Potential Effects of Caffeine on Hepatotoxic Rats Induced by Carbon Tetrachloride

Asmaa Gamal Nour El-Din[1], Rania Adel Eid[2], Heba Y. Nasef [3], Amira Hamdy Darweesh[4]

Abstract

Carbon tetrachloride (CCl_4) is a hepatotoxin; it is frequently employed to cause liver injury in animal models. Supplementing with antioxidants may therefore be beneficial in treating its hepatotoxic effects. Thus, this study's objective 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 control negative (-) was fed a basal diet (BD), and the second group n=24 received two weekly CCl_4 (٢ mg/kg bw) to cause hepatotoxicity, then separated into four subgroups. Subgroup 1 was served as a positive group (+) that received a BD only. Subgroups 2, 3, and 4 received BD and treated with an oral intake of 10, 30 and 50 mg CAF/kg/bw 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 30 and 50 mg/kg bw for 28 days exhibited a decrease in liver enzyme activities compared with control and 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 by 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. Our findings suggest that CAF might protect against oxidative stress and CCl_4-induced liver damage.

Keywords:

Serum albumin, Lipid peroxidation markers, Antioxidant enzymes, Triglycerides

(1,2,4) Nutrition and Food Science Dep., Faculty of Home Economic, Menoufia University, Egypt.
(3) Nutrition and Food Science Dep., Faculty of Home Economic, Helwan University, Egypt.

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 (**Amandeep *et al.*, 2021)**. The term "hepatotoxins" refers to the substances that harm the liver. Overdoses of specific pharmaceutical medications, industrial chemicals, natural compounds such as microcystins, herbal remedies, and dietary 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 an elevated bilirubin levels in the extracellular fluid, symptoms of hepatotoxicity can include jaundice or icterus, which causes skin yellowing, eyes, and mucous membranes; pruritus; severe abdominal pain; nausea or vomiting; weakness; extreme exhaustion; persistent bleeding; skin rashes; widespread itching; swelling of the feet or legs; abnormally high weight gain in a short period of 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 period of 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)**. Among the foods that include caffeine are tea, coffee, chocolate bars, cocoa beverages, and soft drinks. Surprisingly more than sixty different plants have a mass CAF content (**Panchal *et al*., 2012)**. For a variety of reasons, it has been shown that the consumption of CAF protects against liver Disease. **Wadhawan and Anand, (2016)** have examined the clinical data demonstrating the potential benefits of coffee drinking in the treatment of alcoholic liver disease, nonalcoholic fatty liver disease, hepatitis B and C, and similar conditions **(Wadhawan and Anand, 2016). 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. So, determining caffeine impact on CCl_4 hepatotoxicity was the primary goal of this investigation.

Materials and methods

Materials

Caffeine powder, CCl_4 and a component of basal diet 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. We bought TG, TC, HDL cholesterol, and LDL-c from El-Nasr Pharmaceutical Chemicals, a Cairo, Egypt-based company.

Animals

Thirty male white Sprague Dawley rats weighing 150± 10g on average were purchased from the Ministry of Health and Population's Helwan station in Cairo, Egypt. The individual rats were housed in stainless steel cages with enough ventilation and controlled standard conditions, including a 12-hour light-dark cycle, a temperature of 20–23 °C, and a humidity of 50–60%. The animals were given a week to acclimate before the tests started.

Methods

Diet components

The basal diet (BD) is prepared by **Reeves *et al.*, (1993)**. As follows: (69.5%) corn starch, (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.

Induction of liver-hepatotoxicity in rats

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 in order 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 (NRC), 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 basal diet for a week in order to acclimate them. Following a week, rats were divided into two main groups: group (1) (6 rats) (negative group) was fed basal diet and given the same amount of tap water as the other groups. The other main group (24 rats) was used for liver impairment and was injected 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 basal diet and was given the same quantity of tap water as the other groups. Groups (3-5) were fed basal diet and given oral CAF 10, 30, and 50 mg/kg 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

Blood samples were taken after a 12-hour fast, by the abdominal aorta at the conclusion of 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**.**

Biochemical assay

Assessment of liver and kidney functions

An autoanalyzer for biochemistry (Olympus AU2700, Japan) was used to evaluate the serum levels of ALT, AST, and ALP in order to estimate liver function. Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) were determined according to the method 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)**.

