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Therapeutic potential of caffeic acid and coumarin in modifying aluminum-induced hepatic injury in Wistar rats

Zehor CHOUARI^{1*}, Omar KHAROUBI¹, Khadidja KESSAS¹, Wafaa LOUNIS¹, Anne VEJUX^{2,3}, Abdelkader AOUES¹

¹University of Oran 1 Ahmed Ben Bella, Faculty of Natural and Life Sciences, Department of Biology, Laboratory of Experimental Biotoxicology, Biodepollution and Phytoremediation, Oran 31100, Algeria; chouarizhor33@gmail.com (*corresponding author); omarkharoubi@gmail.com; khadoujakess@gmail.com; wflns06@gmail.com; aoues_a@yahoo.fr ²University of Bourgogne Franche-Comté, Team 'Biochemistry of the Peroxisome, Inflammation and Lipid Metabolism' EA7270/Inserm, 21000 Dijon, France; Anne.Vejux@u-bourgogne.fr

³University of Burgundy, Center for Taste and Food Sciences, CNRS, INRAE, Institut Agro, Dijon, 21000, France

Abstract

Antioxidants and phenolic compounds have always been tested as chemoprotective agents to regulate disease progression related to oxidative stress. Through this study, we investigate the possible corrective effects of caffeic acid and coumarin against aluminum- (Al-) induced stress response and liver damage in rats. Male Wistar rats were divided into four groups (10 rats per group), cotreated with caffeic acid (30 mg kg⁻¹) and coumarin (5 mg kg⁻¹) and exposed to aluminum (60 mg kg⁻¹) for 45 days. Al induced significant alterations in body and liver weight over time. It also led to a marked increase in plasma levels of transaminases (aspartate aminotransferase and alanine aminotransferase), alkaline phosphatase, and lactate dehydrogenase. Additionally, there was a substantial change in the lipid profile, characterized by elevated levels of triglycerides and total cholesterol in both serum and liver. Furthermore, the LDL-c level and the HDL-c/total cholesterol ratio in serum were also affected. In contrast, the levels of HDL-c in serum and phospholipids in liver tissues were reduced. The results of this study also showed increased levels of TBARS, protein carbonyls, nitrates/nitrites, and TNF- α . Conversely, the activity of antioxidant enzymes (catalase, GP-x, and SOD) was reduced. These altered parameters were restored after treating rats with caffeic acid and coumarin. Our study strongly suggests that caffeic acid and coumarin possess antioxidant properties and hepatoprotective capacity by attenuating aluminum toxicity-induced liver damage.

Keywords: aluminum; caffeic acid; coumarin; hepatoprotective; oxidative stress

Introduction

Humans and animals interact daily with their surroundings and are exposed to a wide variety of chemical compounds and heavy metals that can bioaccumulate inside the body and collect in tissues with low excretion (Al Dera, 2016). These pollutants are recognized to participate in the pathogenesis of various illnesses in humans. Heavy metals like mercury (Hg), lead (Pb), and cadmium (Cd) were shown to induce

Received: 07 Oct 2023. Received in revised form: 10 Jan 2024. Accepted: 16 Feb 2024. Published online: 11 Mar 2024. From Volume 13, Issue 1, 2021, Notulae Scientia Biologicae journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers. neurodegeneration, carcinogenesis, and anemia (Lisiewicz, 1993; Waalkes and Rehm, 1994; Neal and Guilarte, 2010) and they were also implicated in many disorders, together with type 2 diabetes and weight problems (Desvergne *et al.*, 2009; Latini *et al.*, 2009). This metal is protected in the substance priority list of the Agency for Toxic Substances and Disease Registry (ATSDR) (Abdel Moneim *et al.*, 2013).

Various tissues were shown to accumulate Al over the years, even though the largest quantities of Al are seen in bone, liver, and, to a lesser extent, the brain (Xu *et al.*, 1990; Exley, 2001; Hellstrom *et al.*, 2008). Hepatocytes, the practical units of the liver, depend on a chain of complicated metabolic networks to hold energy homeostasis within the human body. Wang *et al.* (2018) concluded that Al accumulation in liver tissue causes hepatotoxicity, which can occur particularly through oxidative strain reactions.

Various herbal elements have been recommended for steel poisoning (Pari and Prasath, 2008; Lotfi-Ghahramanloo and Baghshani, 2016). In epidemiological and experimental investigations, antioxidants and phenolic compounds were historically explored as chemoprotective retailers to regulate disease progression associated with oxidative pressure. Around one-third of the polyphenolic compounds observed in flora are phenolic acids. The primary class of phenolic acids located in flowers are hydroxybenzoic and hydroxycinnamic acids, respectively. Caffeic acid is a prominent hydroxycinnamic acid that is commonly present in various foods such as fruits, vegetables, wine, olive oil, and coffee (Olthof et al., 2001). Studies in lab and sanatorium settings revealed the useful properties of caffeic acid and its derivatives, which include antibacterial (Magnani et al., 2014), antiatherosclerotic (Magnani et al., 2014; Tosovic, 2017), antioxidant (Genaro-Mattos et al., 2015; Rodrigues et al., 2015; Tosovic, 2017), anti-inflammatory (Genaro-Mattos et al., 2015; Rodrigues et al., 2015; Tosovic, 2017), immunostimulant (Kilani-Jaziri et al., 2017), antidiabetic (Genaro-Mattos et al., 2015; Rodrigues et al., 2015; Tosovic, 2017), cardioprotective (Agunloye et al., 2019), anticancer (Xie et al., 2017), hepatoprotective (Yang et al., 2013; Bispo et al., 2017) effects. Moreover, caffeic acid was found to protect the liver from nickel-induced lesions while reducing the peroxidation of tissue lipids and enzymes that mark hepatic lesions and enhancing the antioxidative mechanism (Pari and Prasath, 2008). Furthermore, the useful impact of caffeic acid on recuperation from chromium (Arivarasu et al., 2012) and lead-precipitated oxidative strain (Lotfi-Ghahramanloo and Baghshani, 2016) were suggested in experimental work.

Conversely, coumarin (1,2-benzopyrone) is also a natural antioxidant compound found in tonka beans, cassia, lavender, yellow candy clover, fruits (e.g., blueberry, cloudberry), inexperienced tea, and chicory (Egan *et al.*, 1990). Coumarin is isolated from many plants, including *Taraxacum officinale, Alchemilla speciosa*, and *Artemisia montana*. These plants have antioxidant, analgesic, and anti-inflammatory activities, with coumarin being their primary energetic element (Naghma *et al.*, 2004).

