Official Journal Issued by Faculty of Veterinary Medicine

Benha Veterinary Medical Journal

Journal homepage: https://bvmj.journals.ekb.eg/

Since 1990

Original Paper

Ameliorative impact of glutathione supplementation on cyclophosphamide-induced hepatic toxicity in male albino rats.

Amira Samir Fetouh [1,2], Ashraf A.A. El Komy [1], Enas Farag [3], Ahmed A. Abdeen [4]

[1] *Department of Pharmacology, Faculty of Veterinary Medicine, Benha University, 13736 Moshtohor, Toukh, Qalyubia, Egypt .*
[2] *Benha University Hospital, Qalyubia, Egypt.*
[3] *Deputy of AHRI for regional laboratories. AHRI. ARC, Egypt .*
[4] *Department of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, Benha University, 13736 Moshtohor, Qalyubia, Egypt.*

ARTICLE INFO

Keywords

Rats

Hepatotoxicity

Glutathione

Cyclophosphamide.

Received 22/07/2024
Accepted 15/08/2024
Available On-Line 01/10/2024

ABSTRACT

Cyclophosphamide (CP) is a bifunctional alkylating and chemotherapeutic agent used to treat cancer. However, its clinical utilization is restricted due to its toxicity to many tissues, most notably the liver and kidneys. Four groups (n = 6) of rats were used. Group I was given normal saline orally for 14 days. Group II received 150 mg/kg of glutathione (GSH) orally once a day for 14 days. Group III was administered 0.5 ml normal saline orally once daily and injected cyclophosphamide on 10th day (200 mg/kg, intraperitoneal). Group IV received GSH (150 mg/kg) orally plus intraperitoneal CP (200 mg/kg). CP induced a significant increase in hepatic serum biomarkers, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) levels, compared with the control group. Additionally, there was a significant increase in total bilirubin (BIT). Also, levels of total protein (T-protein) and albumin (ALB) were significantly decreased in the CP group. Moreover, cyclophosphamide significantly decreased catalase (CAT) and glutathione (GSH) levels in liver tissues, while increasing malondialdehyde (MDA) levels. Interleukin-1β (IL-1β) was substantially upregulated by CP in liver tissues. Furthermore, CP induced severe histopathological and immunochemical changes in liver tissues, and administering GSH concurrently with CP resulted in a significant improvement in estimated parameters compared to the CP-only group. GSH is recommended for use due to its ability to protect rats from CP-induced liver damage..

1. INTRODUCTION

Chemotherapeutic agents have been used for several years and are still used today to treat many types of cancer. Chemotherapy like cyclophosphamide (CP) has an impact on renal and hepatic functions (Ribeiro et al., 2019). Cyclophosphamides are a type of nitrogen mustard alkylating agent (Qian et al., 2022). They are used to treat cancers, such as solid tumors and lymphomas. Additionally, cyclophosphamides play a role in the management of many immunosuppression conditions, such as multiple sclerosis and systemic lupus erythematosus (Madubogwu et al., 2022).

Cyclophosphamides exhibit advantageous applications and a broad range of clinical benefits. However, their use is constrained by adverse effects such as hepatotoxicity, cardiotoxicity, nephrotoxicity, immunotoxicity, and bone marrow suppression (Caglayan, 2019). These side effects stem from CP's limited ability to discriminate between healthy and malignant cells within the body (Madubogwu et al., 2022). Notably, hepatotoxicity stands out as a primary adverse action of CP, given that its metabolism and excretion predominantly occur via the liver and kidneys (Ghareeb et al., 2019).

Anticipated organ protection from cyclophosphamide toxicity includes the kidneys, liver, cardiovascular system, and other vital organs. Therefore, administering antioxidants to patients before or during cyclophosphamide treatment can provide significant value in safeguarding these organs (Zhang et al., 2018).

GSH is a tripeptide containing thiol, comprising glutamate, cysteine, and glycine, connected through adenosine-triphosphate-dependent processes (Park et al., 2023). GSH, as the main antioxidant in all tissues, protects cells from producing too many reactive oxygen species (ROS), free radicals caused by ROS, and oxidative stress (Silvagno et al., 2020). GSH is widely distributed in mammalian cells and predominantly concentrated in the liver, mostly in the forms of reduced glutathione (GSH) and oxidized glutathione disulfide (GSSG) (Tan et al., 2023). The purpose of this study was to assess the efficacy of glutathione in protecting rats from cyclophosphamide-induced hepatic toxicity.

