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Protective effects of chlorogenic acid against glyphosate-induced organ and blood toxicity in Wistar rats

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Glyphosate, a widely used herbicide against broadleaf weeds and grasses, has been associated with various harmful effects. Our study examines the efficacy of chlorogenic acid (CGA) in alleviating the toxicity of a glyphosate-based herbicide (GBH) in 42 Wistar rats across six groups of seven animals receiving either no treatment (control), CGA alone (50 mg/kg), GBH alone (800 mg/kg), or their combinations varying three CGA doses (12.5, 25, or 50 mg/kg) (CGA12.5+GBH, CGA25+GBH, and CGA50+GBH, respectively) by oral gavage over 49 days in a row. At the end of the experiment, samples of blood, brain, heart, liver, and kidney tissues were collected and analysed for oxidative stress indicators (MDA, GSH, SOD, CAT), oxidative DNA damage (8-OHdG), liver and kidney function markers (AST, ALT, ALP, urea, and creatinine) as well as for histopathological changes. As expected, GBH increased AST ALT, ALP, urea, creatinine, 8-OHdG, and MDA levels, and lowered GSH levels and SOD and CAT activities, leaving histopathological changes in the brain, heart, liver, and kidney tissues. CGA dose-dependently improved biochemical and oxidative stress parameters and reversed histopathological changes in GBH-treated albino rats. Our findings consistently confirm the potential of CGA as a promising natural agent against the adverse health effects associated with exposure to glyphosate. Future research should focus on long-term glyphosate exposure and CGA treatment using molecular methods and on the signalling pathways associated with oxidative stress.

KEY WORDS: 8-OHdG; ALP; ALT; AST; blood; brain; CAT; CGA; creatinine; DNA damage; GSH; heart; herbicides; histopathology; kidney; liver; MDA; oxidative stress; SOD; urea

Glyphosate-based herbicides (GBHs) are a class of broad-spectrum herbicides that contain glyphosate as the active ingredient. They are applied extensively in agriculture, horticulture, and urban landscaping (1). Consequently, glyphosate residues have been increasingly detected in animal feed (2).

Glyphosate exerts its toxic effects primarily by disrupting metabolic pathways, the shikimic acid pathway in particular, essential for the synthesis of aromatic amino acids in plants and certain microorganisms (3). This disruption may result in oxidative stress as well as inflammation in exposed organisms, including mammals. The resulting oxidative damage can manifest itself as increased lipid peroxidation levels, decreased antioxidant enzyme activity, and histopathological changes in vital organs (4, 5). In addition, glyphosate has been reported to increase the levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG), a biomarker of oxidative DNA damage associated with carcinogenesis (6–8).

Emerging evidence suggests that certain natural compounds may have the potential to mitigate the harmful effects of herbicides (9, 10). One such compound is chlorogenic acid (CGA), a polyphenol found in various plants, including coffee, apples, and berries. Many *in vivo* and *in vitro* studies have demonstrated its protective effects against various chemicals, fungal and bacterial toxins, pharmaceuticals, metals, and pesticides, as it lowers nitric oxide and reactive oxygen species (ROS) production and inhibit pro-apoptotic signalling pathways (11, 12).

To the best of our knowledge, however, no evidence has been produced so far to show that CGA can protect against glyphosate-based herbicides. The aim of our study was to address this gap in knowledge by determining whether CGA could alleviate oxidative stress and DNA damage induced by GBH exposure.

MATERIALS AND METHODS

Chemicals

We used a Knock-out® glyphosate formulation marketed in Turkey by its producer Hektaş (Gebze, Turkey). The active ingredient in this herbicide is the 48 % isopropylamine salt of glyphosate. We selected this brand due to its popularity in controlling weeds resistant to other high glyphosate herbicides.

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CGA (Cat. No. C3878, purity ≥95 %) was acquired from Sigma (Sigma-Aldrich, Shanghai, China).

For 8-hydroxy-2'-deoxyguanosine (8-OHdG) determination we used the ELISA kit from the Shanghai Sunred Biological Technology (Shanghai, China, Cat. No. 201-11-0032).

All other reagents and chemicals mentioned below were of analytical grade. GBH and CGA were diluted in distilled water.

Animals and experimental design

For the experiment we used 42 adult (10–12 weeks old) male Wistar rats, weighing 200–250 g, acquired from the Experimental Research and Application Centre at Afyon Kocatepe University in Afyonkarahisar, Turkey. The animals were housed in polypropylene cages under standard laboratory conditions of 55±5 % relative humidity, 22±2 °C room temperature, and 12-hour light/dark cycle, with free access to food pellets and tap water.

The animals were randomly divided into six groups of seven rats to receive distilled water or the following daily doses of CGA, GBH, or their combination by gavage for 49 days in a row. The control group received 0.5 mL of distilled water (otherwise used as solvent for CGA). The CGA50 group received 50 mg/kg of CGA dissolved in 0.5 mL of distilled water. The GBH group received 800 mg/kg of GBH (one tenth of their LD_{50}) dissolved in 0.5 mL of distilled water. The CGA12.5+GBH group received 12.5 mg/kg CGA one hour before receiving 800 mg/kg of GBH. The CGA25+GBH group received 25 mg/kg of CGA as described for the previous group. The CGA50+GBH group received 50 mg/kg of CGA as described for the previous group.