Biological effects of coumarin include antibacterial, antiviral, antimutagenic, and antioxidant activities, inhibition of lipoxygenase and polyoxygenase, inhibition of lipid peroxidation, and scavenging of superoxide hydroxyl radicals (Chang *et al.*, 1996; Hoult and Paya, 1996). Various studies confirmed that coumarin possesses strong antioxidant properties, which can be attributed to its ability to effectively scavenge free radicals and chelate metal ions (Tseng, 1991). To the best of our knowledge, the beneficial effects of coumarin on liver damage have not yet been investigated. Thus, the current study evaluates the possible beneficial effects of caffeic acid and coumarin on aluminum-prompted liver damage in Wistar rats.

Materials and Methods

Reagents and chemicals

Aluminum chloride (AlCl₃, 6H₂O), caffeic acid, coumarin, Folin–Ciocalteu reagent, hydrochloric acid (37%), sulphuric acid (H₂SO₄), sodium carbonate (Na₂CO₃), pyrogallol, bovine serum albumin (BSA),

thiobarbituric acid (TBA), trichloroacetic acid (TCA), and 2,4-dinitrophenylhydrazine (DNPH), and all used chemicals were of analytical grade and purchased from Sigma-Aldrich Chemical Co. (St. Louis, France).

Animals and experimental design

A total of 40 male Wistar rats, four weeks old, weighing 70 \pm 10 g, were used in this study. Our experiment was conducted after authorization from the Institutional Ethics Committee for Animal Research (agreement number 47/DGLPAG/DVA, SDA 14). It was also approved by the university's scientific committee. Animals were housed in cages, fed *ad libitum*, exposed to a 12 h light/12 h dark cycle, and room temperature was maintained at 22 \pm 2 °C. Animals were divided into four groups of 10 animals each as follows:

- Group 1 (control) served as an untreated control and received an intraperitoneal injection of 0.9% saline (NaCl).
- Group 2 (Al) consisted of Al-intoxicated rats that received a dose of 60 mg kg⁻¹ body weight (BW) of Al chloride once a week.
- Group 3 (Al-CA) was the caffeic acid-treated group, which received a dose of 30 mg kg⁻¹ BW by gavage three times a week simultaneously with an intraperitoneal injection of 60 mg kg⁻¹ BW of Al chloride once a week.
- Group 4 (Al-Coum) was the coumarin-treated group that received a dose of 5 mg kg⁻¹ BW by gavage three times per week simultaneously with an intraperitoneal injection of 60 mg kg⁻¹ BW of Al once a week.

The current study selected the dose and mode of preparation and administration of chemical agents based on published literature and the method used in our laboratory (Kharoubi *et al.*, 2016; Lahouel *et al.*, 2020). All groups were treated under the same housing conditions for a period of 45 days.

Sample collection

At the conclusion of the experimental period, 24 hours after the final administration, the rats were euthanized under anesthesia using an intraperitoneal injection of a 10% chloral solution (Pentobarbital) at a dosage of 3 ml kg¹ of BW. The animals were weighed before being sacrificed; then, after the sacrifice, their blood was collected and centrifuged to obtain the serum for the biochemical assays and the liver was quickly removed, rinsed with a saline solution (0.9% NaCl), then weighed and saved at -80 °C till use. A portion of the liver was preserved in 37% formalin diluted to 1/10th to generate histological sections. The organ weight ratios were envisioned and their relative weights were calculated in g 100 g⁻¹ BW. Later, the liver became homogenized with 10 volumes of phosphate buffer (0.1 mol l⁻¹, pH 7.4) and centrifuged at 3000 × g for 10 min at 4 °C and the resulting supernatant was centrifuged at 10,000 × g for 10 min at 4 °C. The final supernatant was separated, after which it was used for the estimation of the biochemical parameters.

Lipids extraction and analysis

Total hepatic lipids were extracted with chloroform: methanol (2:1/V:V) according to the method of Folch *et al.* (1957). Then, 1 g of liver tissue was homogenized in 20 mL of extraction solvent and stored overnight at + 4 °C; after filtration, the first extract was obtained. This step was repeated three times to homogenize the residue completely and extract the lipids. The three extracts were combined, and the volume was measured. The lipid extract was stored at +4 °C for subsequent biochemical analysis.

Biochemical assessment

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (γ GT), lactate dehydrogenase (LDH), and triglycerides were estimated using diagnostic kits (ChronoLab, Spain). Total, HDL, and LDL cholesterol levels were measured using the

Spinreact Kit according to the manufacturer's protocol. Concurrently, the phospholipid content was measured according to the method described by Zhou and Gilbert (1992).

Protein estimation

Protein was measured using the method of Lowry *et al.* (1951) with BSA as a standard, and necessary dilutions were obtained to get the correct concentrations of the proteins present in tissues.

Lipid peroxidation (TBARS)

LPO levels in liver homogenates were estimated by measuring malondialdehyde (MDA) formed using substances reactive with thiobarbituric acid, according to the spectrophotometric method of Ohkawa *et al.* (1979). Briefly, 0.2 ml of supernatant prepared from homogenized tissues was added to 0.2 ml of sodium dodecyl sulfate, 1.5 ml of acetic acid, and 1.5 mL of thiobarbituric acid. After making up the volume with 4 ml of distilled water, the samples were heated in a boiling water bath for 60 min; then, the samples were cooled and centrifuged at 4000 rpm for 10 min. Absorbance was measured at 532 nm. The amount of MDA was calculated using a molar extinction coefficient of 1.56×10^5 mol l⁻¹ cm⁻¹.

Measurement of protein carbonyls

The oxidative protein damage was determined using the method of Levine *et al.* (1990). Briefly, 500 μ l of 10 mM 2,4-dinitrophenylhydrazine (DNPH), dissolved in 2.5 M HCl, was added to 200 μ l of the sample and then incubated for 1 h at room temperature in the dark. Samples were vortexed every 15 min. After adding 500 μ l of 20% TCA, the tubes were centrifuged for 3 min at 11000 rpm to collect the protein precipitates. Three washes were performed using 1 ml ethanol-ethyl acetate (1:1 v/v). The pellets were dissolved in 600 μ l of 6 M guanidine hydrochloride and left for 10 min at 37 °C and then centrifuged for 3 min at 11000 rpm. The absorbance of the samples was recorded at 370 nm. The protein carbonyl content was expressed as nmol mg⁻¹ of protein.

Catalase activity (CAT)

CAT was measured using Aebi's method (1994). The decomposition rate of H_2O_2 was followed by monitoring the absorption at 420 nm. Briefly, 250 ml of liver homogenates and 250 mL of 0.03 mol l^{-1} H_2O_2 (prepared in phosphate buffer, 0.066 mol l^{-1} , pH 7.0) were added to a cuvette. After incubation for 5 min, TiOSO₄ was added to the mixture and the absorbance was directly measured against phosphate buffer as a blank, and one unit of CAT equals 1 mmol of degraded H_2O_2 mg⁻¹ protein.

Determination of superoxide dismutase (SOD) activity

The Marklund and Marklund (1974) assay technique was performed in a buffer (50 mM Tris-HCl and 10 mM EDTA) at pH 8.2, with a fraction of brain homogenate and pyrogallol (15 mM) and the change in absorbance was followed for three minutes at a wavelength of 440 nm. Results are expressed as U of SOD mg⁻¹ of protein.

