Oxidative Damage and Reproductive Toxicity Associated with Antouka Super® in Male Japanese Quail (*Coturnix coturnix japonica*): The Protective Effects of Hydroethanolic Leaves Extract of *Persea americana*

Ngoumtsop Victor Herman¹, Ngoula Ferdinand¹*, Ngouateu Kenfack Omer Bebe², Kenfack Augustave¹, Nguemmogne Tamdem Ghislaine¹, Mutwedu Valerie¹, Tchoffo Herve¹, Azafack Kana Dorice¹, Deutcheu Nienga Sorelle¹ and Manjeli Yacouba¹

¹Department of Animal Science, Faculty of Agronomy and Agricultural Sciences, University of Dschang, P.O.Box 222, Dschang, Cameroon.

²Department of Animal Physiology, Faculty of Sciences, University of Yaounde I, P.O.Box 812, Yaounde, Cameroon.

**Authors’ contributions**

This work was carried out in collaboration between all authors. Authors NVH, NF and MY contributed substantially to conception and design of the study, data analysis and interpretation. Authors NVH, NF, NTG, MV, TH, AKD and DNS contributed in data acquisition. Authors NVH, NF and KA contributed in drafting the article or revising it critically for important intellectual content. All authors read and approved the final manuscript.

**Article Information**

DOI: 10.9734/ARRB/2017/34098

Editor(s): (1) Jean-Marie Exbrayat, Universite Catholique de Lyon, France. (2) George Perry, University of Texas at San Antonio, USA.

Reviewers: (1) Eze Ejike Daniel, Kampala International University, Uganda. (2) Martha de Oliveira Guerra, Federal University of Juiz de Fora, MG, Brazil.

Complete Peer review History: [http://www.sciencedomain.org/review-history/19817](http://www.sciencedomain.org/review-history/19817)

**Received 13th May 2017**

**Accepted 28th June 2017**

**Published 3rd July 2017**

**ABSTRACT**

The present study was undertaken to evaluate the protective role of hydroethanolic leaves extract of *Persea americana* (HEPA) against reproductive toxicity induced by Antouka Super® (AS) in male

*Corresponding author: E-mail: ferdinand.ngoula@univ-dschang.org, fngoula@yahoo.fr*
Japanese quail. The study was carried out in the Teaching and Research Farm of University of Dschang between February and May 2016. Forty (40) immature male Japanese quails (28 days old), were divided into five groups of 8 birds each and subjected to the following treatments: Group 1, birds receiving 10 ml/kg b.w of distilled water (negative control group (CO-); Group 2, birds receiving 75 mg of AS/kg (positive control group (CO+). while groups 3, 4 and 5 were administered 50, 100 and 200 mg/kg b.w of HEPA respectively together with AS at 75 mg/kg. All the test solutions were orally administered once a day for 60 days using an endogastric canule. Dissection of the vas deferent was performed to obtain spermatozoa. The protective effects of HEPA on the organ weights, serum hormones, oxidative stress biomarkers, sperm characteristics and histology changes in the testes were evaluated. Results revealed that exposure to AS significantly (p<0.05) decreased reproductive organ weights (testes, epididymis and vas deferens); the levels of testicular proteins and of serum hormones (LH, FSH and Testosterone). This insecticide also significantly (p<0.05) decreased sperm characteristics (mobility, viability and density) and fertility indices (percentage of fertile eggs, hatching rate and chick survival rate after hatching), and increased the sperm abnormalities (minor and major) and the embryonic and post-embryonic mortality rate. In addition, the activities of superoxide dismutase (SOD), total peroxidase (POD) and catalase (CAT) significantly (p<0.05) decreased in the testes of AS treated quails. While the level of malondialdehyde (MDA) significantly (p<0.05) increased compared with the values recorded in the negative control group birds. Histopathological examination of the testes of AS treated quails revealed testicular lesions characterized by moderate to severe degenerative changes of seminiferous tubules and incomplete spermatogenesis. Administration of HEPA to treated birds alleviates the reproductive toxicity and testicular oxidative damage induced by AS. Thus, exposure of male Japanese quails to AS induce oxidative stress and impairment on the reproductive parameters. These effects can be mitigated by the administration of HEPA.

Keywords: Antouka Super® (AS); reproductive toxicity; oxidative damage; hydroethanolic leaves extract; Persea Americana (HEPA); Japanese quail.

1. INTRODUCTION

Pesticides have brought the green revolution in the world and are being widely used to control agriculture pests causing public health hazards including infertility. The infertility rate has increased tremendously in the past few decades [1,2]. The decline in sperm counts by about 50% may be the main cause of male infertility [3]. Exposure to chemical agents including pesticides has contributed to this decline [4]. Owing to the extensive use pesticides in agriculture, there is a high risk of organism exposure to these chemicals [5].