2 .MATERIAL AND METHODS

2.1. Ethical considerations

The study protocol was approved by the departmental committee before the trial began. After completing case collection and obtaining the study outcomes, the Local Ethical Committee received final approval with code BUFVTM 04-04-24.

2.2. Chemicals

Cyclophosphamide (Endoxan® 1 gm vial, bought from Baxter Oncology Chemical Co. in Egypt) was mixed with physiological saline (0.9% NaCl) so that it could be injected into the abdomen. Glutathione (GSH), at a concentration of 500 mg, was obtained from Jarrow Formula Co., CA, USA. GSH was dissolved in 0.9% normal saline. All biochemical

* Correspondence to: amirasamir01@yahoo.com

investigation kits were procured from Bio Diagnostics Company in Giza, Egypt.

2.3. Experimental animals

Twenty-four Wister albino male rats weighing 160–200 grams were bought from the Faculty of Veterinary Medicine, Benha University, Egypt, and kept in metal cages at a temperature of 25 °C. Commercial rat food and constant water availability were provided. Before beginning the experiment, fourteen days of acclimatization were permitted.

2.4. Experimental design and treatment protocol

Twenty-four albino male rats were categorized into four groups, each comprising six rats. The group structure was as follows: Group I (Vehicle Control): Rats received saline orally once daily for 14 consecutive days. Group II (GSH): Rats were orally administered 150 mg/kg body weight of glutathione (GSH) for 14 consecutive days. The dose of GSH was determined according to Ahmadvand et al. (2018). Group III (CP): Rats received a single intraperitoneal injection of cyclophosphamide (CP) at a dose of 200 mg/kg on the 10th day, following the protocol established by Temel et al. (2020). Group IV: Rats were treated with oral GSH for 14 days, concurrently with a single CP dose administered intraperitoneally on the 10th day.

2.5. Blood sampling

Twenty-four hours following the final treatment, rats were humanely euthanized with an intraperitoneal injection of pentobarbital (800 mg/kg), as described by Zatroch et al. (2016). Subsequently, blood samples were collected from the retro-bulbar venous plexus without the use of anticoagulants. The sera were separated by centrifugation at a speed of 2000 xg for 10 minutes, transferred to clean vials, and stored at -20 °C for additional biochemical assays.

2.6. Biochemical analysis

Serum enzymes, including alanine aminotransferase (ALT) and aspartate aminotransferase (AST), were quantified using the method developed by Reitman and Frankel. (1957). Additionally, total protein was determined according to the method described by Lowery et al. (1951). Serum albumin was determined according to the method described by Doumas et al. (1971). Alkaline phosphatase activity was determined according to the method described by Rosalki et al. (1993). Also, total bilirubin was determined according to the method described by Doumas et al. (1985). To assess oxidative stress in liver tissue, levels of malondialdehyde (MDA), a marker for lipid peroxidation, were determined following the procedure outlined by Ohkawa et al. (1979). Furthermore, the activities of catalase (CAT) as described by Aebi (1984) and glutathione (GSH) levels according to Beutler (1963) were assessed using commercial assay kits obtained from Bio Diagnostic Company in Giza, Egypt. These biochemical analyses adhered to established protocols provided by the manufacturer.

2.7. Tissue specimens

The liver was immediately removed and then washed. A portion of the liver was selected and fixed in 10% neutral-buffered formalin for the histological evaluation. Other sections of the liver were used in biochemical analysis to estimate the concentration of CAT, reduced GSH, and MDA as oxidative stress markers.

Other sections of the liver were transferred to isotonic saline for flow cytometry measurements of interleukin-1β.

2.8. Histopathological analysis and immunohistochemistry procedure

After liver samples were properly fixed, they were dried out using increasing amounts of ethyl alcohol, cleaned with xylene, and then embedded in paraffin wax. Paraffin blocks were cut into 4-6 micrometer thick sections and stained with hematoxylin and eosin (H&E) to examine their overall architecture (Bancroft and Stevens, 2016). Slides were viewed and analyzed utilizing a Leica microscope (Leica Microsystems AG, Switzerland).