The dosage of GBH administered to the animals was determined by Turkmen and Dogan (13), whereas the dosage of CGA was taken from Qi et al. (14).

The study protocol was approved by the Ethics Committee for Animal Experiments of the Afyon Kocatepe University (approval No. 2018-49533702/65).

Blood collection and preparation of erythrocyte lysate

On day 50, we took blood from the animals under mild sevoflurane anaesthesia (1–3 % concentration in oxygen) by cardiopuncture into heparinised and non-heparinised tubes. Blood was centrifuged at 600 *g* and 4 °C for 15 min within 30 min of blood collection to precipitate the erythrocytes and extract plasma and serum. Serum was used for biochemical measurements of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), urea, creatinine, malondialdehyde (MDA), and reduced glutathione (GSH), while plasma was used to measure 8-OHdG.

After separating the buffy coat, we washed erythrocytes three times in isotonic saline, placed them into vials with an equal volume of isotonic saline, and stored at approximately -20 °C for 3 days. At the end of the storage period, we obtained the erythrocyte lysate for analysis by inducing haemolysis. To ensure complete cell lysis, a repeated freeze-thaw process was applied. This approach allowed for the preparation of a homogeneous lysate for subsequent analyses. To prevent the erythrocyte suspensions from being disrupted due to osmotic pressure, we added five times the volume of cold deionised water. This ratio ensured controlled haemolysis of the cells, allowing for the preparation of a homogeneous lysate suitable for enzymatic analyses.

The erythrocyte lysate was then stored at 4 °C for 3 days before measuring SOD and CAT activity. This storage period was chosen based on previous studies (15), which found it sufficient to maintain enzyme stability and ensure accurate activity measurements.

Homogenate preparation

The brain, heart, liver, and kidney were promptly dissected, rinsed with ice-cold 0.9 % NaCl solution, and homogenised in a homogeniser (VWR International GmbH, Darmstadt, Germany) using 1.15 % potassium chloride buffer to obtain a 1:10 (w/v) homogenate. Homogenates were then centrifuged at 600 *g* and 4 °C for 15 min for the analysis of MDA, SOD, and CAT. To quantify GSH concentration in homogenates, the samples were centrifuged at 4900 *g* and 4 °C for 20 min.

Preparation of tissues for histopathological examination

Tissue specimens were preserved in 10 % neutral buffered formalin, dehydrated using a graded alcohol series, and embedded in paraffin. Sections were 5 μm thick, stained with haematoxylin and eosin (H&E), and analysed under a light microscope (Olympus BX51 with DP20 Microscopic Digital Image Analysis System, Tokyo, Japan) by a blinded pathologist.

Semi-quantitative scoring of brain, heart, liver, and kidney lesions followed the methodology outlined by Gibson-Corley et al. (16).

Measurement of serum biochemical parameters

Serum levels of AST, ALT, ALP, urea, and creatinine were determined with the COBAS test kits (Roche Diagnostics Systems, Istanbul, Turkey) following the manufacturer's instructions (17).

Measurement of MDA in serum and tissue homogenates

Serum MDA was determined using the method described by Yoshioka et al. (18), while tissue homogenate MDA was determined using the method described by Ohkawa et al. (19). Both methods rely on the spectrophotometry of the colour produced by the reaction between MDA and thiobarbituric acid (TBA) at 532 nm on a Shimadzu 1601 UV-VIS spectrophotometer (Shimadzu, Tokyo, Japan). MDA concentration was determined using the absorbance coefficient of the MDA-TBA complex and the results are reported in nmol/mL of blood or nmol/g of tissue.

Measurement of GSH in serum and tissue homogenates

GSH was quantified in serum and tissue homogenates using the method described by Sedlak and Lindsay (20). Optical density was measured spectrophotometrically (Shimadzu 1601 UV-VIS) at 412 nm and the results are reported as nmol/mL of blood or nmol/g of tissue.

Measurement of SOD activity in erythrocyte lysate and tissue homogenates

SOD in erythrocyte lysate and tissue homogenates was measured as described by Sun et al. (21). The absorbance of blue formazan obtained from the reduction of nitro blue tetrazolium (NBT) by superoxide radicals was determined spectrophotometrically (Shimadzu 1601 UV-VIS) at 560 nm. SOD activity is expressed as U/g of haemoglobin (Hb) for erythrocytes or U/g of protein for tissue.

Measurement of CAT activity in erythrocyte lysate and tissue homogenates

CAT activity in erythrocyte lysates and tissue homogenates was determined as described by Aebi (22). One unit of CAT activity corresponds to the amount of enzyme that decomposes 1 μmol of H_2O_2 per minute at pH 4.5 and 25 °C. CAT activity (k; nmol/min) was expressed as catalase/g of Hb for erythrocytes or catalase/g of protein for tissue.

