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

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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 Ca^{++} concentration, were observed during the course of the experiment. (Hassanen et al., 2020) stated that serum glutathione levels were decreased and intracellular 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 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- κ 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 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 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⁺ 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 Ca²⁺ 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.

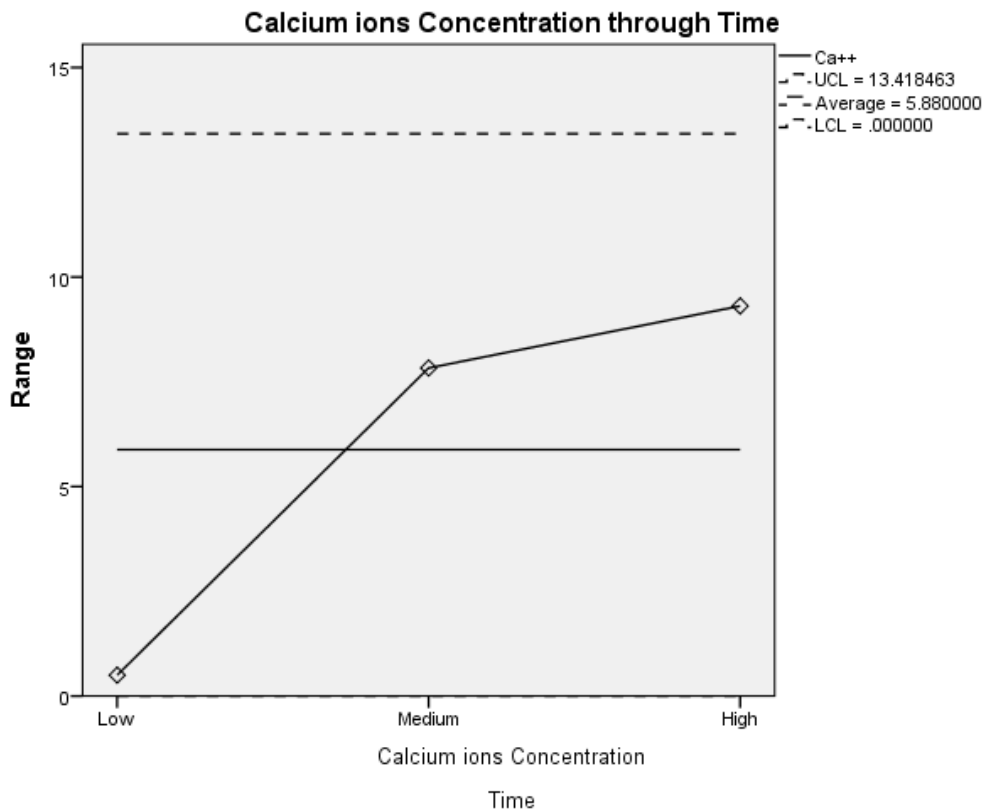


Fig. 1 Shows serum calcium ions concentrations (mg/ dl) vs. Time

Table 1 Shows significance of serum ca⁺⁺ 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 ⁺⁺	6.883	11	.000	20.8233333	14.164497	27.482170
Time	8.124	11	.000	2.0000000	1.458155	2.541845

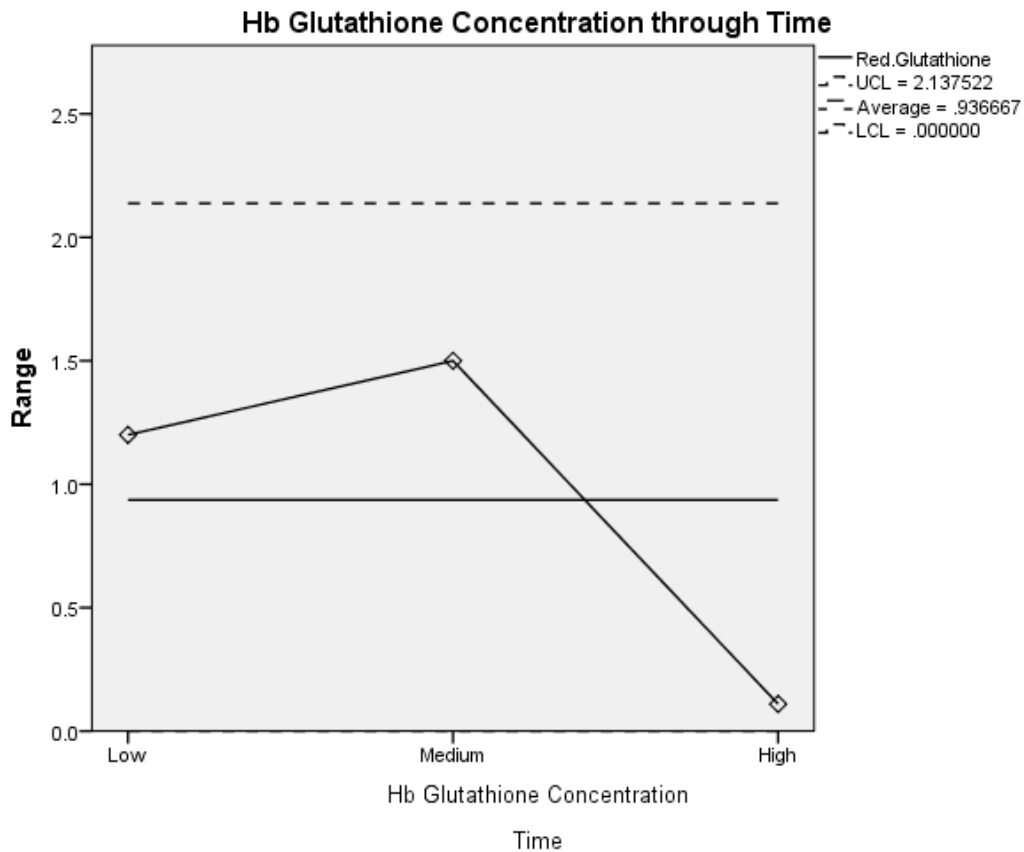


Fig. 2 Shows reduced Hb glutathione concentrations (μM) vs. time

Table 2 Shows significance of reduced Hb glutathione 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
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.

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