For immunohistochemistry analysis, paraffin-embedded tissue sections were placed onto positively charged slides and treated employing the avidin-biotin-peroxidase complex (ABC) approach. An anti-interleukin-1 beta antibody generated against rabbits (Cat #ab283818; Abcam Ltd.) diluted at a ratio of 1:500 was used for this experiment. Afterward, the ABC components were added sequentially (Vector Laboratories, Inc.). Positive reactions were revealed using diaminobenzidine (DAB; Sigma-Aldrich Co.), which is employed to display antigens. Non-specific IgG served as negative control solutions that replaced either primary or secondary antibodies. Digital images were acquired and recorded using a Leica microscope equipped with a color camera, screen, and hard drive connected to a Leica IBM Personal Computer running Leica QWin 500 software. For statistical analysis, three different high-power (400x) fields with brownish immunoreactivity were picked at random on each slide to find out what percentage of the area was occupied (% Area) using Leica QWin 500 software (UK). Results were documented and presented as mean ± standard error values for %Area.

2.9. Statistical analysis

The data obtained from the study was analyzed statistically using SPSS version 20 (SPSS Inc., Chicago, USA). To compare the different study groups, a one-way analysis of variance (ANOVA) was conducted initially. The Duncan's multiple range test (Duncan, 1955) was also carried out subsequent to ANOVA. The data is represented as mean ± standard error $P \leq 0.05$.

3. RESULTS

Serum AST, ALT, ALP, and BIT levels were significantly increased ($P < 0.05$) in the CP-intoxicated group compared to their levels in the control group. Serum albumin and T-protein levels were significantly ($P < 0.05$) decreased in CP-treated groups in comparison to their levels in control rats.

Pretreatment with GSH significantly ($P < 0.05$) reduced serum AST, ALP, BIT, and ALT levels in CP-treated rats. In addition, the albumin and T-protein levels of CP-intoxicated rats pretreated with GSH were significantly higher than those of CP-intoxicated rats ($P < 0.05$) (Figure 1).

Furthermore, rats intoxicated with CP showed a significant ($P < 0.05$) decrease in the hepatic levels of CAT and GSH, with a significant increase ($P < 0.05$) in MDA level compared to the control group. Compared to the CP-treated rats, pretreating with GSH significantly decreased ($P < 0.05$) MDA levels and significantly increased CAT and GSH levels (Figure 2).

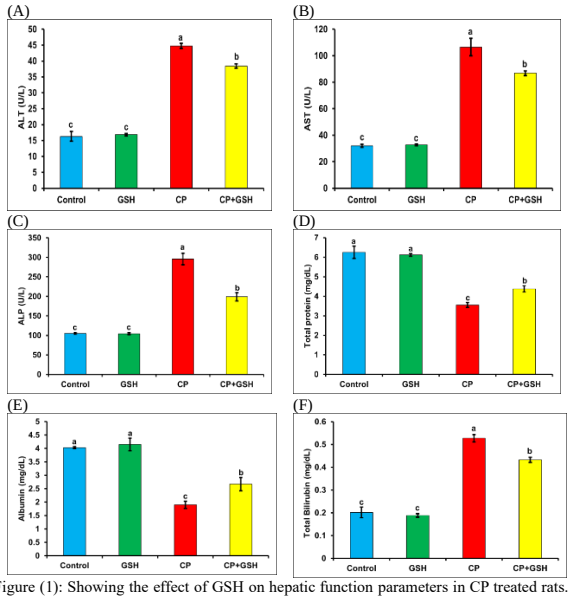


Figure (1): Showing the effect of GSH on hepatic function parameters in CP treated rats.

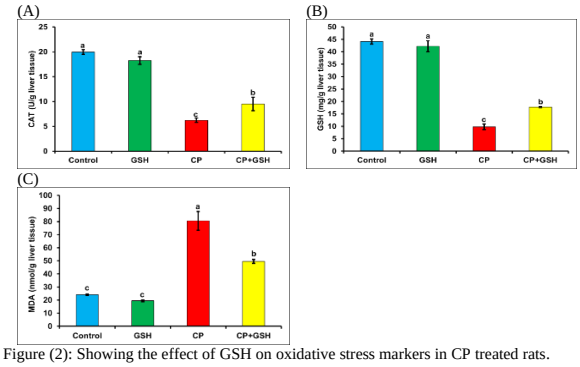


Figure (2): Showing the effect of GSH on oxidative stress markers in CP treated rats.

