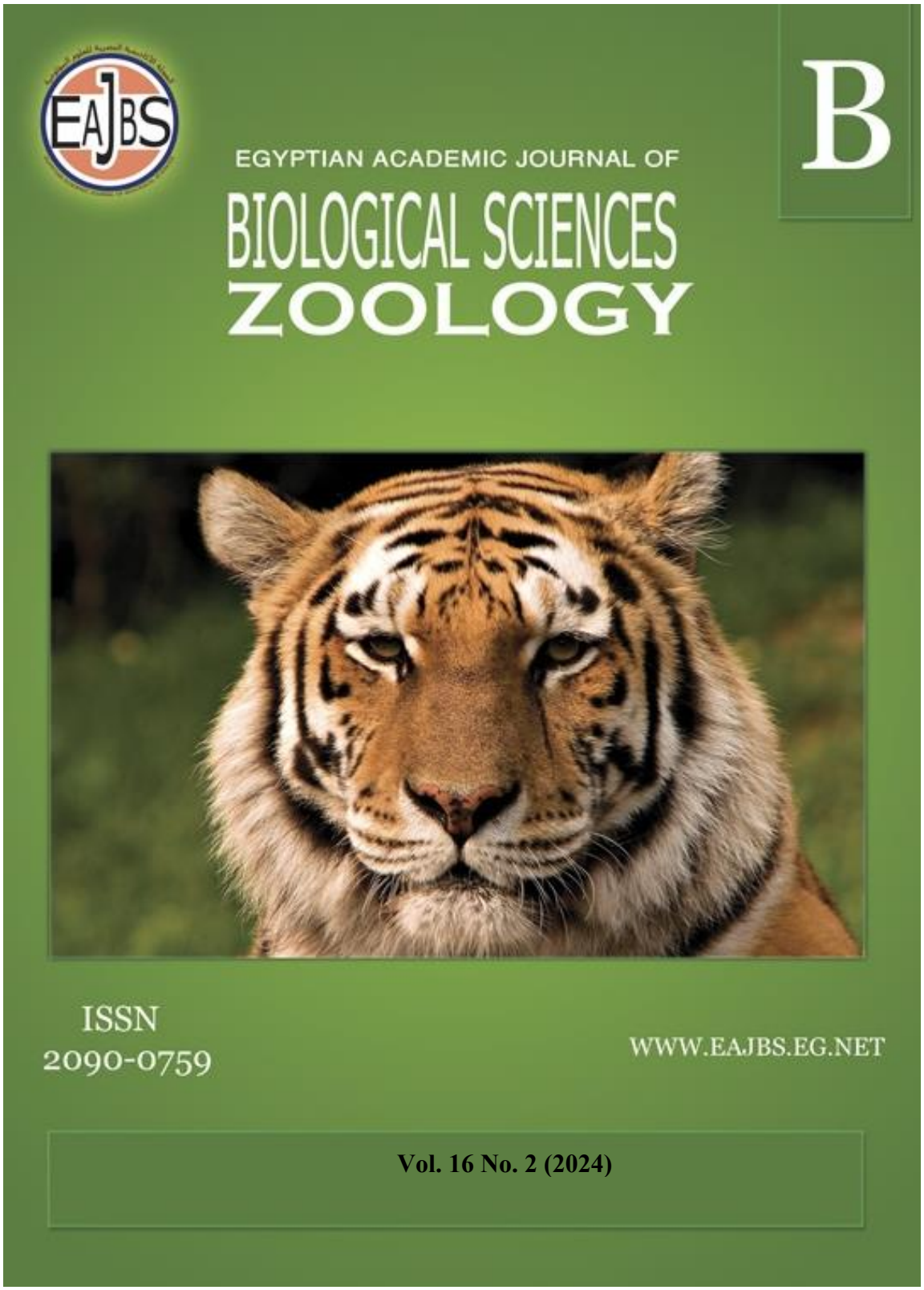


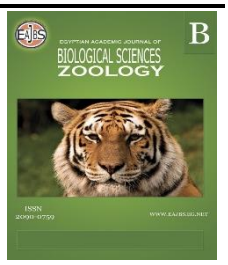
.

www.eajbs.eg.net

Citation: *Egypt. Acad. J. Biolog. Sci. (B. Zoology) Vol. 16(2) pp:39-50 (2024)*
DOI: 10.21608/EAJBSZ.2024.374242

Egypt. Acad. J. Biolog. Sci., 16(2):19-37(2024)

Egyptian Academic Journal of Biological Sciences
B. Zoology
ISSN: 2090 – 0759
http://eajbsz.journals.ekb.eg/



Ameliorative Effect of Vitamin C against Hydroxyurea-Induced Hepatic Toxicity in Male Rats

Faiza Milad; Ebtesam Abdulhadi and Ghaliyah Aqeelah
Department of Zoology, Faculty of Sciences, University of Sirte, Sirte, Libya.
*** E-mail** : ebtes80ah@gmail.com

ARTICLE INFO
Article History
Received:7/7/2024
Accepted:15/8/2024
Available:19/8/2024

Keywords:
Hydroxyurea;
Vitamin C;
Hepatotoxicity;
Histopathological;
Genotoxicity.

ABSTRACT

Background and aim: Hydroxyurea (HU) possesses several characteristics of an ideal drug for various diseases, despite the reported side effects. The current study intends to search for the potential ameliorative effectiveness of vitamin C on hydroxyurea-induced hepatotoxicity in a rat model. **Materials and methods**: 24 adult male albino rats were split into four groups. Group I served as the control; Group II received 5 mg/kg bw of vitamin C; Group III received 100 mg/kg bw of HU; and Group IV received a combination of 5 mg/kg bw VC and 100 mg/kg bw HU, which were administered orally for 30 days to assess liver function tests, oxidative stress, histopathology, and the detection of DNA damage. **Results**: The results demonstrated a significant decline ($p < 0.01$) in the absolute organ weight of rats treated with HU. However, there was no significant contrast in the liver-relative condition. The rats exhibited elevated levels of AST, ALT, and MDA a reduction in albumin, total protein, SOD, CAT, and GSH. The group that received HU displayed signs of degeneration, hydropic deterioration, and fatty degeneration. A comet assay revealed an increase in tail length and DNA content in liver cells. There was no significant difference in olive tail moment but a significant decrease in % tailed, % DNA in the tail, and tail moment. **Conclusion**: The administration of VC with HU improved liver function tests, oxidative stress, and histopathology compared to the HU group.