Total Protein (TP) and Albumin (Alb) serum determination

TP was calculated using bovine serum albumin (BSA) as a standard, as previously reported by **Lowry *et al.*, (1951).** As stated by **Spencer and Price (1977),** serum Alb was measured in g/dl.

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 detremined 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 one that was less than 0.05 ***Peng, (2008).***

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 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 hepato-toxic 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. **Afolabi *et al*., (2023)** showed that serum AST and ALT activity were significantly ($P \leq 0.05$) lower in the 50 mg/kg caffeine treatment group than in the positive control group. Consuming CAF has been associated with adecreased risk of fibrosis and a reduction in liver enzyme levels (**Modi *et al*., 2010)**. In numerous studies, coffee has been linked to lower levels of ALP, gamma-glutamyl transferase according to **Heath *et al*., (2017)**. Additionally, CAF (GGT), ALT, and AST 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}±4	84^{e}±2	194^{e}±2
Positive control	65.33^{a}±3.51	248.33^{a}±3	363.33^{a}±3.51
10 mg CAF	55^{b}±3	185^{b}±3	326.33^{b}±3.51
30 mg CAF	45^{c}±4	155.33^{c}±3.51	265^{c}±3
50 mg CAF	34.33^{d}±4.51	135^{d}±3	246.33^{d}±2.52
LSD	6.98	5.35	5.39

The formula used for mean values is means ± SD. When there are significant differences between treatments ($p \leq 0.05$), the means under the same column are indicated by a different letter. **CAF**: caffeine, **ALT**: alanine aminotransferase, **AST**: aspartate aminotransferase 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 ($p \leq 0.05$) 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 shown that urea and creatinine were significantly ($p \leq 0.05$) 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. These results were consistent with **Afolabi *et al.*, (2023)** who discovered that urea and creatinine levels were significantly ($p \leq 0.05$) lower in the group treated with 50 mg/kg of caffeine than in the positive control group.

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}±0.01	27^{d}±4
Positive control	1.09^{a}±0.1	74.66^{a}±4.5
10 mg CAF	0.96^{ab}±0.07	54.33^{b}±5.68
30 mg CAF	0.85^{bc}±0.07	47^{b}±4
50 mg CAF	0.73^{cd}±0.1	37.66^{c}±2.52
LSD	0.14	7.76

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.

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

normal and Table (3) illustrates the effects of caffeine on TP, ALB and GLB in hepatotoxic rats. The data Showed that the levels of TP, ALB and GLB in the positive control group significantly decreased ($p \leq 0.05$) following CCl_4 induction compared to the negative control group. In contrast, hepatotoxic rats given 10,30 and 50 mg CAF had significantly ($p \leq 0.05$) 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 significantly increased 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. **Afolabi *et al.*, (2023)** discovered that TP levels increased significantly ($p \leq 0.05$) after 50 mg/kg of caffeine treatment in comparison to the positive control group.

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
10 mg CAF	7.35^{c}±0.04	3.52^{c}±0.03	3.8٣^{ab}±0.09
30 mg CAF	7.45^{c}±0.03	3.74^{b}±0.04	3.٧١^{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 treatment ($p \leq 0.05$), 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 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 significantly reduced 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 match 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	$102^{a}\pm5$	$136^{a}\pm4$	$44^{b}\pm1$	$30.8^{a}\pm4.1$	$27.2^{a}\pm0.8$
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. These results were consistent with **Afolabi *et al.*, (2023)** who found that GSH and GPX levels significantly ($p \leq 0.05$) increased after receiving 50 mg/kg of caffeine in comparison to the positive control group. From the previous results, in hepatotoxic rats, CAF may be thought to provide protection 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	**$39.26^{a} \pm 1.1$**	**$65.23^{e} \pm 3.2$**	**$0.76^{d} \pm 0.05$**
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

Photo (1,2,3.4.5) 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 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.