Plasma 8-OHdG measurement

Plasma 8-OHdG levels were measured using a competitive enzyme-linked immunosorbent assay (ELISA) kit and a Multiskan Go microplate spectrophotometer (Thermo Scientific, Waltham, MA, USA), in accordance with the Sunred ELISA kit protocols for rat 8-OHdG (Cat. No. 201-11-0032) assay. The absorbance was measured at 450 nm and the 8-OHdG concentration quantified using a standard curve and reported in ng/mL.

Measurement of haemoglobin and protein concentrations

Hb was quantified in erythrocyte lysates using the cyanmethaemoglobin method as described by Drabkin and Austin (23). The protein concentration of the sample in tissue homogenates was evaluated using the colourimetric method established by Lowry et al. (24).

Statistical analysis

The sample size calculated with a power test using G*Power 3.1 software (Heinrich Heine University, Düsseldorf, Germany) to meet one-way ANOVA or Kruskal-Wallis test requirements for six independent groups. Based on the calculation, the required sample size was 114 animals, assuming an effect size of 0.4, an error probability (alpha) of 0.05, and a power (1-beta) of 0.9. However, due to practical constraints, including limitations in time, resources, and animal availability, a smaller sample size of 42 animals was used in this study. It is recommended to use the smallest number of animals possible in studies, in accordance with ethical principles outlined by the guidelines for animal research, such as those provided by the National Institutes of Health (NIH) and local ethical review boards. Since the sample size obtained with the power test was very large, similar studies were used to determine the sample size (25, 26). Despite the reduced sample size, statistical analyses were conducted with careful consideration of the limitations, and we believe that the findings are still meaningful and provide useful insights under the given conditions.

For statistical analysis of the data we used the SPSS version 20.0 (SPSS, Chicago, IL, USA) software package. The normality of distribution was established for all data with the Shapiro-Wilk test, and one-way ANOVA used for further analysis. In cases where differences were statistically significant ($p<0.05$), we used the post-hoc Duncan test to determine which groups were different. The results are presented as means ± standard deviations (SD). The level of significance was set at $p<0.05$.

RESULTS

Effects of CGA on serum biochemical parameters

AST, ALT, ALP, urea, and creatinine were higher in the GBH group than control ($p<0.05$), but CGA pre-treatment lowered them in a dose-dependent manner. There were no significant changes in the CGA alone group (Figure 1A–E).

Effects of CGA on GBH-induced lipid peroxidation

GBH significantly increased MDA levels in the serum, brain, heart, liver, and kidney tissues relative to the control and CGA50 group ($p<0.05$), but CGA pre-treatment lowered them in a dose-dependent manner, restoring them to normal (control) with the highest, 50 mg/kg dose (Table 1).

Antioxidant enzyme activities and GSH levels

A similar pattern was observed with the antioxidant enzyme activities. GBH alone significantly lowered them ($p<0.05$), whereas CGA pre-treatment increased them in a dose-dependent manner. No significant change was observed in the CGA50 group compared to the control (Tables 2–4).

8-OHdG levels

8-OHdG levels significantly increased in rats receiving GBH alone compared to control ($p<0.05$), but CGA pre-treatment lowered them, although never restoring them to control values. CGA alone treatment did not change them significantly compared to control (Figure 2).

