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Antouka Super[®] induced oxidative stress and reproductive toxicity in male Japanese quail (*Coturnix coturnix japonica*)

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Abstract

Background: Antouka Super[®] (AS), a combination of insecticide (Pirimiphos-methyl 16% and Permethrin 3%), is one of the most widely used pesticides in agriculture, public health, home and garden, with high potential for human and animal exposure.

Objective: The present study was undertaken to evaluate the effect of AS on the serum testosterone, oxidative stress biomarkers, testis histology and fertility of male Japanese quail.

Methods: Thirty-two (32) male Japanese quails twenty-eight (28) days were randomly divided into four groups: C0 (control), T1, T2 and T3, exposed daily (gavage) to 0, 37.5, 56.25 and 75 mg of AS/kg body weight (b.w), respectively, for 49 consecutive days and were analysed for fertility. Control and experimental male quails were cohabited, for two days, with untreated female quails and sperm positive female quail were analysed for paternal-mediated toxicity. After

completion of fertility studies quails were sacrificed and analysed for reproductive endpoints.

Results: There was a dose dependent decrease of the relative weight of testis, epididymis and vas deferens. Additionally, testis total proteins and serum testosterone levels were decreased in AS treated quails ($p < 0.05$). A decrease of sperm motility, viability and concentration per vas deferens, and an increase of sperm anomalies were recorded in AS exposed quails with respect to the controls. The embryonic and post-embryonic mortality rate were significantly ($p < 0.05$) higher in group T3 ($25.00 \pm 3.40\%$ and $31.66 \pm 10.22\%$ respectively) than in control group ($6.25 \pm 3.98\%$ and $9.54 \pm 3.72\%$ respectively). The superoxide dismutase (SOD), total peroxidase (POD) and catalase activity (CAT) were significantly ($p < 0.05$) lower treated than control quails, while the level of malondialdehyde (MDA) was significantly ($p < 0.05$) higher in groups T1, T2 and T3 (13.00 ± 0.96 , 23.50 ± 1.35 and 29.08 ± 1.58 nmol/mg tissues respectively) compared to the control one (9.32 ± 0.67 nmol/mg tissues). Histopathological examination of the testes of AS treated quails revealed testicular lesions characterized by moderate to severe degenerative changes of seminiferous tubules, incomplete spermatogenesis and depletion in the germ layers of seminiferous tubules in which immature spermatozoa were hardly seen.

Conclusion: From the above study, it can be inferred that AS (56.25 and 75) mg/kg b.w decrease body and relative organ weights and induces testicular lesions. Also, AS increases the level of MDA while it reduces the levels of enzymatic antioxidant biomarkers, serum testosterone and reproductive indices of intoxicated quails and their offspring. However, further work is needed to establish the genetic toxicology and immunohistochemistry of caspase-3 and claudin-1.

Keywords: Biological sciences, Toxicology, Physiology

1. Introduction

With the growing demand for food production as a result of the dramatic rise in the human population, a large number of chemical substances have been used either for increasing or maintaining the productivity in agriculture [1, 2]. The lack of sound regulatory laws and necessary enforcement are major reasons for pesticide pollution problems in developing countries [3]. Humans and animals in these countries are exposed to pesticides via contaminated food and drinking water. Exposure to pesticides is known to produce a variety of biochemical changes, which some may be responsible for the adverse physiological and reproductive effects reported in experimental animals [4, 5]. Refs. [6, 7, 8] reported that many pesticides exert their toxic effects via oxidative stress mechanisms. This in turn would lead to the generation of reactive oxygen species (ROS), reactive nitrogen species (RNS) and significant alterations in antioxidants or ROS/RNS scavenging enzyme systems [9, 10]. ROS, RNS and their derived free radicals are believed to

be the cause of DNA and protein damage in vivo, inducing aging and diseases [11]. Oxidative stress is a consequence of an imbalance between the body antioxidant system and pro-oxidant state generated by pesticide toxicity [12]. In addition, oxidative stress conditions may cause alterations in sperm cells due to the high levels of polyunsaturated fatty acid (PUFA) in their plasma membrane [13].