Histopathological findings

The examined liver sections in the control and GSH groups showed normal histoarchitecture of the hepatic parenchyma, where the polygonal hepatic cells were arranged in hepatic cords and appeared with their typical organization (Figures 3a–3b). In contrast, liver sections from rats that had been given CP showed severe damage, including severe hydropic degeneration of the hepatocytes, congestion of the central veins, and microvascular steatosis (Figure 3c). However, the liver section from the CP and GSH-pretreated group marked obvious improvement along the hepatic tissue (Figure 3d).

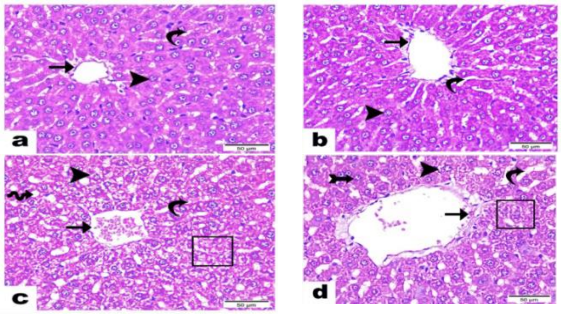
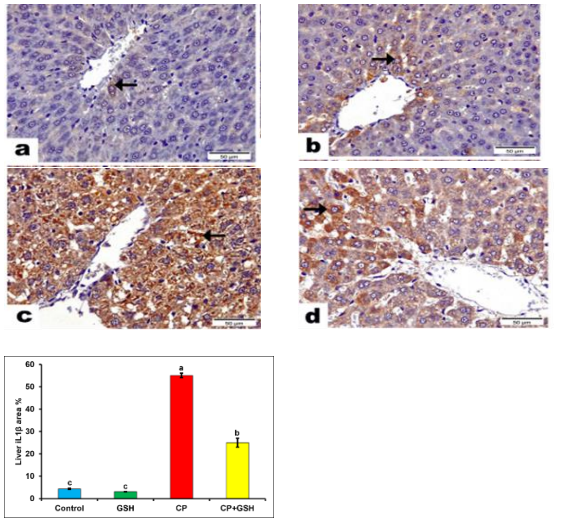


Figure (3): Photomicrographs represented the effect of GSH drug on histopathological along liver tissue (central vein area) in the studied group (H and E stain, magnification power= x400 and scale bar= 50µm) as follows: Section from control group (a) and GSH group (b) revealing the normal structure of central vein area with intact central vein endothelial lining (arrow), hepatic cords appeared with its typical organization and lined by normal hepatocytes with vesicular central nuclei (arrowhead). Hepatic sinusoids emerged between hepatic cords with their normal structure (curvy arrow). Section from CP group (c) highlighting sever degenerative changes along hepatic tissue assembled as diffuse hydropic degeneration along hepatocytes (arrowhead), congested central vein (arrow), micro-vesicular steatosis (wave arrow), some hepatic sinusoids detected with congestion (curvy arrow), while others appear with loss of its normal organization and structure (rectangle). Section from GSH pretreated group (d) marking obvious improvement along hepatic tissue except small area still appeared degenerated along endothelial lining the central vein (arrow), some hepatic cords observed with normal with intact normal vesicular hepatocytes lining (arrow with tail), however, some of them show mild hydropic degeneration (arrowhead).

Immunohistochemical findings.

Liver Sections from the negative control and GSH groups displayed very few cytoplasmic expressions of interleukin 1 beta (IL-1β) along hepatocytes, with non-significant differences ($P \geq 0.05$) between them (Figure 4a–4b). However, the CP group demonstrated strong positive cytoplasmic expressions of IL-1β along hepatocytes, with a significant difference ($P \leq 0.05$) from the negative control group and GSH group (Figure 4c). Sections from the CP and GSH-treated groups exhibited moderate cytoplasmic expression of IL-1β along hepatocytes, with a significant difference ($p \leq 0.05$) from the CP group (Figure 4d). Very little IL-1β immune positivity was observed in the control or GSH groups. This pro-inflammatory activity increased markedly in the CP group compared to the control group. Moreover, IL-1β intensity was significantly decreased in the CP+GSH group compared to the CP group (Figure 4e).