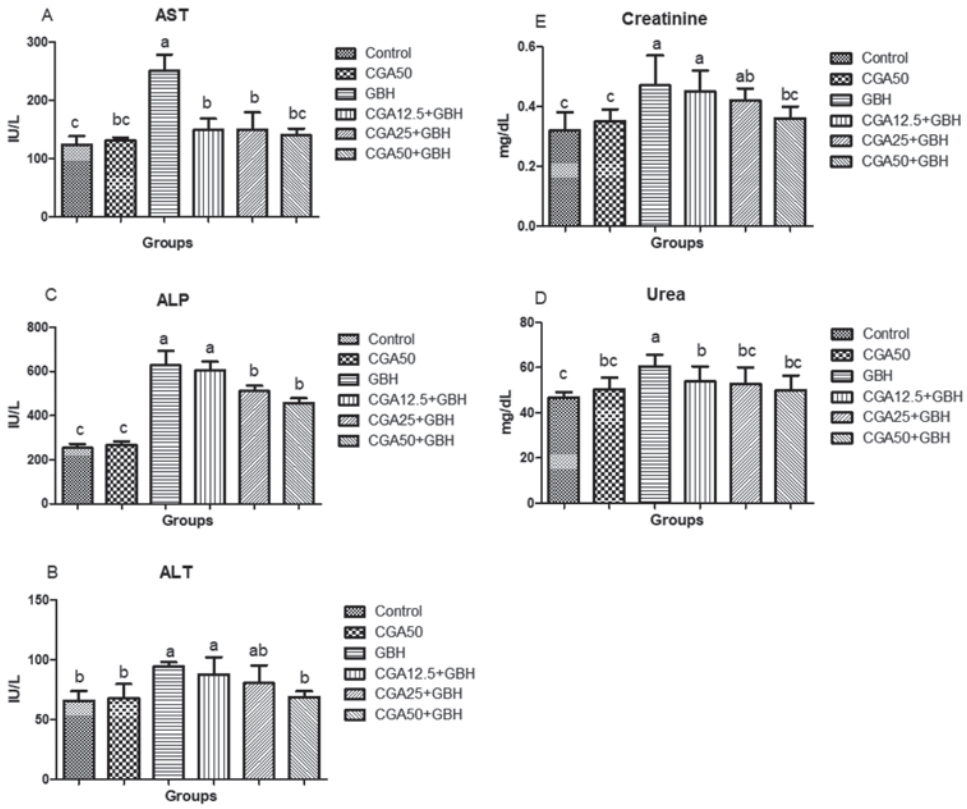

Figure 1 The effects of GBH and CGA treatments on AST (A), ALT (B), ALP (C), urea (D), and creatinine (E) levels in the serum of Wistar rats. Values are expressed as mean ± SD of seven rats per group. Different superscripts (a–c) above the bar indicate significant difference between groups ($p<0.05$)

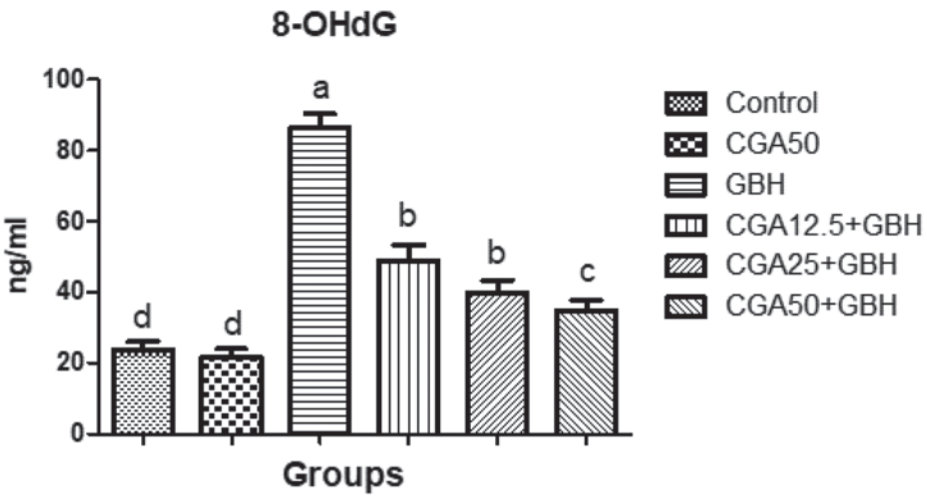

Figure 2 The effects of GBH and CGA treatments on 8-OHdG levels in the plasma of Wistar rats. Values are expressed as mean ± SD of seven rats per group. Different superscripts (a–d) above the bar indicate a significant difference between groups ($p<0.05$)

Table 1 Blood, brain, heart, liver, and kidney tissue MDA levels in Wistar rats treated with CGA and/or GBH by gavage for 49 days

Groups	Blood (nmol/mL)	Brain (nmol/g tissue)	Heart (nmol/g tissue)	Liver (nmol/g tissue)	Kidney (nmol/g tissue)
Control	5.66±0.27^{d}	2.63±0.07^{c}	4.43±0.51^{b}	4.83±0.43^{c}	4.41±0.26^{c}
CGA 50	5.72±0.27^{d}	2.55±0.15^{c}	4.49±0.21^{b}	4.70±0.48^{c}	4.35±0.23^{c}
GBH	20.25±0.95^{a}	6.17±0.43^{a}	6.70±1.30^{a}	9.43±1.20^{a}	9.92±0.70^{a}
CGA12.5+GBH	8.36±0.35^{c}	5.15±0.12^{ab}	6.13±1.10^{ab}	8.81±1.09^{ba}	8.77±0.79^{ba}
CGA25+GBH	6.10±0.69^{b}	4.34±0.24^{b}	5.08±0.90^{b}	7.06±0.97^{b}	6.54±0.57^{b}
CGA50+GBH	5.88±1.56^{d}	3.78±0.10^{c}	4.57±0.39^{b}	5.03±0.54^{c}	5.32±0.48^{c}

Values are expressed as means ± SD of seven rats per group. Different superscripts (a–d) in the same column indicate significant difference between groups ($p<0.05$). GBH – glyphosate-based herbicide (800 mg/kg); CGA12.5 – pre-treatment with chlorogenic acid (12.5 mg/kg) one hour before treatment with GBH (800 mg/kg); CGA25 – pre-treatment with chlorogenic acid (25 mg/kg) one hour before treatment with GBH (800 mg/kg); CGA50 – pre-treatment with chlorogenic acid (50 mg/kg) one hour before treatment with GBH (800 mg/kg)