Antouka Super[®] (AS) is a broad-spectrum insecticide widely used in agriculture and crop's storage applications worldwide. It is made up of two insecticides: Pirimiphos-methyl 16% and Permethrin 3%. Pirimiphos-methyl [acide phosphorothioic, O-(2-(diethylamino)-6-methyl-4-pyridimyl) O, O-diethyl ester)] is a broad-spectrum organophosphate insecticide that distresses the nervous system by inhibiting acetyl cholinesterase activity [14]. It is employed in agriculture to control insects and mites that affect cereals, rice, fruits, stored grains and cotton. [4] reported that treatment of adult male rats with pirimiphos-methyl at the doses of (62.5–125) mg/kg b.w for 90 consecutive days alters semen characteristics following by the testis damage in male rats. Permethrin [(1RS, 3RS)-3-(2, 2-Dichlorovinyl)-2, 2-dimethylcyclopropane-1-carboxylate (3-phenoxyphenyl)] is a pyrethroid insecticide. Permethrin is an axonic poison that affects nerve fibers by binding to a protein that regulates the voltage-gated sodium channel [15]. It is employed in agriculture to control insects and mites that affect cereals, rice, fruits, stored grains and cotton. [16] showed that permethrin dramatically reduces testosterone levels and sperm counts in adult male mice.

In spite of the existence of many research related to the impact of pirimiphos-methyl and permethrin on the male reproductive system, there is no available information on the impact of their combination on the male reproductive system. In addition, the mechanisms by which these insecticides exert their effects on the reproductive system remain poorly understood. Therefore, the purpose of this study was to determine the effects of AS on oxidative stress markers, some sperm parameters, histology of testis and some reproductive performance, in male Japanese quails (*Coturnix coturnix japonica*).

2. Materials and methods

2.1. Birds

Healthy 28-day-old male Japanese quails weighing 109–118 g were used in this study. Birds were housed in specialized cages, eight per cage, in centralized birds care facilities maintained at 22–25 °C with a relative humidity of $76 \pm 5\%$, for 7 weeks. Animals were kept in a 12 h light-dark cycle and provided *ad libitum* with water and a specific diet.

2.2. Chemical

Antouka Super[®] (SYNGENTA, United Kingdom) is a combined insecticide whose active principles are:

- pirimiphos-methyl (0,2-diethylamino-6-methylpyrimidin-4-yl O,O-dimethyl phosphorothioate) concentrated at 19 g/kg,
- permethrin (1RS, 3RS; 1RS, 3SR)-3-(2,2-Dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylate (3- phenoxyphenyl)) concentrated at 3 g/kg.

2.3. Ethical consideration

Experimental protocols used in this study were approved by the Ethical committee of the Department of Animal Science of the University of Dschang (ECDAS-UDs 23/02/2015/UDs/FASA/DSAES) and was in conformity with the internationally accepted standard ethical guidelines for laboratory animal use and care as described in the European Community guidelines; EEC Directive 86/609/EEC, of the 24th November 1986 [17].

2.4. Experimental design

Birds were randomly divided into four groups of 8 quails each and orally treated as follows: Birds of group 1 received 10 ml/kg of distilled water, while birds of groups 2–4 received *the same volume of dilute AS* at the doses 37.5, 56.25 and 75 mg/kg b.w for 49 consecutive days. The distilled water and the test solutions were administered using an endogastric canule. The doses used in this study were selected from a pilot study and represent 1/30, 1/20 and 1/15 of LD₅₀ value obtained in quails (1125 mg/kg b.w) (Personal communication). During the treatment, body weight was weekly measured.

2.5. Clinical signs and behavioral alterations

As stated in previous reports, the salient features of pirimiphos-methyl toxicity include neurotoxicity [14]. Therefore, for the present study, signs suggesting nervous disturbances (depression, decreased attraction towards feed, weakness, anorexia and dizziness) were taken into account and subjectively evaluated daily directly after administration of AS. Depending on the severity and frequency, each clinical sign was scored from 0 to +4 (0 = none, +1 = very weak, +2 = weak, +3 = moderately and +4 = severely).

2.6. Fertility test

At the end of the treatment, male quails were allowed to mate (1:2) untreated proven fertile female quails. Mating was confirmed by the presence of sperm

deposition in the vaginal orifice upon vaginal examination. Two days after mating, eggs were collected, and during 7 days incubated for 19 days and unhatched eggs were opened and examined for fecundation (Presence of embryo or germinal disk). The male fertility rate was determined according the following formulae:

Percentage of fertilized eggs = (Number of fertile eggs/Number of total incubated eggs) \times 100

Hatching rate of total eggs = (Number hatching eggs/Number of incubated eggs) \times 100

Embryonic mortality = (Number of dead chicks in eggs/Number of total fertile eggs) \times 100

Post-mortality embryonic = (Number of dead chicks/Number of total chicks) \times 100

Chicks viability at (14 days) = (Number of viable chicks/Number of total live chicks) \times 100

2.7. Blood and organ collections

At the end of the treatments (49th day), blood was collected after sectioning the jugular vein of each bird. Serum was prepared and stored at $-20\text{ }^{\circ}\text{C}$ for subsequent analysis. After killing the quail by decapitation, testes, epididymis and vas deferens were carefully removed, freed of adipose tissue, blotted dry and weighed separately. The left testis of each bird was then homogenized at 20% (weight/volume) of 0.9% NaCl in cold distilled water solution and aliquots of supernatant were kept at $-20\text{ }^{\circ}\text{C}$ for biochemical analysis.

