LJS Vol. 27, No. 02 (2024) 26-34

The Libyan Journal of Science
University of Tripoli Vol. 27, No. 02
https://uot.ed.ly/journals/index.php/ljs

Hepatoprotective and Hematoprotective Effects of Vitamin C Against Paracetamol Toxicity in Wistar Albino Rats

Wafa F. El Matoni[1*], Mustafa A. Sidoun[1], Alhammaly H. Shabash[1]

[1.] Department of Biology, zoology branch, Faculty of Science, University of Misurata

Corresponding author: Wafa F. El Matoni, elmatonw@sci.misuratau.edu.ly

ARTICLE INFO

Article history:

Received 20/10/2024

Received in revised form 06/07/2024

Accepted 20/07/2024

ABSTRACT

Paracetamol is a commonly prescribed analgesic and antipyretic medication with potentially lethal side effects when used in large doses. The current experiment sought to determine if vitamin C (ascorbic acid) alleviates the hepatotoxicity and hematotoxicity induced by paracetamol in rats. Thirty-two male rats were divided into four groups and treated for 4 weeks: control group; paracetamol treated group (APAP 400 mg/kg, orally); vitamin C treated group (200 mg/kg, orally); vitamin C-paracetamol treated group (vitamin C 200 mg/kg orally and APAP 400 mg/kg orally). The findings of this study showed that paracetamol administration significantly elevated the liver enzymes (AST and ALT), WBCs, and MCHC values while significantly reducing the MCV level. Vitamin C restored the AST, ALT levels, and WBCs count to their normal levels and reversed the paracetamol effects on hematological markers. The current study's findings show that vitamin C greatly reduces APAP -induced hepatotoxicity while only slightly protecting against hematotoxicity.

Keywords: Paracetamol, Vitamin C, Hepatotoxicity, Liver enzymes, Complete blood count.

1. Introduction

Paracetamol (Acetaminophen; N-acetyl-Paminophenol (APAP) is a widely used analgesic and antipyretic both over the counter and on prescription [1]. It was first made available on the market by Von Mering in 1893 as an analgesic medication [2]. Although paracetamol is generally regarded as a safe medication, an excessive amount can have fatal consequences for both humans and lab animals due to hepatotoxicity [3].

Vitamin C (VC), also known as L-ascorbic acid, is an organic compound that exists in nature and has antioxidant properties [4]. Furthermore, it is a crucial

co-factor to produce collagen, the metabolism of carnitine and catecholamines, and the assimilation of dietary iron [5]. V C is a water-soluble vitamin found in many foods, particularly vegetables like cabbage, cauliflower, turnips, potatoes, peas, sweet potatoes, and tomatoes as well as fruits like kiwi, mango, orange, strawberry, and watermelon [6].

The liver is the largest organ in the body. It is responsible for detoxifying medicines and poisons [7], as well as storing nutrients, breaking down erythrocytes, secreting bile, producing plasma proteins, and producing steroid alcohol [8]. Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) are aminotransferases that are involved in the liver's gluconeogenesis. Hepatocytes' cytoplasm and mitochondria both contain AST, however, only the cytosol of these cells contains ALT. Whenever there is hepatocytic damage, these enzymes leak into the circulation [9]. ALT is mostly located in the liver, as opposed to AST, which is also plentiful in other organs such as cardiac muscle and the kidneys. As a result of the concurrent increase in ALT and AST levels, ALT is more specific than AST in diagnosing hepatic [10].

Hematological changes are frequent biomarkers of physiological state [11]. One of the most popular laboratory tests conducted today is the complete blood count (CBC) with differential. The assessment of red blood cell (RBC) indices, hemoglobin (Hb), and hematocrit (HCT), provides details regarding the synthesis of all blood cells and determines the patient's capacity to carry oxygen. Through a differential analysis of the white blood cell (WBC) count, it also offers insight into the immune system. These tests are useful for identifying anemia, some types of cancer, infections, acute hemorrhagic conditions, allergies, and immunodeficiencies. They can also be used to check for the side effects of specific medications that can result in blood dyscrasias [12].

