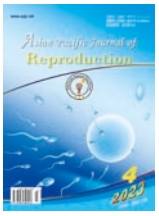

Original Article

Asian Pacific Journal of Reproduction

Journal homepage: www.apjr.net

doi: 10.4103/2305-0500.380984

Plantain-based diet modulates atrazine-induced testicular toxicities in rats

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ABSTRACT

Objective: To assess the potential of plantain-based diet in modulating testicular toxicities in rats exposed to atrazine.

Methods: The plantain-based diet at 50%, 25% and 12.5% were prepared from the basal diet by substituting the corn starch with plantain fruit pulp flour at different percentages. Wistar rats were fed plantain-based diet in varying concentrations ranging from 12.5% to 50% of the basal diet for 21 days before or after atrazine treatment in a two-phase experiment: preventive and therapeutic phases. The therapeutic model (n=35) had seven groups with 5 rats each, including the control, atrazine, atrazine recovery, atrazine plus plantain-based diet 50%, 25%, 12.5%, and atrazine plus quercetin groups. The preventive model (n=30) had six groups of 5 rats, consisting of the control, atrazine, 50%, 25%, 12.5% plantain-based diet plus atrazine, and quercetin plus atrazine groups. Gonadal hormones (testosterone, luteinizing hormone and follicle-stimulating hormone), sperm parameters (sperm motility, viability, morphology and concentration), and testicular function indices (protein, cholesterol, glycogen, acid phosphatase, alkaline phosphatase and lactate dehydrogenase) were measured.

Results: The gonadal hormones, sperm characteristics, and testicular function indices of the rat testis decreased significantly in the atrazine group alongside degeneration of the histoarchitecture. However, plantain-based diet restored the gonadal hormone concentrations, semen parameters, and testicular function indices in both the preventive and therapeutic models.

Conclusions: Treatment with plantain-based diet protects against rat testicular toxicity caused by atrazine *via* the modulation of gonadal hormones, sperm quality, testicular function index as well as histoarchitecture of rat testes.

KEYWORDS: Plantain; Atrazine; Endocrine disruptor; Environmental toxicants; Medicinal biochemistry; Testicular toxicity

1. Introduction

The usage of pesticides has helped increase agricultural output. However, these substances and their metabolites may have negative impacts on the environment and human health despite playing a significant role. The triazine group of herbicides, which account for about 30% of all pesticides in use annually, are among the most widely used pesticides in the world[1,2]. Atrazine is a common herbicide for weed control in corn, sugarcane, and sorghum. Although this herbicide is widely used, it is not authorized in Europe, Brazil, Argentina, or the United States. The resistance of atrazine to biodegradation and its ease of detection in both surface water and groundwater are some of its health hazard properties that fuel concerns[2,3]. Atrazine has also been found in dairy products,

Significance

Plantain has been reported as a possible remedy for testicular dysfunction in traditional medicine. However, there is insufficient empirical information concerning how it positively affects male fertility in rats. This study indicated that plantain-based diets reverse atrazine-induced toxicity in rats by improving gonadal hormone status, and sperm parameters as well as enhancing the testicular functional indices that were impaired by oral administration of atrazine. The findings support the prospects of plantain as a readily available and low-cost nutraceutical for male fertility therapy.

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How to cite this article: Rotimi DE, Adeyemi OS. Plantain-based diet modulates atrazine-induced testicular toxicities in rats. *Asian Pac J Reprod* 2023; 12(4): 170-181.

Article history: Received: 12 January 2023; Revision: 21 March 2023; Accepted: 15 May 2023; Available online: 18 July 2023

including milk and yogurt. Suffice it to state that atrazine is a powerful endocrine disruptor that could impact reproduction in several vertebrate species, including mammals, birds, amphibians, reptiles, and fish[4]. Moreover, men in agricultural areas have been noted to have low semen quality and fertility[5]. Atrazine has been linked to the suppression of male fertility through several processes that affect epididymis, seminal vesicles and prostate leading to altered sperm maturation as a result of inadequate testosterone production in the Leydig cells[6,7].

Several attempts have been explored to reduce the effects of atrazine on male fertility by using natural products[8]. The traditional use of plants and herbs has been engaged by indigenous healers in most of the world for the prevention and treatment of different diseases, especially testicular dysfunction[9]. Plantain (*Musa paradisiaca*), for example, has been reported as a possible remedy for testicular dysfunction[10,11]. However, the action mechanisms for their testicular function-promoting effects are not clear. Studies revealed that plantains increased the reproductive ability of male Wistar rats[11]. Adnan and Noory[10] reported that the ripe green fruit of *Musa paradisiaca* improved semen parameters in male mice, thus making it a viable treatment for male fertility dysfunction. Moreover, plantain pulps have been credited with several pharmacological benefits, including antibacterial, antioxidant, and wound healing[12]. Meanwhile, phytochemical studies showed that plantain has several bioactive substances such as flavonoids, alkaloids, and tannins[1]. However, there is insufficient information in the literature concerning the mechanism of action *via* which plantains could improve fertility in rats. Therefore, this present study was to investigate the action mechanisms of a plantain-based diet as a prospective therapy for testicular dysfunction in rats exposed to an environmental toxicant, atrazine.

2. Materials and methods

2.1. Materials

Unripe plantain bunches were purchased from a local farm in Omu-Aran, Kwara State. The plantains were cleansed with water before the fruit was peeled, and the pulp was sliced and sun-dried to a constant weight. The dried pulp was milled into flour using an electric blender[13]. The flour was sieved and stored in an airtight container.

2.2. Plantain–based diet formulation

Feed preparations were carried out using the method of Ajiboye *et al*[14], Oloyede *et al*[15], Oyegoke and Oladiji[16] with slight modifications. The feed ingredients were weighed out and carefully blended to homogeneity. The feeds were pelletized, packaged in polythene bags, labelled, and kept in the refrigerator. The composition of the diets includes soybean (25 g/100 g), sucrose (10 g/100 g), soybean oil (5 g/100 g), Vitamix (5 g/100 g), rice husk (4 g/100 g), D-methionine (0.4 g/100 g), and corn starch (50.6 g/100 g) for the basal diet. The plantain-based diet at 50%, 25% and 12.5% were prepared from the basal diet by replacing/substituting the corn starch with plantain fruit pulp flour at different percentage (50%, 25% and 12.5%).

2.3. Experimental animals

Sixty-five sexually matured male Wistar-albino rats aged 10 weeks, weighing (120±20) g, were obtained from the Department of Biochemistry, Landmark University, Kwara State. The animals were kept in a well-ventilated room (temperature: 25℃-27℃; 12 h light/dark cycle; humidity: 45%-55%), and were allowed to acclimate while being fed with rat chow (Premier Feed Mill Limited, Ibadan, Nigeria) and water *ad libitum*.