Glutathione peroxidase activity (GSH-Px)

Glutathione peroxidase (GSH-Px) activity in liver and kidney tissues was assessed using the method of Rotruck *et al.* (1973). Briefly, the reaction mixture contained 0.2 ml of Tris-HCl buffer (0.4 mol l^{-1} , pH 7.0), 0.2 ml of reduced GSH (1 mmol l^{-1}), 0.1 ml of sodium azide (10 mmol/L), 0.1 mL of H₂O₂ (1 mmol l^{-1}), and 0.2 ml of tissue sample. After incubation at 37 °C for 10 min, the reaction was stopped by adding 0.4 ml of 10% trichloroacetic acid and the tubes were subjected to centrifugation at 2400 rpm for 10 min. The supernatant (0.2 ml) was then added 0.1 ml of Ellman's reagent (0.019 × 8 g of DTNB prepared in 0.1% sodium citrate). Absorbance was recorded at 340 nm.

Nitrite oxide (NO)

The accumulation of nitrite in the supernatant, an indicator of nitric oxide (NO) production, was determined by a colorimetric assay using Greiss reagent (0.1% N-(1-naphthyl) ethylene diamine dihydrochloride, 1% sulfanilamide, and 2.5% phosphoric acid), as described by Green *et al.* (1982). Equal volumes of supernatant and Greiss reagent were mixed, the mixture was incubated for 10 min at room temperature in the dark, and the absorbance was determined at 540 nm spectrophotometrically. The concentration of nitrite in the supernatant was determined based on the sodium nitrite standard curve and expressed as mmol per mg protein.

Evaluation of the tissue proinflammatory marker TNF-a:

Tumor necrosis factor alpha (TNF- α) levels in liver tissue were measured using a Rat ELISA kit (RAB0479 Sigma-Aldrich) according to the manufacturer's instructions. The results were interpolated from the standard curve derived from the TNF- α standards provided by the manufacturer.

Statistical analysis

Data were expressed as mean \pm standard deviation (SD). Differences between means were analysed with a one-way analysis of variance (ANOVA), followed by Tukey's post hoc test using SPSS version 25. A P-value level of less than 0.05 was considered significant.

Results

Effect of treatment on body weight and liver

The present study reports the variations in BW, liver weight, and relative liver weight of rats subjected to different treatments (Table 1). Notably, Al exposure led to a statistically significant reduction in final BW and absolute tissue weight of the liver (P < 0.001), with a decline of 15.93% and 15.69%, respectively, compared to the control group. However, concomitant administration of caffeic acid and coumarin along with aluminum resulted in a modest improvement, with a significant change (P < 0.05) in BW of 7.36% noted solely in the Al-Coum group. No significant difference was observed in relative liver weight among the experimental groups.

Parameters	Groups			
	Control	Al	Al-CA	Al-Coum
Initial body weight (g)	71.57 ± 6.04	69.90 ± 4.91	68.77 ± 6.31	73.82 ± 5.07
Final body weight (g)	202.98 ± 13.72	170.65 ± 11.54 ***	178.38 ± 6.31	184.21 ± 8.57 #
Absolute liver weight (g)	5.86 ± 0.63	4.94 ± 0.55 ***	5.065 ± 0.692	4.96 ± 0.32
Relative liver weight (g 100 g ⁻¹ of body weight)	2.94 ± 0.09	2.71 ± 0.17	2.86 ± 0.37	2.78 ± 0.18

Table 1. Effects of aluminum chloride and treatment on body weight and absolute and relative liver weight

Values are represented as mean \pm SD for each group. ***: P < 0.001 compared with the control group; #: P < 0.05 compared with the Al group; ANOVA: one-way analysis of variance.

Effect of treatment on biochemical analysis

The mean values of biochemical indicators of liver function for the control and experimental groups are presented in Table 2. Al treatment induced a statistically significant increase (P < 0.001) in AST, ALT, PAL,

and LDH levels compared to the control group, increasing them by 62.44%, 43.83%, 62.71%, and 57.79%, respectively. No significant alteration was noted in the activity levels of γ GT in this group. In contrast, oral administration of caffeic acid and coumarin displayed therapeutic potential by significantly reducing (P < 0.05) the levels of these parameters, except for ALT activity in the Al-Coum group and γ GT activity in the Al-CA group compared to the intoxicated group.

Parameters	Groups			
	Control	Al	Al-CA	Al-Coum
AST (U l ⁻¹)	16.66 ± 6.59	29.66 ± 6.08 ***	21.66 ± 1.36 #	17.52 ± 2.23 ###
ALT (U l ⁻¹)	10.33 ± 1.03	27.5 ± 2.672	13 ± 2.36 ###	25.5 ± 4.81
PAL (U l ⁻¹)	11 ± 1.095	29.5 ± 6.02	17 ± 3.28 #	18.5 ± 7.12 #
γGT (U l ⁻¹)	13 ± 5.44	18 ± 8.55	12.66 ± 1.86	6 ± 2.68 #
LDH (U l ⁻¹)	117.33 ± 26.85	278 ± 24.09 ***	140.33 ± 16.50 ###	186.66 ± 32.79 ###

Table 2. Effects of aluminum chlorid	e and treatment on serum	biochemical parameters
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Values are represented as mean \pm SD for each group. ***: P < 0.001 compared with the control group; #: P < 0.05; ###: P < 0.001 compared with the Al group; ANOVA: one-way analysis of variance.

Effect of treatment on lipid profile

The determination of the lipid panel of the different treatment groups (Table 3) indicates that the serum levels of cholesterol, triglycerides, LDL-c, and total chol/HDL-c ratio were significantly higher in the rats intoxicated with aluminum by 40.96%, 37.93%, 54.16%, and 59.13%, respectively, compared with rats in the control group and other treatment groups. However, a significant decrease was observed in the Al-CA and Al-Coum groups compared to the Al-only group. Conversely, the HDL-c level was reduced in the Al group by 40.96%, but no significant difference was recorded in the treated groups.