Previous research has demonstrated that paracetamol can cause liver toxicity in humans, rats, and mice [1,3,8] and that vitamin C can function as a preventive antioxidant against this toxicity [13-14]. Hassanin and his colleagues [15] claimed that the administration of paracetamol (two doses of 600 mg/kg, orally) resulted in a significant increase in AST and ALT enzymes. However, pre-administration of vitamin C (dose of 500 mg/kg body weight/ day for six successive days (paracetamol was given on the third and fourth days of administration)) attenuated this increase.

El-Ridi and Rahmy [13] reported that giving a single toxic oral dose of APAP (1 g/kg b.wt.) to rats caused a noticeable rise in the levels of the AST and ALT enzymes. On the other hand, injected by a single dose of vitamin C (80, 160, and 320 mg/kg b.wt.) 20 min after paracetamol reduced this rise; the highest dose was the most effective one.

According to Abdulkhaleq et al. [14], pre-treatment of rats with vitamin C (500 mg/kg) for 9 days reduced the hepatotoxicity produced by APAP (2800 mg/kg on days 7 and 8), as seen by lower AST and ALT values.

Previous studies have emphasized APAP-induced hematotoxicity [16-18]; however, the protective role of vitamin C against this toxicity has not been fully understood. To our knowledge, there was only one study that illustrated this effect [19]. Matić et al. [19] claimed that paracetamol caused a reduction in red blood cell indicators while causing elevated levels of white blood cell count in rats receiving a dose of 300 mg/kg/day i.p. for three days. On the other hand, co-treatment of vitamin C (100 mg/kg/day i.p.) reverted alterations in hematological parameters to those measured in the control group, reducing the harmful

effects of APAP. Previous studies have almost exclusively focused on paracetamol acute hepatotoxicity and the role of vitamin C in preventing this toxicity. Thus, the purpose of this study was to investigate the potential protective benefits of vitamin C on altered hepatic and haematological markers in rats given paracetamol for a month.

2. Material and Methods

2.1. Chemicals

Paracetamol was provided by the Faculty of Pharmacy/ Misurata University. VC (Ascorbic Acid 95%Granulation, Roche Company).

2.2. Experimental Animals

A total number of thirty-two male albino Wistar rats, weighing 110-125 g were used in this experiment. Rats were obtained from the animal house of Zoology department, Faculty of Science, Misurata University. Animals were housed in (36x26x22 cm) cages in a constant environment at normal temperature (22 ± 5 °C) under 13:11 h light-dark cycle. The animals had access to diet and water ad libitum throughout the period of the experiment. The international guide for the care and use of laboratory animals was followed when handling and using the animals.

2.3. Experimental design

The rats were randomly divided into four equal groups (8 rats each).

Group 1: Control group: Rats did not receive any treatment.

Group 2: APAP treated group: rats were orally given APAP at a dose of 400 mg/kg body weight for four weeks [15, 20-21].

Group 3: VC treated group: rats were orally administered vitamin C 200 mg/kg body weight for four weeks [15, 21].

Group 4: VC- APAP treated group: rats were orally administered VC at a dose of 200 mg/kg body weight/day for four weeks half hour before the administration of APAP (400 mg/kg) [15,21].

All treatments were given via oral gavage (Gastric Tube) and the solutions for paracetamol and vitamin C were freshly prepared daily.

2.4. Serum biochemical analysis

At the end of the experiment, rats in all groups were fasted overnight. All rats were sacrificed, and blood was taken from the jugular vein. Two blood samples were taken from each rat. One sample was put into a tube containing EDTA as an anticoagulant for hematological assessment. The other sample was put in a plain tube and allowed to coagulate (15 minutes at room temperature), then centrifuged at 3000 for 15 min. The clear sera were collected for biochemical analysis. At MED laboratory (Misurata), the biochemical markers AST and ALT were measured using an autonomic analyzer (Biosystem (BTS-350) in accordance with the manufacturer's methods and instructions.

