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Mature Wistar Rats' Spleen and Liver Histomorphological Changes Related to the Duration of Atrazine Exposure

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Spleen and liver is secondary lymphoid organ that is highly sensitive to different chemicals. Widespread use of pesticides in agriculture has always been a matter of concern. And surprisingly, atrazine distinguishes out for being used more frequently among numerous harmful pesticides. As a result, long term exposure to atrazine and other pesticides is thought to produce metabolic abnormalities; however, little is known about how atrazine affects the spleen and liver and how this relates to its histo-achitectural structure.
Aim: The histopathology of the spleen and liver from rats exposed to atrazine was the subject of our investigation.
Materials and Methods: Twenty male wistar rats ranging from 150-200g were acclimated to laboratory conditions for 14 days, following which they were randomly assigned into 4 groups 1, 2, 3 and 4 of 5 animals each based on average body weight. Groups (2-4) were administered atrazine

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via oral route corresponding to 1237 mg/kg (20/5 LD50), 618 mg/kg (10/5 LD50) and 309 mg/Kg/body weight (1/10 LD50) for 7, 14 and 30 days, while group I (control) received distilled water orally using orogastric canula for 30 days . The liver and spleen from each group of rats were harvested, weighed, and fixed in 10% buffered formal saline fixative before being taken for histological examination 24 hours following the experimental periods of oral administration of the extract.

Results: At the end of the experiment, the histological findings showed increased and numerous area of the white pulp of spleen from rats exposed to atrazine as compared to that from the control. The relative area of germinal centre in the structure of the splenic lymph follicles of rats exposed to atrazine also revealed increased. Also, Histo-pathologically, the liver showed necrotic hepatic cells and congested central vein, with the highest atrazine concentration causing the most adverse effects.

Conclusion: Our data demonstrated that rats exposed to high-dose of atrazine led to hypertrophy of white pulp of the spleen and hepatic cell damage with liver. From this we concluded that both organ are highly sensitive to the debilitating effects of atrazine.

Keywords: Atrazine; toxicity; rats; spleen; liver; histology.

1. INTRODUCTION

The use of herbicides for agricultural activities is constantly on the increase worldwide, with significant increases in food production [1]. However, these herbicides are contributing to environmental contamination, adverse impact on humans and animal health and leading to species extinction [2]. Despite these negative consequences and our poor knowledge of the numerous mechanisms underlying herbicide toxicity at various degrees of biological orientation, new herbicides are routinely produced [3].

The hepatic, renal, neurological system, immunological system and reproductive system are just a few of the organs and systems that are negatively impacted by the toxicity of organophosphorus pesticides [4-6]. Organophosporus insecticides used on humans and animals were always tested for toxicity using histopathological changes to tissues and organs and alterations to biochemical markers [7]. Animals used in experiments are harmed by organophosphorous chemicals, with the kidney being one of its targets [8,9].

Among the most extensively used agricultural herbicides is atrazine (ATZ), a broad-spectrum triazine herbicide (2-chloro-4-ethylamino-6-isopropylamino-5-triazine) [10,11]. Globally, ATZ is used to eliminate weeds during production of maize, sorghum, sugarcane, vines, fruit orchards, chemical fallows and grassland, with its biggest market in maize production [12]. Lipophilicity, slow hydrolysis, poor water solubility, high solubility in organic solvents and rapid absorption by organic matter, clay and fat tissues are some of its chemical properties [13]. Historically, atrazine linked to numerous reports of endocrine system effects on the reproductive and developmental systems, resulting in reduced semen quality and birth abnormalities in mammals [14-16]. Additionally, it has been demonstrated to operate as a disruptor of the neuroendocrine axis and sexual development in male frogs, resulting in the full reversal of sex from male to female [17]. Very few studies on atrazine toxicity have been conducted on endocrine related liver and kidneys, among other organs. However, hepatic and renal toxicity of other herbicides has been extensively studied mostly in mammals, with very few studies done on amphibians [18,19].

To assess the scope of the harmful consequences of atrazine in rats, it is crucial to emphasize the detrimental effects of atrazine at high, medium and low dosages on the histo-morphological structure of the liver and spleen.

2. MATERIALS AND METHODS

2.1 Experimental Animals Model

A total of twenty [20] male wistar rats (weighing 150-200g) between 6-8weeks old were used for the experiment. The animals were bought and housed in a regulated setting. Before the study began, the animals were given 14 days of free access to food and water at their leisure. The National Institutes of Health's Guide for Care and Use of Laboratory Animals was followed as recommended by Enye et al. [20]. Additionally, cautious handling, medical care and animal

euthanasia were employed in an effort to lessen the animals' suffering. For 30 days straight, oral doses were given 30 minutes apart (4 weeks).