Table 3. Effects of aluminum chloride and treatment on serum concentrations of total cholesterol (T Chol), triglycerides (TG), high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), and the ratio of T Chol/HDL-c

Parameters	Groups			
	Control	Al	Al-CA	Al-Coum
Triglycerides	0.26 ± 0.05	0.58 ± 0.03	0.44 ± 0.01	0.42 ± 0.02
$(g l^{-1})$	0.36 ± 0.05	***	###	###
Total cholesterol (g	0 40 + 0 07	0.83 ± 0.13	0.56 ± 0.086	0.62 ± 0.14
l^{-1})	0.49 ± 0.07	***	##	#
LDL-c	0.11 ± 0.008	0.24 ± 0.04	0.2 ± 0.032	0.18 ± 0.05
$(g l^{-1}l)$	0.11 ± 0.008	***	0.2 ± 0.032	
HDL-c	0.32 ± 0.02	0.22 ± 0.02	0.24 ± 0.03	0.23 ± 0.03
$(g l^{-1})$		***	0.24 ± 0.03	0.23 ± 0.03
Total		3.72 ± 0.93	236 ± 0.66	273 ± 0.74
cholesterol/HDL-c	1.52 ± 0.14	5.7 <u>2</u> <u>1</u> 0.75 ***	2.30 ± 0.00	2.75±0.74
ratio			$\pi\pi$	$\pi\pi$

Values are represented as mean \pm SD for each group. ***:P < 0.001 compared with the control group; #: P < 0.05; ##: P < 0.01; ###: P < 0.001 compared with the Al group; ANOVA: one-way analysis of variance.

Furthermore, in liver tissue, we recorded an increase in the level of total cholesterol and triglycerides of 32.24% and 36.25%, respectively, and a reduction of 38.02% in the concentration of phospholipids in rats

intoxicated by aluminum compared to those in the control group rats. The treatment with caffeic acid and coumarin was able to normalize total cholesterol and phospholipid levels. Concerning the hepatic concentration of triglycerides, no significant change was observed.

Parameters	Groups			
	Control	Al	Al-CA	Al-Coum
Total cholesterol	64.99 ± 11.51	05.02 ± 22.69	53.5 ± 25.54 ##	35.1 ± 25.88
$(mg dl^{-1})$		93.92 ± 22.08		###
Triglycerides	1.6 ± 0.57	2.51 ± 0.39	2.24 ± 0.54	214 ± 0.46
(g l ⁻¹)	1.0 ± 0.97	**	2.24 ± 0.94	2.11 ± 0.10
Phospholipides (µg	152.21 ± 9.96	95.02 ± 16.34	$122.82 \pm 2/127$	142.84 ± 8.86
mg ⁻¹ of protein)	$1, j, j, l \pm 0.00$	***	122.05 ± 24.57	###

Table 4. Effect of aluminum chloride and treatment on concentrations of total cholesterol (T Chol), triglycerides (TG), and phospholipids (PL) in liver tissue

Values are represented as mean \pm SD for each group. **: P < 0.01; ***: P < 0.001 compared with the control group; ##: P < 0.01; ###: P < 0.001 compared with the Al group; ANOVA: one-way analysis of variance.

Effects of treatment on the lipid peroxidation and carbonyl levels

Figures 1 and 2 demonstrate the variations in TBARS and carbonyl levels. A significant (P < 0.001) increase of 66.4% and 66.71% was observed in the levels of TBARS and carbonyls, respectively, in the intoxicated rats compared to the controls. Treatment with caffeic acid and coumarin decreased TBARS production in both the Al-CA and Al-Coum groups, with a reduction of 16.25% and 26.07%, respectively. Treatment with these compounds also resulted in a significant decline in carbonyl levels, with the Al-CA and Al-Coum groups exhibiting a reduction of 24.25% and 32.16%, respectively, compared to the intoxicated rats. Caffeic acid resulted in fewer effective outcomes for restoring normal values of modified parameters than coumarin.



Figure 1. Effect of aluminum chloride and treatment on TBARS level (nmol mg⁻¹ of protein) Values are represented as mean \pm SD for each group. ***:P < 0.001 compared with the control group; #:P < 0.05; ###: P < 0.001 compared with the Al group; ANOVA: one-way analysis of variance.



Figure 2. Effect of aluminum chloride and treatment on carbonyl level (nmol mg⁻¹ of protein) Values are represented as mean ± SD for each group. ***:P < 0.001 compared with the control group; ##: P < 0.01; ###: P < 0.001 compared with the Al group; ANOVA: one-way analysis of variance.

Effects of treatment on the antioxidant parameters

The impact of Al exposure on hepatic redox status was assessed in this study, and the results are shown in Figures 3, 4, 5, and 6. CAT, SOD, and GPx levels were significantly decreased by 33.15%, 44.20%, and 61.80%, respectively, in the Al-exposed group compared to the control group. Moreover, NO concentrations increased significantly ($P \le 0.001$; Figure 6). Conversely, treatment with caffeic acid and coumarin during exposure to Al increased CAT, GPx, and SOD levels. GP-x and SOD activities were significantly increased by 42.01% and 71.31% in the Al-CA group, respectively, and 22.58% and 65.23% in the Al-Coum group, respectively, compared to those in the Al-treated group. The Al-CA and Al-Coum groups showed a significant ($P \le 0.001$) reduction in NO content (56.60%, 42.49%) compared with the Al group.



Figure 3. Effect of aluminum chloride and treatment on catalase activity (mmol mg^{-1} of protein) Values are represented as mean \pm SD for each group. **:P < 0.01 compared with control group; ANOVA: one-way analysis of variance.



Figure 4. Effect of aluminum chloride and treatment on SOD Activity (U mg⁻¹ of protein) Values are represented as mean \pm SD for each group. ***: P < 0.001 compared with control group; #: P < 0.05; ###: P < 0.001 compared with the Al group; ANOVA: one-way analysis of variance.



Figure 5. Effect of aluminum chloride and treatment on GP-x activity (μ mol min⁻¹ mg⁻¹ of protein) Values are represented as mean \pm SD for each group. ***: P < 0.001 compared with the control group; ###: P < 0.001 compared with the Al group; ANOVA: one-way analysis of variance.



Figure 6. Effect of aluminum chloride and treatment on NO level (μ mol mg⁻¹ of protein) Values are represented as mean \pm SD for each group. ***: P < 0.001 compared with the control group; ###: P < 0.001 compared with the Al group; ANOVA: one-way analysis of variance.

Effects of treatment on TNF-a level

Aluminum administration resulted in a highly significant (P < 0.001) increase in TNF- α levels by 80.45% in the intoxicated group compared to the control group. However, pretreatment with caffeic acid and coumarin significantly (P < 0.001) reduced the changes in TNF- α levels induced by aluminum (58.94% and 36.99%, respectively).

Parameters	Gropus			
	Control	Al	Al-CA	Al-Coum
TNF- α (pg ml ⁻¹)	103.05 ± 15.19	527.32 ± 68.1	216.56 ± 98.61 ###	332.24 ± 41.17 ###

Table 5. Effect of aluminum chloride and treatment on TNF- α levels

Values are represented as mean \pm SD for each group. ***:P < 0.001 compared with control group; ###: P<0.001 compared with the Al group; ANOVA: one-way analysis of variance.