Table 2 Blood, brain, heart, liver, and kidney tissue GSH levels in Wistar rats treated with CGA and/or GBH by gavage for 49 days

Groups	Blood (nmol/mL)	Brain (nmol/g tissue)	Heart (nmol/g tissue)	Liver (nmol/g tissue)	Kidney (nmol/g tissue)
Control	34.30±3.12^{a}	4.88±0.34^{a}	4.43±0.16^{a}	6.76±0.35^{a}	6.63±0.54^{a}
CGA 50	33.12±1.87^{a}	4.95±0.42^{a}	4.50±0.32^{a}	6.82±0.61^{a}	6.72±0.38^{a}
GBH	19.00±1.53^{d}	3.89±0.21^{b}	3.83±0.19^{b}	4.12±0.19^{c}	4.33±0.26^{c}
CGA 12.5+GBH	28.67±1.35^{c}	4.00±0.53^{b}	4.07±0.41^{b}	4.54±0.48^{bc}	4.62±0.43^{bc}
CGA 25+GBH	30.55±1.64^{b}	4.20±0.58^{ab}	4.23±0.36^{ab}	5.84±0.62^{b}	5.97±0.62^{b}
CGA 50+GBH	32.14±2.05^{a}	4.83±0.71^{a}	4.38±0.22^{a}	6.67±0.34^{a}	6.48±0.46^{a}

Values are expressed as means ± SD of seven rats per group. Different superscripts (a–d) in the same column indicate significant difference between groups ($p<0.05$). GBH – glyphosate-based herbicide (800 mg/kg); CGA12.5 – pre-treatment with chlorogenic acid (12.5 mg/kg) one hour before treatment with GBH (800 mg/kg); CGA25 – pre-treatment with chlorogenic acid (25 mg/kg) one hour before treatment with GBH (800 mg/kg); CGA50 – pre-treatment with chlorogenic acid (50 mg/kg) one hour before treatment with GBH (800 mg/kg)

Table 3 Erythrocyte, brain, heart, liver, and kidney tissue SOD activity in Wistar rats treated with CGA and/or GBH by gavage for 49 days

Groups	Erythrocyte (U/gHb)	Brain (U/g protein)	Heart (U/g protein)	Liver (U/g protein)	Kidney (U/g protein)
Control	17.55±2.44^{a}	1.43±0.14^{a}	2.51±0.42^{a}	6.91±0.44^{a}	6.42±1.37^{a}
CGA50	17.61±2.19^{a}	1.51±0.47^{a}	2.65±0.27^{a}	7.14±0.95^{a}	6.74±1.81^{a}
GBH	6.13±1.04^{c}	0.48±0.13^{b}	1.39±0.32^{b}	1.09±0.12^{d}	0.80±0.27^{c}
CGA12.5+GBH	7.48±1.74^{c}	0.57±0.14^{b}	1.50±0.36^{b}	1.48±0.33^{d}	1.00±0.16^{c}
CGA25+GBH	12.65±2.61^{b}	1.02±0.22^{ab}	1.94±0.41^{ab}	2.47±0.32^{c}	1.54±0.22^{bc}
CGA50+GBH	16.72±2.73^{a}	1.47±0.18^{a}	2.55±0.42^{a}	4.01±0.42^{b}	2.53±0.57^{b}

Values are expressed as means ± SD of seven rats per group. Different superscripts (a–d) in the same column indicate significant difference between groups ($p<0.05$). GBH – glyphosate-based herbicide (800 mg/kg); CGA12.5 – pre-treatment with chlorogenic acid (12.5 mg/kg) one hour before treatment with GBH (800 mg/kg); CGA25: pre-treatment with chlorogenic acid (25 mg/kg) one hour before treatment with GBH (800 mg/kg); CGA50: pre-treatment with chlorogenic acid (50 mg/kg) one hour before treatment with GBH (800 mg/kg)

Table 4 Erythrocyte, brain, heart, liver, and kidney tissue CAT activity in Wistar rats treated with CGA and/or GBH by gavage for 49 days

Groups	Erythrocyte (U/mgHb)	Brain (CAT/g protein)	Heart (CAT/g protein)	Liver (CAT/g protein)	Kidney (CAT/g protein)
Control	15.32±2.40[a]	1.11±0.17[a]	1.15±0.21[a]	6.46±1.34[a]	4.81±0.94[a]
CGA50	13.51±2.08[a]	1.19±0.16[a]	1.22±0.23[a]	6.78±1.52[a]	4.97±1.02[a]
GBH	3.18±1.04[c]	0.41±0.19[c]	0.59±0.12[b]	0.95±0.23[c]	0.81±0.43[d]
CGA12.5+GBH	5.42±0.62[c]	0.69±0.13[b]	0.79±0.19[a]	1.20±0.10[c]	1.11±0.18[cd]
CGA25+GBH	8.31±1.51[b]	0.80±0.19[b]	0.99±0.21[a]	2.78±0.68[b]	1.45±0.27[c]
CGA50+GBH	8.84±2.73[b]	1.05±0.22[a]	1.04±0.24[a]	3.75±0.63[b]	3.02±0.94[b]