2.5. Haematological analysis

Whole blood samples were used immediately after collection on EDTA to estimate hematological parameters such as red blood cell (RBC) count, hemoglobin (Hb) concentration, white blood cell (WBC) count, hematocrit value (HCT%), platelets (Plt) count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). These parameters were determined using an automated hematological analyzer (CBC-SAMSUNG).

2.6. Statistical analyses

All statistical analyses were performed using the GraphPad Prism 5 software (GraphPad Software Inc., San Diego, USA). Results were expressed as the mean ± standard error. $p < 0.05$ was considered

significant. Experimental data were analysed using analysis of variance (ANOVA). The significant differences between means were determined using Tukey's multiple comparison test.

3. Results

3.1. Effects of paracetamol and vitamin C on the live function enzymes

There was a significant increase in AST and ALT activity in the paracetamol-treated group compared to the control group ($p< 0.05$). In contrast, the group treated with vitamin C significantly decreased serum enzymatic activity of AST and ALT when compared to hepatotoxicity induced group (Fig. 1).

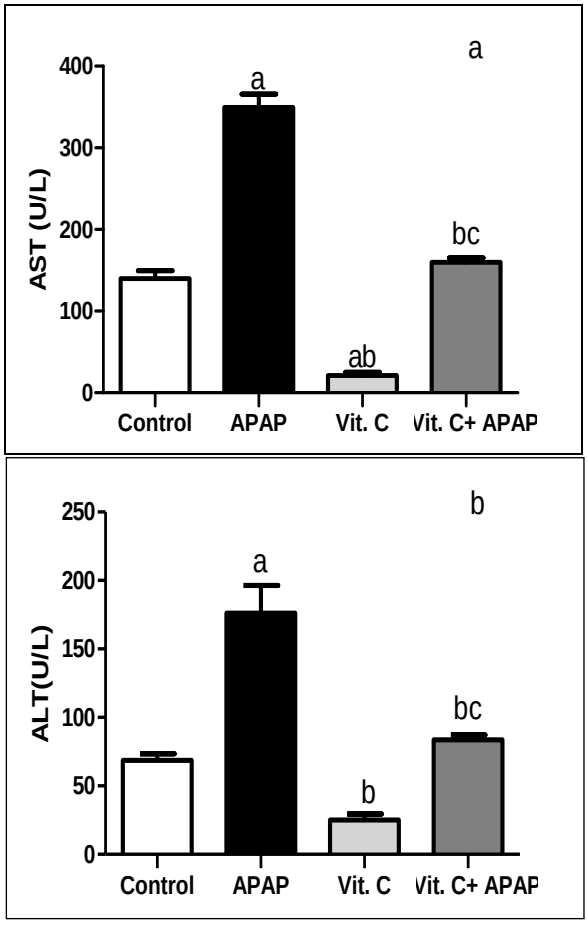

Fig.1. Effects of paracetamol and Vitamin C on liver function enzymes AST (a) and ALT (b)

3.2. Effects of paracetamol and vitamin C on hematological parameters

Administration of paracetamol caused a slight increase in RBCs count compared to the control group. Pre-administration of vitamin C resulted in a higher RBC count compared to the paracetamol-treated group. However, these changes were not statically different ($p>0.05$) (Fig. 2a). There was a significant increase in WBC count in the group treated with APAP compared to the control. On the other hand, there was a decrease in their count when pre-treated with vitamin C ($p < 0.05$) (Fig. 2b).