Discussion

The liver plays a central role in the metabolic processing of xenobiotics, including aluminum, which can cause hepatic dysfunction through degeneration, inflammation, or necrosis of hepatocytes when accumulated (Belaïd-Nouira *et al.*, 2013). Therefore, the present study aims to investigate whether caffeic acid and coumarin could protect liver tissue from aluminum-induced toxicity. Our results revealed that compared to the control group, exposure to aluminum led to changes in BW and liver weight, suggesting a potentially adverse effect of aluminum on weight (Table 1), possibly resulting from the interaction of aluminum with hormonal status and/or protein synthesis. These findings are in line with those of previous research (El-Demerdash *et al.*, 2022), which reported that AlCl₃ administration decreased body and liver weight. Similar results were also reported by Balgoon (2019). The reduction in absolute liver weight after Al administration in rats could be due to hepatocyte inflammation, which may be associated with Al metabolism or direct toxicity. However, our study revealed that rats treated with caffeic acid and coumarin exhibited improved final BW and absolute liver

weight; this is in agreement with the results of Oršolic *et al.* (2021), where caffeic acid treatment altered BW changes in diabetic mice.

In this study, all the Al-intoxicated rats displayed typical hepatotoxicity patterns, as evidenced by elevated serum levels of AST, ALT, ALP, γ GT, and LDH, which are important biomarkers for indicating hepatocellular damage (Chen *et al.*, 2019). These results suggest that hepatocytes might have been altered in their transport function and membrane permeability or that enzymes leaked from the cell into the bloodstream. The study's findings are consistent with those of recent research that has evidenced significant plasma elevations of these enzyme activities in animals consuming aluminum chronically owing to severe membrane damage (Balgoon, 2019; Othman *et al.*, 2020; El-Demerdash *et al.*, 2022). AST and ALT, known as transaminases, are critical enzymes for biological processes. Their increased levels were consistent with those in previous research demonstrating an association between exposure to Al and liver necrosis attributed to possible binding of Al to DNA or RNA, thereby inhibiting the function of critical enzymes such as hexokinase, acids, alkalines, phosphatases, and phosphodiesterases (Ochmanski and Barabasz, 2000). ALP, a membrane-bound enzyme, is responsible for transporting multiple metabolites and is thus considered a biomarker for pathologies. A decrease in its activity may indicate the impairment of cellular metabolic processes and interfere with its transport function (Shakoori *et al.*, 1994).

The results of our study showed that exposure to Al caused a significant increase in the plasma activity of ALP, potentially due to increased membrane permeability or severe damage to the liver tissue membrane (Cheng *et al.*, 2014; Al-Qhtani and Farran, 2017). We also found that LDH activity was significantly increased in AlCl3-intoxicated rats, which is in agreement with the findings of prior research conducted by Cheraghi and Roshanaei (2019) and El-Demerdash *et al.* (2022). This effect may result from cellular impairment, leading to disturbances in carbohydrate and protein metabolism and energy depletion (Sivakumari *et al.*, 1997). More importantly, treatment with caffeic acid and coumarin significantly improved Al-induced liver lesions in rats. These findings are consistent with prior research demonstrating the hepatoprotective activities of caffeic acid in rodents (Olayinka *et al.*, 2017; Vanderson *et al.*, 2017) and confirm that caffeic acid can maintain normal architectural structure and hepatocyte integrity, preventing the leakage of serum marker enzymes (Olayinka *et al.*, 2017). In contrast, Belgin *et al.* (2011) concluded that coumarin (1,2-benzopyrone) has limited hepatoprotective effects against CCl₄-induced liver damage, as it only slightly decreased serum enzyme levels compared to other coumarin derivatives.

The administration of aluminum causes considerable disturbances in lipid metabolism (lipoprotein fractions) in rats. These disturbances present with hypertriglyceridemia and hypercholesterolemia at the serum and tissue level, which may indicate a loss of membrane integrity, a disturbance in lipid metabolism and/or hepatic dysfunction (Sarin *et al.*, 1997). Concomitant with our findings are several studies that reported that Al induced abnormal activities of lipase enzymes, which appear to be one of the main factors responsible for increasing serum cholesterol after AlCl₃ administration (Ghorbel *et al.*, 2017). In liver tissue, phospholipids were found to be reduced following Al intoxication; this result is in agreement with that of Belmokhtar *et al.* (2020). Regarding the effect of caffeic acid and coumarin treatment on the lipid profile, it was observed in this study that these compounds were able to reduce the metal-induced elevation of the lipid profile, which could be attributed to their possible inhibitory effect on endogenous lipid synthesis.

Moreover, we suggest that the toxic effects associated with aluminum exposure may be due to the generation of reactive oxygen species (ROS), leading to lesions via oxidation of cellular macromolecules such as lipids and proteins and ultimately triggering DNA damage (Al Olayan *et al.*, 2020; Mokrane *et al.*, 2020). In rats exposed to Al, we observed disturbances in their antioxidant status, which manifested as a significant increase in TBARS and carbonyl levels. These results are in line with previous findings, where the consumption of Al salts promotes lipid peroxidation and protein oxidation (El-Kholy *et al.*, 2010; Othman *et al.*, 2020; El-Demerdash *et al.*, 2022).

The presence of polyunsaturated fatty acid tails on the phospholipidic components of biological membranes is responsible for their fluidity, as reported by Catala (2015). Excessive free radicals promote lipid peroxidation, leading to the formation of a range of complexes, including MDA, reactive carbonyl compounds, and lipid peroxides (Valko et al., 2006; Ayala, 2014). These effects are accompanied by a reduction in antioxidant enzyme activities, including catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx), which form the front line of defense against oxidative stress and protect cellular biomolecules from oxidative damage. These findings demonstrate the role of free radicals in the oxidative damage induced by Al toxicity. Our observations are consistent with earlier research that has shown that Al uptake stimulates oxidative stress and inhibits antioxidant enzyme activity in hepatic tissues (Mokrane et al., 2020; Othman et al., 2020; El-Demerdash et al., 2022). The inhibition of antioxidant enzyme activity may arise due to the reduced biosynthesis of these enzymes underpinning higher intracellular levels of Al and/or the overproduction of free radicals (Nampoothiri et al., 2015). The estimation of nitrite is an indirect technique to evaluate NO levels. Thus, the high nitrite levels suggest elevated NO levels in the liver. Our findings show a high nitrite level in Al-exposed animals compared to that in the control group, confirming other research results (Mokrane et al., 2020; Othman et al., 2020). NO reacts readily with superoxide radicals to form peroxynitrite, capable of causing cellular damage by instigating lipid peroxidation and protein adduct formation that contribute to cell damage (Kumar et al., 2013). Studies on hepatoprotection highlighted the antioxidant activity of phenolic compounds against free radicals in living systems, protecting against oxidative stress and liver damage in rats (Maheswari et al., 2008).