E

Figure (4): Photomicrographs showing the expression of interleukin 1 beta along liver tissue between inspected groups (interleukin 1 beta, magnification power= x400, scale bar= 50µm) as follow: Sections from negative control group (a) and GSH group (b) displaying very few cytoplasmic expressions of IL-1β along hepatocytes (arrow) with nonsignificant difference ($p \geq 0.05$) between them. Section from CP group (c) demonstrating strong positive cytoplasmic expressions of IL-1β along hepatocytes (arrow) with significant difference ($p \leq 0.05$) from negative control group and GSH group. Section from CP and GSH treated group (d) exhibiting moderate cytoplasmic expression of IL-1β along hepatocytes (arrow) with significant difference ($p \leq 0.05$) from other groups. (e) The effect of therapeutic single dose of or/and GSH on iL1β Area.%

4. DISCUSSION

Cyclophosphamide is used to treat some kidney diseases. However, it has severe side effects on vital organs (Saleh et al., 2023). One of the most vital organs in the body is the liver. Furthermore, the liver is protective in the pathophysiology of illnesses, as well as in the detoxification of different substances and medications (Qian et al., 2022). Hepatotoxicity is one of the main adverse effects of CP, as its metabolism and excretion are mostly via the liver (Ghareeb et al., 2019). The process of tissue destruction is significantly influenced by oxidative stress. The phenomenon happens when there is an unbalance between the generation of reactive oxygen species (ROS) and the body's capacity to counteract or restore their detrimental impacts (Elsayed et al., 2022; Aboubakr et al., 2023; Elsayed et al., 2024; Soliman et al., 2024).

Injury or damage to normal tissues is the major limitation of using CP as a potential chemotherapeutic drug. Antioxidants are beneficial for CP-induced toxicity (Zhang et al., 2018). GSH is an antioxidant that protects the cells from the overproduction of reactive oxygen species (ROS), ROS-induced free radical formation, and oxidative stress

(Silvagno et al., 2020). The current study assessed the potency of GSH to mitigate the hepatic dysfunction induced by CP in rats.

After administering CP to the rats, notably higher levels of hepatic ALT, ALP, and AST were recorded compared to the control group devoid of any intervention. This observation aligns with earlier studies (Yahya et al., 2022). Administering GSH in conjunction with CP successfully mitigated the adverse impacts of CP on liver function. Previous studies have shown that GSH restored AST and AlP levels in rats with renal ischemia-reperfusion injury (Ahmadvand et al., 2018).

The increase in these biomarker enzymes and related metabolites in the bloodstream might be owing to the action of acrolein, a compound formed during CP metabolism, leading to disruption of the body's natural defenses against reactive oxygen species (ROS), culminating in excessive generation of ROS, which subsequently damages the structural integrity of the hepatocyte membrane, potentially releasing these enzymes into the circulatory system (Adikwu and Bokolo, 2018).

Pretreatment with GSH before CP injection attenuated the toxic effect of CP on the liver enzymes. This result goes in parallel with previous studies that reported CP-induced liver toxicity in rats and how the antioxidants attenuated these toxicities (Khordad et al., 2021; Turedi, 2023). Another study showed that GSH administration could reduce the activity of liver markers in vivo (Chen et al., 2015).

In the present work, decreases in serum albumin and total protein levels were recorded in rats administered CP. These observations were compatible with previous findings (Germoush and Mahmoud, 2014). However, the group pretreated with GSH showed a decrease in these levels. These results agreed with those of Helal and Soliman (2008). Produced MDA during lipid peroxidation serves as an indicator for oxidative stress-related damage. Generally, GSH and CAT are essential enzymes of the antioxidant system that remove ROS and preserve the equilibrium of the antioxidant mechanism, safeguarding the liver against oxidative stress (Qian et al., 2022). Therefore, changes in these enzyme levels indicate variations in liver antioxidant capacity. In this investigation, a rise in MDA concentrations and decreased CAT and GSH activities were noted after administering CP. These outcomes correspond to previous reports concerning CP's impact on organ toxicity (Golmohammadi and Abedi, 2022; Qian et al., 2022). In the GSH-pretreated group, there was a considerable decrease in liver tissue MDA content and an enhancement in CAT and GSH activities, resulting in significant reductions in liver injury in CP-intoxicated rodents.

