



# IJEAST

INTERNATIONAL JOURNAL  
OF ENGINEERING APPLIED SCIENCE  
AND TECHNOLOGY



VOLUME : 7    ISSUE : 02    Print / Issue Publication Date: 05-Aug-2022



ISSN : 2455-2143



DOI : 10.33564/IJEAST.2022.v07i02.062

Indexed In



[WWW.IJEAST.COM](http://WWW.IJEAST.COM)

[editor@ijeast.com](mailto:editor@ijeast.com)



# SPLEEN FUNCTIONS INFLUENCED BY THE ENVIRONMENTAL POLLUTANT ATRAZINE HERBICIDE AND ITS CONFLICTS ON HUMAN COMMUNITY HEALTH USING WISTAR RATS AS EXPERIMENTAL ANIMALS

Abdelgadir MI

Department of chemistry, Faculty of Education,  
University of Bakht Alruda, Ed-Duiem, Sudan.

Awad ElGeed.

BA. Department of chemistry, Faculty of science,  
University of Bakht Alruda Ed-Duiem, Sudan.

Mahmood.MH

Department of Food technology,  
Faculty of Agricultural technology and Fish Sciences,  
El Neelain University, Khartoum, Sudan

Omer FI.

Department of Biochemistry and molecular biology,  
Faculty of Science and Technology,  
El Neelain University, Khartoum, Sudan

**Abstract**—this study was focused on assessing an environmental pollutant atrazine herbicide conflicts on spleen and its impacts on human society using Wistar rats as experimental animals. 32 Wistar rats were quarantined, for 1 week in metal cages that were cleaned twice a week. Animals were divided into 4 groups, 1 group served as control, and the remainder 3 groups orally received different doses of atrazine (150, 300, and 600 mg/kg body weight) for 4 weeks. Animals were freely subject to basal diet, and tap-water. Blood samples were taken and kept at 5 °C in suitable containers till sample analysis. Significant decreased ( $P \leq 0.05$ ) Hb reduced glutathione, and increased ( $P \leq 0.05$ ) serum  $\text{Ca}^{++}$  concentration, were observed during the course of the experiment. (Hassanen et al., 2020) stated that serum glutathione levels were decreased and intracellular  $\text{Ca}^{2+}$  within spleen cells were increased due to atrazine treatment.

**Keywords**- atrazine, glutathione, calcium ions, spleen, environment, hormonal imbalance.

## I. INTRODUCTION

Atrazine (2-chloro-4-ethylamino-6-isopropylamine-1, 3, 5-triazine; ATR) is a common potent endocrine disruptor that results in hormonal imbalance. Up regulation of spleen Fas and caspase-III genes was recorded in ATR-exposed rabbits. Clear splenocyte apoptosis was observed in the immunohistochemical examination by the caspase-III technique. GA diminished the ATR-induced splenocyte apoptosis through down regulation of Fas and caspase-III expressions (Morgan et al., 2019). ATR exposure also caused increases in intracellular  $\text{Ca}^{2+}$  within splenocytes. Moreover, ATR treatment led to increased expression of genes for some antioxidant enzymes, such as HO-1 and Gpx1, as well as increased expression of NF- $\kappa$ B and Ref-1 proteins in the spleen. It appears that oxidative stress and disruptions in calcium homeostasis might play an important role in the induction of immunotoxicity in mice by ATR (Gao et al., 2016). The widely used herbicide atrazine (ATR) can cause many adverse effects including immunotoxicity, but the underlying mechanisms are not fully understood. In a previous study, ATR at different doses was administered to Balb/c mice daily for 21 days by