Our findings suggest that prior treatment with caffeic acid and coumarin restores the balance of oxidant and antioxidant molecules. This is indicated by the levels of TBARS, carbonyls, and NO, as well as the increased activities of antioxidant enzymes (CAT, SOD, GP-x) in the Al-CA and Al-Coum groups compared to the Al group. These outcomes are consistent with those of previous reports by Bilgin *et al.* (2011), Rajarajeswari and Pari (2011), Olayinka *et al.* (2017), and Manju and Jagadeesan (2019), which showed that caffeic acid and coumarin can scavenge free radicals and enhance the activities of antioxidant enzymes by decreasing oxidative stress. Particularly, Rajarajeswari and Pari (2011) found that coumarin restores peroxidative lipid markers to normal levels in diabetic rats, and Chang *et al.* (1996) demonstrated its inhibition of lipoxygenase activity and lipid peroxidation and trapping of superoxide and hydroxyl radicals. Furthermore, caffeic acid was found to scavenge free radicals and attenuate lipid peroxidation, possibly due to its antiradical activity. These findings are consistent with those of other studies showing that Al ingestion elevates tissue TNF- α (Othman *et al.*, 2020; El-Demerdash *et al.*, 2022), potentially disrupting the prooxidant/antioxidant balance. However, TNF- α levels were reversed in the groups treated with caffeic acid and coumarin, consistent with the results of Mallik *et al.* (2016), who demonstrated that caffeic acid can decrease lipopolysaccharide-induced neuroinflammation.

Conclusions

The experimental results demonstrated that exposure to aluminum chloride leads to impaired liver function through oxidative damage, disruption of the antioxidant defense system, and alterations in the biomarkers of liver function. Conversely, pretreatment with caffeic acid and coumarin was found to restore liver function and provide protection against the toxic and detrimental effects of aluminum.

Authors' Contributions

OK, AA, and AA proposed and conceived the project and designed the experiments. ZC, KK, and WL performed the experiments. OK and ZC analyzed the data and contributed reagents, materials, and analytical

tools. ZC and AA wrote the manuscript. All authors read, revised, and approved the manuscript before submission. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Our experiment was conducted following approval from the Institutional Ethics Committee for Animal Research (agreement number 45/DGLPAG/DVA, SDA 14).

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

- Abdel Moneim AE, Othman MS, Mohmoud SM, El-Deib KM (2013). Pomegranate peel attenuates aluminum induced hepatorenal toxicity. Toxicology Mechanisms and Methods 23(8):624-633. https://doi.org/10.3109/15376516.2013.823634
- Aebi H (1994). Catalase in vitro. Methods Enzymology 105:121-126. https://doi.org/10.1016/s0076-6879(84)05016-3
- Agunloye OM, Oboh G, Ademiluyi AO, Ademosun AO, Akindahunsi AA, Oyagbemi AA (2019). Cardio-protective and antioxidant properties of caffeic acid and chlorogenic acid: mechanistic role of angiotensin converting enzyme, cholinesterase and arginase activities in cyclosporine induced hypertensive rats. Biomedicine and Pharmacology 109:450-458. https://doi.org/10.1016/j.biopha.2018.10.044
- Al Dera HS (2016). Protective effect of resveratrol against aluminum chloride induced nephrotoxicity in rats. Saudi Medical Journal 37(4):369-378. https://doi.org/10.15537%2Fsmj.2016.4.13611
- Al Olayan EM, Aloufi AS, Al Amri OD, El-Habit OH, Abdel Moneim AE (2020). Protocatechuic acid mitigates cadmium-induced neurotoxicity in rats: role of oxidative stress, inflammation and apoptosis. Science of the Total Environment 723. *https://doi.org/10.1016/j.scitotenv.2020.137969*
- Al-Qhtani, SA, Farran SK (2017). The Protective and therapeutic effect of resveratrol in improving renal and hepatic failure induced by aluminum chloride in experimental animals. Journal of American Science 13(10). http://dx.doi.org/10.7537/marsjas131017.03
- Arivarasu NA, Priyamvada S, Mahmood R (2012). Caffeic acid inhibits chromium (VI)-induced oxidative stress and changes in brush border membrane enzymes in rat intestine. Biological Trace Element Research 148:209-215. https://doi.org/10.1007/s12011-012-9349-1
- Ayala J (2014). Targeting mitochondrial reactive oxygen species as novel therapy for inflammatory diseases and cancers. Journal of Hematology and Oncology 6:19. *https://doi.org/10.1186/1756-8722-6-19*
- Balgoon MJ (2019). Assessment of the protective effect of Lepidium sativum against aluminum induced liver and kidney effects in albino rat. BioMed Research International 2019:9. *https://doi.org/10.1155/2019/4516730*
- Belaïd-Nouira Y, Bakhta H, Haouas Z, Flehi-Slim I, Ben Cheikh H (2013). Fenugreek seeds reduce aluminum toxicity associated with renal failure in rats. Nutrition Research and Practice 7(6):466-474. https://doi.org/10.4162/nrp.2013.7.6.466
- Belmokhtar S, Kharoubi O, Dida N, Bouakline HE, Benglia A, Benyettou I, Benahmed F, Aoues A (2020). Antihypertensive activity and lipid-lowering effect of tree wormwood (Artemisia arborescens) on rats intoxicated

with Aluminium chloride. South Asian Journal of Experimental Biology 10(2):58-69. https://doi.org/10.38150/sajeb.10(2).p58-69

