HISTOPATHOLGICAL EFFECT OF PARAQUAT DICHLORIDE ON THE LIVER OF PREGNANT WISTAR RATS AT VARIOUS TRIMESTERS

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Abstract

Background: Paraquat dichloride, a widely used herbicide, poses health and environmental risks due to its wide range of use in the modern-day Argo life, endangering aquatic life, farm animals, rodents and human health particularly, liver-related ailments, the aim of the study is to evaluate the effect of paraquat on the liver of pregnant Wistar rats at various trimesters. **Material and Method**: The study involved 24 pregnant Wistar rats divided into four groups: A (control), B, C and D (treated with 1mg/kg, 5mg/kg, 10mg/kg concentrations of paraquat dichloride) each group comprised six rats. Throughout each trimester, two rats from each group were euthanize on day 7, 14, and 21 respectively. The concentrations (1mg/kg, 5mg/kg, 10mg/kg) were chosen below the lethal dose of 50mg/kg to induce graded dose histological changes in the liver. **Result**: The liver tissue initially appeared normal at day 7 across all group with varying doses. However, by day 14 and 21, significant histological changes were observed, such as; central vein congestion, hemorrhages, degenerative changes, and bile duct hyperplasia. **Conclusion**: These finding highlight the changes that occurs with varying doses of paraquat exposure in the liver as pregnancy progresses in Wistar rats.

Key words: Paraquat, Liver, Histology, Wistar rats, Trimester, Doses.

INTRODUCTION

The rapid application of pesticide and herbicides across the globe today is on the increase, particularly in the areas of agriculture and industry for the purpose of weed and pest control. Paraquat Dichloride has been noticeably observed to be one of the paramount non-selective used herbicides. Following it wide spectrum use, its abusive effect to farmers and users in respect to its constant use is inevitable (Bagheri *et al.*, 2021). Some studies have proven that its residue encounter water bodies and also intoxicating aquatic lives, which in turn affect human health, especially the liver, over a long duration depending on the concentration of contaminant in the water body (Singh, 2012; Kumar *et al*., 2016; Yeganeh *et al*., 2016; Sribanjam *et al*., 2018). The liver, which is a site for metabolism and detoxification, has made it vulnerable for toxicant and other harmful agent. The liver also supports almost every other organ in the body because of its multidimensional functions, making the liver to be prone to inflammation (Gawarammana and Buckley, 2011; Costa *et al*., 2013).

Previous studies showed that (biochemically), majority of chemicals that causes hepatoxicity share an underlying mechanism which is the production of reactive free oxygen radicals (ROS), which result to oxidative stress and lipid peroxidation (LPO) (Dzoyem, Kuete and Eloff., 2014, Ramachandaran,A and Jaeschke,H. 2018,) Some studies have displayed that the cumulative effect of the aforementioned events became evident as a result of damaged membrane of the hepatocytes causing hepatocyte enlargement, deterioration, necrosis, and fibrosis (Selvam *et al*., 2013; Zhang *et al*., 2018). In same fate Reactive oxygen species are produced when paraquat rapidly reduces and then reoxidizes, which is why it is poisonous. It is suggested that toxic free radicals, can accumulate in different organs especially the liver resulting in paraquat poisoning (Awadalla, 2012; Zeinvand-Lorestani *et al*., 2018). Despite studies on the ameliorative effect of several agents on the hepatotoxic effect of paraquat dichloride and few study displaying its effect on the histology of the liver, (Jaya *et al*., 2017; Ujowundu *et al.*, 2018; Hussein *et al.*, 2021;). The effects of a single dose of paraquat dichloride on the histopathology of the liver in pregnant Wistar rats are still not fully understood in the literature. Therefore, the purpose of this study was to look into any possible hepatotoxic effects that paraquat dichloride exposure may cause the livers of pregnant Wistar rats.

Materials and methods

Ethical consideration

Ethical approval was received with reference number REC/FBMS/DELSU/22/161 before the start of this research from the Delta State University Faculty of Basic Medical Sciences' Ethics and Research Committee.

Animal Model

Twenty-four (24) mature pregnant female Wistar rats, weighing between 180 and 200 grams, were procured from the College of Health Science's animal holding facility at Delta State University of Abraka, Delta State, Nigeria, to serve as the experimental model. The animals were housed in conventional settings with a 12-hour light and dark cycle and a temperature between 28 and 31°C for two weeks while they became acclimated. They were fed twice a day and allowed unlimited access to food and water. Three weeks were allotted to the experiment.

Chemical

Paraquat dichloride was used for this study and the Zenseca AG Products Wilmington, Delaware (1989) manufactured it. It was purchased from a local store in Abraka, Ethiope-East Local Government Area of Delta State.