Histopathological changes in the liver were determined by light microscopy. Histological injuries in the hepatocytes explain the biochemical results obtained in this study.The examined livers of rats injected with CP-induced histological alterations in the liver tissues revealed severe degenerative changes along hepatic tissue, as well as severe hydropic degeneration along hepatocytes and congested central veins. Similar results were documented by Madubogwu et al. (2022). The histological changes observed in the hepatic cells and blood vessels could be attributed to the direct effect of the drug on the cells' structures or may be attributed to the production of free radicals, which caused damage to the membrane integrity of the hepatocytes (Khalil et al., 2020). The results reported that the adverse effects of CP in the hepatic tissue were significantly reversed in the pre-treated GSH group. These data agree with those obtained by Ayza et al. (2022).

Cytokines play crucial roles in the growth of immune responses, the initiation of inflammation, the management of blood cell formation, the regulation of cell division and transformation, and the promotion of wound healing. Studies conducted both inside and outside the body have revealed that exposure to CP causes an inflammatory reaction in a variety of organs (Ayza et al., 2022; Turedi, 2023). The current research used immunohistochemistry to demonstrate that rats given CP had particularly intense, cytoplasmic expression of IL-1 beta within their hepatocytes, which differed substantially from the negative control group and the GSH treatment groups. Turedi (2023) found elevated levels of the pro-inflammatory cytokine IL-1beta in liver tissue exposed to CP. In the liver tissue of GSH-pretreated animals, GSH reduced IL-1 beta immunoreactivity. Also, the percentage of IL-1 beta staining area in liver tissue was considerably higher in the CP-treated group. These findings concurred with Turedi (2023), who argued that GSH could avoid cell death by inhibiting IL-1β activation. As a result, the administration of antioxidants like GSH, which offer favorable impacts on indicators of oxidative stress, may either assist or mitigate the problems linked with drug-induced oxidative stress (Babaeenezhad et al., 2023).

5. CONCLUSIONS

The recorded data revealed cyclophosphamide-induced severe alteration of liver biomarkers, lipid peroxidation biomarkers MDA, antioxidant enzymes CAT and GSH, as well as liver tissue. However, the pre-treatment of glutathione provided a beneficial role in such prior changes in CP-treated rats through its free radical scavenging and antioxidant activities. Thus, the use of antioxidant agents may be useful to inhibit the toxic effects of CP.

6. REFERENCES

1. Aboubakr, M., Elbadawy, M., Ibrahim, S.S., Khalil, E., Darweish, M., Farag, A., Elfadadny, A., Alkafafy, M., Soliman, A., Elsayed, A., 2023. Allicin and lycopene possesses a protective effect against methotrexate-induced testicular toxicity in rats. Pak Vet J 43,3 , 559-566.
2. Adikwu, E., Bokolo, B., 2018. Effect of cimetidine on cyclophosphamide-induced liver toxicity in albino rats. Asian J Med Sci 9,5 , 50-56.
3. Aebi, H., 1984. 'Catalase in vitro'. Methods in Enzymology 105, 121-126.
4. Ahmadvand, H., Babaeenezhad, E., Nasri, M., Jafaripour, L., Khorramabadi, R.M., 2018. Glutathione ameliorates liver markers, oxidative stress and inflammatory indices in rats with renal ischemia reperfusion injury. Journal of Renal Injury Prevention, 8,2 , 91-97.
5. Ayza, M.A., Zewdie, K.A., Yigzaw, E.F., Ayele, S.G., Tesfaye, B.A., Tafere, G.G., Abrha, M.G., 2022. Potential protective effects of antioxidants against cyclophosphamide-induced nephrotoxicity. International Journal of Nephrology 2022, 5096825-5096837.
6. Babaeenezhad, E., Dezfoulian, O., Moradi H.F., Monfared, S.R., Fattahi, MD., Nasri, M., Ahmadvand, H., 2023. Exogenous glutathione protects against gentamicin-induced acute kidney injury by inhibiting NF-κB pathway, oxidative stress, and apoptosis and regulating PCNA. Drug and Chemical Toxicology 46,3 , 441-450.
7. Bancroft, J. D., Stevens, A. 2016. Theory and practice of histological techniques ,7th ed., pp. 120-131 . Churchill Livingstone: London.
8. Beutler, E., 1963. Improved method for the determination of blood glutathione. J lab clin Med 61, 882–888.
9. Caglayan, C., 2019. The effects of naringin on different cyclophosphamide-induced organ toxicities in rats: investigation of changes in some metabolic enzyme

