Potential Effects of Caffeine on Hepatotoxic Rats Induced by Carbon Tetrachloride

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Abstract

Carbon tetrachloride (CCl_4) is a hepatotoxin that is frequently used to induce liver injury in animal models. Supplementing with antioxidants may therefore be beneficial in treating its hepatotoxic effects. Thus, the objective of this study was to assess the impact of caffeine (CAF) as an antioxidant on hepatotoxic rats induced by CCl_4.Thirty male albino rats were divided into two main groups. The first group (n=6), the negative control (-), was fed a basal diet (BD), and the second group (n=24) received CCl_4 twice per week (2 mg/kg bw) to induce hepatotoxicity and was then further divided into four subgroups. Subgroup 1 served as a positive control group (+) that received a BD only. Subgroups 2, 3, and 4 received BD and were treated with oral intakes of 10, 30, and 50 mg CAF/kg body weight daily. Liver and renal function tests, lipid profile, lipid peroxidation markers, antioxidant enzymes, and histological tests were carried out at the end of treatment. Oral daily treatments of CAF at 30 and 50 mg/kg bw for 28 days exhibited a decrease in liver enzyme activities compared with the positive control and significantly restored serum albumin levels. On the other side, the previous two interventions caused a significant ($P \leq 0.05$) decrease in the serum lipid profile of the hepatotoxic rats (positive control group) after they were fed daily for four weeks at different rates: 16, 23.5, 33.8, 41.6, 47, 63.9, 33.8, and 41.9% for total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-c), and very low-density lipoprotein cholesterol (VLDL-c), respectively. These findings suggest that CAF might protect against oxidative stress and CCl_4-induced liver damage, potentially through its antioxidant properties.

Keywords: Serum albumin, Lipid peroxidation markers, Antioxidant enzymes, Triglycerides

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Introduction

Hepatotoxicity, sometimes referred to as liver toxicity, is a disorder marked by liver damage brought on by exposure to dangerous chemicals, including alcohol, some medications, and even incorrect usage of herbal and nutritional supplements. Impaired liver function and, in extreme situations, liver failure may result from this injury **Singh *et al.,* (2021)** . Hepatotoxins refer to the substances that harm the liver. Overdoses of specific pharmaceutical medications, industrial chemicals, natural chemicals such as microcystins, herbal remedies, and nutritional supplements are examples of exogenous molecules of clinical significance that are known as hepatotoxicants **Singh *et al.,* (2011**). Strong environmental hepatotoxins such as CCl_4 have been used as model chemicals to investigate oxidative damage and hepatotoxicity. It has also been used to assess the potential therapeutic benefits of dietary antioxidants and medications **(Basu, 2003; Prasenjit *et al.*,2006)** In addition to direct toxicity of the initial chemical, hepatotoxicity can also be caused by a reactive metabolite or an immunologically mediated reaction that affects the liver vasculature, biliary epithelial cells, and/or hepatocytes **Deng *et al.,* (2009)**. When there is a high level of bilirubin in the extracellular fluid, symptoms of hepatotoxicity can include jaundice or icterus, which causes yellowing of the skin, eyes, and mucous membranes; pruritus; severe abdominal pain; nausea or vomiting; weakness; extreme exhaustion; persistent bleeding; skin rashes; widespread itching; swelling of the feet and/or legs; abnormally high weight gain in a short time; dark urine; and light-colored stool **Chang and Schaino , (2007**). Vaccines, antiviral medications, and steroids used to treat liver illnesses might have negative side effects, particularly if taken for an extended time. Plant-based hepatoprotective medications appear to be appealing substitutes for the limited number of effective liver-protective medications available in contemporary medicine **Gulati *et al* ., (2018)**. Caffeine provides a range of positive benefits. Other coffee species and coffee beans, including Coffea arabica and Coffea canephora, are excellent sources of CAF, which is a known plant alkaloid **Addai, (2010).** Purine-derived caffeine is a white, bitter-tasting powder that is poorly soluble in water and odorless **NCBI , (2020).** Tea, chocolate bars, coffee, cocoa beverages, and energy and soft drinks are some

of the food products that contain CAF. Surprisingly, more than sixty different plants have a mass CAF content **Panchal e*t al.*, (2012)**. CAF consumption has been shown to exert protective effects against liver diseases for multiple reasons. **Wadhawan and Anand (2016)** reviewed clinical evidence supporting the potential benefits of coffee consumption in the management of alcoholic liver disease, nonalcoholic fatty liver disease (NAFLD), hepatitis B, hepatitis C, and other similar conditions. Similarly, **Shim *et al* ., (2013)** conducted two meta-analyses and showed that consuming more than two cups of coffee daily significantly lowers the risk of patients suffering from liver cirrhosis, liver fibrosis, hepatocellular carcinoma, and death. Therefore, the primary goal of this study was to investigate the potential protective effect of caffeine against carbon tetrachloride-induced hepatotoxicity.