INTRODUCTION

In the field of medical research and clinical practice, it is imperative to understand the effects of different substances on physiological systems. Hydroxyurea (HU) is a ribonucleotide reductase inhibitor medication known to be a genotoxic chemical with biological effects on living organisms **(**Rosenthal *et al*., 1928). It is a pharmacological agent predominantly employed in the management of haematological illnesses such as sickle cell disease and specific malignancies *(*Alsalman *et al*., 2021*)*. In 1960, it was discovered to possess anti-tumor abilities (Stearns *et al*., 1963; Adamson, 1965). Additionally, it has evolved into a therapy for a variety of disorders, including myeloproliferative disorders (Spivak and Hasselbalch, 2011), brain tumors **(**Madaan *et al*., 2012), Alzheimer's disease (Brose *et al*., 2018), and HIV **(**Lisziewicz *et al*., 2003). However, it is critical to consider its potential influence on liver function. Its mechanism of action is the suppression of DNA synthesis, thereby impacting cells undergoing fast division (Demchuk *et al*., 2020). The

main focus of this drug is on the formation of blood cells and the management of vaso-occlusive crises in individuals affected by sickle cell disease (Weaver *et al.*, 2021).

Therefore, a theoretical understanding of the precise methods by which HU impacts live cells holds excellent practical relevance **(**Ashley *et al*., 2014; Yazinski and Zou, 2016). Cells with checkpoint abnormalities are highly susceptible to HU, although it does not affect cells that have the S-phase checkpoint intact once the medication is stopped. HU's cytotoxic effects are associated with the formation of DNA strand breaks (Xu *et al*., 2016; Kramara *et al*., 2018 Julius *et al*., 2019). Patients on long-term HU treatment exhibited a higher survival rate with no elevated risk of stroke, infection, or malignancy (Steinberg *et al*., 2010; Hankins *et al*., 2014). Several studies have shown that HU has hepatotoxic effects, including the acute elevation of liver function tests (Hallam and Kolesar, 2008), hepatic dysfunction (Shimizu *et al*., 2019), and hepatitis (Heddle and Calvert, 1980). However, the mechanism underlying this impact has not been well studied.

Vitamin C is an important vitamin that plays a crucial role in various physiological processes, like collagen formation, antioxidant activity, prevention of tissue damage in human disease, and immune system function (Goncharova *et al*., 2021). Ascorbic acid is classified as a water-soluble antioxidant vitamin present both intracellularly and extracellularly (Chihuailaf *et al*., 2002). It is a natural antioxidant that contributes to the stability of cellular and basal membranes, protecting against free radical production. It may protect lipids and lipoproteins in cellular membranes from oxidative damage via its oxidation and reduction properties (Gupta et al., 2004) and exhibit hepatoprotective effects **(**McDowell, 1989 Sies *et al*., 1992).

As the scientific community further investigates the impact of hydroxyurea and vitamin C on liver health, it becomes apparent that a comprehensive and nuanced methodology is required. Although the primary target of hydroxyurea is not the liver, it is necessary to closely monitor any potential side effects on liver function. The potential advantages associated with its use in the management of blood diseases need to be carefully considered in light of any potential negative impact on liver health.

This study investigates the application of HU therapy on hepatic enzymes, oxidative status, histopathology, and DNA damage in liver cells, while also investigating the protective potential of vitamin C against HU-induced hepatotoxicity in rats.

MATERIALS AND METHODS

1- Animals and Management:

Male albino rats with weights between 170 and 220 g were purchased from the National Research Center's (NRC) Animal Breeding House in Dokki, Giza, Egypt. Rats were kept in polypropylene cages with six rats in each cage and had unrestricted access to food, water, and standard pellets while being housed in standardized settings (light cycle of 12 hours, temperature of 23 C, and minimum relative humidity of 48%). Prior to this trial, rats were acclimated for seven days. All of the rats were maintained following the "Guide for the Care and Use of Laboratory Animals," which was authorized by the national research center's Local Ethical Review Committee, and their well-being was also protected.

2- Chemicals:

Hydroxyurea (HU) capsules with an active component of 100 mg hydroxyurea (E.R. Squibb and Sons Ltd., England). HU and Vitamin C 5mg. were dissolved in purified water immediately before delivery. The assay kits used for biochemical measurements of alanine transferase (ALT), aspartate aminotransferase (AST), total protein (TP), albumin, superoxide dismutase (SOD), catalase (CAT), glutathione reduced (GSH), lipid peroxidation biomarker as malondialdehyde (MDA), were purchased from Biodiagnostic Company, Doki, Giza, Egypt.

3- Experimental Design:

After two weeks of acclimatization period, 24 rats were randomly assigned into four experimental groups. Group I was only given water, which served as the control group. Group II was placed on VC at 5mg/kg.bw. Group III was placed on HU at 100mg/kg.bw (based on the pilot research). Group IV was placed on a combination of VC+HU at the same dosage as groups II and III. These substances were administered orally for 30 days, the rats were observed daily for signs of toxicity and mortality.

4- Collection and Processing of Samples:

After the last treatment rats fasted overnight and were anaesthetized using sodium pentobarbital (30 mg kg1, i.p.) (Liu *et al*., 2007). The blood samples were obtained by puncturing the animals' retro-orbital venous plexus with a fine sterilized glass capillary (Kendro Laboratory Products GmbH, Germany). The serum was isolated and stored at 4˚C for future biochemical examination of liver enzymes. Following euthanasia with intraperitoneal injections of 80 mg/kg ketamine (Nimatek; Eurovet, Bladel, the Netherlands) combined with 0.5mg/kg of medetomidine (Domitor, Novartis, Arnhem, The Netherlands) **(**Vermeij *et al*., 2013). The abdomens of all rats were opened, and a portion of their livers were promptly removed following the sacrifice, washed with saline, and weighed. Small portions of each liver were preserved for 48 hours in 10% buffered formalin for analysis of histological studies. Another portion of each rat's liver was stored at 20˚C for analysis of oxidative stress biomarkers studies.