In fact, pesticides are known to increase the production of Reactive Oxygen Species (ROS), which in turn generate oxidative stress in different tissues [6]. Many studies have elucidated oxidative damage as the central mechanism of toxicity [7,8]. Oxidative damage primarily occurs through excessive production of ROS that are generated during the reaction and react with biological molecules, eventually damaging membranes and other tissues [9,10]. Many insecticides are hydrophobic molecules that bind extensively to biological membranes, especially phospholipids bilayers [11] and they may damage membranes by inducing lipid peroxidation (LPO) [12,10].

Antouka Super® is a broad-spectrum insecticide widely used in agricultural and crops storage in many countries including Cameroon. It is made up of two insecticides: (Pirimiphos-methyl 16% and Permethrin 3%). Pirimiphos-methyl is a broad-spectrum organophosphate insecticide that distresses the nervous system by inhibiting acetyl cholinesterase activity [13]. It is employed in agriculture to control insects and mites that affect cereals, fruits, stored grains and cotton. Ngoula et al. [14] 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 is detrimental to the reproduction.

Permethrin is a pyrethroid insecticide class. It is an axonic poisons that affects nerve fibers by binding to a protein that regulates the voltage-gated sodium channel [15]. As pirimiphos-methyl, It is employed in agriculture to control insects and mites that affect cereals, fruits, stored grains...
and cotton. Zhang et al. [16] showed that Permethrin dramatically reduces testosterone levels and sperm counts in adult male mouse.

In our previous study, Antouka Super® (AS) increased the production of ROS which in turn increased lipid peroxidation and decreased the levels of oxidative stress biomarkers, such as superoxide dismutase (SOD), catalase (CAT) and total peroxidase (POD). This insecticide also decreased the sperm characteristics (mobility, viability and density), the level of serum testosterone and the fertility parameters (percentage of fertile eggs, hatching rate and chick survival rate after hatching). The contrary was recorded for the embryonic and post-embryonic mortality rate and the sperm anomalies (major and minor) (Personal communication).

As mechanism of AS toxicity involved oxidative stress, numerous efforts were done to identify dietary compound able to strengthen the cellular antioxidant defense so as to counteract the oxidative stress. In this respect, medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases. More attention has been paid to the protective effects of natural antioxidants against ROS [17,18]. The increasing interest in the health properties of Persea americana and its main quercetin polyphenols have led to a significant rise in scientific investigation for prevention and therapeutics in several diseases [19]. Agomuo et al. [20] and Arukwe et al. [21] reported that the leaves of Persea americana displays antioxidants and free radical scavenger properties. Considerable data on the protective effects of plants extract against pesticides on some reproductive performances in mammal species are documented, but information related to birds are rare. The present study aims to investigate the protective effects of HEPA against AS induced reproductive toxicity and oxidative stress in male quail.

2. MATERIALS AND METHODS

2.1 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 and Permethrin (1RS, 3RS; 1RS, 3SR) - 3-(2, 2-Dichlorovinyl)- 2, 2- dimethylcyclopropane-1-carboxylate (3- phenoxyphenyl)) concentrated at 3 g/kg.

2.2 Plant Harvesting and Extract Preparation

Persea americana leaves were collected in October 2015 at Dschang, West Region of Cameroon and authenticated at the Cameroon National Herbarium under the voucher number 18604/Sfr/Cam. The plant material was dried on the shade, grinded to obtain fine powder which was macerated in the ethanol (70%) for 72 hrs. After filtration, the filtrate was concentrated under vacuum to remove ethanol and further dried using freezer dryer to obtain a fine powder.

2.3 Phytochemical Screening

Chemical screening of the HEPA revealed the presence of alkaloids, tannins, phytosterols, triterpenes, anthraquinones, phenols, saponines, flavonoids, glucosides and coumarin.

2.4 Birds

Healthy twenty-eight (28) days male Japanese quails weighing between 109 and 118 g were used in this study. Birds were housed in specialized wire cages, twelve per cage, in animal care facility, maintained at 22 to 25°C with a relative humidity of 76 ± 5%, for 7 weeks. Birds were kept in a 12 h light-dark cycle and had free access to water and a laboratory diet.

2.5 Experimental Design

Birds were randomly divided into five groups of 8 quails each and treated as follows: Group 1, birds receiving 10 ml/kg b.w of distilled water (negative control group (CO-); Group 2, birds receiving 75 mg of AS/kg b.w (positive control group (CO+). while groups 3, 4 and 5 were administered 50, 100 and 200 mg/ kg b.w of HEPA respectively together with AS at 75 mg/kg b.w. All the test solutions were administered per os once a day for 60 days using an endogastric canule. The doses of AS used in the study were selected from a pilot study and represent 1/15 of LD50 value obtained in quails (1125 mg/kg b.w) (personal communication). During the treatment, body weight was weekly measured.