2.8. Semen characteristics

Immediately after each bird sacrifice, vas deferens were carefully removed, minced in a 10 ml of 0.9% NaCl ($40\text{ }^{\circ}\text{C}$) and used to evaluate the sperm motility, concentration, viability and morphology. The sperm motility was estimated on scale basis as reported by [18], semen viability expressed as percentage of swelled spermatozoa and morphology expressed as percentage of abnormal shape spermatozoa. Sperm viability and morphology were analyzed using hypo-osmotic swelling test [19] and eosin-nigrosin staining respectively. Five microliters of semen were mixed with 5 μl of eosin-nigrosin solution. While 1000 μl of sperm were mixed with 10 μl of hypo-osmotic solution. The morphological defects of head, mid-piece, tail and the proportions of cells affected were evaluated. For each of the both parameters (viability and morphology), a total of 200 spermatozoa were counted in at least five different microscopic fields according to the protocol described by Revell and Mrode [20]. The sperm density was determined using Thoma hemocytometer.

2.9. Biochemical analysis

The levels of proteins in the testis were determined using CHRONOLAB kit following the manufacturer's protocol. Serum testosterone was determined using appropriate kit (ELISA AccuDiagTM, Diagnostic Automation Inc). The levels of SOD and MDA and the activities of CAT and POD were assessed in testicular homogenates using a spectrophotometer (GENESYS 20.0) and according to the methods described respectively by: [21, 22, 23, 24].

2.10. Tissue preparation and histopathology

The right testis of each quail was fixed in Bouin's fluid for 1 week, embedded in paraffin, cut at 5 μ m and stained with Harris haematoxylin and eosin. The tissue sections were observed under a light microscope (Leica DM 750, X10 and X40) for morphology and cellular integrity.

2.11. Statistical analysis

Values are presented as Mean \pm SEM ANOVA was performed for comparison with post-hoc Duncan test to compare the level of significance between the control and experimental groups. A value of $p \leq 0.05$ was considered statistically significant. Statistical analyses were performed with the aid of SPSS for Windows software program (Release 20.0).

3. Results

3.1. Clinical signs and behavioural alterations

The clinical signs recorded on AS treated quails are presented in Table 1. No clinical signs and behavioural changes were observed in animals of the control group (0 mg AS/kg b.w). Depression, excitation, decreased attraction towards feed, anorexia, diarrhea and dizziness started at the 5th week in birds of T3 group (75 mg AS/kg b.w). In group T2 (56.25 mg AS/kg b.w), clinical signs appeared at the 6th week whereas, in the T1 group (37.5 mg AS/kg b.w), half of the birds (4/8) showed a mild degree of depression, decreased attraction towards food, weakness and anorexia at the 7th week.

3.2. Body weight and relative weight of reproductive organs

The final body weight, the body weight gain and the relative organs weight decreased ($p < 0.05$) in a dose-dependent manner (Table 2).

Table 1. Effects of different levels of AS on some qualitative clinical signs and behavioral alterations of male Japanese quails.

Experiment time	Levels of AS (mg/kg b.w) (n = 8)	Depression (n = 8)	Decreased attraction towards food (n = 8)	Weakness (n = 8)	Anorexia (n = 8)	Diarrhea (n = 8)	Dizziness (n = 8)
(0–4 weeks)	0	0	0	0	0	0	0
	37.5	0	0	0	0	0	0
	56.25	0	0	0	0	0	0
	75	0	0	0	0	0	0
(5 weeks)	0	0	0	0	0	0	0
	37.5	0	0	0	0	0	0
	56.25	0	0	0	0	0	0
	75	+1	+1	+1	+1	+1	+1
(6 weeks)	0	0	0	0	0	0	0
	37.5	+1	+1	+1	+1	+1	+1
	56.25	+2	+2	+2	+2	+2	+2
	75	+3	+3	+3	+3	+3	+3
(7 weeks)	0	0	0	0	0	0	0
	37.5	+1 (4/8)	+1 (4/8)	+1 (4/8)	+1 (4/8)	0	0
	56.25	+3	+2	+3	+3	+2	+3
	75	+4	+4	+4	+4	+4	+4

Score from 0 to +4 denotes the severity of clinical signs ((0 = none, +1 = very weak, +2 = weak, +3 = moderately and +4 = severely). Antouka Super[®] levels in groups control, T 1, T2 and T3 were 0, 37.5, 56, 25 and 75 mg/kg b.w, respectively. n = number of animal.

