Article

Effect Of Paracetamol(Acetaminophen) Toxicity On Oxidative Stress and Antioxidant Enzymes In Albino Rats

Abdulazeez Mohammed Hussein1,\*, Siham Wadee2 and Dakheel Hussein Hadree3.

1 Department of Pharmacology, College of Veterinary Medicine, Tikrit University; abdulaziz1994hussein1994@gmail.com.

2 Department of Pharmacology, College of Veterinary Medicine, Tikrit University; Sihamwadee@tu.edu.iq.

3 Department of Pharmacology, College of Veterinary Medicine, Tikrit University; dakeel1981@tu.edu.iq.

**\*** Correspondence: abdulaziz1994hussein1994@gmail.com.

**Abstract:** This study aimed to investigate the impact of paracetamol on oxidative stress and some antioxidant markers in male rabbits.Methods: 30 growing rats reared under high ambient temperature were divided into three equal groups, 10 rats. The control was administered with normal saline in the first group, and the second and third groups were paracetamol-treated rats (1000 & 2000 mg/kg b.w. orally) for 30. Blood samples were withdrawn to measure serum Glutathione (GSH), malondialdehyde (MDA), Superoxide dismutase (SOD), and catalase (CAT), and activities were assayed. Results: In the paracetamol-treated group, significant increases in malondialdehyde (MDA) levels and significant decreases in catalase (CAT), glutathione, SOD, and GSH levels compared to the control group.Conclusion: paracetamol administration produces noticeable biochemical changes in a dose-dependent manner associated with increased oxidative stress markers and decreased antioxidative activity.

|  |
| --- |
| Citation: Hussein, A.M.; Wadee, S.; Hadree, D.H. Effect Of Paracetamol(Acetaminophen) Toxicity On Oxidative Stress and Antioxidant Enzymes In Albino Rats  . Revista Bionatura 2021, 13, x. https://doi.org/10.21931/xxxxx  Academic Editor: Firstname Lastname  Received: date  Accepted: date  Published: date  **Publisher’s Note:** Bionatura stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.    **Copyright:** © 2022 by the authors. Submitted for possible open-access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/). |

**Keywords:** Paracetamol; Malondialdehyde; Toxicity; GSH.

1. Introduction

Minimizing toxicity remains one of the major barriers to bringing a drug to market. Approximately 92% of all developed compounds fail because of adverse effects of the candidate during clinical development. Millions worldwide use paracetamol (acetaminophen – APAP) as a safe analgesic drug at therapeutic doses 1-3. After oral administration, about 63% of paracetamol is metabolized via glucuronidation and 34% via sulphation, primarily in the liver. The water-soluble metabolites consisting of these metabolic pathways are excreted via the kidney. N-acetyl-p-benzoquinone (NAPQI) is a reactive intermediate that occurs when the microsomal P-450 enzyme system4,5 oxidizes the <5% percent of paracetamol. The main reason for the development of such medical complications is that when the toxic dose of paracetamol is ingested, excessive NAPQ1 is produced and consequently causes serious GSH reduction as well as overproduction of reactive metabolites leading to covalent attachment of sulfhydryl groups in cellular proteins. This disrupts homeostasis and starts apoptosis or programmed cell death, leading to tissue necrosis, organ dysfunction and oxidative stress6.

2. Materials and Methods

The experimental study was conducted on 30 rats (200 mg) from 15/3/2022. It has been achieved in the animal house of the College of Veterinary Medicine / Tikrit University. They were left to acclimatize for 1 week. They were housed in metallic cages at room temperature and kept under constant healthy environmental and nutritional conditions. Animals were kept under a schedule of diurnal lighting conditions (12 h of darkness and 12 h of light); they were fed on ordinary food and housed under standard laboratory conditions. The animals were divided into 3 cages of 10 animals each.