oral gavage. In accordance to 24 hr. performance after the final exposure showed that ATR could induce the generation of reactive oxygen species in mice spleen, serum glutathione levels were decreased, each in a dose-related manner. ATR exposure also caused increased intracellular  $\text{Ca}^{2+}$  within spleen cells. Moreover, Oxidative stress and disruptions in calcium homeostasis might play an important role in the induction of immunotoxicity in mice by ATR (Hassanen et al., 2020). The herbicide Atrazine (ATR) can result in immunotoxicity, and may cause unfavorable results for humans. Rabbits of different weight were utilized and appointed into 4 equal groups. Group 1: control; group 2: Received Atrazine at 1/10 LD50 via food; group 3: Received Akropwer at 1ml/1l/day by means of drinking water; group 4: Received both Atrazine and Akropwer associatively by the same said dosage and course. Atrazine and Akropwer exposure were accomplished for 60days. The genotoxic mechanisms of Atrazine- induced humoral immunotoxicity were explained by increased serum total protein and albumin levels, decreased RHDV antibody titer only after four weeks of vaccination and increased level of spleen Fas and Caspase-III genes expression in Atrazine-exposed rabbits. Marked splenocytes apoptosis were detected. Akropwer attenuated ATR-induced apoptosis through down-regulation of Fas and Caspase-III genes expression and suppression of their signaling pathway. Induction of apoptosis by overexpression of Fas and Caspase-III genes gives a new insight into the mechanism of ATR immunotoxicity was concluded. The protective part of Akropwer, on the other hand, was characterized by attenuation of Fas and Caspase-III genes mediated apoptosis (Morgan et al., 2019). Increased interest in ATR studies is due to its adverse environmental effects. Residues of ATR, its primary metabolite, deethylatrazine, and other derivatives of the parent compound can leach from soils and persist in ground and surface water for several years (Dorfler et al., 1997). Occupational exposure of farmers and other agricultural workers to high concentrations of ATR is of particular concern. Levels of ATR exposure can be detected in the saliva and urine of these workers after spraying. It is not only the direct ATR applicators at risk, but also their families through detectable levels in body fluids. Considerably higher amounts of ATR and its metabolites are found in the urine in populations living within proximity of farms that use this herbicide (Hines et al., 2006). Many toxicological studies of ATR focus primarily on the effects of ATR on the endocrine and reproductive systems (Hayes et al., 2009). A decrease of thymus and spleen weights, and total spleen cell numbers are also observed in C57BL/6 mice after daily oral treatment for 14 days at up to 250 mg/kg/day or 500 mg/kg/day [Filipov et al., 2005]. The metabolic pathways of atrazine in humans have not been fully characterized. In rodents the dominant Phase-I metabolic reaction is cytochrome P450-mediated N-dealkylation as demonstrated by urine analysis

and liver fraction studies. Phase II biotransformation of atrazine may also occur: several studies demonstrated GSH conjugation of the parent compounds by rat and mouse liver fractions. In mouse liver, GSH conjugation is likely mediated by a Pi class glutathione S-transferase (GST). In humans, dealkylated metabolites are detected in urine and are formed following human liver microsome incubation with atrazine. However, most human studies have been performed either with microsomes alone or with urine extraction and analysis techniques optimized for Phase-I metabolites. Therefore, the possibility remains that significant Phase-II-mediated biotransformation occurs in humans. In fact, in recent studies utilizing enzyme-linked immunosorbent assay (ELISA) and mass spectrometry techniques, atrazine mercapturates were shown to be significant metabolites of atrazine in human urine, indicating that GSH conjugation may be an important route of biotransformation in humans (Erika et al., 2004).

## II. MATERIALS AND METHODS

Atrazine (ATR; 2-chloro-4-ethylamino-6-isopropylamino-s-triazine; 98% purity; lot 301-49A) was prepared. For the animal treatment, ATR was dissolved in safflower oil and administered for 4 weeks by a daily gavage at doses of (150, 300, or 600 mg/kg body weight). Control animals received the safflower oil vehicle.

### Sample collection and analysis

blood was obtained by a cardiac puncture and immediately transferred into 2 ml vacutainer tubes containing citric buffer (BD Biosciences Pharmingen, San Diego, CA). The tubes were maintained on a rocker platform until PBMC isolation and subsequent analysis for calcium and glutathione concentrations. Glutathione concentrations were detected in accordance to the method described by (Rossi et al., 2002). Calcium ions concentrations were detected in accordance to the method described by (Goldstein, 1990)

## III. STATISTICAL ANALYSIS

Study results were statistically analyzed via Quality Control Charts & Compare Means One - Sample T - Test in accordance to SPSS version 2019.

## IV. RESULTS AND DISCUSSIONS

Significant Increased serum calcium ions and decreased Hb glutathione concentrations were observed through the course of the experiment due oral administration of the herbicide atrazine. Study results are confirmed by (Hassanen et al., 2020) who stated that serum glutathione levels were decreased and intracellular  $\text{Ca}^{2+}$  within spleen cells were increased due to atrazine treatment. Also (Arthur et al., 2018) showed that GST expression, concentrations and activity were increased, along with GSH levels, in animals treated with ATR for 3 and 4 days. Oral administration of ATR (at 250 and 500 mg/kg) for 14 days

was immunotoxic in mice and was manifested by an increase in number of CD8<sup>+</sup> T-cells and significant decreases in spleen cell numbers, spleen weight, and thymus weight. It has been suggested that chemicals can damage immune cells through a variety of biological mechanisms, including oxidative stress, alterations in calcium homeostasis, and apoptosis. In keeping with this premise, a previous study proved that Fas-mediated apoptosis was one

mechanism for ATR toxicity among splenocytes of mice stated (Shuying et al., 2016). Also (Gao et al., 2016) showed that ATR exposure results in increases in intracellular Ca<sup>2+</sup> within splenocytes. Study results are confirmed by (Zhang et al., 2011) who stated that ATR is capable of inducing splenocytic apoptosis mediated by the Fas/FasL pathway in mice, which could be the potential mechanism underlying the immunotoxicity of ATR.