Table 2. Effects of different levels of AS on body and relative reproductive organs weight of male Japanese quails.

Parameters	Doses of Antouka Super [®] (mg/kg b.w)			
	0 (Control) (n = 8)	37.5 (n = 8)	56.25 (n = 8)	75 (n = 8)
Initial body weight (g)	116.00 ± 0.68	116.08 ± 0.58	116.71 ± 0.41	115.48 ± 0.36
Final body weight (g)	172.17 ± 0.66 ^a	162.41 ± 1.46 ^b	155.08 ± 2.07 ^c	145.81 ± 0.77 ^d
Body weight gain (g)	56.17 ± 1.7 ^a	46.33 ± 1.17 ^b	38.37 ± 1.98 ^c	30.33 ± 0.79 ^d
Relative weight of reproductive organs (%)				
Testis	1.76 ± 0.08 ^a	1.42 ± 0.08 ^b	1.33 ± 0.1 ^b	0.94 ± 0.13 ^c
Epididymis	0.028 ± 0.004 ^a	0.027 ± 0.005 ^a	0.020 ± 0.003 ^{ab}	0.010 ± 0.002 ^b
Vas deferens	0.044 ± 0.005 ^a	0.033 ± 0.005 ^{ab}	0.030 ± 0.006 ^{ab}	0.019 ± 0.005 ^b

n = number of animal, each value represents mean ± standard error mean. Means values for each parameter in the same row, with different superscripts (a, b, c, d) differ significantly ($p \leq 0.05$).

3.3. Oxidative stress biomarkers

In male quails treated with AS at the doses of 56.25 and 75.00 mg/kg b.w, the proteins level in the testes significantly decreased as compared to control birds. The opposite trend was recorded for MDA concentration (Table 2). The activities of SOD, CAT and POD were significantly lower ($p < 0.05$) in treated than in control quails (Table 3).

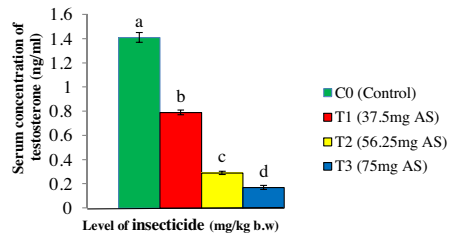
3.4. Serum testosterone

The serum testosterone concentration decreased ($p < 0.05$) in a dose-dependent manner (Fig. 1)

Table 3. Effects of different levels of AS on oxidative stress biomarkers in the testis of male Japanese quails.

Oxidative stress parameters in the testis	Doses of Antouka Super [®] (mg/kg b.w)			
	0 (Control) (n = 8)	37.5 (n = 8)	56.25 (n = 8)	75 (n = 8)
Testicular protein (mg/ml)	10.40 ± 0.33 ^a	9.73 ± 0.34 ^{ab}	7.76 ± 1.36 ^{bc}	7.33 ± 0.33 ^c
MDA (nmol/mg tissues)	9.32 ± 0.67 ^d	13.00 ± 0.96 ^c	23.50 ± 1.35 ^b	29.08 ± 1.58 ^a
SOD (UI/tissues)	24.46 ± 0.90 ^a	17.16 ± 0.68 ^b	12.76 ± 0.34 ^c	9.53 ± 0.2 ^d
CAT (UI/mg tissues)	7.69 ± 0.20 ^a	5.82 ± 0.12 ^b	5.36 ± 0.16 ^b	4.00 ± 0.18 ^c
POD (μmol/mg tissues)	17.73 ± 0.54 ^a	15.84 ± 0.03 ^b	12.86 ± 0.02 ^c	12.35 ± 0.04 ^d

n = number of animal, each value represents mean ± standard error mean. Means values for each parameter in the same row, with different superscripts (a, b, c, d) differ significantly ($p \leq 0.05$).