The procedure for animal research was systematically evaluated and in compliance with the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines. All animals were treated humanely following the guidelines in the publication "Guide for the Care and Use of Laboratory Animals" by the National Institutes of Health[17].

2.4. Experimental design

A two-phase experiment was applied to evaluate the preventive and therapeutic effects of plantain-based diet in rats exposed to atrazine (Figure 1). The therapeutic regimen was undertaken to assess plantain-based diet as a potential therapeutic agent, while the preventive model was undertaken to assess the potential of plantain-based diet as an agent for chemoprevention of reproductive dysfunction.

In the therapeutic regimen, 35 rats were exposed to atrazine [120 mg/kg body weight (b.w.)] for 21 days before treatment with plantain-based diet for another 21 days. The rats were divided into the control, atrazine only, atrazine recovery, atrazine+50% plantain-based diet, atrazine+25% plantain-based diet, atrazine+12.5% plantain-based diet, and atrazine + quercetin (50 mg/kg b.w.) groups, with 5 rats per group. The atrazine recovery group were initially exposed to atrazine for 21 days after which the rats were fed a basal diet only for another 14 days to check for reversal of effects. After 21 days, we successfully established that atrazine-induced testicular dysfunction before the treatment with plantain-based diet was initiated.

In the preventive regimen, 30 rats were initially pretreated with plantain-based diet for 21 days before exposure to atrazine for another 21 days. The rats were divided into the control, atrazine, 50% plantain-based diet + atrazine, 25% plantain-based diet + atrazine, 12.5% plantain-based diet + atrazine, and quercetin + atrazine groups, with 5 rats per group.

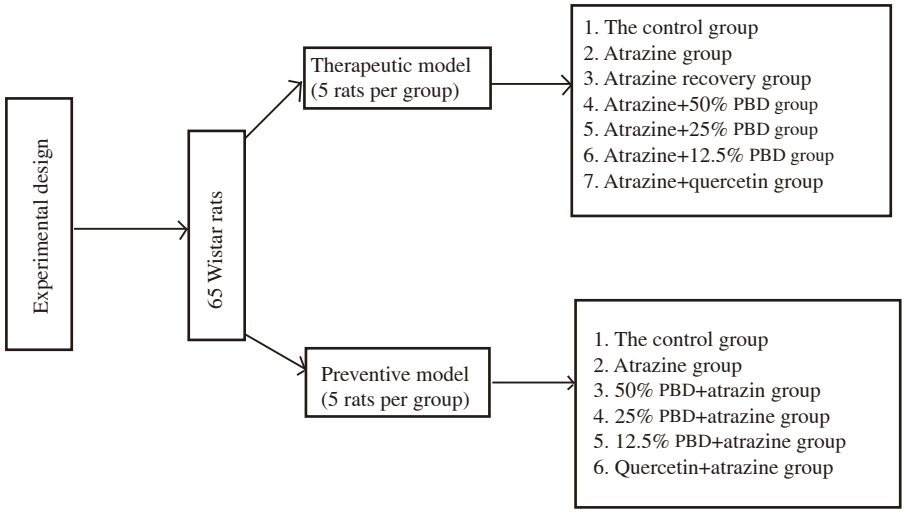

Figure 1. The experimental approach of the study. PBD: plantain-based diet.

The control group were administered the vehicle (olive oil at 2 mL/kg b.w.) while atrazine-administered rats (atrazine only) received daily gavage of 120 mg/kg b.w. atrazine based on a previous report[18]. Quercetin served as a reference drug as reported by Badr *et al*[19]. The diet given to the animals was formulated as described above. Oral administration of atrazine was done orally once daily and the rats were fed plantain-based diet in varying concentrations ranging from 12.5% to 50% of the basal diet.

2.5. Preparation of tissue homogenates and semen

After the termination of experimental treatment, the animals were fasted overnight and sacrificed. Before sacrificing the animals, they were weighed. Briefly, the rats were placed under diethyl ether anaesthesia and the jugular veins were cut with a sterile scalpel blade to get blood. The blood was centrifuged at 4 000 ×*g* for 10 min using a Uniscope Laboratory centrifuge (model C5, LW Scientific, USA). The sera were collected into sample bottles and used for biochemical assays. For assessment of other testicular indices, the testes were excised, weighed, and homogenized using 0.25 M sucrose solution (ice-cold) at a weight-to-volume ratio of 1:5 (1:5 w/v) using a homogenizer positioned in ice to reduce heat due to friction during the process of homogenization. The homogenized organs were then centrifuged at 1 398×*g* for 12 min. The supernatants from the centrifuged homogenates were aspirated into sample bottles and stored frozen until needed[20].

To calculate the body-weight ratio, the organ weight was divided by the final body weight of the animals as described by Kayode *et al*[20].

2.6. Semen analysis

To determine semen parameters, surgical blades were used to open the cauda epididymis and release the sperm cells onto a sterilized, clean microscope slide containing physiological saline (0.9%) in the ratio of 1:5 weight (g) by volume (mL). The semen was used for the evaluation of sperm count, motility, viability, and morphology.

2.6.1. Sperm count

The method by the World Health Organization[21] was used for the sperm cell count. Briefly, under a light microscope (Leica DMD 500, Germany), an aliquot of the sperm solution was placed on the hemocytometer with an improved double Neubauer ruling. The average counts of sperm head were recorded using light microscopy at ×400 magnification.

2.6.2. Sperm morphology

Sperm morphology is divided into normal spermatozoa and abnormal spermatozoa. Normal spermatozoa have an oval-shaped head, a short middle piece, and a long thin tail, while the head of abnormal spermatozoa has a tapering head (pyriform), an abnormal shape, and an absent or abnormally large acrosomal cap.

To check for sperm morphology, a thin smear of semen was placed on a slide. A 95% v/v ethanol was used to fix the smear for 5-10 min and allowed to air-dry. Afterwards, round contour seminiferous tubules were chosen randomly.

2.6.3. Sperm motility

For sperm motility, a semen drop was fixed on a glass slide and with the aid of a light microscope (Leica DMD 500, Germany); several fields were scanned for sperm motility using an ×10 objective lens. A total of 200 spermatozoa were counted and the percentage of sperm motility was reported as motile, non-motile, and sluggish[21].

2.6.4. Sperm viability

To check for the viability of the sperm, 0.5% eosin-nigrosin was used to stain the diluted semen (drop). After 2 min, the preparations were then examined with the aid of a light microscope. The seminal smear was evaluated for sperm viability by determining the relative proportion of live and dead sperm cells. Viable spermatozoa remain unstained, and non-viable spermatozoa are stained red.

