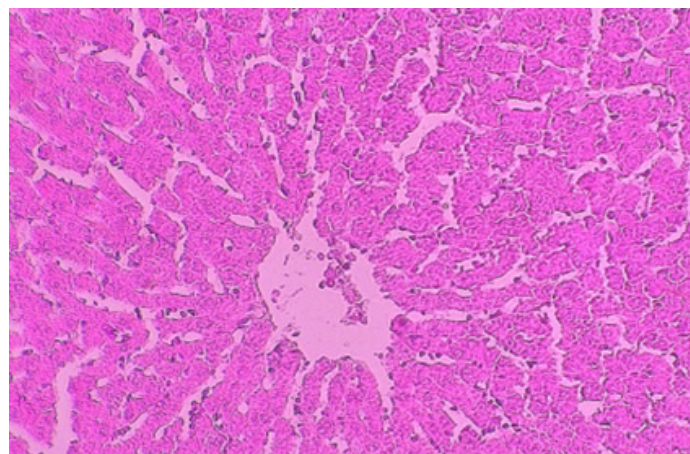


Figure 2: Histophotograph of liver treated with CPZ showing hepatocyte suffering from acute cell swelling. H and E X400.

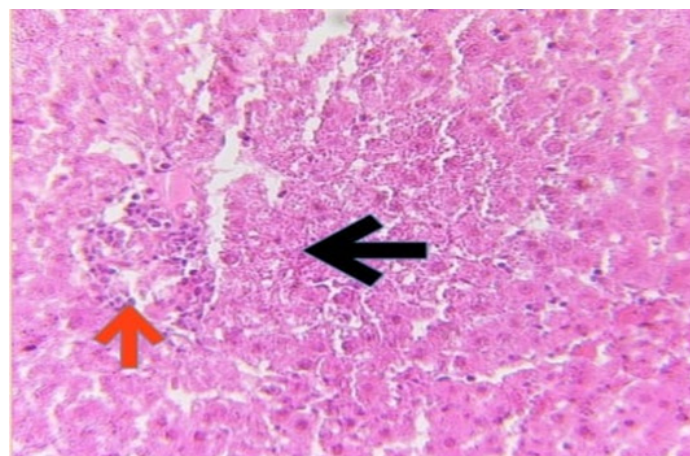


Figure 3: Histophotograph of liver treated with CPZ showing necrotizing hepatocyte (black arrow) and inflammatory cells infiltration (red arrow). H and E X400.

As previously reported, the liver section of CPZ-treated rat had lipid droplets, necrotic cytoplasm, and pyknotic or apoptotic nuclei, as well as congestion and fibrous thickening of the portal tube with extended bile canaliculi (Abdel-Wahhab *et al.*, 2017). These results mirrored those in the liver published by Yang *et al.* (2015). Additionally, this result is consistent with other studies that show the contribution of oxidative stress in the pathogenesis of cholestasis (Parola *et al.*, 1996; Ohta *et al.*, 1999) and can be explained as a consequence of generation of CPZ cation radicals and metabolic activation of CPZ to quinonimine derivatives (Tolar, 2004).

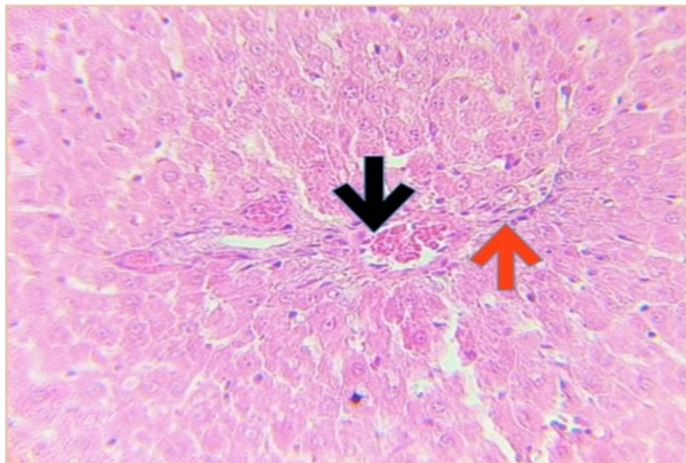


Figure 4: Histophotograph of liver treated with CPZ showing blood vessels congestion (black arrow) and perivascular inflammatory cells infiltration (red arrow). H and E X400.