1. **Control group 1 (G1):** It included 10 adult rats. The animals of this group received 1 ml of normal saline 0.9% for 4 weeks.
2. **Treated group 2 (G2): It** included 10 rats. Each animal received 1000mg/kg/day of paracetamol daily for 30 days.
3. **Group 3 (G3):** It included 10 rats; each animal received 2000mg/kg/day of paracetamol daily for continuous 30 days.

Blood samples (5 ml) were collected through retro-orbital puncture. Serum samples were prepared for biochemical assays by centrifuging for 10 min at 3000 rpm. All serum samples were kept at −80°C until the assays were performed. Serum SOD, GSH, MDA, G-Px, and catalase levels were measured by spectrophotometric kit.

2.1. Statistical Evaluation

Data was expressed as mean ± SD. Differences between groups were compared by ANOVA using the SPSS software (version 16). A p-value of less than 0.05 was considered to be statistically significant.

3. Results

**Table 1.** Effect of paracetamol administration on biochemical parameters of rats

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| SOD | GSH | MDA | CAT | Parameters  Groups |
| 666.0±43.6  A | 290.1±16.0  A | 0.573±0.082  A | 6.110±1.092 | **G1** |
| 384.0±24.6  B | 262.6±18.8  Ab | 0.669±0.173  A | a 2.776±1.197  B | **G2** |
| 97.8±23.5  C | 0.702±0.014B | 0.702±0.014  A | 1.709±1.083  b | **G3** |
| 0.050 \* | 0.049 \* | 0.393 ns | 0.044 \* | **P – Value** |

**Figure 1.** Effects of paracetamol on the serum MDA in adult rats

**Figure 2.** Effects of paracetamol on the serum catalase in adult rats

**Figure 3.** Effects of paracetamol on the serum GSH in adult rats

**Figure 4.** Effects of paracetamol on the serum SOD in adult rats

4. Discussion

Oxidative and reductive stress are dual dynamic phases experienced by the cells adapting to an endogenous or exogenous noxious stimulus. The former arises due to the imbalance between the production of reactive oxygen species (ROS) or free radicals and antioxidant defense, which may induce tissue injury and play a significant role in the pathogenesis of many diseases and mechanisms of complications 7-9. Malondialdehyde is a decomposition product of auto-oxidation of polyunsaturated fatty acids, used as an oxidative damage index. The high concentration of MDA in those patients indicates increased membrane lipid peroxidation. Enhanced lipid peroxidation may occur as a result of the fact that naturally occurring scavenging mechanisms are suppressed, and the free radical generation processes are enhanced10-11. The high concentration of MDA in those patients indicates increased membrane lipid peroxidation. Regarding serum MDA levels, which are decomposition products of auto-oxidation of polyunsaturated fatty acids, the index of lipid peroxidation and oxidative stress significantly increased in the paracetamol group compared to the control group. Glutathione (GSH) is a non-enzymatic tripeptide protecting the tissues and organs against the adverse effects of ROS. It plays a role in eliminating free radical species such as H2O2, superoxide radicals and membrane protein thiols. It is also known as a substrate of the GPx enzyme.GSH significantly contributes to the detoxification of NAPQI and scavenging of peroxynitrite in APAP hepatotoxicity. Nevertheless, excessive NAPQI induces prominent depletion of GSH and subsequently results in lipid peroxidation, which is indicated by MDA accumulation12,13. The infiltrating inflammatory cells also generate ROS and free radicals, including hydroxyl radical superoxide anion, hydrogen peroxide and singlet oxygen. Superoxide dismutase (SOD) is widely distributed in oxygen-metabolizing cells and can catalyze the dismutase conversion of superoxide to oxygen and hydrogen peroxide. Then hydrogen peroxide is catalyzed to water by catalase and glutathione system. SOD and other enzymatic antioxidative agent activities decline in severe inflammatory and oxidative stress conditions14,15. The SOD and catalase levels were significantly decreased in the paracetamol-treated rats, which might be due to the decrease in the capacity to eliminate increased H2O2, which is converted to highly reactive hydroxyl radical through Fenton reaction at the existence of enriched Fe2+/Cu1+ 16. These results were in agreement with a study done by Mervat 17. Catalase, GPx and SOD are the two scavenging enzymes that enhance superoxide anion’s breakdown by converting them into H2O2 and are catalytically converted by catalase into groundstate oxygen and hydroxyl radicals 18,19. GPx serves to prevent oxidative stress. GPx, a selenium-containing enzyme, and GST collaborate with GSH to degrade H2O2 or other organic into non-toxic compounds. The treatment of paracetamol in rats decreased the levels of Catalase, GPx and SOD20.