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 spermatozoa deposition in the vaginal orifice upon vaginal examination. On the other hand, eggs were collected 2 days after the observation of sperm cells in the vaginal orifice and for 7 days. Eggs were incubated for 19 days and unhatched eggs were opened and examined to see whether they were fertilized or not (presence of embryo or germinal disk). The male fertility parameters were calculated according to the following formulae:

\[
\text{Percentage of fertility eggs} = \frac{\text{Number of fertile eggs}}{\text{Number of total incubated eggs}} \times 100
\]

\[
\text{Hatching rate} = \frac{\text{Number hatching eggs}}{\text{Number of incubated eggs}} \times 100
\]

\[
\text{Embryonic mortality} = \frac{\text{Number of dead chicks in eggs}}{\text{Number of total fertile eggs}} \times 100
\]

\[
\text{Post-mortality embryonic} = \frac{\text{Number of dead chicks}}{\text{Number of total chicks}} \times 100
\]

\[
\text{Chicks viability at (14 days)} = \frac{\text{Number of viable chicks}}{\text{Number of total live chicks}} \times 100
\]

2.7 Blood and Organ Collections

At the end of treatment (60th day), blood was collected after sectioning the jugular vein of each bird. Serum was prepared and stored at -20°C prior to analysis. After killing the quail, organs like testes, epididymis and vas deferens were carefully removed, free of adipose tissue, blotted dry and weighed separately. The left testes of each bird was then homogenized at 20% (weight/volume) in 0.9% NaCl solution and aliquots of supematant were kept at -20°C prior to biochemical analysis.

2.8 Evaluation of Sperm Characteristics

Immediately after the sacrifice of each bird, vas deferens were carefully removed, minced in a 10 ml of 0.9% NaCl (40°C) and used to evaluate the sperm motility, concentration, viability and morphology. The sperm motility was estimated on scale basis as reported by Mamun et al. [22], sperms viability expressed as percentage of swelled sperms and the morphology expressed as percentage of abnormal sharp sperm. Sperm viability and morphology were analyzed using respectively hypo-osmotic swelling test Amorim et al. [23] and eosine/nigrosine test. Five microliters of sperm were mixed with 5 microliters of eosine/nigrosine solution. While 10 microliters of sperm were mixed with 1000 microliters 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 microscope fields according to the protocol provided by Revell and Mrod [24]. The sperm density was determined using Thoma hemocytometer.

2.9 Biochemical Analysis

The level of proteins in the testis was determined using CHRONOLAB kit following the manufacturer's protocol. Serum LH, testosterone and FSH levels were determined using appropriate commercially available kits (ELISA AccuDiag™, Diagnostic Automation Inc, 23963 Craftsman Rd Suites: D/E/F Calabas, ca 91305 USA and ELISA EIA, Gmhr, DRG, 1288 Germany). Total peroxidase (POD); superoxide dismutase (SOD); malondialdehyde (MDA) and catalase activity (CAT) were measured in testicular homogenates using spectrophotometer (GENESYS 20.0) and according to the methods described respectively by: Habbu et al. [25] and Dimo et al. [26] and Kodjo et al. [27] and Sajeeth et al. [28].

2.10 Tissue Preparation and Histopathology

The right testis was fixed in Bouin fluid for 1 week, embedded in paraffin, cut at 5 µm and stained with Harris hematoxylin and eosin. Tissue sections were observed under a light microscope (Leica DM 750, X40) for changes in the seminiferous tubules and intertubular spaces.

2.11 Statistical Analysis

Values are presented as Mean ± 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≤0.05 was considered statistically significant. Statistical analyses were performed with the aid of SPSS for Windows software program (Release 20.0).

3. RESULTS AND DISCUSSION

3.1 Relative Weight of Some Reproductive Organs

The relative weight of testis, epididymis and vas deferens of birds in group CO+ was significantly
lower compared to the values of this parameter recorded in groups T1, T2, T3 and CO-.

Furthermore, the relative weight of testis, epididymis and vas deferens of birds in groups treated with HEPA shown no significant differences when compared to group CO-

Table 1. Effects of HEPA on body and reproductive organs weight of male Japanese quails exposed to AS

| Weight parameters       | CO- (n=8)           | CO+ (n=8)           | T1 (n=8)           | T2 (n=8)           | T3 (n=8)           |
|-------------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| Initial body (mg)       | 113.83±4.45         | 113.83±4.95         | 113.00±3.35         | 113.17±4.53         | 114.17±2.48         |
| Final body (mg)         | 228.17±2.85         | 187.17±16.15        | 200.83±10.36        | 211.50±13.38        | 219.00±7.92         |
| Body gain (mg)          | 110.64±14.29        | 71.00±14.95         | 87.83±10.96         | 98.33±17.00         | 103.33±8.89         |
| Relative reproductive organs (%) |
| Testis                  | 1.54±0.14           | 0.54±0.19           | 1.24±0.18           | 1.31±0.12           | 1.35±0.18           |
| Epididymis              | 0.029±0.003         | 0.023±0.008         | 0.031±0.004         | 0.031±0.005         | 0.028±0.007         |
| Vas deferens            | 0.037±0.008         | 0.025±0.007         | 0.043±0.010         | 0.043±0.008         | 0.044±0.015         |

n=number of animals, each value represents mean ± standard error mean
(a,b,c,d) means bearing different letters in a row differ significantly at p< 0.05.

