Scientific Research Publishing

Open Journal of Veterinary Medicine, 2021, 11, 41-56
https://www.scirp.org/journal/ojvm
ISSN Online: 2165-3364
ISSN Print: 2165-3356

Hepato-Preventive Effects of Hydroethanolic Leaves Extract of *Persea americana* Mill. (Lauraceae) "Avocado" against Antouka Super® Induced Damage in Male Japanese Quail (*Coturnix coturnix* Japonica)

Ngoumtsop Victor Herman[1,2*], Tchoffo Herve[2], Guiekep Nounamo Arthénice Jemima[3], Mutwedu Valence[4], Ngoula Ferdinand[2]

[1]Institute of Fisheries and Aquatic Sciences (ISH) at Yabassi, Douala, Cameroon
[2]Animal Physiology and Health Research Unit, Department of Animal Science, Faculty of Agronomy and Agricultural Sciences, University of Dschang, Dschang, Cameroon
[3]Department of Animal Production, College of Technology, University of Bamenda, Bambili, Cameroon
[4]Department of Animal Production, Faculty of Agriculture and Environmental Studies, Université Evangélique en Afrique (UEA), Bukavu, DR Congo
Email: *ngoumtsopherman.jetlee@yahoo.fr, ngoumtsophermanvictor@gmail.com

How to cite this paper: Herman, N.V., Herve, T., Jemima, G.N.A., Valence, M. and Ferdinand, N. (2021) Hepato-Preventive Effects of Hydroethanolic Leaves Extract of *Persea americana* Mill. (Lauraceae) "Avocado" against Antouka Super® Induced Damage in Male Japanese Quail (*Coturnix coturnix* Japonica). *Open Journal of Veterinary Medicine*, **11**, 41-56.
https://doi.org/10.4236/ojvm.2021.111003

Received: May 8, 2020
Accepted: January 26, 2021
Published: January 29, 2021

Abstract

The present study was undertaken to evaluate the protective effects of Hydroethanolic leaves extract of *Persea americana* (HEPA) against Antouka Super® (AS) induced hepatotoxicity in male Japanese quail. In total, 40 immature male Japanese quails aged 28 days were used and divided equally into 5 groups. The groups were designed as the control group (received only a 10 ml/kg of distilled water) and the AS group (75 mg/kg b.w). Other three groups received AS (75 mg of AS/kg b.w) plus HEPA (50, 100, and 200 mg/kg b.w/day respectively) by the oral route. After 60 days of the experiment, the crushed liver was performed to obtain homogenate. The protective effects of HEPA on the biochemical parameters, oxidative stress biomarkers and histology changes in the liver were evaluated. The results indicated that AS treatment caused significant alterations in the clinical signs and behavior. It induces the increase in the content of Urea, Creatinine, Protein, AST and ALT in liver tissues and serum. The activities of enzymatic oxidative stress markers such as Superoxide Dismutase (SOD); Catalase (CAT) and Total Peroxidase (POD) also showed significant perturbations in AS-treated quails. Histopathological examination of the liver of AS-treated quails revealed liver

lesions characterized by moderate to severe degenerative changes showing a number of hepatocytes undergo fatty changes, focal aggregation of the lymphocytes, multiple necrotic changes and inflammatory infiltrate. The administration of HEPA however, markedly ameliorated the toxicity of AS by protecting the levels of aforesaid biomarkers to near normal levels. These results suggested that HEPA due to its phytochemical constituents with antioxidant properties possesses significant effects against AS-induced toxicity. However, these effects were more pronounced at a dose of 200 mg/kg bw.

Keywords

Antouka Super® (AS), Hepatoprotective, Toxicity, Hydroethanolic Leaves Extract, *Persea americana*, Japanese Quail

1. Introduction

Pesticides have been applied in agriculture and household to protect plants, animals and humans from insects and vector diseases. The negligent and random uses of pesticides can cause environmental damage, food, water contamination, and health problems (e.g. cancer, nerve disease, birth defects). Animals and humans are potentially exposed to pesticides either directly through occupational exposure or indirectly via food and water consumption Ngoumtsop *et al.* [1], [2].