2.7. Gonadal hormone level

The serum concentrations of gonadal hormones [testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH)] were measured using rat-specific enzyme-linked immunosorbent assay (ELISA) kits. The assays were performed according to the manufacturer's instructions (Monobind Inc., Lake Forest, USA).

2.8. Testicular indices

2.8.1. Protein concentration

The assay described by Gornall *et al*[22] was used to detect protein concentration. The aliquot (1 mL) of the sample was appropriately diluted and mixed with 4 mL of biuret reagent. The mixture was shaken thoroughly and left undisturbed at 25 ℃ for 30 min for color development. The absorbance at 540 nm was read using UV/Vis spectrophotometer (Jenway, Staffordshire, United Kingdom).

2.8.2. Assay for acid phosphatase (ACP) activity

The procedure described by Wright *et al*[23] was used to determine the activity of acid phosphatase. A known volume (2.2 mL) of sodium acetate buffer (concentrated acetic acid; pH 4.5) was added to 0.2 mL of the appropriately diluted samples in the test tubes. The mixture was equilibrated for 10 min. Then, 0.5 mL of 10 mM p-nitrophenyl phosphate (substrate) was added. The resulting solution was thoroughly mixed and incubated for 30 min at 37 ℃. The reaction was terminated immediately by adding 2 mL of 1 N sodium hydroxide. The absorbance of the mixture was at 400 nm.

2.8.3. Alkaline phosphatase (ALP) activity

The procedure employed was as described by Wright *et al*[24]. Briefly, a 2.2 mL of carbonate buffer (0.1 M) and 0.1 mL of $MgSO_4.7H_2O$ (0.1 M) were added into the test tubes. Thereafter, 0.2 mL of the enzyme source (appropriately diluted) was added and incubated at 37 ℃ for 10 min. A known amount (0.5 mL) of 10 mM p-nitrophenyl phosphate (substrate) was added and the assay mixture was incubated again for 30 min at 37 ℃. The reaction was terminated immediately by adding 2 mL of 1 N sodium hydroxide. The absorbance of the mixture was read at 400 nm.

2.8.4. Testicular glycogen

The determination of glycogen content was described by Kemp and Van Heijningen[25]. Briefly, 0.5 mL of appropriately diluted testis homogenate was pipetted into a test tube containing 5.0 mL of deproteinizing solution (5 g of trichloroacetic acid and 100 mg of $AgNO_3$). The test tubes were placed in a boiling water bath for 15 min and thereafter cooled in running water, filled up to the mark with the deproteinizing solution to compensate for evaporation. The solution was centrifuged at 100.5×*g* for 5 min. Afterwards, 1 mL of clear supernatant fluid was added to 3.0 mL of diluted H_2SO_4 in a test tube and mixed by vigorous shaking. The mixture was heated in a boiling water bath for exactly 6.5 min and subsequently cooled under running tap water. The intensity of the pink color produced was measured at 520 nm on a UV/Vis spectrophotometer.

2.8.5. Lactate dehydrogenase (LDH) assay

LDH activity was determined by monitoring the increase in absorbance at 340 nm resulting from the formation of a reduced form of nicotinamide adenine dinucleotide (NADH) according to the protocol as described by Wahlefeld[26]. An aliquot of the supernatant was added into a cuvette containing 2.4 mL of Tris/L-lactate to make a total of 2.5 mL. The solution was mixed and incubated for 5 min at 30 ℃. Then, 0.1 mL of NAD was added and the absorbance of the mixture was taken at 340 nm at an interval of 30 s for 2.5 min.

2.8.6. Testicular cholesterol

The concentration of total cholesterol in the rat testes was determined using the "CHOD-PAP" reaction as described by Fredrickson *et al*[27]. The testicular cholesterol was determined by using Randox assay kit (Crumlin, UK).

2.9. Histopathological examination

The rat testes were fixed in Bouin's solution and processed according to the normal techniques for histopathological examination. The tissues were then dehydrated in ethanol, cleaned in xylene, and embedded in paraffin wax. Following fixation on slides and hematoxylin and eosin staining (H & E, ×10), sections of tissue were cut at 4–5 μm using a microtome. Testicular tissue sections were assessed for Leydig cell count, percentage of germ cell loss, seminiferous tubule diameter, and seminiferous epithelial height. A pathologist blind to the treatments performed the histological analysis using a light microscope (Olympus CH; Olympus, Tokyo, Japan).

2.10. Statistical analysis

Data were analyzed using GraphPad Prism version 9 (GraphPad Software Inc., San Diego, California, USA). Data analysis was made using one-way analysis of variance (ANOVA) followed by a *post hoc* Tukey test. The data were expressed as mean±standard deviation (mean±SD) of 5 replicates. Values are considered significant at $P<0.05$.

2.11. Ethics statement

This study was approved by the Landmark University Department of Biochemistry Ethical Committee, with experimental protocol No. LUAC/2021/002A.

3. Results

3.1. Body weight parameters in the therapeutic model

There was a significant decrease in the final weight, % weight change, testicular weight and relative testicular weight of rats administered atrazine only compared to the control group. However, the treatment with atrazine + 12.5% plantain-based diet significantly increased the testicular weight, final weight and % weight change when compared to the atrazine only group. Also, there was a significant increase observed in the atrazine + 25% plantain-based diet group in the testicular weight and relative testicular weight compared to the atrazine group (Table 1).

3.2. Body weight parameters in the preventive model

Compared with the control group, the atrazine group had significantly reduced rat body weight, % weight change, testicular weight and relative testicular weight. However, the different plantain-based diets were able to increase the final weight and % weight change compared to the group administered atrazine only (Table 2).

3.3. Sperm analysis in the therapeutic model

There was a significant decrease in the total sperm count in the atrazine group compared to the control group. However, compared with the atrazine group, there were significant increases in sperm count and total sperm concentration in the various treatment groups including atrazine + 50% plantain-based diet, atrazine + 25% plantain-based diet, atrazine + 12.5% plantain-based diet, and atrazine + quercetin group (Table 3).

3.4. Sperm analysis in the preventive model

The atrazine group significantly reduced sperm count compared with the control group (P<0.05). There was a significant (P<0.05) increase in sperm concentration and count and decrease in the % non-motile and slow moving sperm in the various treatment groups including 50% plantain-based diet + atrazine, 25% plantain-based diet + atrazine, 12.5% plantain-based diet + atrazine and quercetin + atrazine group compared to the atrazine group (Table 4).

Table 1. The therapeutic effect of the plantain-based diet on rat weight after exposure to atrazine.