Materials and methods

Materials

Caffeine powder, CCl_4, and a component of BD were acquired from Sigma Chemical Company in St. Louis, Missouri, USA. Furthermore, the assay kits for malondialdehyde (MDA), ALP, AST, ALT, renal function, total protein, and albumin were acquired from Al-Gomhoria Co. for trading chemicals, drugs, and medical equipment in Cairo. GSH and MDA measurement kits were supplied by My Bio-Source, Inc. of San Diego, CA, USA. TG, TC, HDL cholesterol, and LDL-c were brought from El-Nasr Pharmaceutical Chemicals, a Cairo, Egypt-based company.

Animals

Thirty male white Sprague Dawley rats, averaging 150± 10g, were provided by the laboratory animal department of the College of Veterinary Medicine at Cairo College in Egypt.

Diet components

The basal diet (BD) is prepared by **Reeves *et al*., (1993)**. As follows: (69.5%) cornstarch, (10%) protein, (10%) corn oil, (5%) cellulose, (4%) mineral mixture, (1%) vitamin mixture, (0.3%) methionine, and (0.2%) choline

chloride. Vitamin and salt mixture components are developed based on the same reference.

Methods

Induction of liver-hepatotoxicity

Using the technique outlined by **Jayasekhar *et al.*, (1997)**, thirty male albino rats were given an intraperitoneal (IP) injection of CCl_4 in olive oil at a 50% V/V (2 ml/kg bw) twice a week for two weeks to cause chronic liver injury. By selecting four rats at random from the experimental group and analyzing their biochemistry (liver functions), liver intoxication was verified**.**

Experimental design

All biological experiments were subject to decisions made by the Commission on Life Sciences, Institute of Laboratory Animal Resources, and National Research Council **NRC, (1996).** Rats (n =30) were kept in wire cages in individual cages in a room with normal healthy circumstances, including a 12-hour lighting cycle, a relative humidity of 56±4%, and a temperature of 25±3°C. Before the trial began, all rats were given BD for a week to acclimate them. Following a week, rats were divided into two main groups: group 1 (n=6) (negative group) was fed BD and given the same amount of tap water as the other groups. The other main group (n=24) was used for liver impairment and was injected CCl_4 twice a week for two weeks at a dose of 2 mg/kg body weight. The rats were then divided into five subgroups as follows: Group (2), the positive group, was fed only BD and was given the same quantity of tap water as the other groups. Groups (3-5) were fed BD and given oral CAF 10, 30, and 50 mg/kg bw daily, respectively. The CAF concentrations used in the experiments were chosen based on the findings of earlier research according to **Guth *et al.*, (2022).**

Blood samples

After a 12-hour fast, blood samples were taken from the abdominal aorta after the four-week experiment, and rats were sedated with ether. According to **Stroev and Makarova, (1989),** in order to separate the serum, blood samples were collected in dry, clean centrifuge tubes, allowed to clot at room

temperature, and then centrifuged for 10 minutes at 3000 rpm. The clear, non-hemolyzed serum was carefully aspirated, transferred into labeled Eppendorf tubes, and frozen at -20°C for further biochemical analysis. The different liver tissue samples were separated and stored in 10% neutral formalin for histological examinations**.**

Assessment of liver and kidney functions

An auto-analyzer for biochemistry (Olympus AU2700, Japan) was used to evaluate the serum levels of ALT, AST, and ALP to estimate liver function. Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) were determined according to the methods of **Tietz, (1976)**, **Henry, (1974),** respectively. The enzymatic approach outlined by **Patton and Crouch, (1977)** was used to determine urea. Creatinine was measured according to **Young and Friedman, (2001).** Determination of total protein (TP), albumin (Alb), and globulin (GLB) were measured in g/dl as stated by **Buzanovskii, (2017); Srivastava *et al*., (2002).**