CO-: Negative control; CO+: 75 mg AS/kg b.w; T1: 75 mg AS+ 50 mg HEPA/kg b.w; T2: 75 mg AS+100 mg HEPA/kg b.w; T3: 75 mg AS+200 mg HEPA/kg b.w.

Table 2. Effects of HEPA on oxidative stress biomarkers in the testis of male Japanese quail exposed to AS

| Oxidative stress parameters in the testis | CO- (n=8) | CO+ (n=8) | T1 (n=8) | T2 (n=8) | T3 (n=8) |
|-----------------------------------------|-----------|-----------|-----------|-----------|-----------|
| Testicular protein (mg/ml)              | 9.53±0.47 | 4.18±0.11 | 5.45±0.31 | 6.36±0.27 | 8.15±0.37 |
| MDA (nmol/mg tissues)                   | 11.39±1.94| 23.87±1.47| 21.54±0.62| 15.94±1.13| 14.54±0.85|
| SOD (UI/mg tissues)                     | 22.47±1.11| 10.66±0.51| 12.09±0.64| 16.22±0.67| 17.92±1.41|
| CAT (UI/mg tissues)                     | 6.52±0.32 | 4.75±0.12 | 5.08±0.03 | 5.30±0.03 | 5.80±0.12 |
| POD (µM/mg tissues)                     | 19.02±0.40| 12.93±0.33| 13.61±0.45| 14.95±0.70| 18.39±1.23|

n=number of animals, each value represents mean ± standard error mean
(a,b,c,d) means bearing different letters in a row differ significantly at p< 0.05.

MDA: Malondialdehyde; SOD: Superoxide dismutase; CAT: Catalase and POD: total peroxidase

AS: Antouka Super®; CO-: Negative control; CO+: 75 mg AS/kg b.w; T1: 75 mg AS+50 mg HEPA/kg b.w; T2: 75 mg AS+100 mg HEPA/kg b.w; HEPA: hydroethanolic leaves extract of Persea americana
Table 3. Effects of HEPA on serum hormones of male Japanese quails exposed to AS

| Hormones characteristics (ng/ml) | Doses of HEPA (mg/kg b.w) | CO- (n=8) | CO+ (n=8) | T1 (n=8) | T 2 (n=8) | T3 (n=8) |
|---------------------------------|---------------------------|-----------|-----------|----------|----------|----------|
| FSH                             |                           |           |           |          |          |          |
|                                 |                           | 4.08±0.37a| 1.35±0.18c| 1.56±0.10c| 3.00±0.77b| 3.35±0.27b|
| LH                              |                           | 3.46±0.13a| 0.72±0.14a| 1.1±0.08d | 1.75±0.05c| 2.03±0.13b|
| Testosterone                    |                           | 1.91±0.16a| 0.46±0.01d | 0.51±0.01cd| 0.6±0.02c | 1.14±0.06b|

n=number of animals, each value represents mean ± standard error mean
(a,b,c,d,e) means bearing different letters in a row differ significantly at p< 0.05.

AS: Antouka Super®; CO-: Negative control; CO+: 75 mg AS/kg b.w; T1: 75 mg AS+50 mg HEPA/kg b.w; T2: 75 mg AS+100 mg HEPA/kg b.w; T3: 75 mg AS+200 mg HEPA/kg b.w; HEPA: hydroethanolic leaves extract of Persea americana

3.4 Sperm Traits

The sperm motility and viability as well as the number of spermatozoa per vas deferens decreased significantly (p<0.05) in treated birds compared to negative control group birds (CO-) (Table 4). Generally, birds that were given AS together with HEPA had demonstrated a significant (p<0.05) increase in their sperm mobility, viability and density per vas deferens when compared to the positive control group (CO+) values. The inverse was observed with the major and minor abnormalities.

3.5 Fertility

The percentages of fertile eggs, hatching rate and chick survival 14 days after hatching in treated groups dropped significantly (p<0.05) compared to those of the negative control group (CO-) group. Co-administration of AS with HEPA significantly increased the values of these parameters (percentages of fertile eggs, hatching rate and chick survival 14 days after hatching). The inverse was recorded for the percentages of embryonic and post-embryonic mortality (Table 5).