activities. Environmental Science and Pollution Research 26,26 , 26664-26673.
10. Chen, J., Wu, F., Long, Y., Yu, W. 2015. Glutathione supplementation attenuates oxidative stress and improves vascular hyporesponsiveness in experimental obstructive jaundice. Oxidative Medicine and Cellular Longevity 2015, 486148-486158.
11. Doumas, B.T., Ard Watson, W., Biggs, H.G., 1971. Albumin standards and the measurement of serum albumin with bromcresol green. Clinica Chimica Acta 31, 87-96.
12. Doumas, B.T., Kwok-Cheung, P.P., Perry, B.W., Jendrzejczak, B., McComb, R.B., Schaffer, R. and Hause, L.L., 1985. Candidate reference method for determination of total bilirubin in serum: development and validation. Clinical chemistry 31,11 ,1779-1789.
13. Duncan, D.B., 1955. 'Multiple range and multiple F tests'. Biometrics 11, 1–42.
14. Elsayed, A., Aboubakr, M., Hassan, F.W., Zakaria, M., Abdelhiee, E.Y., Soliman, A., El-Shafey, A., Farag, A., Elfadadny, A., Abdelmageed, N., 2024. Testicular injury of acrylamide in rats and potential protection of coenzyme Q10 and rosuvastatin. Pak Vet J 44,2 , 344-351.
15. Elsayed, A., Elkomy, A., Alkafafy, M., Elkammar, R., Fadl, S.E., Abdelhiee, E.Y., Abdeen, A., Shaheen, H., Soliman, A., Aboubakr, M., 2022. Ameliorating effect of lycopene and N-acetylcysteine against cisplatin-induced cardiac injury in rats. Pak Vet J 42,1 , 107-111.
16. Germoush, M.O., Mahmoud, A.M., 2014. Berberine mitigates cyclophosphamide-induced hepatotoxicity by modulating antioxidant status and inflammatory cytokines. Journal of Cancer Research and Clinical Oncology 140, 1103-1109.
17. Ghareeb, M.A., Sobeh, M., El-Maadawy, W.H., Mohammed, H.S., Khalil, H., Botros, S., Wink, M., 2019. Chemical profiling of polyphenolics in Eucalyptus globulus and evaluation of its hepato–renal protective potential against cyclophosphamide induced toxicity in mice. Antioxidants ,Basel 8,9 , 415-434.
18. Golmohammadi, M.G., Abedi, A., 2022. Saponin Protects Against cyclophosphamide- induced kidney and liver damage via Antioxidant and Anti-Inflammatory Actions. Physiol Int 110,2 , 108-120.
19. Helal, E., Soliman, G.Z., 2008. Biochemical studies on the effect of sodium nitrite and/or glutathione treatment on male rats. The Egyptian Journal of Hospital Medicine 30,1 , 25-38.
20. Honda, Y., Kessoku, T., Sumida, Y., Kobayashi, T., Kato, T., Ogawa, Y., Nakajima, A., 2017. Efficacy of glutathione for the treatment of nonalcoholic fatty liver disease: An open-label, single-arm, multicenter, pilot study. BMC gastroenterology 17,1 , 1-8.
21. Khalils, A.M., Kasem, N.R.A., Ali, A.S.A., Salman, M.M.A, 2020. Brown seaweed sargassum cinereum extract ameliorates the hepato and nephrotoxicity induced by Cyclophosphamide in Male Albino Rats: Hematological Biochemical and histobiochemical examinations. Saudi Journal of Pathology and Microbiology 05,02 , 86–94.
22. Khordad, E., Alipour, F., Pourabbas, M., Mansouri, S., Salimnejad, R., 2021. Hepatoprotective impact of ghrelin against cyclophosphamide-induced toxicity in the male mice. Drug Research 71,07 , 407-412.
23. Lowery, O.H., Rosebrough, N.J., Farr, A.L. and Randhll, R.J., 1951. Protein measurement with the folin-phenol reagent. Journal of Biological Chemistry 193, 265-275.
24. Madubogwu, N., Erhirhie, E., Okoye, F.B., 2022. Extracts of gnetumafricanum ,Gnetacae ameliorated liver injuries of cyclophosphamide immunosuppressed rats. Journal of Current Biomedical Research 2,1 , 10-18.