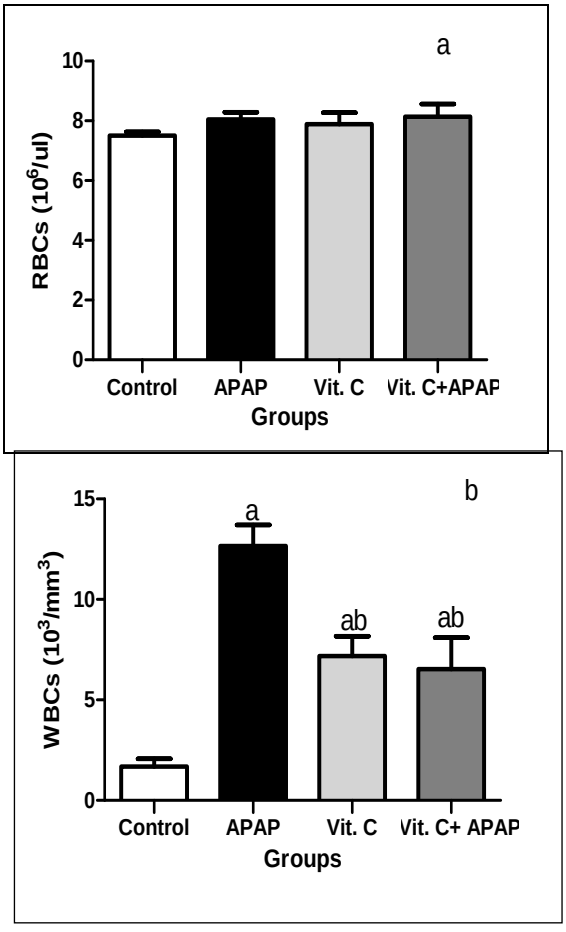

Fig.2. Effects of paracetamol and Vitamin C on RBCs (a) and WBCs (b) count

There was a reduction in PLT numbers in the APAP group and the VC+ APAP group compared to the

control group. These changes were not statistically different ($p > 0.05$) (Fig. 3).

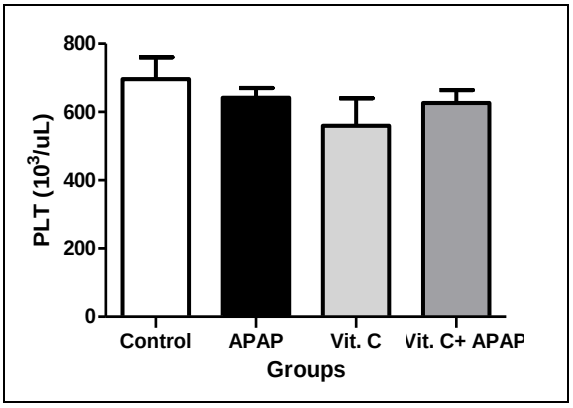

Fig.3. Effects of paracetamol and Vitamin C on PLT count

Exposure to APAP insignificantly ($p > 0.05$) increased Hb and HCT when compared to the control group. Administration of VC slightly decreased the Hb compared to the paracetamol group while significantly elevating the HCT value when compared to the APAP and control groups ($p < 0.05$) (Fig. 4 a, b).

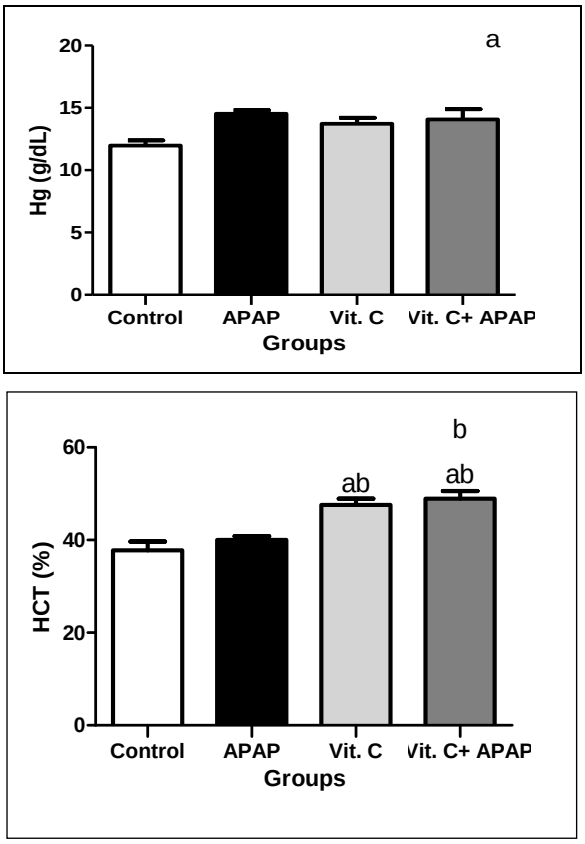

Fig. 4. Effects of paracetamol and Vitamin C on Hb (a) and HCT (b)