Paraquat Administration

Paraquat dichloride administered at graded doses in this study at 1mg/Kg, 5mg/Kg and 10mg/Kg body weight respectively for 21 days before the animals were euthanized (Nair and Lalithakunjamma 2010). The different concentrations were administered through the oral route with the use of an orogastric cannula. The choice of the graded doses of Paraquat dichloride was based on the previous study of Nair, and Lalithakunjamma (2010) which showed the anatomical and physiological changes following administration of Paraquat. The doses of 1mg/Kg. body weight, 5mg/Kg.BW and 10mg/Kg. body weight was calculated as 2%, 10% and 20% of LD_{50} (50mg/Kg) of Paraquat dichloride.

Study Design

This study adopted an experimental study design, which entails twenty-four (24) pregnant Wistar rats, which was divided into four groups (groups A, B, C, and D). Six rats made up the control group (A), while six rats per group made up the treated groups (B (1mg/kg), C (5mg/kg), and D (10mg/kg). Two rats were taken out of each of the groups at the conclusion of each trimester, and they were sacrificed on days 7, 14, and 21, respectively.

Group A: Just food and distilled water were given to the six (6) Wistar rats (Control) **Group B**: Six (6) Wistar rats were given food, water, and 1 mg/kg of paraquat dichloride. **Group C**: Six (6) Wistar rats were given food, water, and 5 mg/kg of paraquat dichloride. **Group D**: six (6) Wistar rats were given food, water, and 10 mg/kg of paraquat dichloride.

Determination of Administered concentration of Paraquat Dichloride

The choice of these concentrations of paraquat dichloride is based on the lethal dose which is 50mg/Kg. BW (Nair *et al.,* 2010). The three different concentrations of the herbicide were administered at separate concentration below 50mg/kg body weight. The gradation in the concentration of paraquat will have the aim of inducing graded dose changes in vital organs assessed in this study.

Histological procedures

When the 7, 14, and 21-day study period came to an end, the animals were sacrifice via cervical dislocations. Liver tissues were inspected thoroughly, put in tissue cassettes, and manually processed using standard histological procedures. These procedures involve multiple steps, starting with fixation using 10% formal saline. The tissue processing consists of four sections: dehydration (a series of alcohol changes in the tissue with time dependency), clearing (a series of xylene changes in the tissue with time dependency), infiltration (a series of paraffin wax changes in the tissue with time dependency), and embedding (a mold-based method of preparing a tissue block). Sectioning (using a microtome at 5 to 7um), staining (using H and E and special stains), mounting (using DPX), and photomicrography.

Photomicrography

Micrograph was read Using a digital microscope with a computer-connected 8.3 mega pixel camera called "CARL ZEISS (Primo Star)," slides were examined and tissue photos were taken. The obtained micrographs were analyzed to determine the effects of paraquat dichloride on the liver's cytology and histology.

Result

The sections of the liver that were shown at days 14 and 21 shows central vein congestion, hemorrhages, degenerative changes, and hyperplasia of the bile ducts. The section also showed the parenchyma sheath encapsulating the hepatic lobule, which is composed of hepatocytes with round to oval vesicular nuclei posited in the cytoplasm of the cell. These cells were separated by a thin fibro vascular stroma. The result obtained at day 7 shows a normal hepatic tissue in all groups, with varying doses.

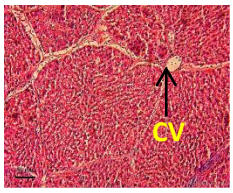
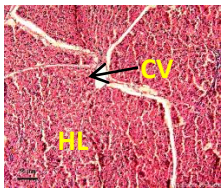
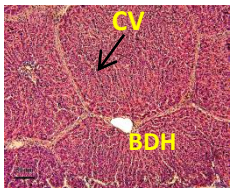
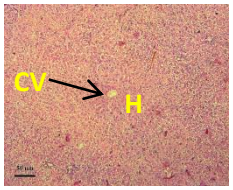
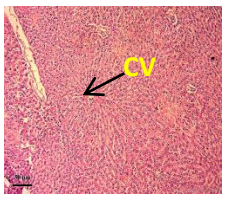
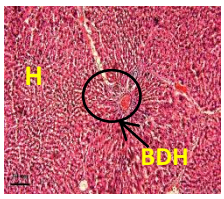
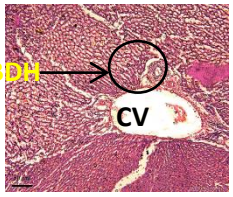
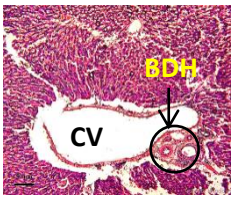
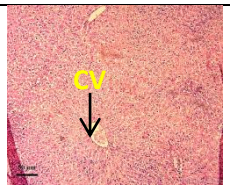
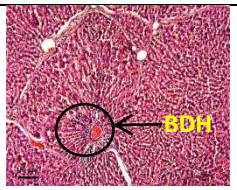
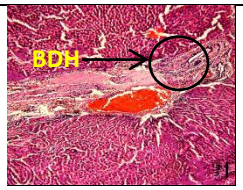
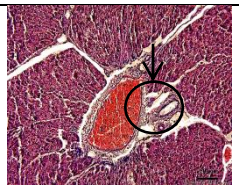
Duration	Group A (Control)	Group B (1mg/kg body weight of paraquat dichloride)	Group C (5mg/kg body weight of paraquat dichloride)	Group D (10mg/kg body weight of paraquat dichloride)
7 Days	 A1	 A2	 A3	 A4
14 Days	 B1	 B2	 B3	 B4
21 Days	 C1	 C2	 C3	 C4