2.2 Chemicals and Mode of Administration

Atrazine dust was procured from Mendel chemical located at No.23 Lagos street, Benin city. The atrazine solution was given to the animals of treatment group orally using 1ml syringe with in tube sterile cannula. The time of administration was between 8:00 am and 10:00 am daily.

2.3 Experimental Procedures

Twenty mature wistar rats overall were randomly assigned into 4 groups 1, 2, 3 and 4 of 5 animals each based on average body weight. Groups (2-4) were administered atrazine via oral route corresponding to 1237 mg/kg (20/5 LD50), 618 mg/kg (10/5 LD50) and 309 mg/Kg/body weight (1/10 LD50) for 7, 14 and 30 days, while group I (control) received distilled water orally using orogastric cannula for 30 days. Rats in all groups were sacrificed 24 hours after the experimental periods of oral administration of the extract and the liver and spleen were harvested, weighed and fixed in 10% buffered formal saline fixative and taken for histological analysis.

2.4 Tissues Collection

At the end of each trial session, the animals had a complete physical examination to determine their general physical condition. The animals were sacrificed by separating the cervical vertebrae. The midline incision was created through the front abdominal walls of the rat. The liver and spleen were removed, weighed and fixed in 10% buffered formal saline fixative before being sent for histological analysis.

2.5 Histological Analyses

The liver and spleen sections were removed for histological processing and inspection. The samples were fixed in neutral formalin buffered at 10%. They were prepared for histological evaluation and examined under a 400x light microscope. A histo-pathology specialist confirmed all modifications.

3. RESULTS

Fig. 1 shows that effect of atrazine on the histological modifications in the rat spleen.

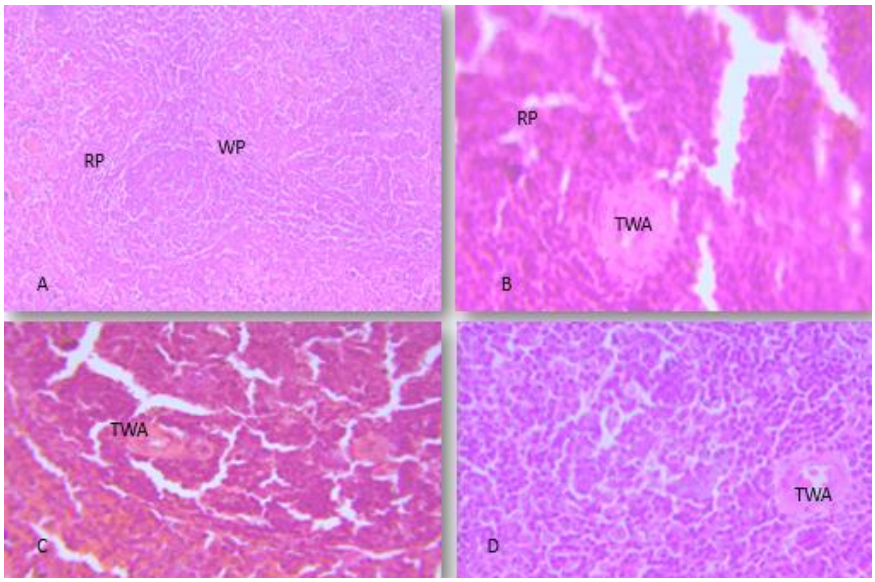

Fig. 1. Demonstrate how atrazine affected the rats' spleen's histological alterations (H&E)

A: Spleen RP and WP appear normal

B: The white pulp (WP) appear with extensive area of active germinal center, moderate mantle and marginal zone with no septa trabeculae seen

C: Photomicrograph of a section of spleen of a rats treated with atrazine (618 mg/kg/day) showing lymphoid follicles of variable sizes disposed within the RP, numerous WP with extensive area of active germinal centre

D: Spleen shown a think wall artery (TWA) vascular channel with mild moderate thickening of the fibrous septa within the splenic tissues and numerous widened white pulp

Fig. 2 shows that effect of atrazine on the histo-pathological changes in the liver of rats.