25. Ohkawa, H., Ohishi, N., Yagi, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95, 351–358.
26. Park, M.K., Choi, B.Y., Kho, A.R., Lee, S.H., Hong, D.K., Kang, B.S., Suh, S.W., 2023. The protective role of glutathione on zinc-induced neuron death after brain injuries. International Journal of Molecular Sciences 24,3 , 2950-2963.
27. Qian, L., Yang, F., Lin, X., Jiang, S., Zhang, Y., Tang, Y., 2022. Pyrroloquinoline quinone ameliorates liver injury in mice induced by cyclophosphamide. Environmental Science and Pollution Research 29,20 , 30383–30393.
28. Reitman, S., Frankel, S., 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. American Journal of Clinical Pathology 28,1 , 56-63.
29. Rezaei, S., Hosseinimehr, S.J., Zargari, M., Malekshah, A.K., Mirzaei, M., Amiri, F.T., 2023. Sinapic acid attenuates cyclophosphamide-induced liver toxicity in mice by modulating oxidative stress, NF-κB, and caspase-3. Iranian Journal of Basic Medical Sciences 26,5 , 526-532.
30. Ribeiro, A.J., Yang, X., Patel, V., Madabushi, R., Strauss, D.G., 2019. Liver microphysiological systems for predicting and evaluating drug effects. Clinical Pharmacology & Therapeutics 106,1 , 139-147.
31. Rosalki, S.B., Foo, A.Y. and Burlina, A., 1993. Multicenter evaluation of iso-ALP test kit for measurement of bone alkaline phosphatase activity in serum and plasma. Clinical Chemistry 39, 648-652.
32. Sarhan, F., El-Naggar, S., El-Said, K., Saleh, A., 2023. Portulaca oleracea leaves extract attenuates cyclophosphamide toxicity in mice. Bulletin of Pharmaceutical Sciences Assiut University 46,2 , 1067-1076.
33. Silvagno, F., Vernone, A., Pescarmona, G.P., 2020. The role of glutathione in protecting against the severe inflammatory response triggered by COVID-19. Antioxidants ,Basel 9,7 , 624-640.
34. Soliman, A., Alkafafy, M., Hassan, F.W., Zakaria, M., Abduljabbar, M.H., Mohamed, K. Aboubakr, M., 2024. Investigating the protective role of L-carnitine and thymoquinone against methotrexate-induced testicular damage in rats. Pak Vet J 44,2 , 314-321.
35. Tan, M., Yin, Y., Ma, X., Zhang, J., Pan, W., Tan, M., Zhao, Y., Yang, T., Jiang, T., Li, H. 2023. Glutathione system enhancement for cardiac protection: pharmacological options against oxidative stress and ferroptosis. Cell Death & Disease 14,2 , 131-150.
36. Temel, Y., Kucukler, S., Yıldırım, S., Caglayan, C., Kandemir, F.M., 2020. Protective effect of chrysin on cyclophosphamide-induced hepatotoxicity and nephrotoxicity via the inhibition of oxidative stress, inflammation, and apoptosis. Naunyn-Schmiedeberg's archives of pharmacology 393, 325-337.
37. Turedi, S., 2023. Protective/preventive effects of quercetin against cyclophosphamide-induced hepatic inflammation, apoptosis and fibrosis in rats. Hepatology Forum 4,3 , 135–141.
38. Yahya, R.A.M., Attia, A.M., Yehia, M.A., Azab, A.E., El.M.Shkal, K., 2022. Cyclophosphamide induces hepatorenal toxicity and attenuation by 5-fluorouracil in male albino rats. Trends Journal of Sciences Research 2,1 , 8–21.
39. Zatroch, K.K., Knight, C.G., Reimer, J.N., Pang, D.S., 2016. Refinement of intraperitoneal injection of sodium pentobarbital for euthanasia in laboratory rats ,Rattus norvegicus . BMC Veterinary Research 13, 1-7.
40. Zhang, Q.Y., Wang, F.X., Jia, K.K., Kong, L.D. 2018. Natural product interventions for chemotherapy and radiotherapy-induced side effects. Frontiers In Pharmacology 9, 1253-1278.