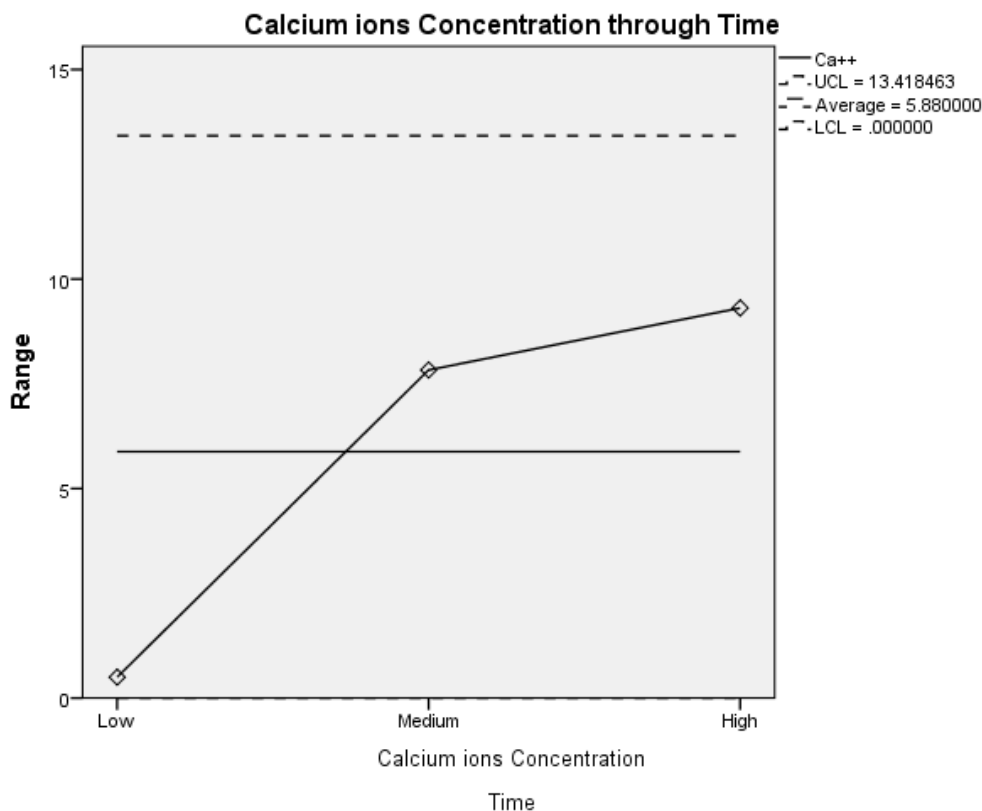


Fig. 1 Shows serum calcium ions concentrations (mg/ dl) vs. Time

**Table 1** Shows significance of serum ca<sup>++</sup> concentrations ( μM) vs. time

**One-Sample Test**

|                  | Test Value = 0 |    |                 |                 |                                           |           |
|------------------|----------------|----|-----------------|-----------------|-------------------------------------------|-----------|
|                  | t              | df | Sig. (2-tailed) | Mean Difference | 95% Confidence Interval of the Difference |           |
|                  |                |    |                 |                 | Lower                                     | Upper     |
| Ca <sup>++</sup> | 6.883          | 11 | .000            | 20.8233333      | 14.164497                                 | 27.482170 |
| Time             | 8.124          | 11 | .000            | 2.0000000       | 1.458155                                  | 2.541845  |