5- Biochemical Analysis of Serum:

5.1- Liver Function Tests:

To measure the toxicity caused by HU, liver functions were studied. The enzymes aspartate aminotransferase (AST), alanine transferase (ALT), albumin, and total protein were the parameters assessed for the liver function test. The Activity of serum aspartate aminotransferase (AST) and alanine transferase (ALT), were estimated using the UV kinetic method given by Reitman and Frankel, (1957), serum albumin was estimated by the method given by Westgard and Poquette, (1972), serum of total proteins were determined by coloremetric method according to Gornall *et al*., (1949). All serum biomarkers were performed in accordance with instructions provided by the Biodiagnostic kit (Biodiagnostic Company, Dokki, Giza, Egypt).

5.2- Oxidative Stress Biomarkers:

The biochemical indicators of oxidative stress, including the levels of SOD, CAT, GSH, and MDA, were assessed in the liver homogenate according to the instructions included with the Biodiagnostic kit. Superoxide dismutase (SOD) levels were measured as described previously by the method given by (Nishikimi *et al*., 1972), and CAT was estimated by the method given by Sinha, (1972). While reduced glutathione (GSH) was estimated by the method given by Beutler *et al*., (1963) and lipid peroxidation biomarker as malondialdehyde (MDA) was estimated by the method given by Ohkawa *et al*., (1979).

6-Histopathological Studies:

All the rats' livers were washed, ethanol-dried, xylene-cleaned, and paraffin wax-fixed. Slices of liver five to six micrometers thick sections were stained with hematoxylin and eosin and prepared for microscopic examination. (Banchroft *et al*., 1996).

7 - Comet Assay:

Single-cell gel electrophoresis (SCGE) was used to measure induced DNA damage, as reported previously (Tice *et al*., 2000). In brief, a lobe of the liver was washed and homogenised in 5ml of homogenising buffer (Hanks' balanced salt solutions containing 25 mmol/l EDTA-2Na and 10% DMSO). The liver lobes were put in ice-cold mincing buffer and washed thoroughly with cold mincing buffer to eliminate any remaining blood. Cell suspensions were cooled on ice for 5 minutes before being centrifuged at 1000 rpm for 5 minutes. After removing the supernatant, the cells were resuspended in homogenising

buffer. 90 ml of 0.5% low-melting agarose gel and 10 microliters of the single-cell suspension were combined, and 90 ml of the resulting combination was applied to a slide that had already been covered with 1% normal melting agarose (NMA). For 30 minutes, the slides were placed on ice to allow the agarose layer to set.

The slides were set on ice for 30 minutes to solidify the agarose coating. The slides were then immersed overnight in a freshly prepared cold lysing solution (2.5 M NaCl, 100mM EDTA-2Na, and 10mM Tris, pH10.0: DMSO: Triton X-100, 89:10:1). the next day. The slides were electrophoresed in a horizontal electrophoresis device for 20 minutes at 1 V/cm and 300 mA, after spending 20 minutes in the electrophoresis buffer (300 mM NaOH, 1 mM EDTA, pH 13). Then the slides were washed three to four times with neutralisation buffer (0.4M Tris), dehydrated in cooled 100% alcohol and saved until analysis. To avoid extra DNA damage, all procedure stages were completed under reduced light conditions.

8 – Statistical Analysis:

The data were subjected to statistical analysis by the Statistical Package for the Social Sciences (SPSS) program (version 20) using one-way ANOVA followed by Duncan's multiple Range test (DMRT). The data were expressed as mean ± standard error (SE). *P* values < 0.01 and 0.05 are significant.

RESULTS

1. Signs of Toxicity:

No mortality was recorded during the experimental period.

3.2. Absolute and Relative Weights:

The data presented in Figure 1, shows the final absolute and relative organ weights of male rats in different treatment groups. There was a significant decrease ($p < 0.01$) in the absolute organ weight of rats treated with HU compared to the control group. Additionally, rats treated with HU+VC showed a significant reduction ($p < 0.05$) in absolute organ weight compared to the control group. However, there were no significant differences in the relative liver weight among all the groups.

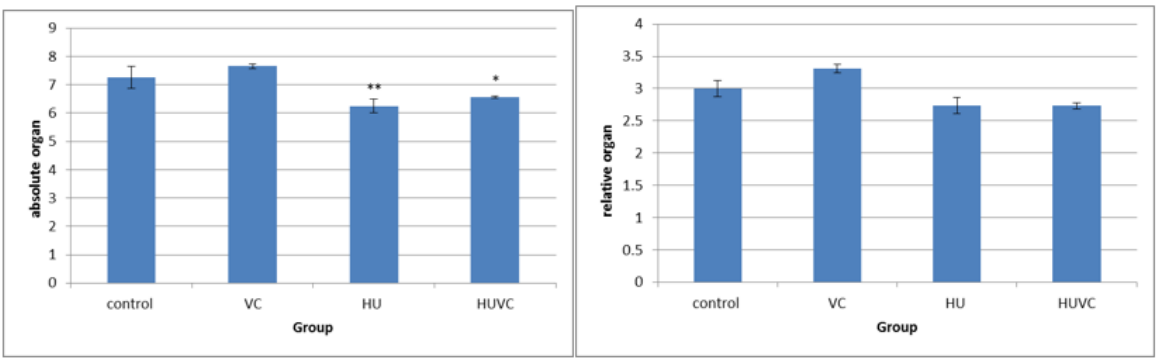


Fig. 1: This figure shows the effect of oral administration of vitamin C (5mg/kg bwt), hydroxyurea (100mg/kg bwt), and a combination of both (100mg/kg bwt of hydroxyurea and 5mg/kg bwt of vitamin C) for 30 days on the absolute and relative liver weight of rats. The data is presented as the mean ± SEM. Compared to the control group, the significance level is denoted as *$p < 0.05$ and **$p < 0.01$ (ANOVA with Duncan's test).