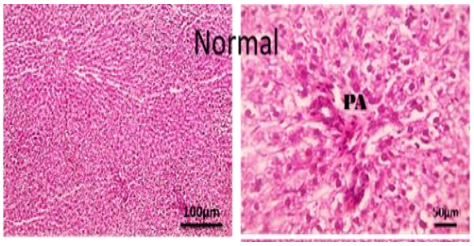

Photo (1): Negative control group

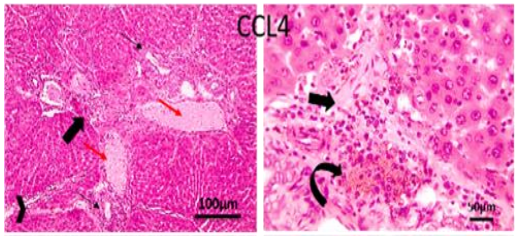

Photo (2): Positive control group

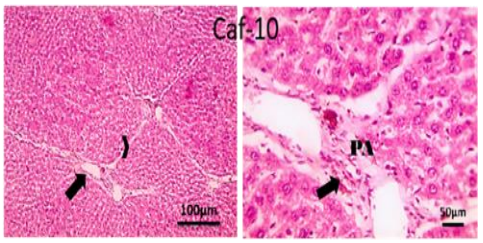

Photo (3): Treated group with 10 mg/caffeine

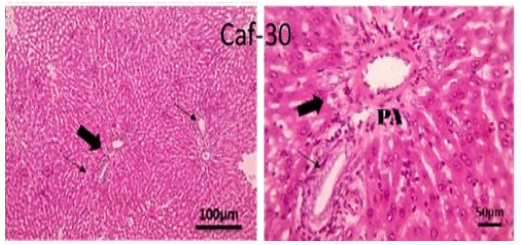

Photo (4): Treated group with 30 mg/caffeine

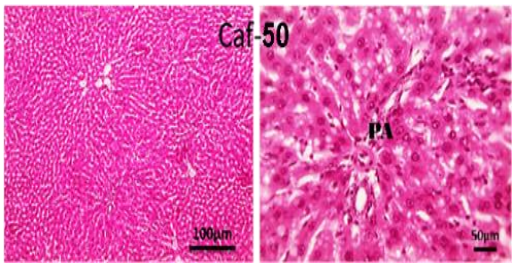

Photo (5): Treated group with 50 mg/caffeine

Conclusion

This study showed that giving rats caffeine in doses of 30 and 50 mg/kg bw for 28 days was able to lessen the CCl_4 induced hepatotoxicity in rats. Specifically, caffeine treatment caused a decrease in liver enzyme activities and an increase in serum albumin levels. Moreover, it considerably reduced the serum lipid profile in hepatotoxic rats. The observed results suggest that caffeine may offer protection against oxidative stress and liver damage caused by CCl_4.

References

Addai F. K. (2010): Natural cocoa as diet-mediated antimalarial prophylaxis, Medical Hypotheses. 74, no. 5, 825–830, https://doi.org/10.1016/j.mehy.2009.12.003, 2-s2.0-77950930027.

Adebayo, J. O.; Akinyinka, A. O.; Odewole, G. A. & Okwusidi, J. I. (2007): Effect of caffeine on the risk of coronary heart disease- A re-evaluation. *Indian Journal of Clinical Biochemistry*, *22*(1), 29–32.

Afolabi, O.B.; Olasehinde, O.R.; Olaoye, O.A.; Jaiyesimi, K.F.; Ekakitie, I.L. and Oloyede, O.I. (2023): Nephroprotective Effects of Caffeine, Vanillin, and Their Combination against Experimental AlCl3-Induced Renal Toxicity in Adult Male Wistar Rats.Hindawi, Biochemistry Research International. Article ID 6615863, 9 pages.

Allen, C.C. (1974): Cholesterol enzymatic colorimetric method. J. of Clin. Chem.; (20): 470.

Amandeep, S.; Sneha, J.; Ashima, J. and Devesh, T. (2021): Drug-induced hepatotoxicity, Tropical Journal of Natural Product Research, Pages 141-157.