3.6 Histological Findings

The testicular histology of treated and untreated quails are illustrated in Figure 1. Typical structure of testis was observed in control Japanese quails; the seminiferous epithelium contained all generations of germinal cells corresponding to the stages of seminiferous epithelium cycle. The lumen contained normal flagellated spermatozoa (1). In the second and third groups (2 and 3), more severe changes were revealed: dramatic depletion in the germ layers of seminiferous tubules with the degeneration of connective tissue between seminiferous tubules and increased intertubular space. While in the fourth and fifth groups (4 and 5), a slight degeneration in the germ layers of seminiferous tubules and intertubular space were observed. The lumen contained normal flagellated spermatozoa.

Table 4. Effects of HEPA on some sperm traits of male Japanese quails exposed to AS

| Semen characteristics | Doses of HEPA (mg/kg b.w) | CO- (n=8) | CO+ (n=8) | T1 (n=8) | T 2 (n=8) | T3 (n=8) |
|-----------------------|---------------------------|-----------|-----------|----------|----------|----------|
| Mobility (%)          |                           | 4.16±0.51a| 2.41±0.66d| 2.83±0.40cd| 3.08±0.20bc| 3.50±0.63b|
| Viability (%)         |                           | 75.67±5.37a| 36.17±3.76d| 40.83±2.48cd| 45.3±9.07c| 64.67±5.35b|
| Number/vas deferens (10⁹) |                   | 2.32±0.12a| 0.67±0.052d | 0.73±0.13d | 1.01±0.07c | 1.58±0.08b |

Semen morphology (%)

| Major anomalies       |                           | 8.83±2.04a| 22.50±3.27b | 20.67±3.20ab | 17.50±3.93abc| 14.83±2.99c|
| Minor anomalies       |                           | 5.83±2.13c| 19.83±1.47a | 12.50±3.56b | 12.67±2.65b | 7.83±3.81c|

n=number of animals, each value represents mean ± standard error mean
(a,b,c,d) means bearing different letters in a row differ significantly at p< 0.05.

AS: Antouka Super®; CO-: Negative control; CO+: 75 mg AS/kg b.w; T1: 75 mg AS+50 mg HEPA/kg b.w; T2: 75 mg AS+100 mg HEPA/kg b.w; T3: 75 mg AS+200 mg HEPA/kg b.w; HEPA: hydroethanolic leaves extract of Persea americana
Table 5. Effects of HEPA on the fertility of male Japanese quails exposed to AS

| Parameters                        | Doses of HEPA (mg/kg b.w) | CO- (n=8) | CO+ (n=8) | T1 (n=8) | T 2 (n=8) | T3 (n=8) |
|-----------------------------------|---------------------------|-----------|-----------|-----------|-----------|-----------|
| Total number of eggs              |                           | 48        | 48        | 48        | 47        | 47        |
| Fertile eggs (%)                  |                           | 89.58±4.50a | 50.00±4.50c | 67.80±4.50b | 66.66±4.50b | 72.53±4.50b |
| Hatching rate (%)                 |                           | 91.06±5.23a | 76.25±5.23ab | 68.75±5.23b | 72.02±5.23b | 85.06±5.23ab |
| Embryonic mortality (%)           |                           | 8.93±5.23b | 23.75±5.23ab | 31.25±5.23a | 27.97±5.23a | 14.93±5.23ab |
| Post-embryonic mortality (%)      |                           | 20.55±5.40b | 42.08±5.40a | 41.66±5.40a | 26.66±5.40b | 20.23±5.40b |
| Viability of chicks at (14 days) (%) |                           | 79.44±5.40a | 57.91±5.40b | 58.33±5.40b | 73.33±5.40ab | 79.76±5.40a |

n=number of animals, each value represents mean ±standard error mean
(a,b,c,d) means bearing different letters in a row differ significantly at p < 0.05.

AS: Antouka Super®; CO-: Negative control; CO+: 75 mg AS/kg b.w; T1: 75 mg AS+ 50 mg HEPA/kg b.w; T2: 75 mg AS+100 mg HEPA/kg b.w; T3: 75 mg AS+200 mg HEPA/kg b.w; HEPA: hydroethanolic leaves extract of Persea americana