Parameters	Control	Atrazine	Atrazine recovery	Atrazine+50% PBD	Atrazine+25% PBD	Atrazine+12.5% PBD	Atrazine+quercetin
Final weight, g	163.5±7.5	144.0±3.6**	141.0±11.6	140.0±4.7**	150.0±17.3	177.0±11.6$^{\#}$	147.5±23.7
% weight change	23.3±1.7	15.3±1.2**	13.5±1.1*	16.0±6.7	11.3±6.1*	24.9±4.9$^{\#\#}$	16.5±5.4
Testicular weight, g	2.2±0.1	1.4±0.1***	1.6±0.0***	1.7±0.1*	2.2±0.3$^{\#\#}$	2.1±0.2$^{\#\#}$	2.0±0.4
Relative testicular weight, (g/100 g body weight)	1.4±0.1	0.9±0.0**	1.1±0.1	1.3±0.1	1.5±0.4$^{\#\#}$	1.2±0.0	1.3±0.1

Data are expressed as mean±SD, n=5. $^{*}P$<0.05, $^{**}P$<0.01, $^{***}P$<0.001: compared to the control group; $^{\#}P$<0.05, $^{\#\#}P$<0.01, $^{\#\#\#}P$<0.001: compared to the atrazine group. PBD: plantain-based diet.

Table 2. The preventive effect of the plantain-based diet on rat weight after exposure to atrazine.

Parameters	Control	Atrazine	50% PBD+atrazine	25% PBD+atrazine	12.5% PBD+atrazine	Quercetin+atrazine
Final weight, g	163.5±7.5	131.0±1.2*	189.5±19.1$^{\#\#\#}$	202.5±6.4$^{**\#\#\#}$	179.5±7.5$^{\#\#\#}$	197.0±15.0$^{**\#\#\#}$
% weight change	19.9±0.9	6.2±0.0**	29.2±11.7$^{\#\#\#}$	32.3±0.9$^{\#\#\#}$	26.6±7.0$^{\#\#\#}$	31.4±4.2$^{\#\#\#}$
Testicular weight, g	2.2±0.1	0.9±0.1**	2.1±0.2$^{\#}$	1.7±0.5	1.9±0.5	2.3±0.0$^{\#\#}$
Relative testicular weight, (g/100 g body weight)	1.4±0.1	0.7±0.1**	1.2±0.2	0.8±0.3*	1.1±0.3	1.2±0.10

Data are expressed as mean±SD, n=5. $^{*}P$<0.05, $^{**}P$<0.01, $^{***}P$<0.001: compared to the control group; $^{\#}P$<0.05, $^{\#\#}P$<0.01, $^{\#\#\#}P$<0.001: compared to the atrazine group. PBD: plantain-based diet.

Table 3. The therapeutic effect of the plantain-based diet on rat semen after atrazine exposure.

Parameters	Control	Atrazine	Atrazine recovery	Aatrazine+50% PBD	Atrazine+25% PBD	Atrazine+12.5% PBD	Atrazine+quercetin
Total sperm concentration, ($\times10^6$)/mL	125.3±3.5	118.7±18.5	61.5±1.0$^{***\#\#\#}$	125.0±4.6$^{\#\#\#}$	127.5±4.0$^{\#\#\#}$	125.7±7.5$^{\#\#\#}$	123.7±16.2$^{\#\#\#}$
Sperm count, (10^6)/total volume	279.5±10.0	168.6±18.9***	96.1±0.7$^{***\#}$	214.6±15.9$^{**\#\#\#}$	278.4±53.2$^{\#\#\#}$	265.9±34.8$^{\#\#\#}$	224.7±12.7$^{\#\#\#}$
Motility (Fast), %	38.8±2.5	56.7±14.4	33.8±2.5	36.7±23.1	60.0±5.8*	62.5±2.9*	60.0±11.6*
Motility (Slow), %	28.8±2.5	17.50±2.9**	26.3±2.5	25.0±5.8	13.8±2.5****	17.5±2.9**	15.0±5.8***
Motility (Non-motile), %	28.8±2.5	33.3±11.6	38.8±2.5	36.7±28.9	25.0±5.8	18.8±2.5	25.0±5.8
Normal morphology, %	57.5±5.0	67.50±2.9	61.3±2.5	60.0±5.8	61.3±2.5	60.0±5.8	65.0±11.6
Head defect	13.8±2.5	11.3±2.5	13.8±2.5	12.5±2.9	12.5±2.9	13.3±5.8	10.0±8.7
Neck defect	8.8±2.5	8.8±2.5	13.8±2.5	12.5±2.9	6.3±2.5	8.8±2.5	10.0±5.8
Tail defect	18.8±2.5	13.3±2.9	8.8±2.5***	13.8±2.5	22.5±2.9$^{\#\#}$	13.8±2.5	12.5±2.9***

Data are expressed as mean±SD, n=5. $^{*}P$<0.05, $^{**}P$<0.01, $^{***}P$<0.001: compared to the control group; $^{\#}P$<0.05, $^{\#\#}P$<0.01, $^{\#\#\#}P$<0.001: compared to the atrazine group. PBD: plantain-based diet.

Table 4. The preventive effects of the plantain-based diet on rat semen after exposure to atrazine.

Parameters	Control	Atrazine	50% PBD+atrazine	25% PBD+atrazine	12.5% PBD+atrazine	Quercetin+atrazine
Total sperm concentration, ($\times10^6$)/mL	125.3±3.5	118.7±18.5	192.3±1.5$^{*\#\#\#}$	138.5±5.2$^{\#}$	134.0±88.3$^{\#}$	147.0±2.3$^{\#\#\#}$
Sperm count, (10^6)/total volume	283.4±2.2$^{\#\#\#}$	107.6±8.7*	393.7±37.9$^{\#\#\#}$	235.2±80.6$^{\#\#\#}$	278.0±204.2$^{\#\#\#}$	340.2±8.2$^{\#\#\#}$
Motility (Fast), %	38.7±2.5	48.3±14.4	68.8±2.5$^{***\#}$	53.3±11.6	55.0±17.3	57.5±2.9*
Motility (Slow), %	28.8±2.5$^{\#\#\#}$	17.5±2.9*	18.8±2.5*	28.8±2.5$^{\#\#\#}$	23.3±14.4$^{\#\#}$	22.5±2.9$^{\#\#}$
Motility (Non-motile), %	28.8±2.5	33.3±11.6	8.8±2.5$^{***\#\#\#}$	16.7±11.6$^{\#\#\#}$	22.5±2.9$^{\#\#\#}$	18.8±2.5$^{\#\#\#}$
Normal morphology, %	53.8±2.5	67.5±2.9***	53.8±2.5	55.0±5.8	60.0±5.8	67.5±2.9***
Head defect	13.8±2.5	8.8±2.5	8.8±2.5	13.8±2.5	12.5±2.9	12.5±2.9
Neck defect	8.8±2.5	8.8±2.5	8.8±2.5	12.5±2.9	12.5±2.9	6.3±2.5
Tail defect	18.8±2.5$^{\#\#\#}$	12.5±2.9*	26.3±2.5$^{**\#\#\#}$	17.5±2.9	16.3±2.5	16.3±2.5

Data are expressed as mean±SD, n=5. $^{*}P$<0.05, $^{**}P$<0.01, $^{***}P$<0.001: compared to the control group; $^{\#}P$<0.05, $^{\#\#}P$<0.01, $^{\#\#\#}P$<0.001: compared to the atrazine group.