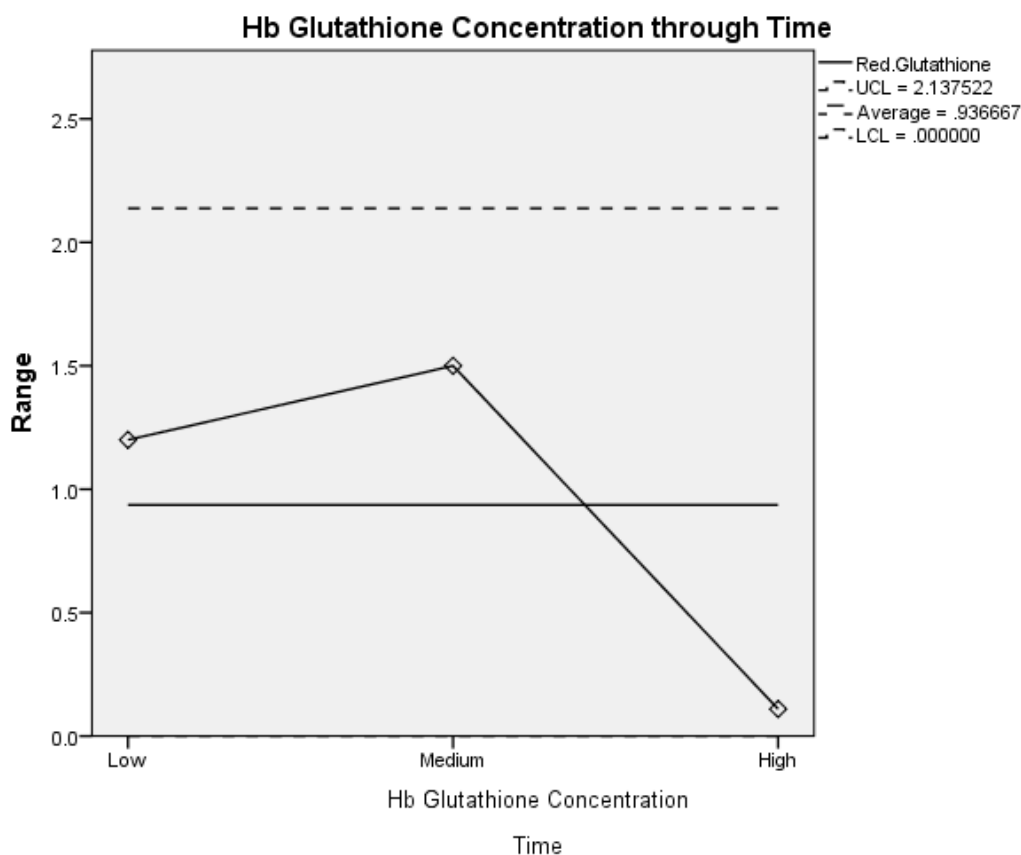


Fig. 2 Shows reduced Hb glutathione concentrations (  $\mu\text{M}$ ) vs. time

**Table 2** Shows significance of reduced Hb glutathione concentrations (  $\mu\text{M}$ ) vs. time

**One-Sample Test**

|                 | Test Value = 0 |    |                 |                 |                                           |          |
|-----------------|----------------|----|-----------------|-----------------|-------------------------------------------|----------|
|                 | t              | df | Sig. (2-tailed) | Mean Difference | 95% Confidence Interval of the Difference |          |
|                 |                |    |                 |                 | Lower                                     | Upper    |
| Time            | 8.124          | 11 | .000            | 2.0000000       | 1.458155                                  | 2.541845 |
| Red.Glutathione | 3.722          | 11 | .003            | 1.6908333       | .691003                                   | 2.690664 |

**V. CONCLUSIONS AND RECOMMENDATIONS**

It is clearly observed the detrimental impacts of atrazine on cellular endocrine system reflected by changes in serum calcium ions and reduced Hb glutathione concentrations, besides the obvious negative effects on man and environment. Firm restrictions concerning safety and use of atrazine herbicide is strongly recommended, accompanied by further research about potential health and environmental effects.

**VI. ACKNOWLEDGEMENTS**

Authors gratefully thank the family of Meck Nimir Research Center, Khartoum, Sudan, for encaging experimental rats, & Family of Khartoum hospital for sample analysis. & Dr. Eljack M.Elamin., (the Head administrator of Zagross company for import/ export of agricultural requirements), for valuable providing atrazine reagent, & Dr. Mohamed H. Bani Khaled for great financial participation and academic encouragement.