5. Conclusion

Paracetamol administration produces noticeable biochemical changes in a dose-dependent manner associated with increased MDAm and decreased Catalase, GSH and SOD.

References

1. Sarhat, E. R.; Wadee, S. A.; Sedeeq, B. I.; Sarhat, T. R.; Abass, K. S. Biochemical and histological evaluation of indomethacin-induced hepatotoxicity in rats. *Indian Journal of Forensic Medicine & Toxicology*, **2018**, 10.4: 733-735.
2. Abdullah, B. A.; Wadi, S. A.; Sarhat, E. Histological Study Effects of Paracetamol on Livers and Kidneys of Adult Mice. *Tikrit Journal for Agricultural Sciences,* **2017**, 17. Special: 97-103., 6th Scientific Conference for Agricultural Researches, 28-29 March, 2017 ISSN-1813-1646
3. Bkhairia, I.; Dhibi, S.; Nasri, R.; Elfeki, A.; Hfaiyedh, N.; Amara, I. B.; Nasri, M. Bioactive properties: enhancement of hepatoprotective, antioxidant and DNA damage protective effects of golden grey mullet protein hydrolysates against paracetamol toxicity. *RSC advances*, **2018**, 8.41: 23230-23240. DOI: [10.1039/ C8RA02178C](https://doi.org/10.1039/C8RA02178C).
4. Canayakin, D.; Bayir, Y.; Kilic Baygutalp, N.; Sezen Karaoglan, E.; Atmaca, H. T.; Kocak Ozgeris, F. B.; Keles, M. S.; Halici, Z. Paracetamol-induced nephrotoxicity and oxidative stress in rats: the protective role of Nigella sativa. *Pharmaceutical biology*, **2016**, 54.10: 2082-209. DOI: [10.3109/13880209.2016.1145701](https://doi.org/10.3109/13880209.2016.1145701)
5. Zakaria, Z. A.; Kamisan, F. H.; Kek, T. L.; Salleh, M. Z. Hepatoprotective and antioxidant activities of Dicranopteris linearis leaf extract against paracetamol-induced liver intoxication in rats. *Pharmaceutical Biology*, 2020, 58.1: 478-489. DOI: [10.1080/13880209.2020.1764058](https://doi.org/10.1080/13880209.2020.1764058)
6. Latif , A. A. E.; Assar, D. H.; Elkaw, E. M.; Hamza, H. A.; Alkhalifah, D. H. M.; Hozzein, W. N.; Hamouda, R. A. 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*, **2021**, 11.1: 1-16. https://doi.org/10.1038/s41598-021-83316-8
7. Sarhata, E. R.; Al Anzyb, M. M.; Ahmedb, T. S. Study of oxidant-antioxidant status in cerebrospinal fluid of children with meningitis. *Eurasian Chemical Communications*, **2022**, 4.9: 863-869. Link: http://www.echemcom.com/article\_148799. html
8. Sarhat, E. R.; Wadi, S. A.; Ahmed, M. S.; Mustafa, S. N.; Sarhat, T. R. Evaluation of Serum Malondialdehyde, Glutathione peroxidase, Superoxide dismutase, and Catalase levels in Hormonal Contraceptives in Tikrit City. *Tikrit Medical Journal*, **2018**, 24.1:10-20.
9. Sarhat, E. R.; Zbaar, S. A.; Ahmed, S. E.; Ahmed, T. S.; Sarhat, T. R. Salivary biochemical variables of Liver Function in among Individuals with COVID-19 in Thi-Qar Province. *Egyptian Journal of Chemistry*, **2022**, 65.6: 1-2.