Oxidative stress assessment

All groups' liver samples were homogenized using cold phosphate buffer (pH 7.4) and centrifuged for 30 minutes at 4°C at 4000 rpm. Malondialdehyde (MDA), an indicator of lipid peroxidation, the activity of glutathione peroxidase+ (GSH-Px), and reduced glutathione (GSH) were assessed in tissue homogenate, and the supernatant was utilized to evaluate liver tissue. To ascertain the oxidative state of the liver specimens, measurements were carried out as previously mentioned **(Ohkawa and Ohishi, 1979; Moron *et al.*, 1979)**.

Estimation of lipid profile

In accordance with **Fassati and Prencipe (1982),** TG was conducted. TC was determined in accordance with **Allen, (1974)**. HDL-c: using the same technique as TC, as **Lopez, (1977)** states. The following is the calculation of VLDL-c and LDL-c using **Lee and Nieman, (1996)** method**:**

VLDL-c (mg/dl) = TG/5

LDL-c (mg/dl) = Total cholesterol - (HDL-c + VLDL-c)

Histological analysis

According to **Drury *et al*., (1976)** liver was processed for histological investigations. The tissues were precisely preserved in a 10% neutral formalin solution. They underwent an increasing series of ethanol dehydration, xylene clearing, paraffin wax embedding, microtome sectioning, eosin and hematoxylin staining, and examination to identify any histological alterations.

Ethical considerations

The Scientific Research Ethics Committee (Animal Care and Use): The biological experiments conducted in this study received ethical approval from the Menoufia University Faculty of Home Economics in Shebin El-Kom, Egypt **(Approval no. MUFHE /F/ NFS / 33/24)**.

Statistical analysis

The results were presented as mean ± standard deviation, and one-way analysis of variance (ANOVA) was used to assess statistical significance. A statistically significant p-value was less than 0.05. Data analysis was conducted using the SAS user's guide **SAS, (2000).**

Results and Discussion

Effect of CAF on liver enzymes in normal and hepatotoxic rats

Information about how CAF affects liver functions in both normal and hepatotoxic rats was included in Table 1. The results showed that the ALT, AST, and ALP enzymes in the positive control group and other groups differed significantly ($P \leq 0.05$). With substantial differences ($P \leq 0.05$), the negative control group had the lowest levels of ALT, AST, and ALP, while the positive control group had the highest. The release of the liver enzymes ALT and AST into the bloodstream indicates damage to the liver parenchyma and serves as a measure of the toxicity and extent of liver damage **Bona *et al*., (2012)**. According to reports, CCl_4 metabolism produces trichloromethyl (CCl_3•) and peroxy trichloromethyl (•$OOCCl_3$) free radicals, which can lead to hepatotoxic effects such as fibrosis, steatosis, necrosis, and hepatocarcinoma **Fang and Lin, (2008)**. ALT, AST, and ALP enzyme levels significantly decreased in hepatotoxic rats administered CAF, in contrast to the positive control group. Based on previous results, it may be concluded that rats given 50 mg CAF showed the greatest decrease in ALT, AST, and ALP values. In comparison to the positive control group, the current data revealed a significant

decrease in AST in hepatotoxic rats administered 10, 30, and 50 mg CAF by 25.4, 37.36, and 45.56%, respectively. These results are completely in line with those of **Shin *et al*., (2010),** who showed that CAF therapy demonstrated a hepatoprotective effect and potential improvement in the liver architecture by reducing the release of those enzymes into the circulation. CAF consumption has been linked to a decrease in liver enzyme levels and a lower risk of fibrosis **Modi *et al*., (2010)**. In numerous studies, coffee has been linked to lower levels of ALP, gamma-glutamyl transferase (GGT), ALT, and AST according to **Heath *et al*. (2017)**. Additionally, CAF activates Nrf2, an enzyme that controls the expression of antioxidant proteins. Thus, it works by lowering oxidative stress, one of the factors that contribute to liver fibrosis **Gordillo-Bastidas *et al*., (2013).** Furthermore, CAF has a hepatoprotective effect, which reduces liver damage and, consequently, the amount of transaminases released into the bloodstream **Guth *et al*., (2022)**.