Figure 1. Micrographs of the section of quail testis (H&E X 400), 1: normal testis (control), showing normal structure with more spermatozoa in the lumen of seminiferous tubules (lst); 2:(75 mg AS/kg) showing dramatic depletion in the germ layers of seminiferous tubes (St) and degeneration of connective tissue between germ layers (Deg), seminiferous tubes are poor in spermatoza (lst) and increased intertubular space (it); 3:(50 mg HEPA/kg) showing severe degeneration (Deg) and space formation in the germ layers of seminiferous tubes (St) the lumen of these seminiferous tubes also present less spermatoza (lst) and increased intertubular space (it); 4:(100 mg HEPA/kg) showing slight degeneration in the germs layers of seminiferous tubules (Deg) and intertubular space (it), the lumen of the seminiferous tubes present more spermatoza (lst) and increased intertubular space (it); 5:(200 mg HEPA/kg) showing slight degeneration in the germs layers of seminiferous tubules (Deg), lumen of the seminiferous tubes present more spermatoza (lst)
4. DISCUSSION
The reproductive toxicity of Antouka Super® (AS) in Japanese male quail was characterized by a low fertility indices, a decrease of testes, epididymis and vas deferens weight, and low sperm characteristics such as sperms motility, viability and concentration. These findings are in agreement with those of [29,30] who reported reduction in fertility indices, a decrease of testes, epididymis and vas deferens weight, and low sperm characteristics such as sperms motility, viability and concentration after chronic exposure of male rats to Cypermethrin and Fenitrothion respectively. The reduction in the testicular weight reflects deleterious changes in seminiferous tubules. Since sperm motility is an important parameter to predict sperm fertilizing capacity, any negative impact on motility would seriously affect fertility. In this respect, marked inhibition of sperm motility in AS treated group may be due to a rapid loss of ATP, causing axonemal damage [31]. Full ATP pool is crucial for normal sperm movement and a slight axonemal damage may be due to a rapid loss of ATP, causing inhibition of sperm motility in AS treated group.

The correction of reproductive damage in intoxicated male quails treated with HEPA can be due to the phytosterols, saponins, polyphenols and flavonoids present in the extract. Many studies have shown that these compounds increase the level of testosterone, the main hormone that controls sexual behavior [37,38,39].

A significant increase in the lipid peroxidation (LPO) level was observed in the present study. These results are in line with the observations of previous researchers after Cypermethrin (insecticide) administration [40]. Oxidative stress refers to disrupted redox equilibrium between the production of free radicals and the ability of cells to protect against damage caused by these species. The main cellular components susceptible to be damage by free radicals are lipids (peroxidation of unsaturated fatty acids in cell membrane); these free radicals can impair cellular structure and function [41,42]. It has been indicated that LPO is one of the molecular mechanism involved in pesticide-induced toxicity. Defense against oxidative stress are maintained using several mechanisms which include antioxidant machinery [43,44].

Reproductive toxicity could also be explained by the impaired antioxidant enzyme activities in the testes of the quails. This study also found a decrease of the activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and total peroxidase (POD) after exposure of male quail to AS. Similar results were reported by [45,46,47]. These enzymes work together to eliminate active oxygen species. In this respect, SOD accelerates the dismutation of superoxide radicals \( \text{O}_2^- \) into molecular oxygen \( \text{O}_2 \) and hydrogen peroxide \( \text{H}_2\text{O}_2 \) while \( \text{H}_2\text{O}_2 \) is neutralized by the combined action of CAT and POD in all organisms [43,44]. POD is a group of antioxidant enzymes such as glutathione peroxidase (GPx) and glutathione S transferase (GST). The major function of these enzymes, which use glutathione as a substrate, is to reduce soluble hydrogen peroxide and alkyl peroxidases [48]. Pesticides have been reported to significantly decrease GPx and GST activities in male testicular tissues [48,10]. In the present study...
study, the drop of POD activities might reflect cellular oxidative stress due to pesticides exposure.

As regards to the histopathological results, testicular damage induced by AS exposure in intoxicated quails, as demonstrated in this study, is in agreement with that of many previous investigators who reported variable degrees of degenerative changes after exposure of male to insecticides [49,29]. Testicular damage induced by AS in this study confirms the reported lowered fertilizing capacity of the treated quails. The toxic effects of AS on male reproductive system of the Japanese quails could be explained by its direct cytotoxic effect and/or indirectly by the decrease serum hormones level.

Most of the biochemical alterations accompanied by histopathological changes were alleviated after administration of HEPA. This could be attributed to the antioxidant compound (polyphenols and flavonoids) found in this extract that reduces the LPO which in turn restore the integrity of the cell membrane and improve the disturbance in permeability. Since the oxidative damage as the central mechanism of pesticides toxicity occurs primarily through production of ROS, the use of antioxidants to counteract the formed ROS is the corner stone in alleviating such hazards. So, the major bioactive compounds in HEPA are the quercetin (polyphenol) that has the most effective antioxidant activity [19]. Quercetin is an efficient free radical scavenger due to their one electron reduction potential [50,19]. In addition, HEPA contains some co-factors of antioxidant enzymes: zinc, selenium, iron, vitamins (A, B1,2,3, C et E) and manganese [20,21]. Polyphenols and flavonoids have additional mechanisms in which they reduce oxidation level through the inhibition of metal ions such as iron and copper and preventing their participation in oxidation reactions (leading to the formation of hydroxyl radical). Polyphenol and flavonoids also react through the suppression of oxidation stimulants such as xanthine oxidase and induction of antioxidant enzymes such as glutathione S-transferase and super oxide dismutase [51,52].