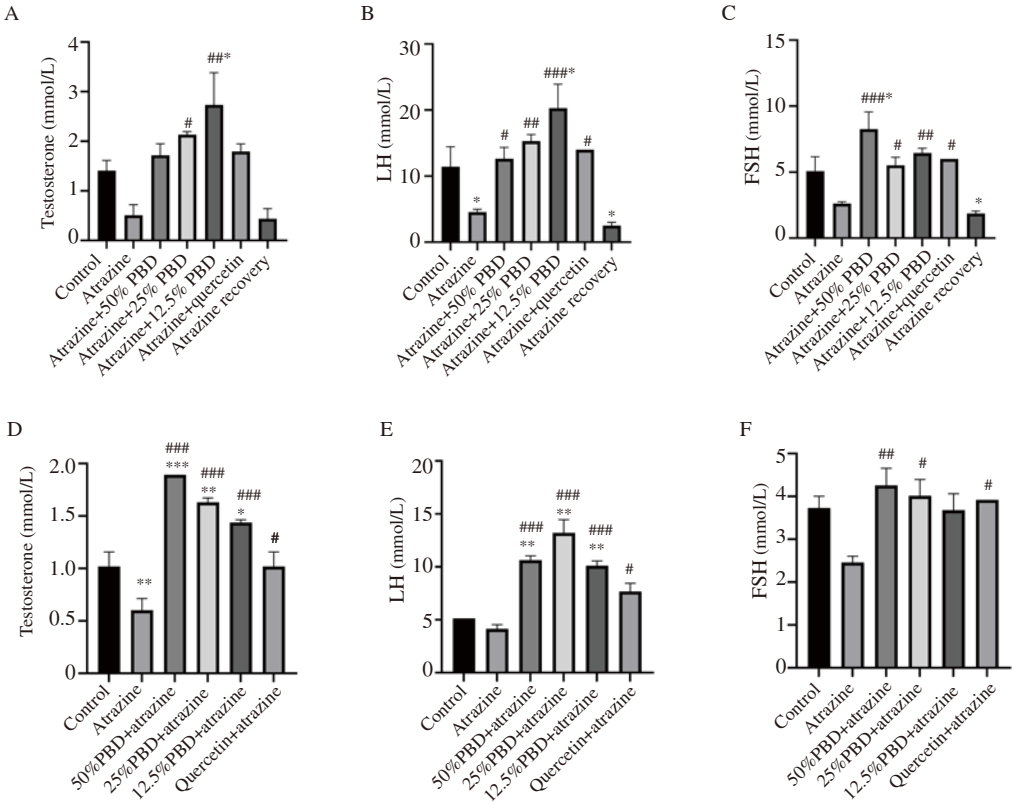

Figure 2 A–C. Therapeutic potential of the plantain-based diet (PBD) on the gonadal hormone level in atrazine-exposed rats. (A) Serum testosterone level, (B) Serum luteinizing hormone (LH) level, (C) Serum follicle-stimulating hormone (FSH) level. **D–F.** Preventive potential of PBD on the serum gonadal hormone level in atrazine-exposed rats. (D) Serum testosterone level, (E) Serum LH level, (F) Serum FSH level. Values are presented as mean±SD, n=5. $^{*}P$<0.05, $^{**}P$<0.01, $^{***}P$<0.001: compared to the control group; $^{\#}P$<0.05, $^{\#\#}P$<0.01, $^{\#\#\#}P$<0.001: compared to the atrazine group.

3.5. Serum gonadal hormone levels in the therapeutic model

Compared with the control, there was non-significant decrease in the testosterone level in the rats administered atrazine only. Compared with the atrazine group, there is an increase in the serum testosterone level by at least 318% in the groups administered with atrazine + 25% plantain-based diet and atrazine + 12.5% plantain-based diet. However, compared with the control, there was a significant (P<0.05) increase of 80% in the serum testosterone level in the atrazine + 12.5% plantain-based diet group (Figure 2A).

Compared with the control group, there was significant decrease in the serum LH level in the rats administered atrazine only. There was a significant (P<0.05) increase of more than 176% in the serum LH levels in atrazine + 50% plantain-based diet, atrazine + 25% plantain-based diet, atrazine + 12.5% plantain-based diet and atrazine + quercetin compared with the atrazine group (Figure 2B).

Compared with the control group, there was non-significant decrease in the serum FSH level in the rats administered atrazine only. There were significant (P<0.05) increases by at least 111% in the serum FSH levels in atrazine + 50% plantain-based diet, atrazine + 25% plantain-based diet, atrazine + 12.5% plantain-based diet and atrazine + quercetin groups compared with the atrazine group. Consequently, compared with the control group, there was a decrease in the serum FSH level in the atrazine recovery group by 63%, while there was an increase in the atrazine + 50% plantain-based diet group by 62% (Figure 2C).