Table (1): Effect of CAF on liver enzymes in normal and hepatotoxic rats

Group		ALT (U/L)	AST (U/L)	ALP (U/L)
Negative control		$33^{d}\pm4$	$84^{e}\pm2$	$194^{e}\pm2$
Positive control		$65.33^{a}\pm3.51$	$248.33^{a}\pm3$	$363.33^{a}\pm3.51$
Hepatotoxic treated groups	10 mg CAF	$55^{b}\pm3$	$185^{b}\pm3$	$326.33^{b}\pm3.51$
	30 mg CAF	$45^{c}\pm4$	$155.33^{c}\pm3.51$	$265^{c}\pm3$
	50 mg CAF	$34.33^{d}\pm4.51$	$135^{d}\pm3$	$246.33^{d}\pm2.52$
LSD		6.98	5.35	5.39

The formula used for mean values is means ± SD. When there are significant differences between treatments ($P\leq0.05$), the means under the same column are indicated by a different letter. **CAF**: caffeine, **AST**: Aspartate aminotransferase, **ALT**: Alanine transaminase and **ALP**: Alkaline phosphatase.

Effect of CAF on creatinine and urea in normal and hepatotoxic rats

The impact of CAF on creatinine and urea in normal and hepatotoxic rats is shown in Table 2. The positive control group's levels of urea and creatinine increased significantly ($P\leq0.05$) following CCl_4 induction compared to the negative control group. One possible explanation for the rise in urea and creatinine levels is the oxidative stress brought on by exposure to CCl_4. These results are completely in line with those of **Waring *et al*., (2008)** who showed that low serum urea concentrations

may correlate with certain risk factors for hepatotoxicity. **Duan *et al*., (2023)** found that hepatotoxic substances can lead to acute kidney injury (AKI) through mechanisms like oxidative stress and mitochondrial dysfunction, resulting in elevated serum creatinine levels. It is noteworthy that the present investigation showed that urea and creatinine were significantly (*P≤0.05*) lower in hepatotoxic rats given 10, 30, and 50 mg CAF by (27.33, 37.05, and 49.56%) and by (11.92, 22.02, and 33.03%), respectively, than in the positive control group. Rats with hepatotoxicity that received 50 mg CAF showed the greatest decrease in urea and creatinine levels.

Table (2): Effect of CAF on creatinine and urea in normal and hepatotoxic rats

Group		Creatinine (mg/dl)	Urea (mg/dl)
Negative control		$0.59^{d}\pm0.01$	$27.00^{d}\pm4$
Positive control		$1.09^{a}\pm0.1$	$74.66^{a}\pm4.5$
Hepatotoxic treated groups	**10 mg CAF**	$0.96^{ab}\pm0.07$	$54.33^{b}\pm5.68$
	30 mg CAF	$0.85^{bc}\pm0.07$	$47.00^{b}\pm4$
	50 mg CAF	$0.73^{cd}\pm0.1$	$37.66^{c}\pm2.52$
LSD		0.14	7.76

The formula used for mean values is means ± SD. When there are significant differences between treatments (*P ≤0.05*), the means under the same column are indicated by a different letter. **CAF**: caffeine.

Effect of CAF on TP, ALB, and GLB in normal and hepatotoxic rats

Table 3 illustrates the effects of caffeine on TP, ALB, and GLB in normal and hepatotoxic rats. The data showed that the levels of TP, ALB, and GLB in the positive control group significantly decreased (*P≤0.05*) following CCl_4 induction compared to the negative control group. In contrast, hepatotoxic rats given 10,30, and 50 mg CAF had significantly (*P≤0.05*) higher levels of TP and ALB by (11.03, 12.54, and 18.28%), and by (12.46, 19.49, and 22.68%), respectively, in comparison to the positive control group. For GLB, the data showed a significant increase in hepatotoxic rats treated with 10,30, and 50 mg CAF by 9.74, 6.3, and 14.33%, respectively. From the previous results, it can be considered that the highest increase in GLB values was observed in the rats treated with 50 mg CAF. The results of the current study were in agreement with the study of **Osz *et al*., (2022),** which reported that animals given 20 or 30 mg/kg of CAF for eight weeks showed increased low

albumin levels. **Birkner *et al.*, (2006)** demonstrated that total protein levels rose following caffeine administration.