Antouka Super® (AS) is a broad-spectrum insecticide widely used in agriculture and crop's 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 accumulated in adipose tissue, brain and liver and has hepatotoxic potential in rat [3] [4]. Permethrin, is a pyrethroid insecticide class; due to their lipophilicity, it is a favor absorption through the gastrointestinal and confer preferential distribution into lipid-rich internal tissues, including body fat, skin, liver and kidney [5]. Hallenbeck *et al.* [6] reported that exposure to permethrin causes enlargement of the liver.

In fact, one possible mechanism by AS-induced toxicity is the production of reactive oxygen species (ROS) in the cell. The imbalance between ROS synthesis and the amounts of antioxidants causes oxidative stress. The presence of oxidative stress damages lipids, proteins and DNA [7] [8]. It has been reported that ROS were involved in the toxicity of organophosphate insecticides (OPIs) [9] and pyrethroid insecticides [7] [10]. Also, a positive correlation with the liver damage has been reported. ROS, especially superoxide anion and hydrogen peroxide, are important signaling molecules in developing and proliferating cells, but also in the induction of programmed cell death [11] [12]. ROS are transient species due to their high chemical reactivity that leads to the LPO and a massive protein oxidation and degradation [13] [14]. These authors reported that ROS cause DNA damage and strand breaks as a result of modifying purines and py-

rimidines bases by superoxide anion radical ($O^{2\bullet-}$), hydrogen peroxide (H^2O^2), and hydroxyl radical ($HO^\bullet$).

The *Persea americana* Mill. tree belongs to the family Lauraceae, genus *Persea* and is a plant native of Central America. Apart from its use as food, the avocado is traditionally utilized for various medicinal purposes including anti-inflammatory [15]; and anti-aging agents [16], and is applied for the treatment of ulcers and cardiovascular diseases [17] [18] [19].

Previous investigations of the skin, leaves and seed revealed a predominance of compounds belonging to the group of flavonoids, proanthocyanidins, and hydrocinnamic acids [19]. Phenolics and flavonoids are bioactive compounds that have been related with a decrement of different deteriorative processes in the human body owing to their ability to reduce the formation and to scavange free radicals [20]. Rodríguez-Carpena *et al.* [21] ascribed the high antioxidant activity exhibited by avocado extracts in various *in vitro* assays to these phenolic compounds. Ekor *et al.* [22] and Owolabi *et al.* [23] reported the protective effect of *P. americana* against toxicity. In addition, phytochemical screening of the leaf extract of *P. americana* revealed the presence of flavonoids which are playing an essential role in neutralizing free radical, quenching singlet and triplet oxygen, decomposing peroxides, stabilizing lipid peroxidation and protecting the cells against oxidative damage [24] [25] [26] by donating a hydrogen atom or electron to stabilize the radical species [27].

Currently, some synthetic antioxidant use to prevent free radical damage can induce side effects Conwell *et al.* [28]. So, the dietary intake of natural products is considered very important for preventing a wide variety of diseases such as allergies, cardiovascular disease, certain forms of cancer, hepatic diseases, and inflammation, which involve free radical-mediated damage in pathologically generating processes [29]. Therefore, that is an essential research about suitable herbal drugs that could replace the chemical ones Owolobi *et al.* [30]. However, the widespread use of *Persea americana* in traditional medicine stimulated us to explore its potential biological activity. To the best of our knowledge, no previous study of the antioxidant and protective activities of hydroethanolic leaves extract of *Persea americana* (HEPA) have been reported. Therefore, the current study was designed to evaluate the antioxidant activity and protective effect of HEPA leaves against AS induced biochemical parameters, oxidative stress biomarkers and histology changes in the liver damage in male Japanese quail.

2. Materials and Methods

2.1. Birds

Forty healthy male Japanese quails aged 28 days and weighing 112 - 118 g were used in this study. Birds were housed in specialized wire cages, eight per cage, in a centralized birds care facility maintained at 22˚C - 25˚C with a relative humidity of 76% ± 5%, for 8 weeks. Animals were kept in a 12 h light-dark cycle and

provided *ad libitum* with water and a specific diet.