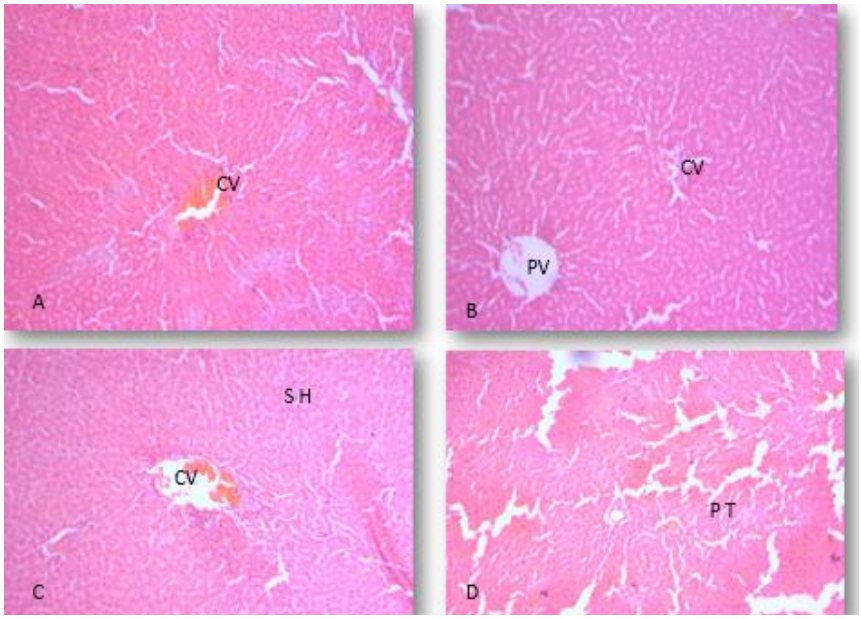

Fig. 2. Histopathological slides of liver (H&E)
A: In the hepatic cells (HC) and the central vein (CV) of the liver appear normal
B: Liver showing areas of necrotic hepatic cells and congested central vein
C: Liver appear relatively preserved architectural morphology of the hepatic cells (HC) and the central vein (CV). It also shows areas of necrotic hepatic cells and congested central vein
D: Liver showing relatively preserved architectural morphology of the hepatic cells (HC) and the central vein (C). It also shows diffused hepatic steatosis

4. DISCUSSION

4.1 Spleen Histopathological Alterations

The white pulp of the spleen from rats exposed to atrazine had a larger and more numerous region in the current study's histo-pathological analysis when compared to that from the control group. Rats treated to atrazine showed increased relative area of the germinal center in the formation of the splenic lymph follicles. These alterations could be linked with relation to oxidative stress brought on by atrazine exposure. Rats within the control group did not exhibit any detectable microscopic alterations within the spleens. Atrazine seems to be a strong immuno-suppressive drug under the current experimental conditions and time period on the basis of the observed degenerative alterations of spleen cells. Earlier reports by Abarikwu and others suggested that atrazine may have an immunosuppressive impact [21]. This outcome is similarly consistent with that reported by Udi et al. [22] who also noted splenic cell degeneration in response to garlic extract. This may be caused by an increase in the rate of erythrocyte breakdown following pesticide exposure [23,24]. Other writers who have identified an increase in lymphoid tissue proliferation in the immune system's peripheral organs under conditions of stress factors also published the facts regarding atrazine-induced alterations to the spleen, as evidenced by the increased area of the white pulp and its compartments [25].

4.2 Histo-Pathological Changes in Liver

The liver is a target organ, the primary site of detoxification, the primary site of intense metabolism and is therefore susceptible to a variety of disorders as a result of exposure to toxins in both extrinsic and intrinsic forms. The liver also plays a crucial role in metabolism to maintain the body's energy levels and structural stability [26]. Additionally, it is where a poisonous molecule is bio-transformed into a less toxic form to lessen toxicity [26]. The liver samples from the control group, however, revealed a normal histological appearance during the histopathological testing. In the current study, Rats exposed to atrazine developed degeneration, necrotic to diffusely degraded hepatocytes in the portal sections of their livers, which may be caused by oxidative stress brought

on by atrazine's toxic effects. The central vein is located at the center of the lobule and is surrounded by hepatocytes. The rats treated to the low dose of atrazine exhibited scattered hepatocyte steatosis and a mildly visible microscopic lesion. These findings are consistent with earlier research [21], which showed that atrazine-treated rats' hepatic sinusoids had an increase in Kupffer cells.

5. CONCLUSION

The findings of this study showed that high doses of atrazine caused liver damage and hepatic cell hypertrophy in rats. From this, we deduced that both organs are extremely susceptible to the incapacitating effects of atrazine.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The ethics committee of the Faculty of Basic Medical Sciences at Delta State University, Abraka, granted approval for this study in a letter with reference number: RBC/ FBMS/ DELSU/ 17/04 on the use and care of animals for research.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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