Figure A1 – C1: Sections of the liver, H & E (x100) for group 1 (control), A2 – C2: Sections of the liver, H & E (x100) for Group 2 (1mg/kg body weight of paraquat dichloride) showing (CV, H, HL, BDH), A3 - C3: Sections of the liver, H & E (x100) for Group 3 (5mg/kg body weight of paraquat dichloride) showing (CV, BDH) and A4 – C4: Sections of the liver for H and E (x100) for Group 4 (10mg/kg body weight of paraquat dichloride) showing (CV, H, BDH)

Key: CV – central vein, HL – hepatic lobule, H – hepatocyte, BDH – bile duct hyperplasia,

Discussion

The liver which is known as a metabolic organ has been proven as a target organ of several toxic substances, which is associated with several pass effect on these agents (Wang *et al.,* 2017). Paraquat dichloride application during farming activities and its poor management have proven to cause significant damage to vital organs in fishes (Channa punctatus) following acute exposure (Badroo *et al.,* 2020). Though some researchers have elucidated the upshot of the varying doses of paraquat dichloride on the liver (Norhan *et al.,* 2022; Hussein *et al.,* 2021; Aribisala *et al.,* 2022), but none has elucidated the histologic effect of the single use of paraquat dichloride exposure to the liver.

Miniature exposure of paraquat dichloride reveals no significant change in the histoarchitecture, of the liver. This may be attributed to the nature of the organ owing to its speedy regenerative property after a period of injury or tissue loss (John and Michael 2021). More also considering the dose and short time of administration of paraquat dichloride, it can be attributed to the unremarkable effect seen in the histology of the liver at day 7. This was contrary to some previous studies conducted where a higher dose of paraquat dichloride was administered for a long period of time (Lalruatfela *et al.,* 2014; Jaya *et al.,* 2017; Shalaby *et al.,* 2020). However, the route of administration of paraquat in relation to the dose administered following miniature exposure might also be a factor. Studies have proven intravenous route of administration are faster as compared to oral route of administration, since it is believed to bypass the process of solute absorption and overcome potential major first pass impact in the liver (Ahama and Odokuma, 2022; Jensen and DeVito, 2016; Jensen *et al*., 2016)

Following subsequent administration of paraquat for 14 and 21 days with high dose of paraquat, displays anatomic defection in the histology of the liver, which is synonymous with the metabolic distortion in cellular function in the liver. The investigation observed at these days can be attributed to the dose of paraquat administered subsequently for 14 and 21day. This is obtainable in a study conducted by Lalruatfela *et al.,* 2014, where focal area of necrosis and periportal hyperplasia of bile ducts around central vein were noticed. More or also liver parenchyma display congestion, granular and prominent vacuolar changes, which were also seen in the current study. Similar findings were also seen in a study conducted by Shalaby *et al.,* 2020, where animals intoxicated with paraquat dichloride at 5mg/kg and 10mg/kg display hepatic congestion, vacuolation of hepatocytes and induced fibrotic connective tissue associated with inflammatory cells infiltration and sinusoidal dilation respectively, which were also clear histologic features seen at day 14 and 21 of the index study. Collaborative biochemical report at day 14 and 21 displays similar deleterious effect where an observable significant increase of decrease of liver enzyme which was also seen in the anatomic architecture of the liver.

CONCLUSION AND RECOMMENDATIONS

This underscores the trimester-specific susceptibility of pregnant Wistar rats to paraquat induced liver injury, with the third trimester being particularly, vulnerable. These findings highlight the importance of considering both dosage and gestational timing in assessing the hepatotoxic effects of paraquat during pregnancy. To clarify the underlying mechanisms and investigate potential strategies to lessen such unfavourable effects, more research is necessary.

CONFLICT OF INTEREST

There was no conflict of interest in this study.

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