2.2. Origin of Pesticide

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

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

2.3. Plant Harvesting and Extract

Persea americana leaves were from Dschang, West Region of Cameroon and authenticated at the Cameroon National Herbarium under the voucher number 18,604/Sfr/Cam. They were shade-dried, ground 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.

2.4. Phytochemical Screening of HEPA

The phytochemical screening of the HEPA was done as described by Tiendrebeogo *et al.* [31] and revealed the presence of tannins, anthraquinones, phenols, alkaloids, sterols and flavonoids.

2.5. 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 [32].

2.6. Experimental Design

In total, 40 immature male Japanese quails aged 28 days were used and divided equally into 5 groups. The groups were designed as the control group (received only a 10 ml/kg of distilled water) and the AS group (75 mg /kg b.w) by the oral route. Other three groups received AS (75 mg of AS/kg b.w) plus HEPA (50, 100, and 200 mg/kg b.w/day respectively) by the oral route. After 60 days of the experiment, the crushed liver was performed to obtain homogenate. 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 measured weekly.

Clinical signs and behavioral alterations: As stated in previous reports, the salient features of pirimiphos-methyl toxicity include neurotoxicity [33]. There-

fore, 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).

2.7. Blood and Organ Collections

At the end of the treatments (8 weeks), blood was collected after sectioning the jugular vein of each bird. Serum was prepared and stored at −20˚C for subsequent analysis. After scarification of the quail by decapitation, liver was carefully removed, freed of adipose tissue, blotted dry and weighed separately. The fragment liver of each bird was then homogenized at 15% (weight/volume) of cold 0.9% NaCl followed by a centrifugation (3000 rpm, 30 min) and aliquots of supernatant were kept at −20˚C for biochemical analysis (Tchoffo *et al.* [34]).

2.8. Biochemical Analysis

All biochemical measurements (total proteins in the liver, total protein in the serum, cholesterol, AST, ALT, Urea and Creatinine) were determined using CHRONOLAB kit following the manufacturer's protocol. The levels of SOD and MDA and the activities of CAT and POD were assessed in liver homogenates using a spectrophotometer (GENESYS 20.0) and according to the methods described respectively by: [35] [36] [37] and [38].

2.9. Tissue Preparation and Histopathology

The same lobe of liver samples randomly selected from each treatment was fixed in Bouin's fluid for 1 week, embedded in paraffin, cut at 5 µm and stained with Harris haematoxylin and eosin. The tissue sections were observed under a light microscope (Leica DM 750, ×10 and ×40) for morphology and cellular integrity.

2.10. Statistical Analysis

Differences between groups were assessed using one-way ANOVA followed by Duncan post hoc test with the significance level set at 0.05. 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 21.0) and results expressed as mean ± standard deviation.

3. Results

3.1. Clinical Signs and Behavioural Alterations

The comparison of the clinical signs, subjectively evaluated is presented in **Table 1**. No clinical signs and behavioural changes were observed in animals of group 1 (0 mg AS/kg bw) and group 5 (75 mg AS/kg bw + 200 mg HEPA/kg bw). Depression, decreased attraction towards feed, weakness, anorexia, diarrhea and dizziness started at the 5th week in group 2 (75 mg AS/kg bw) and 3 (75 mg

AS/kg bw + 50 mg HEPA/kg bw). In addition at the 8th week, in group 2 (75 mg AS/kg bw), all the birds showed a degree of depression, decreased attraction towards food, weakness and anorexia week, while in group 3 (75 mg AS/kg bw + 50 mg HEPA/kg bw), half the birds (4/8) showed a mild degree of depression, decreased attraction towards food, weakness and anorexia.

3.2. Growth Parameters

The final body weight and body weight gain decreased ($p < 0.05$) in a dose-dependent manner. The opposite trend was recorded for the relative liver weight (Table 2). In the reference to the positive control (G2), the final body weight, body weight gain show significant ($p < 0.05$) increase in quails co-exposed to 75 mg of AS/kg bw and HEPA whatever the dose. Inversely, the relative weight of the liver decreased significantly significant ($p < 0.05$).

Table 1. Effects of different levels of HEPA on some qualitative clinical signs and behavioral of male Japanese quail.