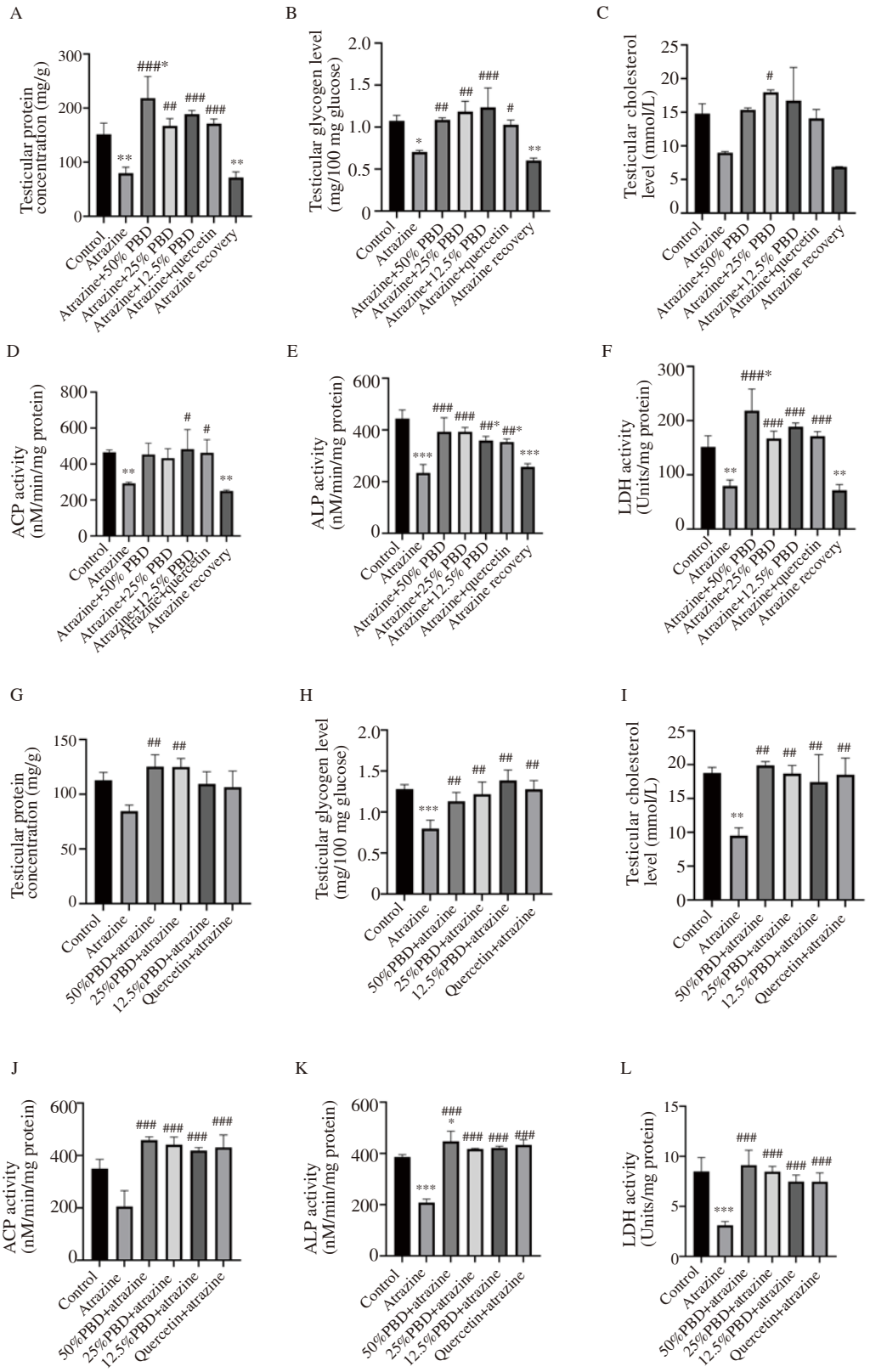

Figure 3 A–F. Therapeutic potential of the plantain-based diet (PBD) on testicular function indices in atrazine-exposed rats. (A) Testicular protein concentration, (B) Testicular glycogen level, (C) Testicular cholesterol level, (D) Testicular acid phosphatase (ACP) activity, (E) Testicular alkaline phosphatase (ALP) activity, (F) Testicular lactate dehydrogenase (LDH) activity. **G–L.** Preventive potential of PBD on testicular function indices in atrazine-exposed rats. (G) Testicular protein concentration, (H) Testicular glycogen level, (I) Testicular cholesterol level, (J) Testicular ACP activity, (K) Testicular ALP activity, (L) Testicular LDH activity. Values are presented as mean±SD, n=5. $^{*}P$<0.05, $^{**}P$<0.01, $^{***}P$<0.001: compared to the control group; $^{\#}P$<0.05, $^{\#\#}P$<0.01, $^{\#\#\#}P$<0.001: compared to the atrazine group.

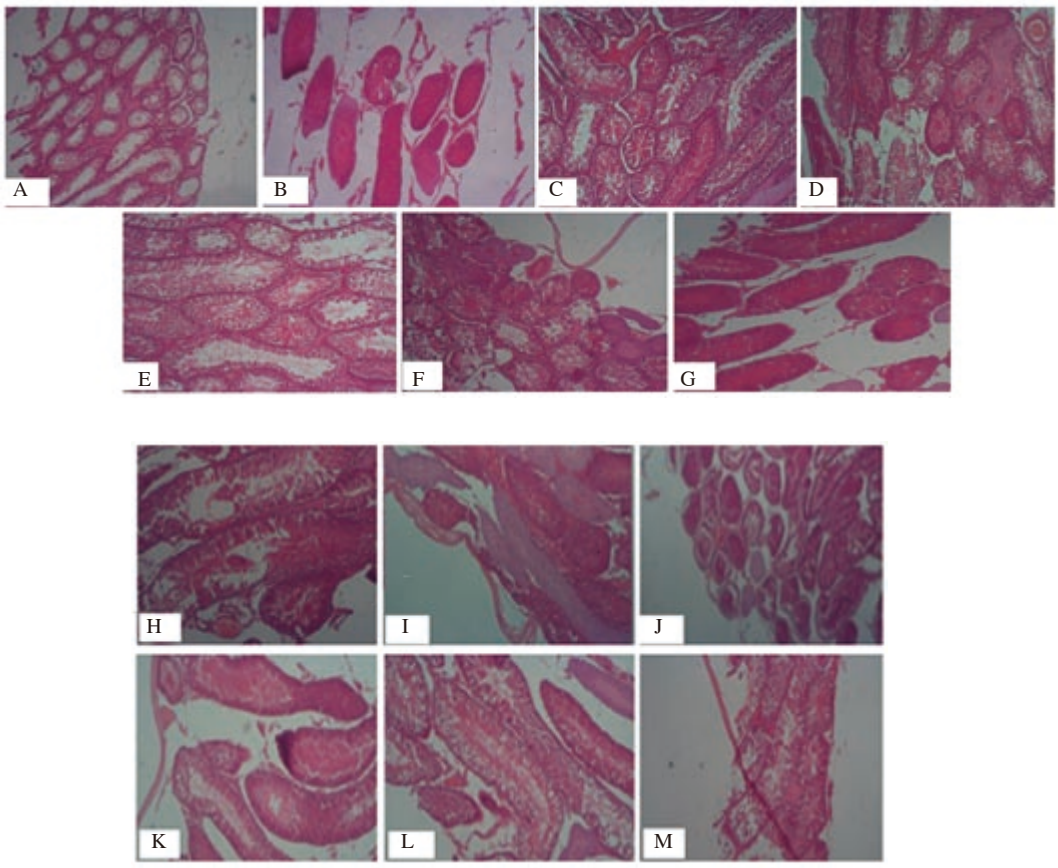

Figure 4 A–G. Representative histopathological sections of the testes in atrazine-exposed rats before plantain-based diet treatment (H & E stain, magnification: ×10). The control (A), 50%, 25% or 12.5% plantain-based diet treatments (C, D, E), and quercetin (F) groups show the normal histological pattern of the testis. The seminiferous tubule exhibits normal morphology with complete spermatogenic profiles and full population of germ cells. The atrazine (B) and atrazine recovery (G) groups show mild to moderate atrophilc degenerative changes, with reduced spermatogenic activities. Necrotinised tubules and cellular disorientations are also observed. **H–M.** Representative histopathological sections of the testes in rats pretreated with plantain-based diet before exposure to atrazine (H & E stain, magnification: ×10). The control (H) and quercetin (M) groups show normal histological pattern of the testis. The seminiferous tubule exhibits normal morphology with complete spermatogenic profiles and full population of germ cells. The 50%, 25% or 12.5% plantain-based diet groups (J, K, L) show mild to moderate atrophilic degenerative changes, with reduced spermatogenic activities. Necrotinised tubules and cellular disorientations are also observed. The atrazine group (I), however, shows severe degenerative and necrosis changes.