3. Serum Hepatocellular Enzymes:

Table 1, shows a significant increase ($p < 0.01$) in the level of serum hepatocellular enzymes (AST & ALT) in rats treated with HU and HU+VC groups in comparison with control. However, there was a significant decrease ($p < 0.01$) in the levels of (Albumin &

Total protein) in both HU and HU+VC groups in comparison to the control group.

Table 1. Effect of HU on AST, ALT, Albumin and Total protein levels in sera of male rats.

Treatments	AST	ALT	Albumin	Total Protein
Control	31.33±2.86	25.00±1.47	4.16±.04	7.13±.11
VC	41.33±1.02	31.00±1.87	4.10±.04	7.00±.04
HU	112.66±5.43**	85.66±4.98**	3.63±.06**	6.73±.06**
HU+VC	63.66±2.71**	48.33±3.79**	3.66±.12**	6.63±.06**

Values are means ± SE
* Significantly different from control (P< 0.05).
** Significantly different from control (P< 0.01).
SE: standard error.

4. Hepatic Oxidative Stress:

The results of hepatic oxidative stress (SOD, CAT, GSH, and MDA) of the rats were presented in Table 2. The level of (SOD, CAT, and GSH) significantly decreased ($p < 0.01$) in rats treated with HU compared to control. There was a significant decrease ($p < 0.05$) observed in rats treated with the combination group (VC+HU) compared to the control group. Rats given HU and VC+HU showed a significant ($p < 0.01$) increase in MDA content compared to the control.

Table 2. Effect of HU on Oxidative Stress biomarkers in liver tissues of male rats.

Treatments	SOD	CAT	GSH	MDA
Control	1045.00±45.14	84.00±5.09	3.92±.32	71.33±4.55
VC	963.33±22.75	93.00±3.08	3.76±.05	87.66±1.24
HU	682.66±13.45**	48.00±1.47**	1.69±.19**	166.33±2.71**
HU+VC	932.66±14.94*	58.66±1.24**	2.57±.16**	122.66±1.64**

Values are means ± SE
* Significantly different from control (P< 0.05).
** Significantly different from control (P< 0.01).
SE: standard error.

3.5. Hepatic Histopathology:

A photomicrograph of liver sections stained by H&E for histopathological changes is shown in Figure 2. The control group (A) displayed the normal histopathological structure of the hepatic lobule (H). The 100 mg/kg bw of HU group (C) exhibited hydropically deteriorated cells (notch arrow), changed lobular shape, nuclear degradation in several areas, necrosis (N), and severe fatty degeneration. Enlargement and congestion of the hepatic portal vein (PV) were observed, along with infiltration of lymphocytes (*) in the portal area and around the central vein (CV). Some hepatic cells showed degenerated alteration (dh) (pyknosis (head arrow) and karyolitic nuclei (bold arrow)) as well as the appearance of cytoplasmic vacuoles (bifid arrow). The VC group (B), treated with 5 mg/kg bw, showed a typical arrangement of hepatic cells (H), with no necrosis or fatty degeneration seen in liver sections. The (VC+HU) group (D) displayed evidence of improvement in the liver (H), moderate congestion, and a mild level of fatty deterioration (wavy arrow), but no necrotic areas or mononuclear cells were observed, although some hepatic cells showed a degree of degeneration (dh).

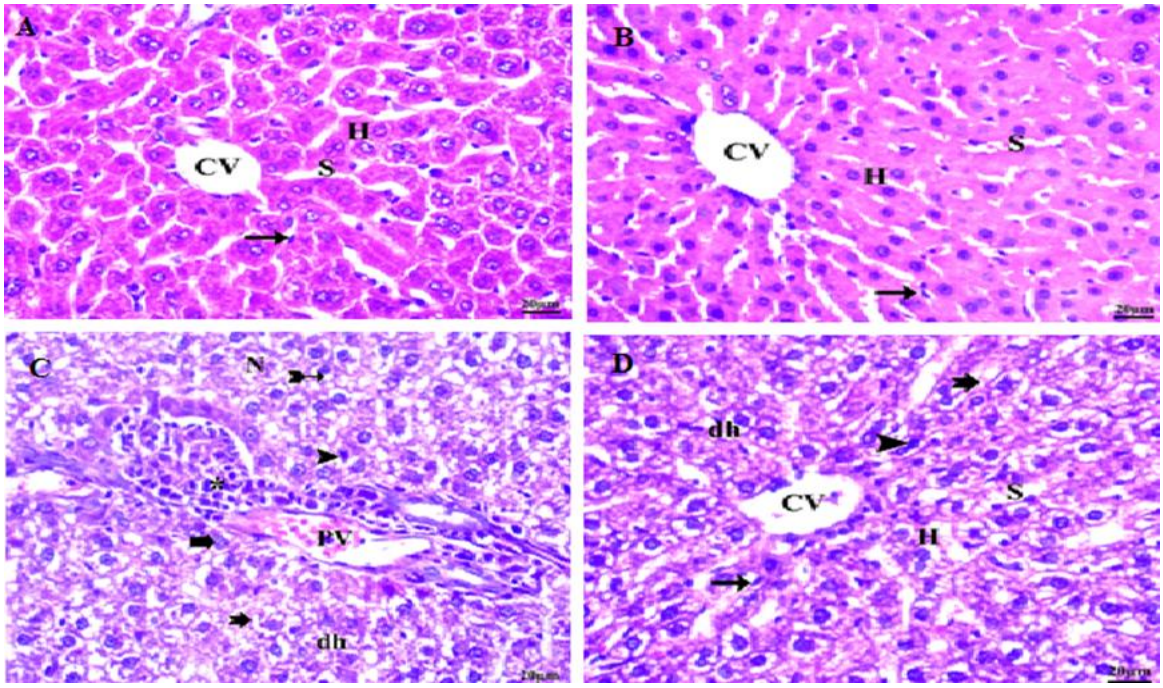


Fig. 2: Liver histopathology images of repeated dose group rat (A) Non-treated control, (B) VC treated group with 5mg/kg bw, (C) HU treated group with 100 mg/kg bw and (D) HU+VC treated group with (100+5 mg/kg bw).