Each value represents mean \pm standard error mean, (a, b, c, d) means bearing different letters on a bar differ significantly ($p \leq 0.05$)

Fig. 1. Effects of different levels of AS on serum testosterone of male Japanese quails. Each value represents mean \pm standard error mean, (a, b, c, d) means bearing different letters on a bar differ significantly ($p \leq 0.05$).

3.5. Sperm characteristics

The sperm motility and viability were significantly low ($p < 0.05$) in quails treated with AS at the doses of 56.25 and 75.00 mg/kg b.w compared to controls. The opposite trend was recorded with the percentages of major and minor anomalies. Furthermore, regardless of the dose of AS, the number of spermatozoa per vas deferens decreased in a dose-dependent manner (Table 4). Negative and significant ($p < 0.05$) correlations ($r = -0.526$) were found between the rates of MDA and spermatozoa concentration ($r = -0.553$) on the one hand and the motility ($r = -0.547$) on the other hand; and between the rate of viability and MDA level ($r = -0.543$). In addition, negative and significant ($p < 0.05$) correlations were found between the level of MDA and majors sperm anomalies ($r = -0.500$).

Table 4. Effects of different levels of AS on sperm characteristics of male Japanese quails.

Sperm characteristics	Doses of Antouka Super®(mg/kg)			
	0 (Control) (n = 8)	37.5 (n = 8)	56.25 (n = 8)	75 (n = 8)
Motility	4.50 \pm 0.12 ^a	4.23 \pm 0.10 ^a	3.75 \pm 0.11 ^b	2.16 \pm 0.10 ^c
Viability (%)	84.33 \pm 1.65 ^a	80.50 \pm 0.99 ^a	65.83 \pm 3.44 ^b	49.33 \pm 2.67 ^c
Number/vas deferens (10 ⁶)	1.27 \pm 0.63 ^a	1.06 \pm 0.37 ^b	0.79 \pm 0.07 ^c	0.17 \pm 0.01 ^d
Number/gr of vas deferens (10 ⁶)	12.64 \pm 1.44 ^a	22.11 \pm 9.13 ^a	15.81 \pm 4.92 ^a	8.96 \pm 2.57 ^a
Sperm morphology (%)				
Major anomalies	6.33 \pm 1.78 ^c	5.67 \pm 0.88 ^c	15.50 \pm 1.78 ^b	23.67 \pm 1.11 ^a
Minor anomalies	9.17 \pm 0.60 ^c	10.50 \pm 0.62 ^c	19.67 \pm 1.62 ^b	23.50 \pm 1.47 ^a

n = number of animal, each value represents mean \pm standard error mean. Means values for each parameter in the same row, with different superscripts (a, b, c, d) differ significantly ($p \leq 0.05$).

3.6. Fertility

The percentages of fertile eggs, hatching rate of fertile eggs and chick survival 14 days after hatching were significantly lower ($p < 0.05$) in birds treated with the doses 75.00 mg of AS/kg bw compared to birds which received (37.5 and 56.25) mg of AS/kg b.w and to the controls. The inverse was observed with the percentages of embryonic and post-embryonic mortalities (Table 5). Negative and significant ($p < 0.05$) correlations were found between the spermatozoa concentration and eggs fertility ($r = -0.526$); sperms motility and eggs fertility ($r = -0.465$); sperms viability and hatching eggs ($r = -0.870$); the level of MDA and hatching eggs ($r = -0.630$). In addition, negative and significant ($p < 0.05$) correlations were found between the rates of major sperm anomalies and embryonic mortalities ($r = -0.547$) and chick survival time after hatching (14 days) ($r = -0.601$).

3.7. Histological analysis

Typical structure of testis was observed in control Japanese quails: The seminiferous epithelium with all generations of germinal cells corresponding to the stages of seminiferous epithelium cycle and the lumen with normal flagellated spermatozoa. In between the tubules the interstitial connective tissue had fibroblasts, blood vessels and Leydig cells (Fig. 2–1). The seminiferous epithelium contained all generations of germinal cells corresponding to the stages of seminiferous epithelium cycle. The lumen contained normal flagellated spermatozoa, slight degeneration in the germ layers of seminiferous tubules and expansion of interstitial space were observed (Fig. 2–2). Severe depletion in the germ layers of seminiferous tubules in which immature spermatozoa were hardly seen, degeneration of connective tissue between seminiferous tubules and

Table 5. Effects of different levels of AS on the fertility of male Japanese quails.