- Bilgin HM, Atmaca M, Obay BD, O"zekinci S, Tas-demir E, Ketani A (2011). Protective effects of coumarin and coumarin derivatives against carbon tetrachloride-induced acute hepatotoxicity in rats. Experimental and Toxicologic Pathology 63:325-330. https://doi.org/10.1016/j.etp.2010.02.006
- Bispo VS, Dantas LS, Chaves Filho AB, Pinto IFD, Silva Rp DA, Otsuka FAM (2017). Reduction of the DNA damages, hepatoprotective effect and antioxidant potential of the coconut water, ascorbic and caffeic acids in oxidative stress mediated by ethanol. Anais Academia Brasileira de Ciencias 89:1095-1109. https://doi.org/10.1590/0001-3765201720160581
- Catala A (2015). Lipid peroxidation modifies the assembly of biological membranes. The Lipid Whisker Model. Frontiers in Physiology 5:520. https://doi.org/10.3389%2Ffphys.2014.00520
- Chang WS, Lin CC, Chuang SC, Chiang HC (1996). Superoxide anion scavenging effect of coumarins. American Journal of Chinese Medicine 24:11-17. *https://doi.org/10.1142/s0192415x96000037*
- Chen C, Lin B, Qi S, He J, Zheng H (2019). Protective effects of salidroside on lead acetate-induced oxidative stress and hepatotoxicity in Sprague-Dawley rats. Biological Trace Element Research 191(2):426-434. https://doi.org/10.1007/s12011-019-1635-8
- Cheng D, Zhu C, Wang C, Xu H, Cao J, Jiang W (2014). Hepatoprotective effects of apple polyphenol extract on aluminum induced liver oxidative stress in the rat. Canadian Journal of Physiology and Pharmacology 92: 109-116. https://doi.org/10.1139/cjpp-2013-0366
- Cheraghi E, Roshanaei K (2019). The protective effect of curcumin against aluminum chloride-induced oxidative stress and hepatotoxicity in rats. Pharmaceutical Biomedical Research 5(1):6-13. http://dx.doi.org/10.18502/pbr.v5i1.761
- Desvergne B, Feige JN, Casals-Casas C (2009). PPAR-mediated activity of phthalates: a link to the obesity epidemic. Molecular and Cellular Endocrinology 304:43-48. *https://doi.org/10.1016/j.mce.2009.02.017*
- Egan DA, Kennedy R, moran E, Thornes RD (1990). The pharmacology, metabolism, analysis and applications of coumarin and coumarin related compounds. Drug Metabolism Reviews 22:503-529. *Https://doi.org/10.3109/03602539008991449*
- El-Demerdash FM, Hussien DM, Ghanem NF, AL-Farga AM (2022). Bromelain modulates liver injury, hematological, molecular, and biochemical perturbations induced by aluminum via oxidative stress inhibition. BioMed Research International Volume 2022, 5342559: 12 pages. https://doi.org/10.1155/2022/5342559
- El-Kholy WM, EL-Habibi EM, Mousa AT (2010). Oxidative stress in brains of rats intoxicated with aluminum and the neuromodulating effect of different forms of sage. Journal of American Science 6 (12): 1462-1474.
- Exley C (2001). Aluminum and alzheimer's disease. Journal of Alzheimer's Disease 3:551-552. https://doi.org/10.3233/jad-2001-3605
- Folch J, Lees M, Sloane Stanley GH (1957) A simple method for the isolation and purification of total lipides from animal tissues. The Journal of Biological Chemistry 226(1):497-509. *http://dx.doi.org/10.1016/S0021-9258(18)64849-5*
- Genaro-Mattos TC, Maurício ÂQ, Rettori D, Alonso A, Hermes-Lima M (2015). Antioxidant activity of caffeic acid against ironinduced free radical generation-A chemical approach. PLoS one 10(6). https://doi.org/10.1371/journal.pone.0129963
- Ghorbel I, Elwej A, Chaabane M, Jamoussi K, Zeghal N (2017). Protective effect of selenium against aluminium chloride induced cardiotoxicity in rats. Pharmaceutical and Biomedical Research 3(2):19-25. http://dx.doi.org/10.29252/pbr.3.2.19
- Green LC, Wagner DA, Glgowski J, Skipper PL, Wishnok JS, Tannebaum SR (1982). Analysis of nitrate, nitrite and (¹⁵N) nitrate in biological fluids. Analitical Biochemistry 126:131-135. *https://doi.org/10.1016/0003-2697(82)90118-x*
- Hellstrom HO, Michaelsson K, Mallmin H, Mjoberg B (2008). The aluminium content of bone, and mortality risk. Age Ageing 37:217-220. *https://doi.org/10.1093/ageing/afm152*
- Hoult JR, Paya M (1996). Pharmacological and biochemical actions of simple coumarins: natural products with therapeutic potential. General Pharmacology 27:713-722. *https://doi.org/10.1016/0306-3623(95)02112-4*

- Kharoubi O, Benyamina A, Hallal N, Benyattou I, Aoues A, Slimani M (2016). Aluminum induced the oxidative stress modification and behavioral variation in rat. International Journal of Preventive Medicine Research 2(1):1-7.
- Kilani-Jaziri S, Mokdad-Bzeouich I, Krifa M, Nasr N, Ghedira K, Chekir-Ghedira L (2017). Immunomodulatory and cellular antioxidant activities of caffeic, ferulic, and p-coumaric phenolic acids: a structure-activity relationship study. Drug and Chemical Toxicology 40:416-424. https://doi.org/10.1080/01480545.2016.1252919
- Kumar A, Lalitha S, Mishra J (2013). Possible nitric oxide mechanism in the protective effect of hesperidin against pentylenetetrazole (PTZ)-induced kindling and associated cognitive dysfunction in mice. Epilepsy and Behavor 29:103-111. https://doi.org/10.1016/j.yebeb.2013.06.007
- Lahouel Z, Kharoubi O, Boussadia A, Bekkouche Z, Aoues A (2020). Effect of Aluminium and Aqueous extract of *Rosmarinus officinalis* on rat Brain: Impact on Neurobehavioral and Histological study. Journal of Drug Delivery & Therapeutics 10 (4):179-187. http://dx.doi.org/10.22270/jddt.v10i4.4252
- Latini G, Marcovecchio ML, Del Vecchio A, Gallo F, Bertino E, Chiarelli F (2009). Influence of environment on insulin sensitivity. Environment International 35:987-993. https://doi.org/10.1016/j.envint.2009.03.008
- Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz AG, et al (1990). Determination of carbonyl content in oxidatively modified proteins. Methods in Enzymology 186:464-478. https://doi.org/10.1016/0076-6879(90)86141-h
- Lisiewicz J (1993). Immunotoxic and hematotoxic effects of occupational exposures. Folia Medica Cracoviensia 34:29-47.
- Lotfi-Ghahramanloo M, Baghshani H (2016). Ameliorative effects of caffeic acid on lead accumulation and oxidative stress in leadexposed mice. Zahedan Journal of Research in Medical Science 18(5):e6674. http://dx.doi.org/10.17795/zjrms-6674
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951). Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry 193(1):265-275. https://pubmed.ncbi.nlm.nih.gov/14907713/
- Magnani C, Isaac VLB, Correa MA, Salgado HRN (2014). Caffeic acid: a review of its potential use in medications and cosmetics. Analytical methods 6:3203-3210. *http://dx.doi.org/10.1039/c3ay41807c*
- Maheswari C, Maryammal R, Venkatanarayanan R (2008). Hepatoprotective activity of "Orthosiphonstami-neus" on liver damage caused by paracetamol in rats. Jordan Journal of Biological Sciences 1:105-108.
- Mallik SB, Mudgal J, Nampoothiri M, Hall S, Anoopkumar Dukie S, Grant G, Rao CM, Arora D (2016). Caffeic acid attenuates lipopolysaccharide-induced sickness behaviour and neuroinflammation in mice. Neuroscience Letters 6(632):218-223. https://doi.org/10.1016/j.neulet.2016.08.044
- Manju M, Jagadeesan G (2019). *In vivo* hepatoprotective effect of caffeic acid on mercuric chloride-induced biochemical changes in albino Wistar rats. Asian Journal of Pharmaceutical and Clinical Research 12(4):119-124. http://dx.doi.org/10.22159/ajpcr.2019.v12i4.31609
- Marklund S, Marklund G (1974). Involvement of the superoxide anion radical in the autooxidation of pyrogallol and a convenient assay for superoxide dismutase. European Journal of Biochemistry 47:469-474. https://doi.org/10.1111/j.1432-1033.1974.tb03714.x
- Mokrane N, Kharoubi O, Tahari FZ, Guenzet A, Aoues A (2020). The effect of *Thymus vulgaris* L. on renal and liver toxicity in wistar rats exposed to aluminum. Journal of Medicinal Plants Research 14(1):13-23. https://doi.org/10.5897/JMPR2019.6819
- Naghma K, Sonia S, Sarwat S (2004). Amelioration of ferric nitrilotriacetate (Fe-NTA) induced renel oxidative stress and tumor promotion response by coumarin (1,2 benzopyrone) in Wistar rats. Cancer Letters 210:17-26. https://doi.org/10.1016/j.canlet.2004.01.011
- Nampoothiri M, John J, Kumar N, Mudgal J, Nampurath GK, Chamallamudi MR (2015). Modulatory role of simvastatin against aluminium chloride-induced behavioural and biochemical changes in rats. Behavioural Neurology vol 2015:1-9. https://doi.org/10.1155/2015/210169
- Neal AP, Guilarte TR (2010). Molecular neurobiology of lead (Pb⁽²⁺⁾): effects on synaptic function. Molecular Neurobiology 42:151-160. *https://doi.org/10.1007/s12035-010-8146-0*
- Ochmanski W, Barabasz W (2000). Aluminum occurrence and toxicity for organisms. Przeglad lekarski 57:665-668.
- Ohkawa H, Ohishi N, Yagi K (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry 95:351-358. *https://doi.org/10.1016/0003-2697(79)90738-3*