Table (3): Effect of CAF on TP, ALB, and GLB in normal and hepatotoxic rats

Group		TP (g/dl)	ALB (g/dl)	GLB (g/dl)
Negative control		8.43^{a}±0.03	4.34^{a}±0.03	4.09^{a}±0.27
Positive control		6.62^{d}±0.04	3.13^{d}±0.07	3.49^{b}±0.11
Hepatoxic treated groups	**10 mg CAF**	7.35^{c}±0.04	3.52^{c}±0.03	3.83^{ab}±0.09
	30 mg CAF	7.45^{c}±0.03	3.74^{b}±0.04	3.71^{ab}±0.11
	50 mg CAF	7.83^{b}±0.12	3.84^{b}±0.08	3.99^{a}±0.28
LSD		0.12	0.1	0.37

The formula used for mean values is means ± SD. When there are significant differences between treatments ($P \leq 0.05$), the means under the same column are indicated by a different letter. **CAF**: caffeine.**TP**: Total protein, **ALB**: Albumin, and **GLB**: Globulin.

Effect of CAF on the serum lipid profile of hepatotoxic rats in normal and hepatotoxic rats

The impact of CAF on the serum lipid profiles of hepatotoxic rats is shown in Table 4. Using these data, it was possible to find that, in comparison to negative control groups, the positive control group had significantly higher serum levels of TG, TC, LDL-c, and VLDL-c ($P \leq 0.05$). However, when compared to other groups, the level of HDL-c decreased significantly ($P \leq 0.05$) in the positive control group. CAF intervention at 30 and 50 mg/kg bw/day for 28 days, the levels of TC, TG decreased significantly ($P \leq 0.05$) at different rates compared to the positive control group at ratios of (16.08 and 23.5%) and (33.8 and 41.69%), respectively. For LDL and VLDL, the data showed significant reduction in hepatotoxic rats treated with 30 and 50 mg CAF by (47.07and 63.96%) and (33.8 and 41.91%), respectively. On the other hand, HDL increased significantly in hepatotoxic rats treated with 30 and 50 mg CAF by 16.59 and 15.9 %, respectively. Rats who received 50 mg/kg bw/day had the lowest levels, almost equal to those of normal rats. According to **Yang *et al.*, (2011)**, the liver is the primary location for cholesterol synthesis and metabolism. Rats with CCl_4-induced hepatotoxicity have been shown to exhibit distinct changes in lipid metabolism **Singhal and Gupta, (2012)**. The current findings showed that in rats

given CCl_4, serum levels of TG, TC, LDL-C, and VLDL-C significantly increased while HDL-C levels decreased. This present study matches with **Adebayo *et al.*, (2007),** who found that administering CAF at a low dose(10mg/kg) significantly increased serum LDL cholesterol concentrations in rats, suggesting a potential risk for coronary heart disease. Conversely, another study indicated that higher CAF intake was associated with increased HDL cholesterol levels, suggesting a protective cardiovascular effect **(Kim *et al.*, 2008).**

Table (4): Effect of CAF on serum lipid profile of hepatotoxic rats in normal and hepatotoxic rats

Group		TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Negative control		$72.3^{d}\pm4.04$	$72^{c}\pm3.6$	$52.6^{a}\pm3.5$	$5.1^{d}\pm0.9$	$14.4^{c}\pm0.72$
Positive control		$102^{a}\pm5$	$136^{a}\pm4$	$44^{b}\pm1$	$30.8^{a}\pm4.1$	$27.2^{a}\pm0.8$
Hepatotoxic treated groups	**10 mg CAF**	$95^{ab}\pm6$	$96^{b}\pm3.6$	$48.33^{ab}\pm3.05$	$27.4^{a}\pm3.2$	$19.2^{b}\pm0.72$
	30 mg CAF	$85.6^{b}\pm5.5$	$90^{b}\pm3.6$	$51.3^{a}\pm4.1$	$16.3^{b}\pm1.5$	$18^{b}\pm0.72$
	50 mg CAF	$78c^{d}\pm3.6$	$79.3^{c}\pm3.21$	$51^{a}\pm3.6$	$11.1^{c}\pm2.6$	$15.8^{c}\pm0.64$
LSD		9.5	5.8	6.2	5	1.5

The formula used for mean values is means ± SD. When there are significant differences between treatments ($P \leq 0.05$), the means under the same column are indicated by a different letter. **CAF**: caffeine, **TC**: total cholesterol, **TG**: Triglyceride, **HDL**: High-density lipoprotein, **LDL**: low-density lipoprotein, and **VLDL**: Very Low-density Lipoprotein.