Values are expressed as means ± SD of seven rats per group. Different superscripts (a–d) in the same column indicate significant difference between groups ($p<0.05$). GBH – glyphosate-based herbicide (800 mg/kg); CAT – catalase; CGA12.5 – pre-treatment with chlorogenic acid (12.5 mg/kg) one hour before treatment with GBH (800 mg/kg); CGA25 – pre-treatment with chlorogenic acid (25 mg/kg) one hour before treatment with GBH (800 mg/kg); CGA50 – pre-treatment with chlorogenic acid (50 mg/kg) one hour before treatment with GBH (800 mg/kg)

Histopathological findings

Figure 3A–D shows histopathological changes in the brain, heart, liver, and kidney tissues. In the control and CGA50 groups their structure is normal, whereas the GBH group shows neuronal degeneration and vacuolation, vessel hyperaemia, and focal gliosis in the brain (Figure 3A3), myocardial hyaline degeneration and focal haemorrhage in the heart(Figure 3B3), expansion of sinusoids, binucleated hepatocytes in periportal areas, and infiltration by mononuclear cells in the liver (Figure 3C3), and hyaline cylinder formations within the lumens of the tubules in the kidney, accompanied by degenerative and necrobiotic changes in tubular epithelial cells (Figure 3D3).

In the CGA pre-treated groups (particularly CGA50+GBH), only slight histopathological changes were observed in the brain, heart, liver, and kidney compared to the GBH group (Figures 3A4–6, 3B4–6, 3C4-6, and 3D4–6, respectively).

Semi-quantitative scoring of tissue lesions (0 – no, 1 – mild, 2 – moderate, and 3 – severe) in Table 5 reflects the patterns reported above, with no scores in the control and CGA50 alone groups and the highest scores in the GBH alone group.

DISCUSSION

Our haematological, biochemical, and histopathological findings in the brain, heart, liver, and kidney tissues confirm that CGA protects against the glyphosate toxicity in Wistar rats and are in agreement with previous reports of glyphosate exposure leading to significant changes in liver and kidney function (27–30).

The same is true for oxidative stress in rats exposed to glyphosate, which has already been reported *in vivo* (29) and *in vitro* (31). In contrast to our results, Novaes et al. (32) reported an increase in SOD and CAT activities in rats after four days of exposure to paraquat, another herbicide. This discrepancy can partly be explained by acute exposure to paraquat as opposed to the subchronic one to glyphosate in our study. As time passes and the production of free radicals continues, so does decrease intracellular antioxidant enzyme activity. However, CGA pre-treatment increased the activity of SOD and CAT enzymes in GBH-exposed animals, nearly annulling its effects. A similar protective role of CGA has been demonstrated in the liver and kidney of rats acutely exposed to potassium dichromate (33), aflatoxin B1 (34), and methotrexate (35). Part of these protective effects can be attributed to lower production of superoxide radicals and part to enhanced intracellular antioxidant defences through increased mRNA expression of SOD and CAT (36).

As concerns our 8-OHdG findings, subchronic GBH administration in our study induced high levels of plasma 8-OHdG, which are indicative DNA damage. CGA administration reversed these effects in a dose-dependent manner, suggesting that CGA may also protect against glyphosate-induced DNA damage.

Our histopathological findings are consistent with the biochemical and oxidative stress outcomes. Compared to the control group, neuronal degeneration and vacuolation in the brain, myocardial hyaline degeneration and focal haemorrhage in the heart, mononuclear cell infiltration and binucleated hepatocytes in the liver, and degenerative and necrobiotic changes and hyaline cylinders in the kidney were observed only in the rats treated with GBH alone. Again, CGA pre-treatment prevented these histopathological changes in a dose-dependent manner, similar to findings reported by Namratha et al. (37) for GBH hepatotoxicity and vitamin C counteraction or those by Larki-Harchegani et al. (12) for CGA protective effects against paraquat in rat lungs.

To conclude, our findings consistently confirm the potential of CGA as a promising natural agent against the adverse health effects associated with exposure to glyphosate. Future research should focus on long-term glyphosate exposure and CGA treatment using molecular methods and on the signalling pathways associated with oxidative stress.

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Conflict of interests

None to declare.