Amer, M.G.; Mazen, N.F. and Mohamed, A.M. (2017): Caffeine intake decreases oxidative stress and inflammatory biomarkers in experimental liver diseases induced by thioacetamide: Biochemical and histological study. International Journal of Immunopathology and Pharmacology. Vol. 30(1) 13–24.

Basu, S. (2003): Carbon tetrachloride-induced lipid peroxidation: Eicosanoid formation and their regulation by antioxidant nutrients. Toxicology, 189(1-2): 113–127.

Birkner, E.; Grucka-Mamczar, E.; Zwirska-Korczala, K.; ZalejskaFiolka, J.; Stawiarska-Pieta, B.; Kasperczyk, S. and Kasperczyk, A. (2006): Influence of sodium fluoride and caffeine on the kidney function and free-radical processes in that organ in adult rats. Biological Trace Element Research, 109: 35-47.

Bona, S.; Filippin, L.I.; Di Naso, F.C.; David, C.D.; Valiatti, B.; Schaun, M.I.; Xavier, R.M. and Marroni, N.P. (2012): Effect of antioxidant treatment on fibrogenesis in rats with carbon tetrachloride induced cirrhosis. International Scholarly Research Network. ISRN Gastroenterology. Article ID 762920, 7 pages. 87, 159-169.

Chang, C.Y. and Schaino, T.D. (2007): Review article: Drug hepatoxicity . Aliment pharmacol.Ther 25: 1135-1152.

Deng, X.; Luyendyk, JP.; Ganey, P.E. and Roth, R.A. (2009): Inflammatory stress and idiosyncratic hepatoxicity: Hints from animal models. Pharmacol Rev 61: 262-282.

Drury, R.; Wallington, E.; and Cancerson, R. (1976): Histopathological techniques. 4th ed. Oxford/London, UK: Oxford University Press.

Duan, Z.R.S.; Dalal, U.; Dagati, V. and Kudose, S. (2023): Acute tubular injury in a patient with acetaminophen toxicity. Kidney International.

Fang, H. L. and Lin, W. C. (2008): Lipid peroxidation products do notactivate hepatic stellate cells. Toxicology, vol. 253, no. 1–3, pp.36–45.

Fassati, P. and Prencipe, L. (1982): Triglyceride enzymatic colorimetric method. J. of Clin. Chem., (28): 2077.

Gordillo-Bastidas, D.; Oceguera-Contreras, E. and Salazar-Montes, A. (2013): Nrf2 and Snail-1 in the prevention of experimental liver fibrosis by caffeine. World J Gastroenterol.; 19:9020.

Gulati, K.; Reshi, M.R.; Rai, N. and Ray, A. (2018): Hepatotoxicity: Its Mechanisms, Experimental Evaluation and Protective Strategies. American Journal of Pharmacology.; 1(1): 1004.

Guth, I.; Matos-Pardal, C.F.; Ferreira-Limaa, R.; Loureiro-Reboucas, R.; Sobral, A.C.; Moraes-Marques, C.A. and Kubrusly, L.F. (2022): Caffeine attenuates liver damage and improves neurologic signs in a rat model of hepatic encephalopathy. REVISTA DE GASTROENTEROLOGIA DE MEXICO 87. 159-169.

Heath, R.D.; Brahmbhatt, M.; Tahan, A.C.; Ibdah, J.A. and Tahan, V. (2017): Coffee: The magical bean for liver diseases. World J Hepatol;9(15): 689-696.

Henry, R.J. (1974): Clinical Chemistry Principal and Techniques. 2nd ed. Hagerston (MD), Harcer, Row, P. 882-885.

Huber, W.; Scharf, G. and Rossmanith, W. (2002): The coffee components kahweol and cafestol induce γ-glutamyl cysteine synthetase, the rate limiting enzyme of chemoprotective glutathione synthesis, in several organs of the rat. Archives of Toxicology 75: 685–694.

Jayasekhar, P.; Mohan, P.V. and Rahinam, K (1997): Hepatoprotective activity of ethylacetate extract of Acacia catechu. Indian. J. of Pharmacology, 29, 426-428.

Kim, M.; Kim, Y.; Lee, J.; Park, B.; Kim, M.; Choi, M. and Kim, A. (2008): The Effects of Caffeine on Lipid and Mineral Content in the Serum of Rats. *The Korean Journal of Food and Nutrition, 21*, 336-343.