6. Comet Parameters:

The results of comet assays using the liver cells of rats given VC, HU, and VC+HU are summarized in Table 3. The values for % Tailed, % DNA in Tail, and Tail Moment were significantly increased ($p < 0.01$) in both the HU and VC+HU groups compared to the control group. Additionally, we observed a significant increase in rats treated with HU and a substantial decrease in rats treated with VC+HU at ($p < 0.05$) as shown in Figure 3. No significant differences were observed in Olive tail moment in all treated groups.

Table 3. Comet parameters; % Tailed, Tail Length, % DNA in Tail, Tail Moment, and Olive tail moment of different treatment groups.

Treatment	% Tailed	Tail length	% DNA in tail	Tail moment	Olive tail moment
Control	8.88±.26	7.89±.26	5.83±.74	.40±.05	1.02±.06
VC	18.05±.30	9.19±.50	8.64±.94	.75±.08	1.21±.09
HU	15.90±.21**	10.20±.57*	6.88±.25**	.67±.07**	1.24±.06
HU+VC	22.73±.24**	7.60±.98*	8.66±.36**	.60±.08**	1.30±.06

Values are means ± SE
* Significantly different from control (P< 0.05).
** Significantly different from control (P< 0.01).
SE: standard error.

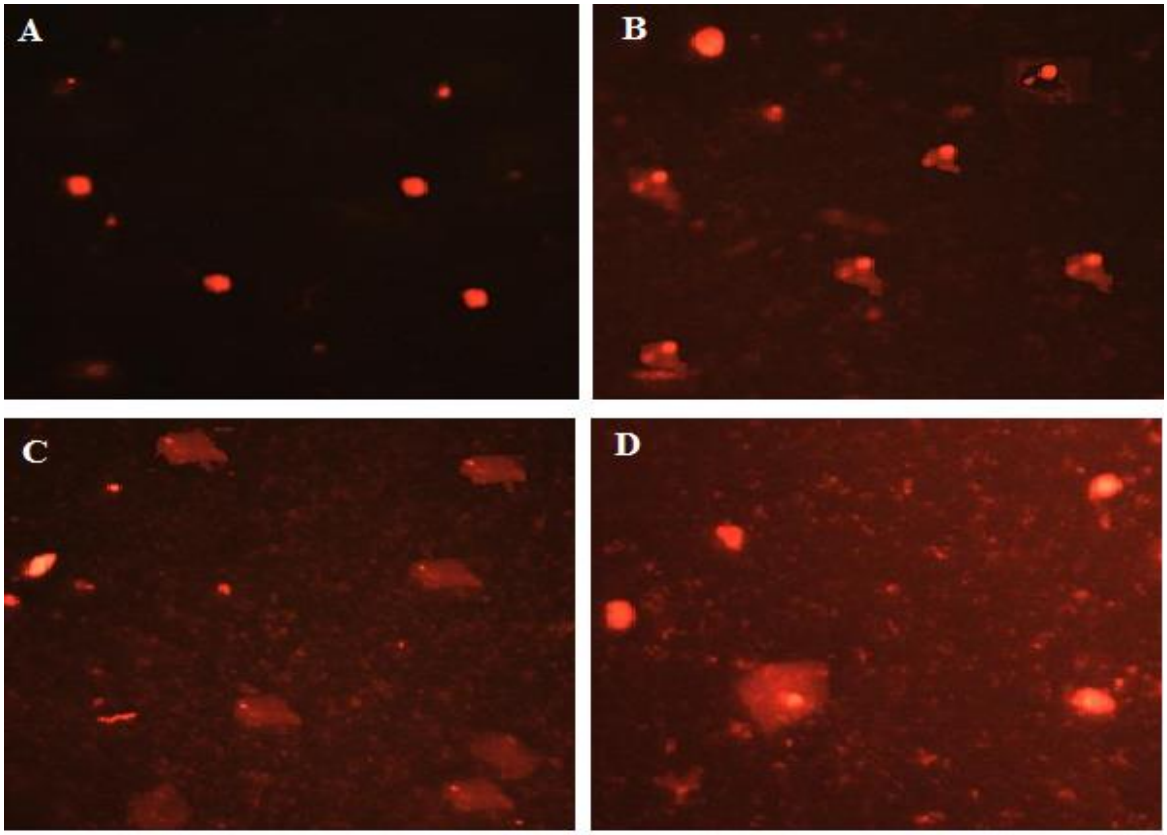


Fig. 3: Photographs of cells tested using the comet assay analysis. This figure displays images captured during the comet assay analysis. The spots in the images depict the DNA of individual cells. The intact DNA is represented by a "dark/white" round spot. Intact DNA is a large molecule that doesn't move much in the electrophoretic field. The less dark, "comet-shaped" area next to the nucleus represents small DNA breaks that can move in the gel. (A) control group, (B) VC group, (C) HU group, and (D) VC+HU group.

DISCUSSION

The study investigates the ameliorative effect of vitamin C against hydroxyurea-induced hepatic toxicity in male rats. Findings revealed a significant decrease in absolute organ weight was seen in rats treated with HU and a significant decrease in rats treated with HU+VC in comparison with the control group. However, all groups had no significant difference in relative liver weight. Khamis and co-workers found that hydroxyurea treatment led to weight loss in rats after 20 days of administration at different doses. (Khamis *et al*., 2017).

Helvaci and colleagues' study on hydroxyurea therapy's effects on sickle cell disorders (SCD) revealed that it leads to weight loss in SCD patients (Helvaci *et al*., 2021). This aligns with previous investigations into the harmful effects of hydroxyurea on various organs and tissues, including hepatotoxicity in rats leading to fatty infiltration, necrosis, and inflammation (Morton *et al*., 2015). In addition, hydroxyurea therapy in mice reduced the testicular weight and sperm count (Jones et al., 2009). It also caused a decrease in body weight of juvenile rats (Huang *et al*., 2021). These results imply that hydroxyurea may have harmful effects on several organs and tissues, which may be a factor in the observed reduction in absolute organ weight in rats given HU and HU+VC treatment. As a result, the lack of a significant difference in relative liver weight between any of the groups is consistent with the findings and implies that the therapies had no impact on relative liver weight.