5. CONCLUSION

This study revealed that AS induces reproductive toxicity in male Japanese quail characterized by a decrease in the fertility indices, weights of sexual organs, some sperm characteristics and serum hormones level as well as testicular damage (induction of lipid peroxidation and depletion of antioxidant enzymes in testes). In contrast, treatment with HEPA inhibit the reproductive toxicity and oxidative damages induced by the insecticide. Finally, we can say that HEPA leaves may provide a cushion for prolonged therapeutic option against toxins-induced reproductive toxicity and oxidative damage without harmful side effects.

ETHICAL APPROVAL

Experimental protocols used in this study were strictly conformed 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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Oehninger S. Strategies for the infertile man. Semin Reprod Med. 2001;19:231-237.
2. Venkatesh S, Deecaraman M, Kumar R, Shamsi BM. Role of reactive oxygen species in the pathogenesis of mitochondrial DNA (mtDNA) mutations in male infertility. Indian J Med Res. 2009;129:127-137.
3. Carlsen E, Giwercman A, Keiding N, Skakkebaek NE. Evidence for decreasing quality of semen during past 50 years. Br Med J. 1992;305:609-613.
4. Cox C. Chlorpyrifos, part 1: Toxicology. J Pesticides Reform. 1994;14:15-20.
5. Sarkar R, Mohanakumar KP, Chowdhury M. Effects of organophosphate pesticide, quinalphos, on the hypothalamo-pituitary-gonadal axis in adult male rats. J Reprod Fertil. 2000;118:29-38.
6. Heikal TM, Ghanem HZ, Soliman MS. Protective effect of green tea extracts against dimethoate induced DNA damage and oxidant/antioxidant status in male rats. Biohealth Sci Bull. 2011;3:1-11.
7. Verma RS, Mehta A, Srivastava N. In vivo chlorpyrifos induced oxidative stress: Attenuation by antioxidant vitamins. Pestic Biochem Phys. 2007;88(2):191-6.
8. Amin K, Hashem K. Deltamethrin-induced oxidative stress and biochemical changes in tissues and blood of catfish (Clarias gariepinus): Antioxidant defense and role of alpha-tocopherol. BMC Vet Res. 2012;8:45-51.

9. Kalender Y, Kaya S, Durak D, Uzun FG, Demir F. Protective effects of catechin and quercetin on antioxidant status, lipid peroxidation and testis-histoarchitecture induced by chlorpyrifos in male rats. Environ Toxicol Pharmacol. 2010;3(2):141-8.

10. Heikal MT, Mossa ATH, Abdel Raoul MA, Marei KGK. The ameliorating effects of green tea extract against cyromazine and chlorpyrifos induced liver toxicity in male rats. Asian J Pharm Clin Res. 2013;6:48-55.

11. Ogutcu A, Suludere Z, Kalender Y. Dichlorvos-induced hepatotoxicity in rats and the protective effects of vitamin C and E. Environ Toxicol Pharmacol. 2008;26:355-361.

12. Mossa ATH, Heikal MT, Omara EAA. Physiological and histopathological changes in the liver of male rats exposed to paracetamol and diazinon. Asian Pacific J Trop Biomed. 2012;2:S1683-S1690.

13. Hayes WJ, Laws ER. Handbook of pesticide toxicology. Academic Press, San Diego, CA. 1998;185:15.

14. Ngoula F, Pierre W, Marie-Chantal D, Augustave K, Kamtochoing J, Joseph T. Effects of pirimiphos-methyl (an organophosphate insecticide) on fertility of adult male rats. Afril Health Scie. 2007;7(1):3-9.

15. National Coalition against the Misuse of pesticides (NCAMP). aglaser@beyondpesticides.org; 2006.

16. Sajeeth CI, Manna PK, Manavalan R. Antioxidant effect of aqueous extract of Curcuma longa rhizomes (Zingiberaceae) in the typhoid ferder induced in wistar rats model. J Adv med Phar and Scie. 2016;7(3):1-13. ISSN: 2394-1111

17. Izatus ST, Siti BB, Ahmad RG, Putri AJ, Santhana RL, et al. Fenitrothion induced oxidative stress and morphological alterations of sperm and testes in male rats. Environ Toxicol Pharmacol. 2012;3(2):220-226.

18. Sajeeth CI, Manna PK, Manavalan R. Antioxidant activity of polyherbal formulation on streptozotocin induced diabetes in experimental animals; Der Pharmacia Sinica. 2011;2(2):220-226.
30. Poonam S, Amir UH, Rambir S. Cypermethrin induced reproductive toxicity in male wistar rats: Protective role of Tribulus terrestris. J Env Biol. 2013;34:857-862.

31. De Lamirande E, Ganong C. Reactive oxygen species and human spermatozoa. II. Depletion of adenosine triphosphate plays an important role in the inhibition of sperm motility. J. Andro. 1992;13:379-386.