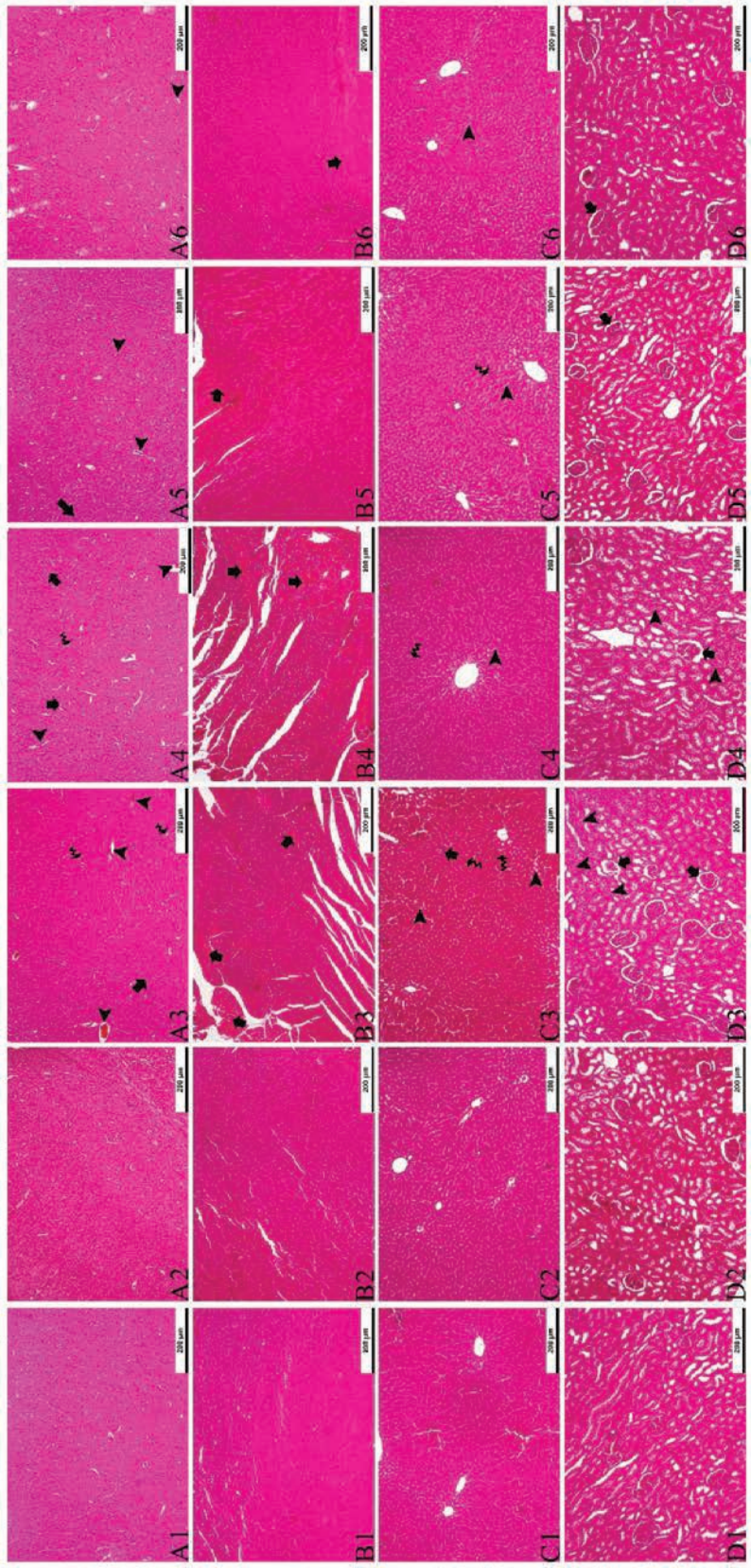

Figure 3 H&E-stained slides showing the protective effects of CGA against GBH-induced damage in the brain (A), heart (B), liver (C), and kidney (D) of Wistar rats(20× magnification; scale bar 200 μm). 1 – control; 2 – CGA50; 3 – GBH; 4 – CGA12.5+GBH; 5 – CGA25+GBH; 6 – CGA50+GBH. Slides A3–A6: arrows indicate neuronal degeneration and focal gliosis; arrowheads indicate hyperaemia in the vessels and curved arrows indicate neuronal vacuolation in the brain. Slides B3–B6: arrows indicate myocardial hyaline degeneration; arrowheads indicate focal haemorrhage in the heart. Slides C3–C6: arrows indicate mononuclear cells infiltration in periportal areas; arrowheads indicate sinusoidal dilatation and curved arrows indicate binucleated hepatocytes in periportal areas in the liver. Slides D3–D6: arrows indicate degenerative and necrobiotic changes in tubular epithelial cells; arrowheads indicate hyaline cylinder formations within the lumens of tubules in kidney tissue

Table 5 Injury scores* in the brain, heart, liver, and kidney tissues of Wistar rats treated with CGA and/or GBH by gavage for 49 days