Moreover, this study explored the potential protective effect of vitamin C against hydroxyurea-induced hepatic toxicity. We observed significant changes in hepatic oxidative stress markers, with levels of superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) significantly decreased in rats treated with hydroxyurea alone, while a combination of vitamin C and hydroxyurea resulted in a significant increase in these antioxidant levels compared to the control group. Conversely, malondialdehyde (MDA) content, an indicator of lipid peroxidation, significantly increased in rats treated with HU and HU+VC compared to the control group.

Tohamy and co-workers' findings were consistent with the results of this study, showing increased oxidative biomarkers and reduced hepatic antioxidant molecules in HU-treated rats (Tohamy *et al*., 2022). Additionally, Suleiman and co-workers observed that lead-induced AST, ALT, and ALP serum concentrations decreased with vitamin C administration, suggesting a potential protective effect against lead-induced liver damage (Suleiman *et al*., 2013). Antioxidants and oxidative stress have been extensively studied among liver conditions, and non-alcoholic fatty liver disease (NAFLD) is included. A randomized clinical experiment by He and co-workers demonstrated improved liver function tests in NAFLD patients with oral vitamin C supplementation (He *et al*., 2021). Similar protective effects were observed in rats treated with 5-fluorouracil and lead suggesting a potential role for vitamin C in preventing drug-induced liver injury. **(**El-Neweshy and El-Sayed, 2011; Al-Asmari *et al*., 2016**).**

Furthermore, in this study, histopathological analysis revealed severe hepatic lesions in rats treated with hydroxyurea, including cellular degeneration, necrosis, fatty degeneration, and inflammatory cell infiltration which were consistent with previous studies (Gao *et al*., 2021). However, co-treatment with vitamin C showed evidence of improvement, emphasizing the potential protective role of vitamin C against hydroxyurea-induced liver damage. However, several studies have explored vitamin C's protective effects against hepatotoxicity induced by different drugs, including emamectin benzoate (Khaldoun Oularbi *et al.,* 2017), alcohol **(**Okamura *et al.,* 2018), and UVC (Attia *et al.,* 2023). These studies consistently demonstrated that vitamin C attenuates liver damage and oxidative stress caused by these substances (Khaldoun Oularbi *et al.,* 2017; Okamura *et al.,* 2018 and Attia *et al.,* 2023).

The findings of this study also demonstrated notable alterations in the comet assay parameters. Specifically, the percentages of cells exhibiting comet tails, the amount of DNA in the comet tails, and the magnitude of the comet tails (Tail Moment) exhibited significant increases in both the hydroxyurea (HU) group and the group treated with a combination of vitamin C and hydroxyurea (VC+HU), when compared to the control group. However, in the VC+HU group, these parameters showed a significant decrease compared to the HU group. On the other hand, no significant disparity was observed in the Olive tail moment across all the treated groups. These findings highlight the impact of hydroxyurea and the potential mitigating effect of vitamin C on the comet assay parameters, underscoring the importance of further investigation in this area.

Prior in vivo studies on chromosome aberrations in SCA patients receiving hydroxyurea showed mixed results, with some studies showing increased DNA damage **(**Friedrisch *et al*., 2018), while a study by McGann *et al*. (2012)was not associated with any significant increases in genotoxicity.

In conclusion, our study provides comprehensive evidence of the potential defensive impact of vitamin C in opposition to hydroxyurea-induced hepatic toxicity in male rats. Vitamin C supplementation demonstrated benefits in mitigating changes in body weight, liver parameters, serum hepatocellular enzymes, oxidative stress indicators, histopathological alterations, and DNA damage. Further research is recommended to elucidate the underlying mechanisms and therapeutic implications of vitamin C in

hydroxyurea-induced liver damage. Mathematical modelling can be employed to advance understanding of hydroxyurea's mechanism of action.

Declarations:

Ethical Approval: The animal experiments were approved by the ethical committee of the Faculty of Science, University of Sirte, Sirte, Libya. (NO. 7/2023).

Competing interests: The authors have declared that no competing interests exist.

Authors Contributions. FM, EA, and GS collaborated on study design, implementation, analysis of results, and manuscript writing.

Funding: This study was not funded.

Acknowledgements: The authors thank Dr. Heba Ali Abd El-Rahman from Cairo University's Department of Zoology, Faculty of Science for her support and encouragement, and Alsaidi M. Altaher Associate Prof. at Sebha University-Department of Statistics. No financial assistance has been provided to support this study.

The authors are commissioners and have all contributed to the drafting and final version of the paper.

REFERENCES

Adamson, R.H. (1965). Activity of Congeners of Hydroxyurea Against Advanced Leukemia L1210. *Proceedings of the Society for Experimental Biology and Medicine*. 119, pp. 456–458.

Al-Asmari, A.K., Khan, A.Q. and Al-Masri, N. (2016). Mitigation of 5-fluorouracil–induced liver damage in rats by vitamin C via targeting redox–sensitive transcription factors. *Human & experimental toxicology*, 35(11), pp. 1203-1213.

Alsalman, M., Alkhalifa, H., Alkhalifa, A., Mustafa, A., Nora, A., Abdulaziz, A., Mohammed, A., Abdulrahman, A. and Abdul, Sattar, K. (2021). Hydroxyurea usage awareness among patients with sickle-cell disease in Saudi Arabia. *Health Science Reports.* 4(4): e437

Ashley, A.K., Shrivastav, M., Nie, J., Amerin, C., Troksa, K., Glanzer, J.G., Liu, S., Opiyo, S.O., Dimitrova, D.D., Le, P., *et al*. (2014). DNA-PK phosphorylation of RPA32 Ser4/Ser8 regulates replication stress checkpoint activation, fork restart, homologous recombination and mitotic catastrophe. *DNA Repair*; 21, pp.131–139.