APAP treatment considerably reduced MCV values and significantly increased MCHC values compared to the control group ($p < 0.05$). However, MCH values did not show substantial changes in the treated groups compared to the control group. When compared to the APAP-treated group, VC administration dramatically reduced MCHC ($p < 0.05$) (Fig.5).

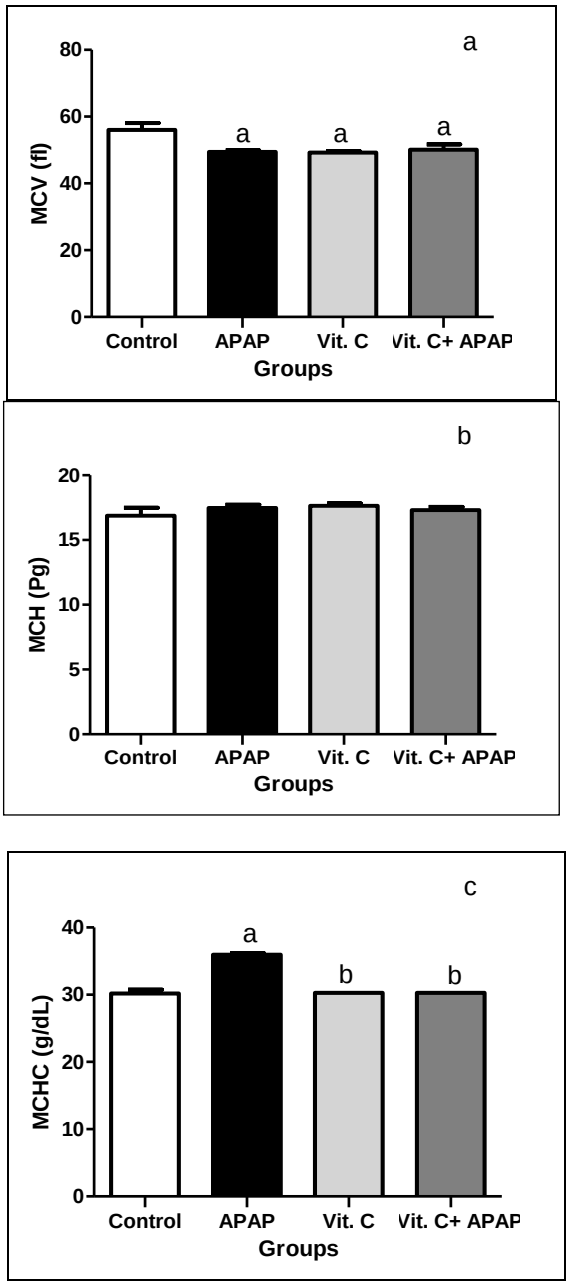

Fig. 5. Effects of paracetamol and Vitamin C on MCV (a), MCH (b), and MCHC (c)

4. Discussion

The current study sought to determine the protective effect of VC against APAP-induced hepatotoxicity and hematotoxicity in Wistar rats. The results showed that VC dramatically decreased liver damage caused by APAP- in albino Wistar rats. The study also found that APAP administration to the rats at the dose of 400 mg/kg body weight increased the levels of AST and ALT. Similar results have been reported; that APAP considerably elevated the levels of these enzymes [15]. The same results were also consistent with [14]. These results can show that the liver's structural integrity has been compromised. After cellular destruction, it is released into the bloodstream, indicating the onset of liver toxicity [20,22]. On the other hand, treatment of rats with VC alleviated the AST and ALT levels. These results are in agreement with previous studies [13,14,15,23].