Parameters	Doses of Antouka Super®(mg/kg)			
	0 (Control) (n = 4)	37.5 (n = 4)	56.25 (n = 4)	75 (n = 4)
Total number of eggs	47	48	47	48
Percentage of fertile eggs	97.91 ± 3.72 ^{ab}	100 ± 0.00 ^a	89.58 ± 4.12 ^b	64.58 ± 10.22 ^c
Hatching rate	93.75 ± 4.81 ^a	89.58 ± 8.33 ^a	70.00 ± 4.16 ^b	75.00 ± 10.48 ^b
Embryonic mortality (%)	6.25 ± 3.98 ^b	10.41 ± 3.98 ^b	30.00 ± 3.96 ^a	25.00 ± 3.40 ^a
Post-embryonic mortality (%)	9.54 ± 3.72 ^b	9.58 ± 4.10 ^b	7.14 ± 4.12 ^b	31.66 ± 10.22 ^a
Chick survival time after hatching (14 days) (%)	90.45 ± 3.72 ^a	90.41 ± 4.10 ^a	92.85 ± 4.12 ^a	68.33 ± 10.12 ^b

n = number of animal, each value represents mean ± standard error mean, Means values for each parameter in the same row, with different superscripts (a, b, c, d) differ significantly ($p \leq 0.05$).

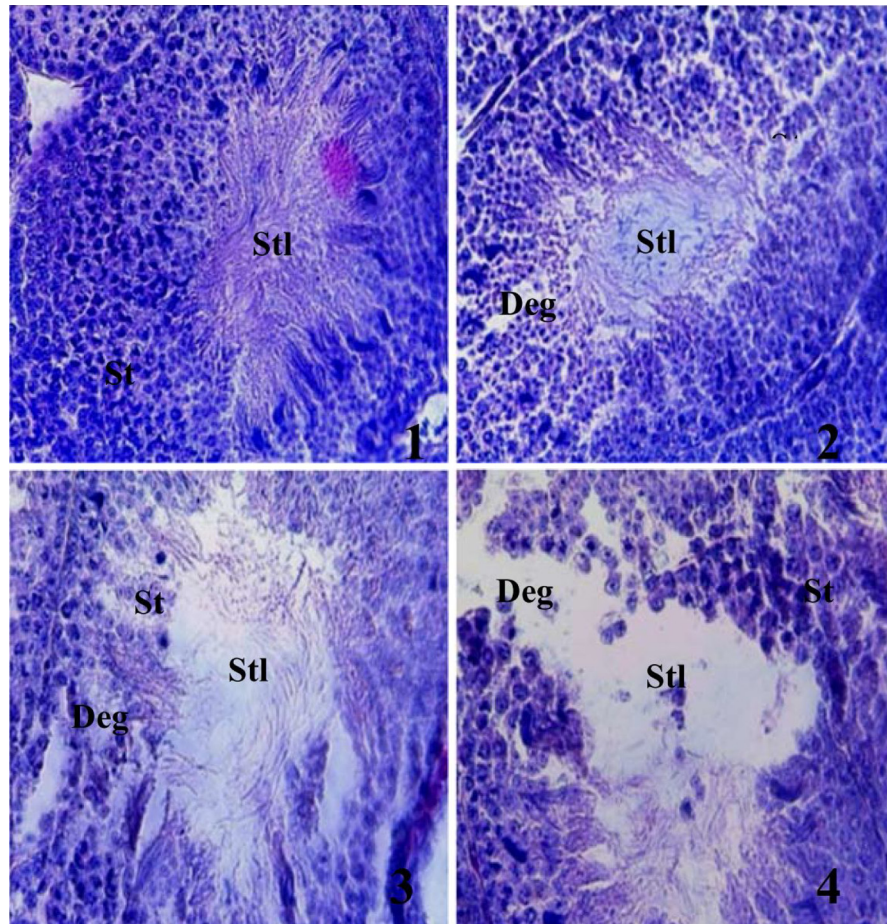


Fig. 2. Photomicrographs of section of quail testis (H&E \times 400), 1: normal testis (control), showing normal structure with more spermatozoa in the seminiferous tubules lumen (Stl); 2 (37.5 mg/kg) showing slight degeneration in the germs layers of seminiferous tubules, lumen of the seminiferous tubes present spermatozoa; 3 (56.25 mg/kg) showing severe degeneration (Deg) and space formation in the germ layers of seminiferous tubes (St) with clear dilation of the lumen which contain very few spermatozoa; 4 (75 mg/kg) showing severe degeneration (Deg) in the germs layers of seminiferous tubules (\rightarrow) and dramatic depletion in the germ layers of seminiferous tubes (Deg) and degeneration of connective tissue between (St), seminiferous tubes are poor in spermatozoa (Stl).