Attia, A.A., Hamad, H.A., Fawzy, M.A. and Saleh, S.R. (2023). The Prophylactic Effect of Vitamin C and Vitamin B12 against Ultraviolet-C-Induced Hepatotoxicity in Male Rats. *Molecules*; 28(11), 4302

Banchroft, J.D., Stevens, A. and Turner, D.R. (1996). Theory and Practice of Histological Techniques. 4th ed.New York, NY, USA: *Churchill Livingstone*

Beutler, E., Duron, O. and Kelly, B.M. (1963). Improved method for the determination of blood glutathione. *Journal of Laboratory and Clinical Medicine.* 61:882

Brose, R.D., Lehrmann, E., Zhang, Y., Reeves, R.H., Smith, K.D. and Mattson, M.P. (2018). Hydroxyurea attenuates oxidative, metabolic, and excitotoxic stress in rat hippocampal neurons and improves spatial memory in a mouse model of Alzheimer's disease. *Neurobiology of Aging*, 72, pp. 121–133.

Chihuailaf, R., Contreras, P. and Wittwer, F. (2002). Pathogenesis of oxidative stress: Consequences and evaluation in animal health. *Veterinaria Mexico journal*.33(3), pp. 265-283

Demchuk, O.G., Hrytsyna, M.R., Kobryn, L.O., Kalytovska, M.B. & Gutyj, B. (2020). The mechanism of action and modified synthesis of hydroxyurea. *Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies.Series: Veterinary sciences*. 22(100), pp. 94-102.

El-Neweshy, M.S. and El-Sayed, Y.S. (2011). Influence of vitamin C supplementation on

lead-induced histopathological alterations in male rats. *Experimental and Toxicologic Pathology*, 63(3), pp. 221-227.

Friedrisch, J.R., Pra, D., Maluf, S.W., *et al*. (2018). DNA damage in blood leukocytes of individuals with sickle cell disease treated with hydroxyurea. *Mutation Research*; 649, pp. 213–220.

Gao, W., Jin, Y., Hao, J., Huang, S., Wang, D., Quan, F., *et al*. (2021). Hydroxyurea affects in vitro porcine oocyte maturation through increased apoptosis and oxidative stress. *Bioscience Reports*. 41(4):BSR20203091.

Goncharova, P.S., Davydova, T.K. and Zhukova, N.G. (2021). Prospects for studying the pharmacokinetics, pharmacodynamics and pharmacogenetics of vitamin C in patients with neurological diseases and mental disorders. *Personalized Psychiatry and Neurology*. 1 (2), pp. 63-82.

Gornall, A.G., Bardawill, C.J. and David, M.M. (1949). Determination of serum proteins by means of the biuret reaction. *Journal of Biological Chemistry*.177, pp. 751-766.

Gupta, R.S., Gupta, E.S., Dhakal, B.K.,Thakur, A.R. and Ahnn, J. (2004). Vitamin C and vitamin E protect the rat testes from cadmium-induced reactive oxygen species. *Molecules and Cells*.17, pp. 132-139.

Hallam, M.J. and Kolesar, J.M. (2008). Hydroxyurea induced acute elevations in liver function tests. *Journal of Oncology Pharmacy Practice*. 14(1), pp. 61–3.

Hankins, J.S., Aygun, B., Nottage, K., *et al*. (2014). From infancy to adolescence: fifteen years of continuous treatment with hydroxyurea in sickle cell anemia. *Medicine (Baltimore)* ;93:e215

He, Z., Li, X., Yang, H., Wu, P., Wang, S., Cao, D. and Luo, X. (2021). Effects of oral vitamin C supplementation on liver health and associated parameters in patients with non-alcoholic fatty liver disease: a randomized clinical trial. *Frontiers in Nutrition*, 8, 745609

Heddle, R. and Calvert, A. (1980). Hydroxyurea induced hepatitis. *Medical Journal of Australia*. 1(3),121

Helvaci, M. R., Altintas, E., Yalcin, A., Muftuoglu, O. E., Abyad, A., and Pocock, L. (2021). Disappearance of hepatitis C virus antibodies with hydroxyure therapy in sickle cell diseases. *Middle East Journal of Family Medicine* 7(10), 43.

Huang, M.C., Turner, K.J., Vallant, M., Robinson, V.G., Lu, Y., Price, C.J., Fennell, T.R., Stilinski, M.A., Waidyanatha, S., Ryan, K.R., Black, S.R., Fernando, R.A. and McIntyre, B.S. (2021). Tolerability and age-dependent toxicokinetics following perinatal hydroxyurea treatment in Sprague Dawley rats. *Journal of applied toxicology:* JAT,41(7), pp. 1007– 1020.

Jones, K.M., Niaz, M.S., Brooks, C.M., Roberson, S.I., Aguinaga, M.P., Hills, E.R. and Archibong, A.E. (2009). Adverse effects of a clinically relevant dose of hydroxyurea used for the treatment of sickle cell disease on male fertility endpoints. *International journal of environmental research and public health* 6(3), pp. 1124-1144.

Julius, J., Peng, J., McCulley, A., Caridi, C., Arnak, R., See, C., Nugent, C.I., Feng, W. and Bachant, J. (2019). Inhibition of spindle extension through the yeast S phase checkpoint is c oupled to replication fork stability and the integrity of centromeric DNA. *Molecular Biology of the Cell*, 30, pp. 2771–2789.

Khaldoun Oularbi, H., Richeval, C., Lebaili, N., Zerrouki-Daoudi, N., Baha, M., Djennas, N. and Allorge, D. (2017). Ameliorative effect of vitamin C against hepatotoxicity induced by emamectin benzoate in rats. *Human & experimental toxicology,* 36(7), pp. 709–717.

Khamis, A. M., Oda, S. S., Khafaga, A. F., and Hashem, M. A. (2017). Hemato Cytological Evaluation of Hydroxyurea-Induced Toxicity in Male Rats. *Alexandria Journal for*

Veterinary Sciences, 55(2).

Kramara, J., Osia, B. and Malkova, A. (2018). Break Induced Replication: The where, the why, and the how. *HHS Public Access. Trends in Genetics***,** 34, pp. 518–531.