Lee, R. and Nieman, D. (1996): National Assessment. 2nd Ed., Mosby, Missouri, USA.

Lopez, M.F. (1977): HDL- cholesterol colorimetric method. J. of Clin. Chem., (230: 282).

Lowry, O.; Roserbrough, N.; Farr, A. and Randall, R. (1951): Protein measurement with the Folin phenol reagent. J. Biol Chem, 193 (1) (1951), pp. 265-275.

Modi, A.A.; Feld, J.J. and Park, Y. (2010): Increased caffeine consumption is associated with reduced hepatic fibrosis. Hepatology.51:201-9.

Moron, M.S.; Despierre, J.W. and Minnervik, B. (1979): Levels of glutathione, glutathione reductase and glutathione-S-transferase activities in rat lung and liver. Biochimica et Biophysica Acta 582: 67e78.

N.C.B.I. (2020): National Center for Biotechnology Information, PubChem compound summary for CID 2519, Caffeine. **https://pubchem.ncbi.nlm.nih.gov/compound/Caffeine**.

N.R.C. (1996): National Research Council. Guide for the care and use oflaboratory animals. Washington DC: National Academy Press.

Ohkawa, H.; Ohishi, N. and Yagi, K. (1979): Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry 95: 351e358.

Osz, B.E.; Jîtca, G.; Ruxandra-Emilia, S.; Pus, T.; cas, A.; Tero-Vescan, A. and Vari, C.E. (2022): Caffeine and Its Antioxidant Properties—It Is All about Dose and Source. Int. J. Mol. Sci., 23, 13074.

Panchal, S. K.; Poudyal, H.; Waanders, J. and Brown, L. (2012): Coffee extract attenuates changes in cardiovascular and hepatic structure and function without decreasing obesity in high-carbohydrate, high-fat diet-fed male rats. The Journal of Nutrition, 142(4), 690–697.

Pasaoglu, H.; Demir, Fatma; Yilmaz-demirtas, C.; Hussein, A. and Pasaoglu, Ö. (2011): "The effect of caffeine on oxidative stress in liver and heart tissues of rats," Turkish Journal of Medical Sciences: 41: (4) 14. https://doi.org/10.3906/ sag-0911-4.

Patton. C.J. and Croush, S.R. (1977): Enzymatic Determination of Urea. J. Anal. Chem. 49: 464-469.

Peng, C. Y. J. (2008). Data analysis using SAS. Sage Publications.

Prasenjit, M.; Mahua, S. and Parames, C. S. (2006): Aqueous extract of Terminalia arjunaprevents carbon tetrachloride induced hepatic and renal disorders. BMC Complementary and Alternative Medicine,6:33.

Reeves, P. G.; Nielsen, F. H. and Fahey, G. C. (1993): AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition adhoc writing committee on the reformulation of the AIN- 76 A rodent diet. Journal of Nutrition, 123: 1939–1951.

Shan, L.; Wang, F.; Zhai, D.; Meng, X.; Liu, J. and Lv, X. (2022): Caffeine in liver diseases: Pharmacology and toxicology. *Frontiers in pharmacology*, *13*, 1030173.

Shim, S. G.; Jun, D. W.; Kim, E. K.; Saeed, W. K. and Yoon, B. C. (2013): Caffeine attenuates liver fibrosis via defective adhesion of hepatic stellate cells in cirrhotic model. *Journal of Gastroenterology and Hepatology*, 28(12), 1877-1884.

Shin, J. W.; Wang, J.H. and Kang, J. K. (2010): Experimental evidence for the protective effects of coffee against liver fibrosis in SD rats. J Sci Food Agric.; 90:450-5.

Singh, A.; Bhat, T.K. and Sharma, O.P. (2011): ClinicalBiochemi try of Hepatotoxicity.J Clinic Toxicol, S:4.

Singhal, G. and Gupta, D. (2012): Hepatoprotective and antioxidant activity of methanolic extract of flowers of Nerium oleander against CCl_4-induced liver injury in rats. Asian Pacific Journal of Tropical Medicine, 5(9): 677-685.