## VII. REFERENCES

- [1]. Arthur DZ., Charles B., Breckenridge KD., Yi Pragati SC., Desiree W., Robert LJ., & Chad DF. (2018). Changes in hepatic phase I and phase II biotransformation enzyme expression and glutathione levels following atrazine exposure in female rats, *Xenobiotica*, 48 (9), 867-881, DOI: 10.1080/00498254.2017.1374486
- [2]. Dorfner U., & Feicht EA., (1997). Scheunert I. S-triazine residues in groundwater. *Chemos.*, 35, 99-106. 10.1016/S0045-6535(97)00142-2.
- [3]. Erika LA., Shaun MO., Christophe LV., Theo KB., & David LE., (2004). Characterization of Atrazine Biotransformation by Human and Murine Glutathione S-Transferases, *Toxicological Sciences*, 80 (2), 230-238, <https://doi.org/10.1093/toxsci/kfh152>
- [4]. Filipov NM., Pinchuk LM., Boyd BL., & Crittenden PL., (2005). Immunotoxic effects of short-term atrazine exposure in young male C57BL/6 mice. *Toxicol Sci.*, 86, 324-332. 10.1093/toxsci/kfi188.
- [5]. Gao S., Wang Z., Zhang C., Jia L., & Zhang, Y. (2016). Oral Exposure to Atrazine Induces Oxidative Stress and Calcium Homeostasis Disruption in Spleen of Mice. *Oxidative medicine and cellular longevity*, 2016, 7978219. <https://doi.org/10.1155/2016/7978219>
- [6]. Goldstein DA., (1990). Serum Calcium. In: Walker HK, Hall WD, Hurst JW, editors. *Clinical Methods: The History, Physical, and Laboratory Examinations*. 3rd edition. Boston: Butterworths; Chapter 143. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK250/>
- [7]. Hassanen EI., Morsy EA., Hussien AM., Ibrahim MA., & Farroh KY., (2020). The effect of different concentrations of gold nanoparticles on growth performance, toxicopathological and immunological parameters of broiler chickens. *Biosci Rep.* 40 (3).
- [8]. Hayes T., (2009). More feedback on whether atrazine is a potent endocrine disruptor chemical. *Environ Sci Technol.*, 43, 6115-10.1021/es901511h.
- [9]. Hines CJ., Deddens JA., Lu C., Fenske R., & Striley CA., (2006). Mixed-effect models for evaluating multiple measures of atrazine exposure among custom applicators. *J Occup Environ Hyg.*, 3, 274-283. 10.1080/15459620600637366.
- [10]. Jessica LR., Claudia H., Vijay G., Steve B., & Heather MB., (2012). Atrazine Exposure in Public Drinking Water and Preterm Birth. *Public Health Rep.* 127 (1), 72–80.
- [11]. Morgan AM., Ibrahim MA., & Hussien AM., (2019). Glycyrrhizic acid modulates the atrazine-induced apoptosis in rabbit spleen. *Environ Sci Pollut Res Int.* 26 (34), 34924-34930.
- [12]. Rossi R., et al., (2002). Blood glutathione disulfide: in vivo factor or in vitro artifact? *Clin. Chem.* 48, 5742–5753.
- [13]. Shuying G., Zhichun W., Chonghua Z., Liming J., & Yang Z., (2016). Oral Exposure to Atrazine Induces Oxidative Stress and Calcium Homeostasis Disruption in Spleen of Mice. *Oxidative Medicine and Cellular Longevity*, 2016, 1-9. <https://doi.org/10.1155/2016/7978219>
- [14]. Taylor EL., Armstrong KR., Perrett D., Hattersley AT., & Winyard PG., (2015). Optimisation of an Advanced Oxidation Protein Products Assay: Its Application to Studies of Oxidative Stress in Diabetes Mellitus. *Oxid Med Cell Longev.* 2015:496271. doi:10.1155/2015/496271
- [15]. Zhang X., Wang M., Gao S., Zheng J., & Zhang Y., (2011). Atrazine-induced apoptosis of splenocytes in BALB/C mice. *BMC Medicine*. volume 9, Article number: 117.

# IJEAST

INTERNATIONAL JOURNAL  
OF ENGINEERING APPLIED SCIENCE  
AND TECHNOLOGY

## ABOUT IJEAST

International Journal of Engineering Applied Science and Technology (IJEAST) is a peer-reviewed, open access journal that publishes high-quality research papers in the field of Engineering, Applied Science and Technology.

IJEAST aims to provide a platform for researchers, academicians, and professionals to share their innovative ideas, research findings, and practical experiences with the global scientific community.

## FOCUS AREAS

- Engineering
- Applied Science
- Technology
- Innovation & Development
- Interdisciplinary Studies



### PEER REVIEWED

All submissions are rigorously peer reviewed to ensure quality.



### OPEN ACCESS

Free and unrestricted access to research for all.



### GLOBAL REACH

Connecting researchers and professionals worldwide.



### TIMELY PUBLICATION

We ensure a swift and efficient publication process.



For more information, visit our website

**[www.ijeast.com](http://www.ijeast.com)**



**INTERNATIONAL JOURNAL**  
OF ENGINEERING APPLIED SCIENCE  
AND TECHNOLOGY

✉ [editor@ijeast.com](mailto:editor@ijeast.com)

🌐 [www.ijeast.com](http://www.ijeast.com)

📍 India



2455-2143