Toxicological effects of paraquat on the histology of the stomach, small intestine and testis of male albino rat (*Rattus norvegicus*)

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Abstract

**Background:** Paraquat is a herbicide, commonly used by farmers in agriculture to prevent weed infestation. The non-target organic toxicity effect of this chemical motivated this study.

**Methods:** Utilizing male albino rats of 0.2 kg average body weight, the LD$_{100}$ (lethal dose that gave 100% death) for Paraquat was obtained as 1g/kg body weight. Furthermore, LD$_{50}$ (median lethal dose of paraquat, ip) was obtained as 0.45 g/kg body weight using arithmetic method of karber. Six dose levels of paraquat 0, 0.09, 0.18, 0.35, 0.70, 1.00 g/kg body weight were administered intraperitoneally into the various groups of the male rats. Within 24hrs, histopathological examination in the stomach, in the small intestine and testis of the rats were performed.

**Results:** Histopathological examination of the organs studied revealed that the stomach had mild mucosal ulceration, muscular coat atrophy, stromal oedema and tubular hyalinization which were dose-dependent. The small intestine showed mucosal ulceration, loss of villi, luminal and stromal oedema and glandular necrosis which were also dose dependent. Furthermore, the testis had classical central fibrosis, cellular polarization, tubular disorganization, necrosis and lack of mitotic figures (no cell division), oligospermia, azoospermia and hyperchromasia which were dose dependent.

**Conclusion:** Paraquat, a notable herbicide used in agricultural weed control, had deleterious effects on such organs as stomach, small intestine and testes. Need therefore arises for caution in the handling of these chemicals as the danger of impairment of the gastrointestinal tract and indeed the reproductive system in males is a possibility.

**Key words:** Paraquat, Acute toxic effects

Introduction

Paraquat (1,1$^\prime$ dimethyl, 4,4$^\prime$ bipyridyl) is a non-selective contact herbicide used worldwide in approximately 130 countries$^1$. Both its herbicidal and toxicological properties are dependent on the ability of the parent cation to undergo a single electron addition to form a free radical, which reacts with molecular oxygen to reform the cation and concomitantly produce a superoxide anion. This radical may directly or indirectly cause cell death$^1$.

The mechanisms of the toxic effects of paraquat are largely the result of a metabolically catalyzed single electron reduction oxidation reaction, resulting in deletion of cellular NADPH and the generation of potentially toxic forms of oxygen such as the super-oxide radical$^2$. Absorbed paraquat is distributed via the blood stream to practically all organs and tissues of the body, but no prolonged storage takes place in any tissue. The lung selectively accumulates paraquat from the plasma by an energy-dependent process. Consequently, this organ contains higher concentrations than other tissues$^3$. Minor toxic effects of paraquat have been noted only at high doses in the nervous, cardiovascular, blood, adrenal...
and male reproductive systems. The current study has used acute toxicity tests to investigate the effects of paraquat on the histology of the stomach, small intestine and testes of male albino rats (Rattus norvegicus).

Materials and Methods

Twenty-four male albino rats were obtained from the Animal House of the Department of Pharmacology and Toxicology, College of Health Sciences, University of Port Harcourt, Rivers State. The animals were fed ad libitum and acclimatized for two weeks in metabolic cages before the commencement of the study.

Source of paraquat samples

The paraquat used was purchased, as a litre volume of 20% w/v solution with the trade name Dizmazone, from Dizengoff W.A. Ltd, properly sealed in an opaque plastic container, with a shelf life of two years. It was kept at room temperature and during use proper caution was taken to avoid spillage, fire or poisoning.

Source of diluent

A litre of 0.9M, Normal saline was purchased at Akoltem Pharmacy, Aba Road, Port Harcourt, Rivers State, Nigeria a product of Pfizer Industries, Ikeja, Lagos, Nigeria.

Acute toxicity study

Twenty four male albino rats of 0.2kg average body weight were arranged in six groups of four rats per group (categorized as A, B, C, D, E and F) and were administered, with paraquat, intraperitoneally, representing 0g, 0.09g, 0.18g, 0.35g, 0.70g and 1.00g per kilogram (kg) body weight of rat respectively.

The animals were closely observed within 24 hours for signs and symptoms of toxicity and possible death. The organs (stomach, small intestine and testes) were harvested and prepared for histopathological examination.

Histopathological examination

The stomach, small intestine and testes, of each animal sacrificed were placed in labelled plastic universal containers containing 10% formalin preservative.