The protective effect of VC appears to be due to its role as a cofactor in a variety of enzymatic activities and may also function as an antioxidant, protecting against oxidative damage caused by paracetamol [14]. Treatment with VC in conjunction with APAP has been found to protect the liver against paracetamol toxicity by protecting cellular membranes and limiting the influence of free radicals [13].

It was previously documented that paracetamol intoxication resulted in lower RBC counts, Hb concentration, HCT, and MCH [16,24]. However, the results of this study indicate that APAP intoxication increased these factors, including RBC count, Hb concentration and HCT compared to the control group. These results are in line with previously reported studies [25,17].

The effect of paracetamol on RCB count, Hb, and HCT appears to vary according to the treatment time; when the treatment lasted less than one week, these biomarkers declined [24], however when the treatment lasted two weeks or more, these indicators increased [25,17]. According to Khattab et al. [26], APAP treatment of rats resulted in a decline in RBC indicators in the first week while boosting these indicators in the second and third weeks of treatment. These results require further investigation to confirm the current observation. Conversely, co-administration of VC to rats enhanced RCB count, Hb, and HCT compared to the control and APAP-treated group. This outcome is consistent with the Matić et al. [19] study.

This study clearly demonstrates that chronic acetaminophen toxicity enhanced the WBCs count. This rise in WBC counts could be related to stress combined with inflammatory alterations in the tissue responsible for toxic substances phagocytosis [27]. The liver damage caused by APAP overdose and the consequent formation of toxic metabolites have been shown to elicit an innate immune response, resulting in leukocyte activation and immune cell function dysregulation [16]. This result is in harmony with previous study [18]. The current data revealed that the treatment of rats with V C attenuated the toxicity effect of APAP [19]. This effect may be due to the antioxidant properties of VC [28].

The data obtained in this study revealed a significant decrease in the number of Plt, this result is consistent with those of Simeon et al. [18], Talaat et al. [24] and Latif et al. [16]. Platelets are generated by the bone marrow through stimulating myeloid stem cells with thrombopoietin [29]. As a result, the observed decrease in Plt due to high or cumulative toxic effects of APAP, thrombocytopenia is prevalent in severe acetaminophen hepatotoxicity and acute liver failure [30]. In this study thrombocytopenia induced by paracetamol was effectively reversed or prevented in the groups that received doses of vitamin C.

The current data revealed that APAP treatment caused a significant reduction in MCV and a

significant increase in MCHC. These results consistently with [17,24]. The reduction in RBC size may interfere with their ability to carry oxygen to tissues [29]. APAP consumption induced considerable alterations in various hematological parameters due to the disturbance of the pro-oxidative/antioxidative balance in the blood of rats. V C, on the other hand, served as an antioxidant to minimize this [19,31].

5. Conclusion

Importantly, the findings of the current work evidenced that VC significantly prevents APAP-induced hepatotoxicity, but partially protects against its heamatotoxicity. Therefore, future investigations are needed to validate the possible protective benefits of vitamin C on APAP-induced damage in different time frames.

6. Conflict of Interest

The authors declare that there are no conflicts of interest.

7. References

1. Badr, N. S., Elbalakousy, H. H., Mohamed, H. F., Elgendy, H. A., Gabr, M. A., Elmezaien, M. S., ... & El, N. N. (2023): Unveiling the Hepatoprotective and Ameliorative Potential of Natural Products in Paracetamol Overdose. Journal of Medical and Life Science, 5(2),76-95.

2. Prescott, L. F. (2000). Paracetamol: past, present, and future. *American journal of therapeutics*, 7(2), 143-148.

3. Chidiac, A. S., Buckley, N. A., Noghrehchi, F., & Cairns, R. (2023). Paracetamol (acetaminophen) overdose and hepatotoxicity: mechanism, treatment, prevention measures, and estimates of burden of disease. *Expert Opinion on Drug Metabolism & Toxicology*, *19*(5), 297-317.