expansion of interstitial space were observed (Fig. 2–3). Seminiferous tubules displayed variables grades of degenerative changes from cloudy swelling to complete cellular destruction. Most of the tubules showed disorganization with sloughing of their cells in the lumina. We are also observed the exhibited proportional spermatogenic hypoplasia with marked thinning of the spermatogenic cell rows. Primary spermatocytes revealed areas of swelling or even ballooning with ill-defined nuclear membrane. Spermatids showed the highest degree of destruction among the spermatogenic cells, most of the cells were swollen (Fig. 2–4).

4. Discussion

Antouka Super[®] (AS) is a broad-spectrum insecticide widely used in agriculture and crop's storage applications worldwide. The present study revealed that the oral daily administration of AS at the doses of 56.25 and 75 mg/kg b.w generates depression, anorexia, diarrhea and dizziness. Similar results were observed by [25] in Japanese quails fed with food contaminated with endosulfan insecticide. The appearance of these clinical signs and behavioural alterations may be explained by the capacity of AS to inhibit acetyl cholinesterase enzymes (AChE), which cause acetylcholine accumulation in cholinergic synapses. The increased acetylcholine in pituitary gland and hypothalamus by organophosphate induced inhibition of acetylcholine esterase could variably affect anterior pituitary functions and the release of secondary neurotransmitters, especially dopamine or gonadotrophins [26]. A significant decrease in body weight, body weight gain and testicular protein was observed in the AS treated groups. This decrease might be associated to the toxic symptoms, such as cholinergic signs. The reduction of body weight, body weight gain and testicular protein could be attributed to systemic toxicity in Japanese quail. Refs. [26, 27] reported that many environmental toxicants inducing alteration of reproductive functions concurrently with impact on the central nervous system and behavior, the so called neuroendocrine disrupters operating through hypothalamo-pituitary-gonadal axis.

Our results showed that the relative weight of vas deferens, epididymis and testes as well as the level of testicular protein and testosterone were significantly decreased in AS treated group as compared to the control. This was in accordance with previous findings showing a decrease of relative testes weight after 90 days exposure of rats to different levels of pirimiphos-methyl (62.5 and 125 mg/kg b.w) [4]. Similar results were recorded by [5] who reported significant reduction in the testes weight after 90 days exposure of rats to chlorpyrifos-ethyl at the dose of 10.5 mg/kg b.w. The weight, size and secretory function of testes, epididymis and vas deferens are closely regulated by androgens [28]. In fact, androgens, especially testosterone have anabolic properties which are characterized by an increase synthesis of proteins and therefore muscle mass. Androgens then contribute to the increased volume and weight of the testis and epididymis by stimulating protein synthesis [29, 30]. The decrease of these organs weight and proteins could then be ascribed to a reduction in androgen's production or a decline of AChE activity. The decrease in testicular weight in the treated quails may be also due to reduced tubular size as confirmed by the histological observations of the testes which show degeneration and atrophy. In the present study, administration of AS (56.5 and 75 mg/kg b.w) for 49 consecutive days resulted in a noticeable diminution of spermatogenic cellular population with widening of the intertubular space. The most striking histological changes were spermatogenic hypoplasia with marked thinning of spermatogenic cells that showed variable degrees of degenerative

changes. The atrophy and degenerative changes of Leydig cells was also observed. Ref. [31] reported that one of the most common morphological responses of Sertoli cells to injuries was vacuolation and germ cell degeneration, disorganization or exfoliation. Sloughing is attributed to the effects of the chemical on microtubules and intermediate filaments of Sertoli cells [32]. These effects spread to the dividing germ cells and naturally tubular atrophy. Testis of quails that received 75 mg/kg AS showed round spermatids multinucleated clusters called giant cells. All these alterations may be due to cytotoxic and apoptotic effect on AS.