The tissues were sliced and dehydrated with different, increasing concentrations (50, 70, 80, 95 and 100%) of ethanol for about 24 hours. After which, they were cleared with xylene, to remove the alcohol and improve their refractive index. They were then imbedded in the molten paraffin wax, allowed to solidify inside the wax. The resulting blocks were sectioned with a Shandon AS 325 rotary microtome, and after wards, slides were prepared with the best of the sections.

The slides were stained with haematoxylin/eosin solution, and the stained slides were carefully studied for any histopathological lesions as a result of the toxicant (Paraquat). Photomicrographs were made using a leitz Wetzlar (Model Dialux-20) at 100, 200 and 400 magnifications, depending on the size of the organs under examination.

Results

Histopathological examination of the stomach, small intestine and testes of rats injected, with paraquat, ip, under acute toxicity study were carried out and the results were as presented in the figures.

The stomach had mild mucosal ulceration, muscular coat atrophy, stromal oedema and tubular hyalinization, which were also dose dependent (Figures 1 and 2). The small intestine showed mucosal ulceration, loss of villi, luminal oedema, stromal oedema, luminal exudates and glandular necrosis. These changes became increased as the dose of paraquat increased (Figures 3 and 4).

The slides of the testes were also affected in the acute toxicity study from 0.18g/kg dose, showing cellular alterations such as, cellular polarization, central fibrosis and necrosis, tubular disorganization, oligospermia, azoospermia and...
hyperchromasia. These distortions were more noticeable at high doses (0.35, 0.70 and 1.00g/kg) respectively (Figures 5 and 6).

Figure 1. Male albino rat injected with 0.9% saline. Notice normal stomach architecture

Figure 2. Male albino rat injected with 1.00g/kg body weight paraquat. Notice (a) Tubular hyalinization (b) Necrosis (c) Stromal oedema

Figure 3. Male albino rat injected with 0.9% saline. Notice normal small intestine architecture

Figure 4. Male albino rat injected with 1.00g/kg body weight paraquat. Notice (a) Massive mucosal ulceration (b) Loss of villi (c) Glandular necrosis

Figure 5. Male albino rat injected with 0.9% saline. Notice normal testis architecture

Figure 6. Male albino rat injected with 1.00g/kg body weight paraquat. Notice (a) Massive cellular polarization (b) Central fibrosis (C) Azoospermia, necrosis
Discussion

Paraquat is a bipyridyl compound, and studies on the toxicity effects of this chemical on the lung, liver and kidney including its mechanism of toxicity have been reported. Furthermore reports all presented paraquat as a hepatotoxin.

The stomach and the small intestine slides exhibited similar lesions, from ulceration, loss of villi to oedema, which became more pronounced with increased dose. It has been observed that systemic paraquat poisoning was characterized by burns of the upper digestive tract when ingested, and by multi-organ failure, fibrosis and necrosis.

This study has shown that the testis was adversely affected by paraquat. It was conceivable that the rest of the male genital tract may be similarly affected. Spermatogenesis, occurs in the testis, specifically in the seminiferous tubules of the testis. In this study, the disappearance of the mitotic figures was an indication that the toxicant invariably affected cell division of the sperm cell (spermatogonia) at the primary stage and hence spermatogenesis.

The tubular disorganization, hyperchromasia and central fibrosis, all confirmed the destructive effect of paraquat on the seminiferous tubule, vas deferens and the tunica albuginea.

The results corroborated the report by Chapin et al, which implicated the target organ for the toxicity effect of paraquat in male animals to be the spermatocytes. However, the work by Butler and Kleinerman observed no difference in the spermatozoa count or motility, of rats, orally administered with paraquat 4mg/kg body weight for 60 days. It is note worthy that 4mg/kg dose was at least twenty times lower than the dose of paraquat used in the current study. The organ toxicity effect of paraquat was dose dependent.

Conclusion

This study has demonstrated the acute toxicity effects of paraquat on organs such as the stomach, small intestine and testes of male albino rats. Considering the lesions observed in the photomicrograph slides of the organs, we inferred that paraquat toxicity was dose dependent. However, further work is needed particularly at the chronic toxicity level to shed some light on the mechanism of the toxic effect of paraquat on the organs studied.

Finally, there is need for caution in the use of paraquat in occupational, recreational, industrial areas, as accidental exposure to man or animal could cause possible toxic effect as indicated in the study.

References

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