4. Lucock, M., Yates, Z., Boyd, L., Naylor, C., Choi, J. H., Ng, X., ... & Veysey, M. (2013). Vitamin C-related nutrient–nutrient and nutrient–gene interactions that modify folate status. *European journal of nutrition*, 52, 569-582.

5. Abdullah, M., Jamil, R. T., & Attia, F. N. (2023). Vitamin C (ascorbic acid). In *StatPearls [Internet]*. StatPearls Publishing.

6. Adikwu, E. & Deo, O (2013): Hepatoprotective Effect of Vitamin C (Ascorbic Acid). *Pharmacology & Pharmacy*; 4: 84-92.

7. Cichoż-Lach, H., & Michalak, A. (2014). Oxidative stress as a crucial factor in liver diseases. *World journal of gastroenterology: WJG*, *20*(25), 8082-8091.

8. Bharatia, R., Jaiswal, A., & Jaiswal, H. (2023). Different experimental models for hepatotoxicity; a review. *International Journal of Pharma Professional's Research (IJPPR)*, *14*(2), 129-144.

9. Agrawal, S., Dhiman, R. K., & Limdi, J. K. (2016). Evaluation of abnormal liver function tests. *Postgraduate medical journal*, *92*(1086), 223-234.

10. Gwaltney-Brant, S. M. (2016). Veterinary forensic toxicology. *Veterinary pathology*, *53*(5), 1067-1077.

11. Seo, I.-H., & Lee, Y.-J. (2022). Usefulness of Complete Blood Count (CBC) to Assess

Cardiovascular and Metabolic Diseases in Clinical Settings: A Comprehensive Literature Review. *Biomedicines*, *10*(11), 2697. https://doi.org/10.3390/biomedicines10112697

12. George-Gay, B., & Parker, K. (2003). Understanding the complete blood count with differential. *Journal of PeriAnesthesia Nursing*, *18*(2), 96-117.

13. El-Ridi, M. R., & Rahmy, T. R. (2000). Action of vitamin C against acetaminophen-induced hepatorenal toxicity in rats. *Journal of Toxicology: Toxin Reviews*, *19*(3-4), 275-304.

14. Abdulkhaleq, F. M., Alhussainy, T. M., Badr, M. M., Khalil, A. A. A., Gammoh, O., Ghanim, B. Y., & Qinna, N. A. (2018). Antioxidative stress effects of vitamins C, E, and B12, and their combination can protect the liver against acetaminophen-induced hepatotoxicity in rats. *Drug design, development and therapy*, 3525-3533.

15. Hassanin, K.; Hashem, K. & Abdel-Kawi, S. (2013): Hepatoprotective Effects of Vitamin C and Micronized Vitamin C Against Paracetamol Induced Hepatotoxicity in Rats: A Comparative Study. International Journal of Biochemistry and Biotechnology; 2 (7): 474-483.

16. Latif, A. A. E., Assar, D. H., Elkaw, E. M., Hamza, H. A., Alkhalifah, D. H. M., Hozzein, W. N., & Hamouda, R. A. (2021). Protective role of Chlorella vulgaris with Thiamine against Paracetamol induced toxic effects on haematological, biochemical, oxidative stress parameters and histopathological changes in Wistar rats. *Scientific Reports*, *11*(1), 3911.

17. Yousef, M. I., Omar, S. A., El-Guendi, M. I., & Abdelmegid, L. A. (2010). Potential protective effects of quercetin and curcumin on paracetamol-induced histological changes, oxidative stress, impaired liver and kidney functions and haematotoxicity in rat. *Food and Chemical Toxicology*, *48*(11), 3246-3261.

18. Simeon, J. O., Zubairu, S. A., & Tosin, J. O. (2023). Nephroprotective effect of Anacardium occidentale (cashew) apple juice on kidney of paracetamol-induced injury in Albino rats. *Future Natural Products*, *9*(1), 31-37.