32. Peters SO, Shoyebo OD, Ilori BM, Ozoje MO, Ikeobi CON, et al. Semen quality traits of seven strains of chickens raised in the humid tropics. Inter J Poult Scie. 2008;7(10):949-953.

33. Parker HM, McDaniel CD. The optimum semen dilution for the sperm quality index that is most predictive of breeder fertility. Inter J Poult Scie. 2004;3(9):588-592.

34. Joshi SC, Mathur R, Gulati N. Testicular toxicity of chlorpyrifos in albinos rats. Toxicol Ind Health. 2007;23:439-444.

35. Bedwal RS, Edwards MS, Katoch M, Bahuguna A, Dewan R. Histological and biochemical changes tests of zinc deficient BALB/c strain of mice. India J Exp Biol. 1994;32:243-247.

36. Oyewopo AO, Saalu LC, Osinubi AA, Imosemi IO, Omotoso GO, et al. The attenuating effect of zinc on propoxur-induced oxidative stress, impaired spermatogenesis and deranged steroidogenesis in wistar rat. J Med and Pharm Scie. 2010;4(2). ISSN: 2250-0480.

37. Sikka SC, Rajasekaram M, Hellstromw J. Role of oxidative stress and antioxidants in male infertility. J Andro. 1995;16:464-468.

38. Yuling MI, Caiqiao Zhang, Chun Mei Li, Shinji T, Gen W, et al. Protective effect of quercetin on the reproductive toxicity of 4-nitrophenol in diesel exhaust particles on male embryonic chickens. J Reprod and Devel. 2010;56(2):195-199.

39. Kougan GB, Miyamoto T, Tanaka C, Paululat T, Mirjolet JF, et al. Steroidal saponins from two species of Dracaena. J Nat Prod. 2010;73(7):1266-1270.

40. Madhubanti B, Pralay M, Tuhina D, Sujata MC. Zinc and alpha-lipoic acid alleviate cypermethrin induced reproductive toxicity in mature male wistar rat. Inter J Life Scie and Pharm Research. 2014;4(2). ISSN: 2250-0480.

41. Baumber J, Ball BA, Gravance CG, Medina V, Davies-Morel MC. The effect of reactive oxygen species on equine sperm mobility, viability, acrosomal intergrity, mitochondrial membrane potential, and membrane lipid peroxidation. J Andro. 2000;21:895-902.

42. Bucak MN, Ateshahin A, Varisli, Yuce A, Tekin N, et al. The influence of trehalose, taurine, cysteamine and hyluronan on ram semen microscopic and oxidative stress parameters after freeze-thawing process. Theriogenology. 2007;67:1060-1067.

43. Eva T, Zuzana K, Laszlo B, Peter M, Nobeht L. Impact of oxidative stress on male fertility a review. Acta Veterinaria Hungarica. 2011;59(4):465-484. DOI: 10.1556/Avet.034

44. Dare BJ, Oyeniyi F, Olaniyan OT. Role of antioxidant in testicular intergrity. Scie Dom Inter. 2014;4(7):998-1023.

45. Afaf A, El-Kashouy, Hanan A, Tag El-Din Chlorpyrifos (from different sources): Effects on testicular biochemistry of male albinos rats. J Amer Scie. 2010;6(7).

46. Cemek M, Buyukben A, Buyukokuroglu ME, Aymelek F, Tur L. Protective roles of vitamin E, selenium and vitamin E plus selenium in organophosphate toxicity in vivo: A comparative study. Pest Biochem Physiol. 2010;96(3):113-8. Available:http://dx.doi.org/10.1016/j.pestbp.2009.09.009

47. Sikka SC, Rajasekaram M, Hellstromw J. Role of oxidative stress and antioxidants in male infertility. J Andro. 1995;16:464-468.

48. Yuling MI, Caiqiao Zhang, Chun Mei Li, Shinji T, Gen W, et al. Protective effect of quercetin on the reproductive toxicity of 4-nitrophenol in diesel exhaust particles on male embryonic chickens. J Reprod and Devel. 2010;56(2):195-199.

49. Azza M, Gawish. The protective role of alph lipoic acid against pesticides induced testicular toxicity. (Histopathological and histochemical studies). Life Scie J. 2010;7(3):117-124. ISSN: 1097-8135.

50. Rice-Evans CA, Cheng SJ, Klauning JE. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic. Biol Med. 1996;20:933-956.
51. Leopoldini MN, Russo, Toscano M. The molecular basis of working mechanism of natural polyphenolic antioxidants. Food Chemistry. 2011; 125(2):288-306.

52. Indumathi P, Vijayalakshmi KM. Quantification of phytochemicals and antioxidant potential of Persea americana and Actinidia deliciosa. Inter J Biol and Pharm Research. 2015;6(1):6-11.

© 2017 Herman et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/19817