Tissue	Parameters	Control	CGA50	GBH	CGA12.5+GBH	CGA25+GBH	CGA50+GBH
Brain	Hyperaemia in the vessels	0.00±0.00^{e}	0.00±0.00^{e}	1.77±0.82^{a}	0.73±0.56^{b}	0.36±0.07^{bc}	0.18±0.05^{bc}
	Degenerative changes of neurons and focal gliosis	0.00±0.00^{b}	0.00±0.00^{b}	1.43±0.52^{a}	1.07±0.93^{a}	0.90±0.19^{a}	0.18±0.04^{b}
	Neuronal vacuolation	0.00±0.00^{e}	0.00±0.00^{e}	1.27±0.41^{a}	0.90±0.79^{b}	0.71±0.16^{bc}	0.36±0.06^{c}
Heart	Hyaline degeneration	0.00±0.00^{e}	0.00±0.00^{e}	1.76±0.53^{a}	1.06±0.93^{b}	0.55±0.10^{bc}	0.18±0.04^{c}
	Focal haemorrhage	0.00±0.00^{e}	0.00±0.00^{e}	1.60±0.54^{a}	1.05±0.15^{ab}	0.55±0.20^{bc}	0.36±0.03^{bc}
Liver	Dilatation of sinusoids	0.00±0.00^{b}	0.00±0.00^{b}	1.26±0.40^{a}	1.05±0.15^{a}	0.55±0.10^{ab}	0.18±0.04^{b}
	Mononuclear cell infiltration in periportal areas	0.00±0.00^{b}	0.00±0.00^{b}	1.43±0.52^{a}	0.53±0.18^{b}	0.18±0.04^{b}	0.16±0.03^{b}
	Binucleated hepatocyte formations in periportal areas	0.00±0.00^{e}	0.00±0.00^{e}	1.93±0.41^{a}	0.88±0.13^{b}	0.70±0.18^{bc}	0.18±0.04^{bc}
Kidney	Hyaline cast formations within the lumens of the tubules	0.00±0.00^{e}	0.00±0.00^{e}	1.25±0.78^{a}	0.72±0.16^{ab}	0.36±0.12^{bc}	0.00±0.00^{e}
	Degenerative and necrobiotic alterations in the tubular epithelial cells	0.00±0.00^{b}	0.00±0.00^{b}	1.76±0.51^{a}	0.70±0.18^{b}	0.36±0.16^{b}	0.18±0.04^{b}

*Scoring system according to Gibson-Corley et al. (22): 0 – no lesion; 1 – mild lesion; 2 – moderate lesion; 3 – severe lesion. Values are expressed as means ± SD of seven rats per group. Different superscripts (a–d) in the same column indicate significant difference between groups ($p<0.05$). GBH – glyphosate-based herbicide (800 mg/kg); CGA12.5 – pre-treatment with chlorogenic acid (12.5 mg/kg) one hour before treatment with GBH (800 mg/kg); CGA25 – pre-treatment with chlorogenic acid (25 mg/kg) one hour before treatment with GBH (800 mg/kg); CGA50 – pre-treatment with chlorogenic acid (50 mg/kg) one hour before treatment with GBH (800 mg/kg)

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Zaštitno djelovanje klorogenske kiseline protiv hematološke i organske toksičnosti glifosata u Wistar štakora

Izloženost glifosatu, herbicidu koji se rabi za suzbijanje rasta širokolisnih korova i trava, povezana je s raznim štetnim učincima, uključujući hematološku i organsku toksičnost. Cilj ovog istraživanja bio je utvrditi djelotvornost klorogenske kiseline u ublažavanju štetnih učinaka herbicidnoga preparata na bazi glifosata u 42 Wistar štakora, podijeljena u šest skupina po sedam životinja. Kontrolna skupina primala je samo destiliranu vodu, dok su ostale skupine primale samo klorogensku kiselinu (50 mg/kg), samo GBH (800 mg/kg) ili njihove kombinacije s trima različitim dozama klorogenske kiseline (12,5, 25 ili 50 mg/kg) (CGA12,5+GBH, CGA25+GBH ili CGA50+GBH) na usta (oralnom sondom) 49 dana. Na kraju eksperimenta uzeti su uzorci krvi, mozga, srca, jetre i bubrega te analizirani pokazatelji oksidacijskoga stresa (MDA, GSH, SOD, CAT), oštećenja DNA (8-OHdG), funkcije jetre i bubrega (AST, ALT, ALP, urea i kreatinin), kao i histopatološke promjene. U skladu s očekivanjima, primjena glifosatskoga preparata značajno je povisila razine enzima AST, ALT i ALP, kao i bubrežnih markera (ureje i kreatinina). Također su se značajno povećale razine 8-OHdG i MDA, a razine GSH smanjile, kao i aktivnost SOD i CAT. Osim toga, glifosatski preparat prouzročio je histopatološke promjene u tkivima mozga, srca, jetre i bubrega. Klorogenska kiselina poboljšala je biokemijske i parametre oksidacijskoga stresa te ublažila histopatološke promjene, ovisno o primijenjenoj dozi, pokazavši se kao obećavajući spoj za sprječavanje i ublažavanje štetnog djelovanja glifosata i mogući temelj strategije očuvanja zdravlja u populacijama izloženima ovom rasprostranjenom herbicidu.

KLJUČNE RIJEČI: 8-OHdG; ALP; ALT; AST; bubrezi; CAT; CGA; GSH; herbicidi; histopatologija; jetra; kreatinin; krv; MDA; mozak; oksidacijski stres; oštećenje DNA; SOD; srce; urea