19. Matić, M. M., Paunović, M. G., Milošević, M. D., Ognjanović, B. I., & Saičić, Z. S. (2021). Hematoprotective effects and antioxidant properties of β-glucan and vitamin C against acetaminophen-induced toxicity: an experimental study in rats. *Drug and Chemical Toxicology*, *44*(3), 302-309.

20. Salem, G. A., Shaban, A., Diab, H. A., Elsaghayer, W. A., Mjedib, M. D., Hnesh, A. M., & Sahu, R. P. (2018). Phoenix dactylifera protects against oxidative stress and hepatic injury induced by paracetamol intoxication in rats. *Biomedicine & Pharmacotherapy*, *104*, 366-374.

21. Saheed, S.; Hendrik, O.& Tom, A. (2016): Zea Mays, Stigma Maydis Prevents and Extenuates Acetaminophen-Perturbed Oxidative Onslaughts in Rat Hepatocytes. Pharmaceutical Biology; 54 (11): 2664–2673.

22. Shabash, A. H., El Matoni, W. F., Alzedani, A. K., ben Saleh, F. A., Eljadi, N. M., & Ahnish, F. A. (2019). The Therapeutic Effect of Vitamin C on The Liver of Albino Rats (Albino Wistar) Treated with Paracetamol. *Third Conference on Theories and Applications of Basic and Life Sciences (Special Issue within the Journal of Science)*, 9:333-340.

23. Abraham, P. (2005). Vitamin C may be beneficial in the prevention of paracetamol-induced renal damage. *Clinical and experimental nephrology*, *9*, 24-30.

24. Talaat, A., Elgendy, Y. A., Mohamed, H. F., Saed, N. M., Abd Elrouf, N. A., Elgendy, H. A., ... & Badr, N. S. (2023). Ameliorative effects of frankincense oil on rats treated with a minimum toxic dose of paracetamol. *Journal of Medical and Life Science*,5(3),155-175.

25. Gomaa, S. (2017). Immunomodulatory and hematological effects induced by diclofenac, ibuprofen or paracetamol toxicity in Swiss albino mice. *European Journal of Biological Research*, *7*(4), 348-359.

26. Khattab, A. A., Taweek, A. M., Abo-EL-Sooud, K., Ahmed, K. A., El-Gendy, A. N., & Ahmed, A. R. (2020). Elettaria cardamomum essential oil rescues paracetamol-induced hepatorenal damage via modulating oxidative stress in rats. *Adv. Anim. Vet. Sci*, *8*(s2), 24-33.

27. Nithiyanandam, S., & Prince, S. E. (2023). Caesalpinia bonducella counteracts paracetamol-instigated hepatic toxicity via modulating TNF-α and IL-6/10 expression and Bcl-2 and caspase-8/3 signalling. *Applied Biochemistry and Biotechnology*, 1-20.

28. Doğan, M. F., Kaya, K., Demirel, H. H., Başeğmez, M., Şahin, Y., & Çiftçi, O. (2023). The effect of vitamin C supplementation on favipiravir-induced oxidative stress and proinflammatory damage in livers and kidneys of rats. *Immunopharmacology and Immunotoxicology*, 1-6.

29. Arika, W. M., & Nyamai, D. W. (2016). Hematological markers of in vivo toxicity. *Journal of Hematology & Thromboembolic Diseases*, *4*(02), 1.

30. Scharf, R. E. (2021). Thrombocytopenia and hemostatic changes in acute and chronic liver disease: pathophysiology, clinical and laboratory features, and management. *Journal of Clinical Medicine*, *10*(7), 1530.

31. Fetoui, H., Garoui, E. M., Makni-Ayadi, F., & Zeghal, N. (2008). Oxidative stress induced by lambda-cyhalothrin (LTC) in rat erythrocytes and brain: attenuation by vitamin C. *Environmental Toxicology and Pharmacology*, *26*(2), 225-231.