Effect of CAF on malondialdehyde, Glutathione, and glutathione peroxidase in normal and hepatotoxic rats

The effects of different CAF concentrations on the levels of GSH, MDA, and GPX in the normal and hepatotoxic groups were shown in Table 5. Following CCl_4 induction, the positive control group's levels of GSH and GPX significantly ($p \leq 0.05$) decreased, but MDA showed the reverse trend. These results were consistent with **Bona *et al.*, (2012),** who found that rat liver homogenates from CCl_4 intoxication had a noticeably greater MDA level than those from the control group. A major factor in liver damage and hepatic fibrogenesis is oxidative stress brought on by the liver's metabolism of CCl_4.

The study's findings on the decrease in GSH in the liver could be explained by GPx directly requisitioning GSH to scavenge the generation of free radicals from the

metabolism of CCl_4. However, when compared to the positive control group, CAF treatment significantly reduced MDA and increased GSH and GPX in hepatotoxic rats. Hepatotoxic rats given 50 mg CAF showed the greatest decrease in MDA value and the greatest increase in GSH and GPX value. The protective properties of caffeine's kahweol and cafestol phenolic diterpenes, which inhibit lipid peroxidation, may be the cause of its preventive effects. **Huber *et al*., (2002)**. These results come in harmony with **Pasaoglu *et al.*, (2011),** who demonstrated that MDA levels are lowered when caffeine is administered at doses of 30 mg/kg and 100 mg/kg daily because it reduces oxidative stress. Therefore, in male rats with liver illness, daily treatment of 37.5 mg/kg markedly elevated GSH and hepatic GPx **Amer *et al*., (2017).** In comparison to the positive control group, hepatotoxic rats given 10, 30, and 50mg CAF had significantly ($p \leq 0.05$) higher levels of GSH and GPX by (35.53, 117.1, and 140.79%) and by (17.28, 29.85, and 48.59%), respectively. However, hepatotoxic rats given 10, 30, and 50 mg CAF had considerably (*$P \leq 0.05$*) lower MDA levels by 14.67, 34.46, and 71.98%, respectively, as compared to the positive control group. Furthermore, it was found that giving 30 and 50 mg CAF to hepatotoxic rats increased their GSH and GPX levels more effectively ($P \leq 0.05$) than giving them 10 mg CAF. From the previous results, in hepatotoxic rats, CAF may be thought to protect against oxidative stress.

Table (5): Effect of CAF on malondialdehyde, Glutathione, and glutathione peroxidase in normal and hepatotoxic rats

Group		MDA (nmol/g.tissue)	GPX (U/g.tissue)	GSH (mmol/g.tissue)
Negative control		$9.56^{d} \pm 1.6$	$133.43^{a} \pm 2.8$	$2.58^{a} \pm 0.09$
Positive control		$39.26^{a} \pm 1.1$	$65.23^{e} \pm 3.2$	$0.76^{d} \pm 0.05$
Hepatotoxic treated groups	**10 mg CAF**	$33.5^{b} \pm 2.13$	$76.5^{d} \pm 3.25$	$1.03^{c} \pm 0.09$
	30 mg CAF	$25.73^{c} \pm 3.63$	$84.7^{c} \pm 2.35$	$1.65^{b} \pm 0.12$
	50 mg CAF	$11^{d} \pm 1.7$	$96.93^{b} \pm 5.6$	$1.83^{b} \pm 0.13$
LSD		3.39	6.59	0.19

The formula used for mean values is means ± SD. When there are significant differences between treatments (*$P \leq 0.05$*), the means under the same column are indicated by a different letter. **CAF**: caffeine, **MDA**: malondialdehyde, **GPX**: glutathione peroxidase, and **GSH**: Glutathione.