The link between oxidative stress and adverse health effects has been suggested for several diseases such as infertility as well as for the general aging process. Pesticides may induce oxidative stress, leading to generation of free radicals and alteration in antioxidants, oxygen free radicals, the scavenging enzyme system, and lipid peroxidation [33]. Testis is the main organ of male reproduction. So, the main objective of the present study was to assess the oxidative damage sustained by testes following sub-acute exposure to AS a compound containing pirimiphos-methyl 16% (Organophosphate) and permethrin 3% (Pyrethroid). AS treatment also resulted in a dose-dependant increase of malondialdehyde (MDA) levels following by pathological changes in seminiferous tubules. These findings support a previous study that reported that most of the organophosphates and pyrethroid insecticides altered MDA levels in experimental animals [34, 35]. The increase of MDA levels is used as important biomarker of lipid peroxidation. It is recorded that pesticides have the ability to cross the blood-testis barrier inducing oxidative stress and lipid peroxidation that damage the biological membranes in the testes [36, 37]. As a consequence, cellular injury, necrosis, inflammation and degeneration of the tissues may take place [38]. The above condition might explain the alterations of testes structure in the AS treated group.

In this study, the activities of superoxide dismutase (SOD); total peroxidase (POD) and catalase (CAT) significantly decreased in testicular tissues of the AS treated groups. Similar results were reported by [39, 40] on the tissues of rat exposed to organophosphate insecticides and by [7] who indicated that deltamethrin causes a significant reduction in SOD and CAT activities. Due to the lack of the cytoplasmic enzyme in mature sperm, the male genital tract, as well as the testis and semen, are rich in both enzymatic and non-enzymatic antioxidants such as SOD; POD and CAT activities in animals which are used to counteract the harmful effects caused by ROS [12]. The severe decrease of the antioxidant enzymes SOD; POD and CAT in this study may be due to the Co-formulation of this insecticide. The decrease in antioxidant enzymes by induction of ROS production causes an increase in the levels of oxidative stress. This in turn may cause degeneration of spermatogenic and Leydig cells, which disrupt spermatogenesis. Ref. [41] reported that H_2O_2 produce during oxidative stress can diffuses across the cell membrane in to the cells and inhibit the activity of enzymes such as glucose 6-phosphate dehydrogenase (G6PD). Inhibition of G6PD leads to a decrease in the availability

of NADPH and a concomitant accumulation of oxidized glutathione, which in turn can reduce the antioxidant defenses of the spermatozoa and increase lipid peroxidation of spermatozoa membrane [42, 43]. Spermatozoa membrane are rich in poly-unsaturated fatty acid (PUFA) and thus, are highly susceptible to ROS attack, which results in a decrease of sperm motility, presumably by a rapid loss of intracellular ATP leading to axonemal damage, decrease of sperm viability, and increase of midpiece morphology defects with deleterious effects on sperm capacitation and acrosome reaction [44, 45]. The above condition might explain the alteration of sperm characteristics of the AS treated quails and these alterations may be due to cytotoxic and apoptotic effect on AS.

The decrease of the percentages of fertile eggs, hatching rate of fertile eggs, chick survival 14 days after hatching and increase of the percentages of embryonic and post-embryonic mortalities was observed in untreated females mated by males of groups T1 and T2. Similar results were observed in the red-legged partridges (*Alectoris rufa*) exposed to seeds coated with imidacloprid, thiram and difenoconazole by [46]. These indirect effects on chicks were likely mediated via paternal effects, because offspring were never exposed themselves to AS. Physiological effects on adults such as reduced fitness and oxidative stress may have further effects on reproduction [46]. In this study, negative correlations between the rates of hatching eggs and MDA ($r = -0.630$), between the rates of major sperm anomalies and embryonic mortality ($r = -0.547$) and between the rates of major sperm anomalies and chick survival ($r = -0.601$) were observed. Similar results have been described in red-legged partridges by [47]. The decrease of the fertility characteristics observed in the treated groups, in this study, could be due to the alteration of antioxidant systems which increased the total spermatozoa abnormalities and decreased the spermatozoa motility, viability and concentration.

5. Conclusion

According to the results of this study, it can be concluded that, AS (56.25 and 75) mg/kg b.w decreases body weight and relative organs weight and induces testicular lesions. Also, AS increases the level of MDA while it reduces the levels of enzymatic antioxidant biomarkers, serum testosterone and reproductive performances of intoxicated quails and their offspring. However, further work is needed to establish the genetic toxicology and immunohistochemistry of caspase-3 and claudin-1.

Declarations

Author contribution statement

Ferdinand Ngoula: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Ngoumtsop Victor Herman: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Ngouateu Kenfack Omer Bebe, Kenfack Augustave: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Mutwedu Bwana Valence, Nguemmogne Tamdem Ghislaine, Tchoffo Herve, Azafack Kana Dorice, Deutcheu Nienga Sorelle: Contributed reagents, materials, analysis tools or data.

Manjeli Yacouba: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Competing interest statement

The authors declare no conflict of interest.

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Additional information

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