Lisziewicz, J., Foli, A., Wainberg, M, and Lori, F. (2003). Hydroxyurea in the Treatment of HIV Infection. *Drug Safety*. 26(9), pp. 605–24.

Liu, I.M., Tzeng, T.F., Liou, S.S. and Lan, T.W. (2007). Myricetin, a naturally occurring flavonol, ameliorates insulin resistance induced by a high-fructose diet in rats. *Life sciences*. 81(21–22), pp.1479–88

Madaan, K., Kaushik, D. & Verma T. (2012). Hydroxyurea: a key player in cancer chemotherapy. *Expert review of anticancer therapy*. 12(1), pp.19–29

McDowell, L.R. (1989). Vitamins in animal nutrition-comparative aspects to human nutrition: vitamin C. *Academic Press, London*. pp. 93-131.

McGann P.T., Flanagan, J.M., Howard, T.A., Dertinger, S.D., He, J., Kulharya,A.S., Thompson, B.W. and Ware, R.E. (2012). BABY HUG Investigators. Genotoxicity associated with hydroxyurea exposure in infants with sickle cell anemia: results from the BABY-HUG Phase III Clinical Trial. *Pediatric blood & cancer*. 59(2), pp. 254-7.

Morton, D., Reed, L., Huang, W., Marcek, J. M., Austin-LaFrance, R., Northcott, C. A., Schelling, S. H., Enerson, B. E., and Tomlinson, L. (2015). Toxicity of hydroxyurea in rats and dogs. Toxicologic pathology, 43(4), pp. 498– 512.

Nishikimi, M., Rao, N.A. and Yagi, K. (1972). The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. *Biochemical Biophysical Research Communications*. 46(2), pp. 849–54.

Ohkawa, H., Ohishi, N. and Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*. 95(2), pp. 351–8

Okamura, Y., Omori, A., Asada, N and Ono, A. (2018). Effects of vitamin C and E on toxic action of alcohol on partial hepatectomy-induced liver regeneration in rats. *Journal of clinical biochemistry and nutrition*, 63(1), pp. 50–57

Reitman, S. and Frankel, S. (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *American Journal of Clinical Pathology* 28(1), pp. 56–63

Rosenthal, F., Wislicki, L. and Kollek, L. (1928). Über die Beziehungen von Schwersten Blutgiften zu Abbauprodukten des Eiweiss Ein Beitrag zum Entstehungsmechanismus der perniziösen Anämie. *Klinische Wochenschrift***,**7, pp. 972–977

Shimizu, T., Mori, T., Karigane, D., Kikuchi, T., Koda, Y., Toyama, T., *et al*. (2014). Hydroxyurea (hydroxycarbamide)- induced hepatic dysfunction confirmed by drug-induced lymphocyte stimulation test. *[Rinsho ketsueki] The Japanese journal of clinical hematology*. 55(1), pp. 125–9.

Sies, H., Stahl, W. and Sundquist, A.R. (1992). Antioxidant functions of vitamins. Vitamin E and C, β-carotene, and other carotenoids. *Annals of the New York Academy of Sciences*, 669(1), pp.7- 20

Sinha, K.A. (1972). Colorimetric assay of catalase. *Analytical Biochemistry*, 47, pp. 389.

Spivak, J.L. and Hasselbalch, H. (2011). Hydroxycarbamide: A user's guide for chronic myeloproliferative disorders. *Expert Review of Anticancer Therapy*. 11, pp. 403–414.

Stearns, B., Losee, K.A. and Bernstein, J. (1963). Hydroxyurea: A New Type of Potential Antitumor Agent. *Journal of Medicinal Chemistry*. 6, 201

Steinberg, M.H., McCarthy, W.F., Castro, O., *et al*. (2010). The risks and benefits of long-term use of hydroxyurea in sickle cell anemia: A 17.5 year follow-up. *American Journal of Hematology:* 85, pp. 403-8.

Suleiman, J.B., Eze, E.D., Momoh, I.J., Usman, W., Hedima, N.C., Zip Ele, H.M., and Isa, A. (2013). Ameliorative effect of vitamin C on serum liver enzymes in lead-induced toxicity in Wistar rats. Journal of Science, 3(1), pp. 188-192.

Tice, R.R., Agurell, E., Anderson, D., Burlinson, B., Hartmann, A., Kobayashi, H., Miyamae, Y., Rojas, E., Ryu, J.C. and Sasaki, Y.F. (2000). Single Cell Gel Comet Assay: Guidelines for In-Vitro and In-Vivo Genetic Toxicology Testing. *Environmental and Molecular Mutagenesis*, 35, pp. 206-221

Tohamy, H.G., El-Neweshy, M.S., Soliman, M.M., Sayed, S., Shukry, M., Ghamry, H.I. and Abd-Ellatieff, H. (2022). Protective potential of royal jelly against hydroxyurea-induced hepatic injury in rats via antioxidant, anti-inflammatory, and anti-apoptosis properties. *Plos one*. 18;17(3):e0265261.

Vermeij, J.D., Aslami, H., Fluiter, K., Roelofs, J.J., van den Bergh WM, Juffermans N.P., *et al*. (2013). Traumatic brain injury in rats induces lung injury and systemic immune suppression. *Journal of neurotrauma*. 30 (24), pp. 2073–9.

Weaver, S.B., Rungkitwattanakul, D. & Singh, D. (2021). Contemporary Management and Prevention of Vaso-Occlusive Crises (VOCs) in Adults with Sickle Cell Disease. *Journal of Pharmacy Practice*, 36(1), pp. 139-148

Westgard, J.O. and Poquette, M.A. (1972). Determination of serum albumin with the "SMA 12-60" by a bromocresol green dye-binding method, Clinical Chemistry. 18 (18) , pp. 647–653.

Xu Y.J., Singh A. and Alter G.M. (2016). Hydroxyurea induces cytokinesis arrest in cells expressing a mutated sterol-14α-demethylase in the ergosterol biosynthesis pathway. *Genetics.* 204, pp. 959 –973.

Yazinski, S.A. and Zou, L. (2016). Functions, Regulation, and Therapeutic Implications of the ATR Checkpoint Pathway. *Annual Review of Genetics*, 50, pp. 155–173.