Effect of CAF on liver histological examinations in normal and hepatotoxic rats

Photos (1,2,3,4, and 5) demonstrated how CAF affected the histological examination of the liver of both normal and hepatotoxic rats. Microscopy pictures of H&E-stained liver slices reveal normal portal regions (PA) and sinusoids in the control negative group, along with normal hepatic cords radially distributed around central veins. Hepatic sections from the CCl_4 group showing markedly disrupted hepatic parenchyma due to vascular dilation (thin red arrow), fibrosis and leukocytic cell infiltration (thick black arrow), and hemosiderosis (curved black arrow) in portal areas with dilation and proliferation of bile ductules (thin black arrow). Long anastomosing fibrous tissue extensions from portal areas are seen (arrowhead). Hepatic sections from the treated group with 10 mg CAF show moderately disrupted hepatic parenchyma due to mild vascular dilation (thin red arrow) and decreased portal fibrosis with fewer leukocytic cell infiltrations (thick black arrow). Long anastomosing fibrous tissue extensions from portal areas are still seen (arrowhead). Hepatic sections from the treated group with 30 mg CAF show moderately disrupted hepatic parenchyma due to mild portal fibrosis (thick black arrow) with dilation of bile ductules (thin black arrow). Finally, hepatic sections from the treated group with 50 mg CAF show a normalized histological picture of hepatic parenchyma. Magnifications: X100 bar 100 and X400 bar 50. This is in line with the results of **Amer *et al*., (2017),** who found that the rat liver's H&E-stained sections from the caffeine and control groups showed normal hepatic architecture, with hepatic cords extending from transparent central veins and divided by sinusoids, free of necrosis or inflammation. The induced group, on the other hand, displayed notable alterations in liver structure along with vascular congestion of the blood sinusoids and central and portal veins. Additionally, it was clear that inflammatory cells had infiltrated the centrilobular areas. However, CAF therapy brought back normal liver histology, albeit with some vascular congestion and inflammatory cell infiltration. **Shan *et al*., (2022)** found that several clinical studies have further confirmed caffeine's effect on liver fibrosis, and in three animal models of liver fibrosis, Dimethyl nitrosamine (DMN), CCl_4, and Thioacetamide (TAA), it has been demonstrated to lessen the amount of hepatic fibrosis brought on by chemical toxicants. Finally, the recovery of

hepatic histological and functional changes following hepatotoxicity was linked to caffeine's anti-fibrogenic, anti-inflammatory, and antioxidant effects.