- Olayinka ET, Ola SO, Ore A, Adeyemo OA (2017). Ameliorative effect of caffeic acid on capecitabine-induced hepatic and renal dysfunction: involvement of the antioxidant defence system. Medicines 4:78. https://doi.org/10.3390%2Fmedicines4040078
- Olthof RM, Hollman HCP, Katan BM (2001). Chlorogenic acid and caffeic acid are absorbed in humans. The Journal of Nutrition 13:66-71. *https://doi.org/10.1093/jn/131.1.66*
- Oršolic N, Sirovina D, Odeh D, Gajski G, Balta V, Šver L, Jazvinš 'c Jembrek M (2021). Efficacy of caffeic acid on diabetes and its complications in the mouse. Molecules 26:3262. https://doi.org/10.3390/molecules26113262
- Othman MS Fareid MA, Abdel Hameed RS, Abdel Moneim AE (2020). The protective effects of melatonin on aluminum-induced hepatotoxicity and nephrotoxicity in rats. Oxidative Medicine and Cellular Longevity 1-12. https://doi.org/10.1155/2020/7375136
- Pari L, Prasath A (2008). Efficacy of caffeic acid in preventing nickel induced oxidative damage in liver of rats. Chemico-Biological Interactions 173:77-83. https://doi.org/10.1016/j.cbi.2008.02.010
- Rajarajeswari N, Pari L (2011). Antioxidant role of coumarin on streptozotocin–nicotinamide-induced type 2 diabetic rats. Journal Biochemical and Molecular Toxicology 25:6. *https://doi.org/10.1002/jbt.20395*
- Rodrigues JL, Araújo RG, Prather KLJ, Kluskens LD, Rodrigues LR (2015). Heterologous production of caffeic acid from tyrosine in Escherichia coli. Enzyme and Microbial Technology 71:36-44. https://doi.org/10.1016/j.enzmictec.2015.01.001
- Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG (1973). Selenium: biochemical role as a component of glutathione peroxidase. Science 179:588-90. *https://doi.org/10.1126/science.179.4073.588*
- Sarin S, Gupta V, Gill KD (1997). Alterations in lipid composition and neuronal injury in primates following chronic Aluminum exposure. Biological Trace Elem Research 59:133-143. *https://doi.org/10.1007/bf02783238*
- Shakoori AR, Butt U, Riffat R, Aziz F (1994). Hematological and biochemical effects of danitol administered for two months on the blood and liver of rabbits. Zeitschrift Fuer Angewandte Zoologie 80:165-180.
- Sivakumari K, Manavalaramanujam R, Ramesh M, Lakshmi R (1997). Cypermethrin toxicity: sublethal effects on enzyme activities in a freshwater fish, Cyprinus carpio var. communis. Journal of Environmental Biology 18:121-125.
- Tosovic J (2017). Spectroscopic features of caffeic acid: theoretical study. Kragujevac Journal Science 39:99-108. http://dx.doi.org/10.5937/KgJSci1739099T
- Tseng A (1991). Chemoprevention of tumors in MTV-H ras transgenic mice with coumarin. Proceedings for the American Association for Cancer 32:2257.
- Valko M, Rhodes C, Moncol J, Izakovic MM, Mazur M (2006). Free radicals, metals and antioxidants in oxidative stressinduced cancer. Chemico-Biological Interactions 160:1-40. https://doi.org/10.1016/j.cbi.2005.12.009
- Vanderson SB, Lucas SD, Adriano BC, Isabella FDP, Railmara PDS, Felipe AMO (2017). Reduction of the DNA damages, Hepatoprotective effect and antioxidant potential of the coconut water, ascorbic and caffeic acids in oxidative stress mediated by ethanol. Anais da Academia Brasileira de Ciências 89(2):1095-1109. https://doi.org/10.1590/0001-3765201720160581
- Waalkes MP, Rehm S (1994). Cadmium and prostate cancer. Journal of Toxicology and Environmental Health 43:251-269. https://doi.org/10.1080/15287399409531920
- Wang X, Gong J, Gui Z, Hu T, Xu X (2018). Halloysite nanotubes-induced Al accumulation and oxidative damage in liver of mice after 30-day repeated oral administration. Environmental Toxicology 33(6):623-630. https://doi.org/10.1002/tox.22543
- Xie J, Yang F, Zhang M, Lam C, Qiao Y, Xiao J (2017). Antiproliferative activity and SARs of caffeic acid esters with mono-substituted phenylethanols moiety. Bioorganic and Medicinal Chemistry Letters 27:131-134. https://doi.org/10.1016/j.bmcl.2016.12.007
- Xu ZX, Fox L, Melethil S, Winberg L, Badr M (1990). Mechanism of aluminum-induced inhibition of hepatic glycolysis: inactivation of phosphofructokinase. Journal of Pharmacology and Experimental Therapeutics 254:301-305.
- Yang SY, Hong CO, Lee GP, Kim CT, Lee KW (2013). The hepatoprotection of caffeic acid and rosmarinic acid, major compounds of Perilla frutescens, against t-BHP-induced oxidative liver damage. Food and Chemical Toxicology 55:92-99. https://doi.org/10.1016/j.fct.2012.12.042
- Zhou X, Gilbert A (1992). Improved procedures for the determination of lipid phosphorus by malachite green. Journal of Lipid Research 33(8):1233-1236. *https://doi.org/10.1016/S0022-2275(20)40776-X*



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