3.6. Serum gonadal hormone levels in the preventive model

Compared with the control group, the atrazine group was able to significantly reduce the testosterone level by 40% (P<0.01). Compared with the atrazine group, there was a significant (P<0.05) increase by a minimum of 69% in the level of serum testosterone across the different treatment groups. Moreover, there were increases by at least 31% in the 50% plantain-based diet + atrazine, 25% plantain-based diet + atrazine, and 12.5% plantain-based diet + atrazine groups compared with the control group (Figure 2D).

There was a non-significant change in the LH level in the atrazine group when compared with the control group. For LH levels, there was a significant increase across the treatment groups by a minimum of 82% when compared with the atrazine group (P<0.05). However, the plantain-based diet treatment improved the effects of the atrazine exposure by at least 51% *versus* the control group (Figure 2E).

There was a non-significant change in the FSH level in the group administered atrazine compared to the control group. However, compared with the atrazine group, there was a significant (P<0.05) increase in the serum FSH level across the groups except the 12.5% + atrazine treatment group (Figure 2F).

3.7. Biochemical assessment of testicular function in the therapeutic model

Compared to the control group, there was a significant decrease in the testicular protein concentration, levels of glycogen, as well as the activities of ACP, ALP, and LDH in the atrazine groups. Compared to the atrazine group, significant increases were observed in the concentration of testicular protein, glycogen level, ALP and LDH activities in the atrazine plus 50%, 25%, 12.5% plantain-based diet groups and the atrazine + quercetin group (P<0.05). Moreover, the atrazine + 25% plantain-based diet group had a significantly increased testicular cholesterol level compared to the atrazine group

(P<0.05), while the ACP activity was increased in the atrazine + 12.5% plantain-based diet, atrazine + quercetin groups compared to the atrazine group (P<0.05) (Figure 3 A-F).

3.8. Biochemical assessment of testicular function in the preventive model

Compared to the control group, there was a significant decrease in the testicular glycogen level, cholesterol level, as well as the activities of ALP, and LDH in the atrazine and atrazine recovery groups. Compared to the atrazine group, there were significant increases in testicular function indices (testicular glycogen, cholesterol, ACP, ALP, LDH) across the plantain-based diet and quercetin treatment groups. Similarly, the protein concentration also increased in the 50% plantain-based diet + atrazine and 25% plantain-based diet + atrazine compared with the atrazine group (Figure 3 G-L).

3.9. Histopathology of rat testes in the therapeutic model

In the the therapeutic model, rat testes in the control group, plantain-based diet treatment (50%, 25% and 12.5%), and quercetin groups showed a normal histological pattern of the testis. The seminiferous tubule exhibited normal morphology with a complete spermatogenic profile and a full population of germ cells. The atrazine exposure and atrazine recovery groups showed mild to moderate atrophic degenerative changes, with reduced spermatogenic activity, necrotised tubules and cellular disorientations (Figure 4 A-G).

3.10. Histopathology of rat testes in the preventive model

In the preventive model, rat testes in the control and quercetin plus atrazine groups showed a normal histological pattern of the testis. The seminiferous tubule exhibited normal morphology with a complete spermatogenic profile and a full population of germ cells. The 50%, 25% and 12.5% plantain-based diet plus atrazine treatment groups showed mild to moderate atrophic degenerative changes, with reduced spermatogenic activity, necrotised tubules and cellular disorientations. However, the atrazine exposure group showed severe degenerative and necrosis changes (Figure 4 H-M).

4. Discussion

This study investigated the protective effect of plantain-based diet against toxicities caused by atrazine in male rats. Several studies have shown that atrazine has a deleterious effect on male fertility[3,18,28,29]. To more accurately reflect how this commonly used herbicide enters the body, oral exposure was adopted in this investigation. We assessed the therapeutic and preventive potential of plantain-based diet to mitigate experimental testicular toxicity in rats exposed to atrazine. As expected, atrazine exposure caused testicular toxicity in male rats. However, plantain-based diet modulated and improved the rat testicular toxicity caused by atrazine in both the preventive and therapeutic models of assessment. It is worthy of note that plantain-based diet greatly improved the rat reproductive indices including sperm parameters, rat gonadal hormones, testicular function indices and the structural integrity of the testes.

We found that testicular weight, testicular index, and the percentage of weight change all decreased after atrazine exposure. The data we present for total body weight and testicular index agree with those from other reports[6,9,29]. In addition, the rats treated with plantain-based diet had lower weight parameters in the therapeutic model but not in the preventive model where plantain-based diet increased the weight parameters.

The male fertility function can be evaluated by determining sperm concentration, morphology, and motility. Spermatogenesis is a complex cyclic process dependent upon testosterone levels[30,31]. As expected, atrazine treatment decreased sperm concentration and count in both therapeutic and preventive models, consistent with an earlier report[7]. Potentially, the decrease in sperm concentration, motility and morphology in this study might be related to hypothalamic-pituitary-gonadal axis suppression due to atrazine administration. This is further corroborated by the reports that atrazine impairs sperm function[7,28,29]. The trends toward lower values suggest that the ability of atrazine-treated animals to secrete the male hormone, androgen might have been compromised[29]. Male infertility is linked to a decline in sperm parameters and, by extension, gonadal hormone production[32]. Spermatozoa abundance in the seminiferous tubules indicates effective spermatogenesis. Furthermore, the improvement of atrazine toxicity by plantain-based diet may be a result of the carbohydrate content in the diet that could provide an adequate supply of energy. An increase in the number of sperm cells, its motility and concentration leads to enhanced fertility in animals. Therefore, the plantain-based diet enhanced sperm count and concentration and possibly boosted the fertility capacity of the spermatozoa by increasing testicular function potency in the animals. Withdrawal of atrazine from the animals for 14 days did not reverse the negative effects caused by atrazine treatment.