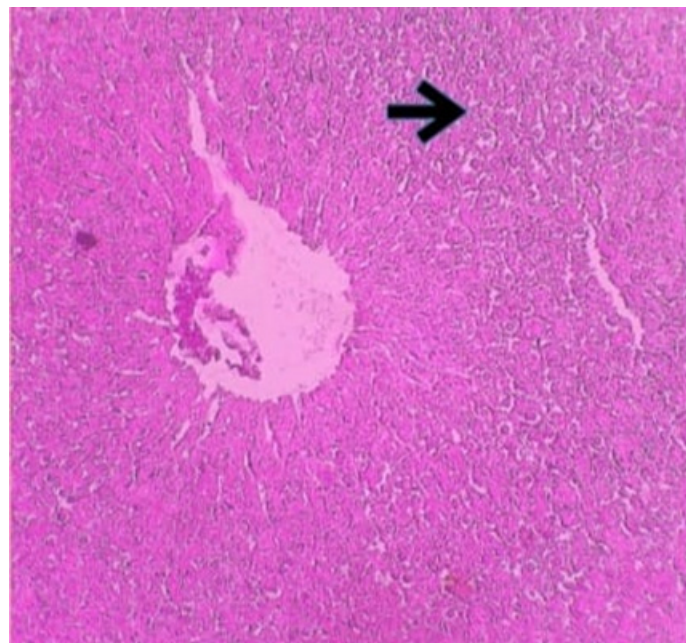


Figure 7: Histophotograph of liver showing peri central area of Hydropic Degeneration (arrow). H and E X 100.

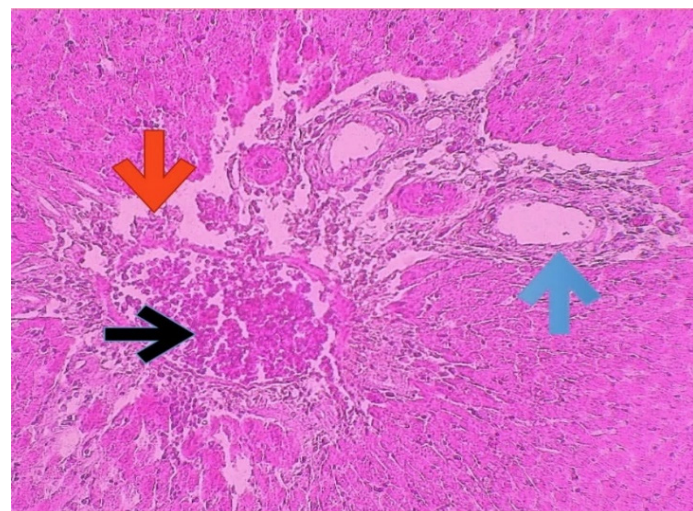


Figure 5: Histophotography of liver treated with CPZ, showing portal vein dilation (black arrow) with perivascular edema (red arrow) and bile ducts epithelial hyperplasia (blue arrow). H and E X100.

Recent investigation has shown that pathological alterations of varied severity in liver tissue occur when CPZ is administered at different doses (Bailo *et al.*, 2023).

In this work, the ethanolic costus extract at a dosage of 5 mg/kg effectively corrected the changes in liver tissue structure caused by CPZ, therefore providing a stronger hepatoprotective effect against CPZ-induced liver damage (Figure 8). By removing too many free radicals, where a great number of earlier research have demonstrated that costus can donate electrons to reactive radical cells and transform them into more stable, non-reactive species (Shediwah *et al.*, 2019; Benedetto *et al.*, 2019; El-Kholie *et al.*, 2022; AL-Zayadi *et al.*, 2023).