Spencer, K. and Price, C.P. (1977): Determination of serum albumin. Annals Clin.Biol,14:105.

Stroev and Makarova, (1989): Textbook of clinical chemistry. Carl A. Burtis. 3rd ed. Philadelphia, USA., WBSaunders.

Tietz, N.W (1976): "Fundamentals of Clinical Chemistry". W.B. Saunders, Philad and Elphia.

Wadhawan, M. and Anand, A. C. (2016): Coffee and liver disease. *Journal of Clinical and Experimental Hepatology*, 6 (1), 40–46.

Waring, W.S.; Stephen, A.F.L.; Robinson, O.D.G.; Dow, M.A. and Pettie, J.M. (2008): Serum urea concentration and the risk of hepatotoxicity after paracetamol overdose. Q J Med; 101:359–363.

Yang, L.; Wang, C.; Ye, Z. and Li, T. (2011): Hepatoprotective effects of polyprenols from Ginkgo biloba L. leaves on CCl_4-induced hepatotoxicity in rats. Fitoterapia, 82(6): 834-840.

Young, D.S. and Friedman, R.B. (2001): Effects of Disease on Clinical Laboratory. Testes, 4th ed. AACC press, Washington, USA.

الملخص العربي

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

أسماء جمال نور الدين[١] ،رانيا عادل عيد[٢] ،هبه يونس ناصف[٣] ،أميرة حمدى درويش[٤]

الملخص

يعتبر رابع كلوريد الكربون سماً كبدياً، وغالبا ما يستخدم لإحداث إصابة كبدية فى الحيوانات. لذا قد يكون من المفيد تناول مضادات الأكسدة فى علاج آثارها السامة على الكبد. لذلك كان الهدف من الدراسة هو تقييم تأثير الكافيين كمضاد للأكسدة على الفئران المصابة بتسمم الكبد الناتج عن رابع كلوريد الكربون. تم تقسيم ثلاثين فأر ذكر من نوع الألبينو إلى مجموعتين رئيسيتين. كانت المجموعة الأولى (٦ فئران) المجموعة الضابطة السالبة تتلقى نظاماً غذائياً أساسياً بينما تلقت المجموعة الثانية (٢٤ فأر) حقنتين من رابع كلوريد الكربون أسبوعيا بما تعادل 2ملج لكل كجم من وزن الجسم للتسبب فى تسمم الكبد، ثم تم تقسيمها إلى أربع مجموعات فرعية. كانت المجموعة الفرعية الأولى بمثابة المجموعة الضابطة الموجبة التى تغذت فقط على الوجبة القياسية. بينما تلقت المجموعات الفرعية ٢، ٣، ٤ الوجبة القياسية مع إضافة ١٠، 30، 50 ملجم كافيين لكل كجم من وزن الجسم يومياً. تم إجراء التحاليل الخاصة بوظائف الكبد والكلى، والدهون، وعلامات أكسدة الدهون، والإنزيمات المضادة للأكسدة، والفحص الهستولوجى فى نهاية التجربة. أظهر التدخل اليومى من الكافيين يوميا لمدة ٢٨ يوم بجرعات30، 50 ملجم لكل كجم من وزن الجسم انخفاضا ملحوظاً فى نشاط إنزيمات الكبد وذلك مقارنة بالمجموعة الضابطة الموجبة كما استعادت مستويات الألبيومين فى السيرم. من جهة أخرى، أدى التدخل اليومي من الكافيين فى بالجرعات السابقة إلى انخفاض ملحوظ فى الدهون للفئران المصابة بتسمم الكبد وذلك بمعدل ١٦،٢٣,٥، ٣٣,٨، ٤١,٦، ٤٧، ٦٣,٩، ٣٣,٨ و٤١,٩ % لكلا من الكوليسترول الكلى، الجلسريدات الثلاثية، الليبوبروتينات منخفضة الكثافة، الليبوبروتينات منخفضة الكثافة جدا على التوالى. أظهرت نتائجنا إلى أن الكافيين قد يحمي من الإجهاد التأكسدي وتلف الكبد الناتج عن رابع كلوريد الكربون.

الكلمات المفتاحية

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