In humans and animals, reproduction, development, and aging are regulated by the hypothalamic-pituitary-gonadal axis. Gonadal steroids influence the hypothalamus to control pulses of gonadotropin-releasing hormone and the pituitary to control the release of gonadotropins (LH and FSH). To enhance spermatogenic cell survival, FSH and LH decrease proapoptotic signals, which control the process of spermatogenesis[33,34]. Because they contain receptors for both testosterone and FSH, Sertoli cells are often the focus of hormonal signals that regulate spermatogenesis. The Sertoli cell-integrated signal transduction pathways for LH and testosterone play a vital role in spermatogenesis[35]. Alterations in gonadal hormonal levels could be used to evaluate testicular function in both animals and humans. Suppressed testicular functions are

linked to marked decreases in serum gonadal hormone levels. Fertility dysfunctions, such as low FSH, LH, and testosterone levels, have been linked to exposure to multiple toxic agents, including pesticides[36]. Atrazine-treated rats had lower gonadal hormone levels, which suggests that the pituitary-testicular axis, which is essential for spermatogenesis, might be impaired in atrazine-exposed rats. Since FSH is heavily involved in spermatogenesis, the low sperm count observed in the atrazine group in the present study may be related to the low FSH concentration observed in the same group. The effect of atrazine on FSH and LH levels may result from its ability to inhibit steroidogenesis or from its effect on gonadotropin synthesis[29,37]. Results from Adedara *et al*[37] corroborated the significant decrease in testosterone concentration seen in the atrazine group. As atrazine inhibits pituitary gonadotropin levels, which control testosterone biosynthesis, low sperm count and testosterone levels are plausible effects. Plantain-based diets seem to have androgenic effects, as shown by the elevated levels of LH, FSH, and testosterone in atrazine-exposed rats, as well as their capacity to sustain the complex control of testicular function.

In this study, we examined the modulatory effects of plantain-based diet on selected testicular function indices in atrazine-treated rats. Testicular proteins are required for sperm production and maturation[32]. In the present study, in atrazine-exposed rats of the therapeutic model, the testicular protein level decreased, which might subsequently affect the production of spermatozoa. However, the reduced testicular protein concentration was improved by plantain-based diet treatment, in support of a boost in sperm production and maturation. Testicular cholesterol is an essential metabolite for the testes and is responsible for the synthesis of steroid hormones, bile acids, and steroidogenesis in men[20]. Furthermore, the decreased levels of testicular cholesterol in the atrazine-treated rats in the therapeutic model may indicate a lipotoxic influence of atrazine in rats. However, this was improved partially with the treatment of plantain-based diet in the therapeutic model, while the preventive plantain-based diet treatment completely averted the atrazine-induced toxicity on the testicular cholesterol level. Moreover, there could be a correlation between the increased level of testicular cholesterol and the enhanced level of gonadotropin hormones noted in the plantain-based diet-treated rats in both models. Testicular glycogen provides an energy source for the effective functioning of testicular tissue and spermatogenetic processes in seminiferous tubular cells[38]. Moreover, sperm growth is aided by dietary glycogen, which acts as a nutrient for the formation of more mature, mobile sperm. In the therapeutic and preventive models, atrazine-treated rats had a reduced testicular glycogen content, which might impose testicular restrictions. The plantain-based diet and quercetin treatment, however, improved testicular glycogen content, which may support the increased glucose transport and/or metabolism needed for sperm production. Improved glycogen levels may also support enhanced synthesis of enzymes involved in hormonal production in manner that underscore the restoration of testicular function.

ACP and ALP are biomarkers for primordial germ cells[1]. Testicular ACP and ALP activities in atrazine-treated rats in both models were reduced, suggesting that these enzymes might have been inhibited in the phosphorylation pathway of testicular glucose reutilization in germ cells. The plantain-based diet therapy boosted phosphatase activity in the testicles and hence may enhance their participation in glucose consumption in the germ cells. Androgen levels have been linked to testicular ALP and ACP activity, and this activity rises when testicular steroidogenesis is increased. The increasing activity of these enzymes in plantain-based diet-treated rats in the current investigation may be linked to elevated levels of testosterone and gonadotropins. The maturation and energy metabolism of spermatogenic cells are closely linked, and LDH is an important marker enzyme for both processes. LDH activity, a germ cell marker enzyme, decreased in the testes of atrazine-treated rats, implying a suppression of testicular lactate metabolism that might lead to inadequate adenosine triphosphatase (ATP) synthesis[37]. Plantain-based diet and quercetin treatments, on the other hand, protected the testicular cells from atrazine-induced toxicity by improving the level of LDH. The increased activity of testicular LDH in plantain-based diet-treated rats may support lactate metabolism involvement in the development of spermatogenic cells, and the synthesis of ATP. These results suggest the modulatory potential of plantain-based diet to protect and prevent against testicular assault. Withdrawal of atrazine from the animals for 14 days did not reverse the negative effects caused by atrazine treatment, underscoring the need for therapeutic assistance to improve atrazine testicular assault.

The histopathological examinations showed that plantain-based diet treatment had a positive effect on the structural integrity of the testes and this corroborates the improvement in biochemical indices afforded by plantain-based diet. Meanwhile, increased testosterone synthesis by the testes may contribute to the preservation of the structural morphology of the testes and epididymis since testosterone is known to control Sertoli cell physiological activity and histological integrity[39].

Meanwhile, a major limitation of the current study is that we did not investigate how the individual or synergistic action of the biomolecules present in the pulp and how these affected beneficial effects of the plantain-based diet.

In conclusion, collectively, the findings demonstrate that plantain-based diet possesses a modulatory potential to protect and prevent against rat testicular damage caused by atrazine exposure. The modulatory and toxicity-attenuating capability of plantain-based diet is depicted by improved rat gonadal hormone levels, sperm parameters, and testicular function indices as well as structural integrity of the testes. Moreover, after withdrawal of atrazine, the negative effects persisted without plantain-based diet or quercetin therapy. The findings support the prospects of plantain as a readily available and low-cost nutraceutical for male fertility therapy.

Conflict of interest statement

The authors declare no conflicts of interest.

Acknowledgements

Authors' appreciation goes to Landmark University, Nigeria.

Funding

This study received no extramural funding.

Data availability statement

The data supporting the findings of this study are available from the corresponding authors upon request.

Authors' contributions

Damilare Emmanuel Rotimi conceived and designed the study, Damilare Emmanuel Rotimi and Oluyomi Stephen Adeyemi collaborated in the discussion of the results. All authors wrote the manuscript and provided administrative support and critically revised the manuscript. All the authors have approved the final manuscript.

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