10. Mohammed, I. J.; Sarhat, E. R.; Wadee, S. A.; Al-Shiakhani, S. M. Shiakhani Histological and biochemical evaluation of the effect of desloratadine drug in parotid gland tissues. *Al-Anbar Medical Journal*, **2021**, 17.2:72−77.
11. Sarhat, E. R.; Mohammed, I. J.; Mohammed, N. Y.; Khairy, B. S.; Hassan, F. F. Evaluation of Salivary Oxidative Stress Marker (Lipid Peroxidation), and Non-Enzymatic Antioxidants (Vitamin C and Vitamin E) in Patients with Acute Myocardial Infarction. *Tikrit Journal for Dental Sciences*, **2019**, 7.1: 20-26.
12. Sreevallabhan, S.; Mohanan, R.; Jose, S. P.; Sukumaran, S.; Jagmag, T.; Tilwani, J.; Kulkarni, A. Hepatoprotective effect of essential phospholipids enriched with virgin coconut oil (Phoscoliv) on paracetamol‐induced liver toxicity. *Journal of Food Biochemistry*, **2021**, 45.2: e13606. <https://doi.org/10.1111/jfbc.13606>
13. Mohamed, K. G. A.; Harahsheh, E.; Hussein, S. Diacerein ameliorates acetaminophen hepatotoxicity in rats via inhibiting HMGB1/TLR4/NF-κB and upregulating PPAR-γ signal. *Molecular Biology Reports*, **2022**, 1-12. https://doi.org/10.1007/s11033-022-07366-5
14. Abd-Allah, A. A. M.; El-Deen, N. A. M. N.; Mohamed, W. A. M.; Naguib, F. M. Mast cells and pro-inflammatory cytokines roles in assessment of grape seeds extract anti-inflammatory activity in rat model of carrageenan-induced paw edema. *Iranian journal of basic medical sciences*, **2018**, 21.1: 97. doi: 10.22038/IJBMS.2017.25067.6219.
15. Awni, N.; Ahmed, T. S.; Sarhat, E. R. Ali, N. H.; Abass, K. S. Altered serum levels of melatonin, antioxidant enzymes and oxidative stress in individuals with diabetes mellitus type 2. *Revista Latinoamericana de Hipertensión*, **2022**, 17. Nº 2:138-141.
16. Mohammed, I. J.; Sarhat, E. R.; Wadee, S. A.; Al-Shiakhani, S. M. Histological and biochemical evaluation of the effect of desloratadine drug in parotid gland tissues. *Al-Anbar Medical Journal*, **2021**, 17.2.
17. Taha, M. E. S.; Kamal, A. M.; Ibrahim, D. R. Possible protective effect of olive leaves extract on paracetamol induced hepatotoxicity in male albino rats. *Biosci. j.(Online)*, **2020**, 245-255.
18. Sarhat, E. R.; Wadi, S. A.; Mahmood, A. R. Effect of ethanolic extraction of moringa oleifera on paraoxonase and arylesterase enzyme activity in high fat diet-induced obesity in rats. *Res. J. Pharm. Technol*, **2018**, 11.10: 4601-4604.
19. Entedhar, R. S. Effect of Ginger on the activity of some antioxidant enzymes (Superoxide dismutase, and catalase) of Alloxan Experimental Induced-Diabetic Rabbits. *Al-Mustansiriyah J. Sci*, **2011**, 22.5: 192-200.
20. Fayed, A.; Ramadan, H. O.; Hassan, S. A.; Hussein, M. A.; Roshdy, T. Thioredoxin1 Gene Modulates Bcl2/p53/NF-KB Signaling Pathways in Strawberry Extract/Paracetamol-treated Rat Model of Acute Liver Injury. *Biomedical and Pharmacology Journal*, **2022**, 15.2: 1025-1037.