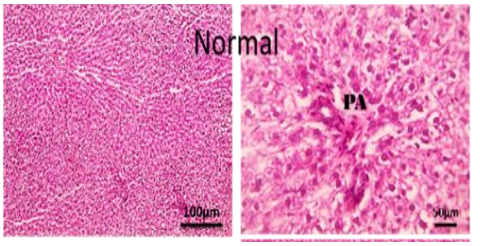

Photo (1): Negative control group

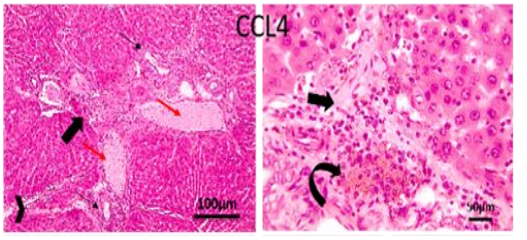

Photo (2): Positive control group

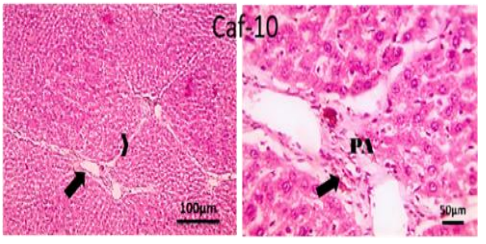

Photo (3): Treated group with 10 mg/CAF

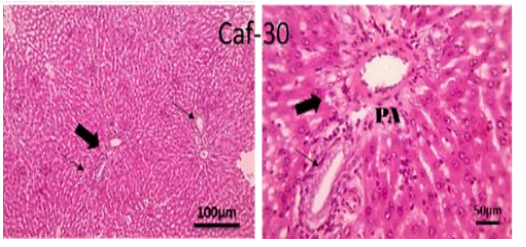

Photo (4): Treated group with 30 mg/CAF

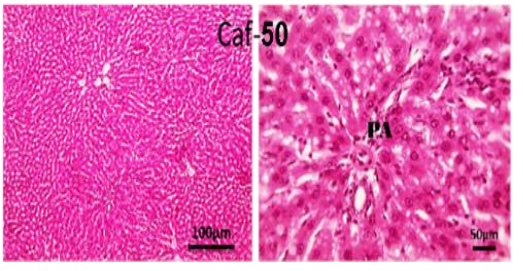

Photo (5): Treated group with 50 mg/CAF

Conclusion

This study showed that administration of caffeine at doses of 30 and 50 mg/kg body weight for 28 consecutive days significantly mitigated CCl_4-induced hepatotoxicity in rats. The treatment resulted in reduced liver enzyme activity and elevated serum albumin levels, suggesting a potential protective role against oxidative liver injury. Furthermore, it markedly decreased the serum lipid profile in hepatotoxic rats. The observed results suggest that CAF may offer protection against oxidative stress and

liver damage caused by CCl_4.These biochemical improvements were corroborated by histological analysis, which revealed considerable recovery of normal liver architecture in caffeine-treated groups. Further studies are needed to determine the exact molecular mechanisms and evaluate the clinical therapeutic potential of caffeine.

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التأثيرات المحتملة للكافيين على الفئران المصابة بتسمم الكبد الناتج عن رابع كلوريد الكربون

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الملخص :

يعتبر رابع كلوريد الكربون مادة سامة للكبد، تستخدم بشكل شائع لإحداث إصابة كبدية فى الحيوانات. ومن ثم، قد يكون لتناول مضادات الأكسدة دوراً مفيداً في علاج آثاره السُمية على الكبد. لذا هدفَت هذه الدراسة إلى تقييم تأثير الكافيين CAF كمضاد للأكسدة على الفئران المصابة بتسمم كبدى ناتج عن رابع كلوريد الكربونCCL4. تم تقسيم ثلاثين فأر من الذكورإلى مجموعتين رئيسيتين. كانت المجموعة الأولى (عددها =6)، المجموعة الضابطة السالبة تم تغذيتها على غذاء أساسي(BD) فى حين تلقت المجموعة الثانية (عددها = 24) جرعتين أسبوعيا منCCL4 بتركيز 2 ملجم لكل كجم من وزن الجسم لإحداث الإصابة الكبدية، ثم تم تقسيمها إلى أربع مجموعات فرعية. كانت المجموعة الفرعية الأولى بمثابة المجموعة الضابطة الموجبة التى تغذت فقط على الوجبة القياسية. أما المجموعات الفرعية 2، 3، 4 فقد تلقت غذاءً أساسيًا مع جرعات فموية يومية من الكافيين بتركيزات 10، 30 و 50 ملجم لكل كجم من وزن الجسم على التوالى. في نهاية فترة العلاج تم إجراء اختبارات لوظائف الكبد والكلى، وتحليل مكونات الدهون فى الدم، ومؤشرات أكسدة الدهون، والإنزيمات المضادة للأكسدة بالإضافة إلى الفحص الهستولوجى. أظهرت المعالجات اليومية بالكافيين بجرعتي 30 و50 ملجم لكل كجم من وزن الجسم انخفاضا ملحوظاً فى مستويات إنزيمات الكبد مقارنة بالمجموعة الضابطة الموجبة كما أدى ذلك إلى تحسن ملحوظ في مستويات الألبومين في السيرم. من ناحية أخرى، تسببت الجرعتان المذكورتان في انخفاض معنوى ($P\leq0.05$) في مؤشرات الدهون فى الدم لدي الفئران المصابة بالتسمم الكبدى بعد تغذيتها بالكافيين يوميًا لمدة أربعة أسابيع وقد كانت نسب الانخفاض كما يلي:16،33.8،23.5، 41.6، 47، 63.9، 33.8 و41.9 % بالنسبة للكوليسترول الكلى(TC)، الجلسريدات الثلاثية(TG)، الليبوبروتينات منخفضة الكثافة(LDL-c)، الليبوبروتينات منخفضة الكثافة جدا(VLDL-c) على التوالى. تشير هذه النتائج إلىأن الكافيين قد يحمي من الإجهاد التأكسدي وتلف الكبد الناتج عن CCL4 وذلك من خلال خصائصه المضادة للأكسدة .

الكلمات المفتاحية: سيرم الالبيومين، مؤشرات أكسدة الدهون، الإنزيمات المضادة للأكسدة، الجليسريدات الثلاثية.