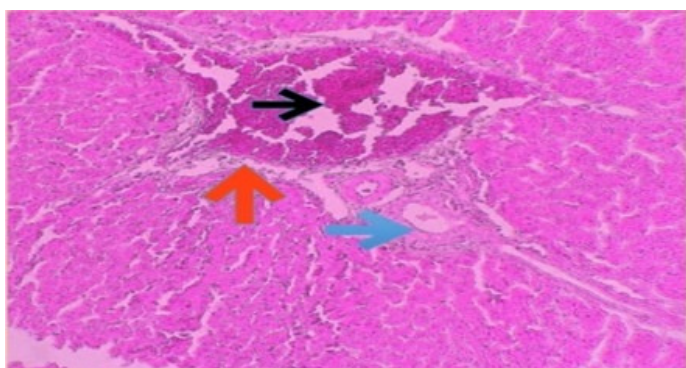


Figure 6: Histophotography of liver treated with CPZ, showing portal vein dilation (black arrow) with perivascular edema (red arrow), bile ducts epithelial hyperplasia (blue arrow) and hemosiderin deposition (blue arrow) H and E X100.

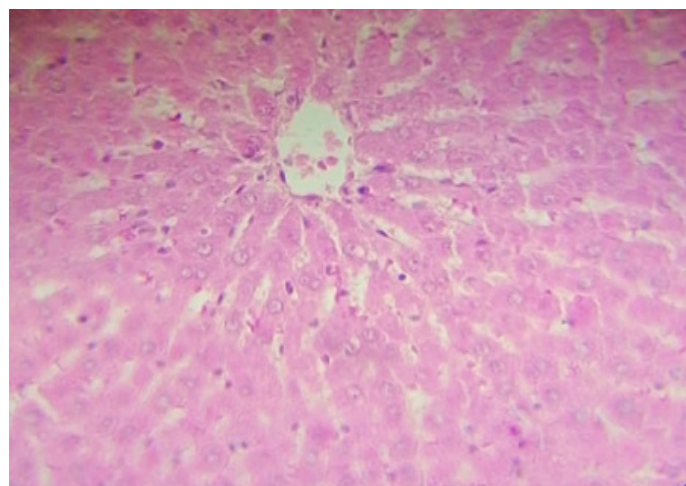


Figure 8: Histophotograph showing the improvement of liver histology by prophylactic group treated with alcoholic extract of *S.costus* (5 ml) H and E 400X.

Ayaz (2017) report that costus extract is rich in bioactive compounds including; dihydrocostus lactone, costonolide, cinnarobicrin, monoterpenes, sesquiterpenoids, flavonoids, lignans, triterpene steroids, and glycosides that are produced as antioxidants. As such, costus extract can be regarded as a suppressor of the negative effects of CPZ in rats and as an inhibitor of the generation of radicals that cause liver damage.

CONCLUSIONS AND RECOMMENDATIONS

The published results confirm that CPZ leads to changes in Weight and responsible for remarkable functional and structural abnormalities in the liver. The pathological changes involve the hepatocytes, and the hepatic connective tissues as well as induced inflammation of the hepatic tissues. Also, treatment with costus root extract significantly reduces CPZ-induced hepatotoxicity histologically in hepatic rats. Therefore, this encourages the use of Indian costus in future studies as therapeutic alternatives.

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NOVELTY STATEMENT

The study demonstrates that ethanolic extract of costus root offers effective hepatic protection against the adverse effects of chlorpromazine, contributing to improved functional and histological liver parameters in rats.

AUTHOR'S CONTRIBUTIONS

Nbaa Mutea Abid AL-Alh and Nagam Khudhair contributed to the conceptualization, methodology, investigation, writing of the original draft, and visualization. Nuha Hatem Khalaf and Ahmed Khalid reviewed, and edited the manuscript. All authors contributed to the article and approved the submitted version.

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Nil.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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