Experiment time	Levels of HEPA (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)	G1	0	0	0	0	0	0
	G2	+1	+1	+1	+1	+1	+1
	G3	+1	+1	+1	+1	+1	+1
	G4	+1	0	+1	0	0	0
	G5	0	0	0	0	0	0
(5 weeks)	G1	0	0	0	0	0	0
	G2	+2	+1	+2	+1	+1	+1
	G3	+1	+1	+1	+1	+1	+1
	G4	0	0	0	0	0	0
	G5	0	0	0	0	0	0
(6 weeks)	G1	0	0	0	0	0	0
	G2	+2	+1	+2	+1	+2	+1
	G3	+2	+1	+1	+1	+1	+2
	G4	0	0	0	0	0	0
	G5	0	0	0	0	0	0
(8 weeks)	G1	0	0	0	0	0	0
	G2	+4	+4	+4	+4	+4	+3
	G3	+3 (4/8)	+2 (4/8)	+2 (5/8)	+3 (4/8)	+2 (4/8)	+2 (2/8)
	G4	0	+1	0	+1	0	0
	G5	0	0	0	0	0	0

Score from 0 to +4 denotes the severity of clinical signs (0: none, +1: very weak, +2: weak, +3: moderately and +4: severely);. n: number of animal; Group 1:10 ml/kg of distilled water only negative control group; Group 2: intoxicated birds receiving 75 mg of AS/kg b.w only positive control group; Group 3: intoxicated birds treated with 50 mg/kg b.w of HEPA; Group 4: intoxicated birds treated with 100 mg/kg b.w of HEPA; Group 5: intoxicated birds treated with 200 mg/kg b.w of HEPA.

3.3. Oxidative Stress Biomarker

As recorded in Table 3, oral administration of AS at 75 mg/kg b.w for 60 consecutive days caused a significant decrease in the levels of proteins in the liver, and the activities of SOD, CAT and POD, as compared to group 1. However, the co-administration of HEPA at different levels with 75 mg/kg b.w increased in a dose-dependent manner the values of all these oxidative stress parameters. The inverse was recorded for MDA concentration (Table 3).

3.4. Biochemical Parameters

The concentration of ALT, AST, Urea, Creatinine, protein and cholesterol in birds exposed to AS and treated with HEPA are reported in Table 4. Oral administration of AS at 75 mg/kg b.w induced a significant ($p < 0.05$) increase in serum ALT, AST, Urea, Creatinine concentration. The opposite trend was recorded for total protein and cholesterol concentration. In general, HEPA administration significantly ($p < 0.05$) decreased levels of hepato and nephrotoxicity markers. As compared to group 2; the inverse was observed with the total protein and cholesterol concentration.

3.5. Histological Analysis

Histological alteration of liver of control and treated quails are reported in Figure 1.

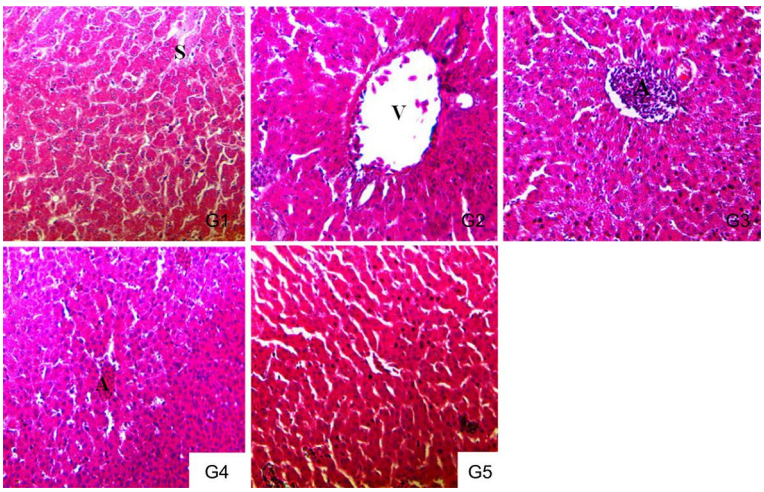

Figure 1. Histopathological alteration of liver of control and treated quails. 1) Liver section of control quail showing a normal hepatocytes. Notice the hepatic sinusoid (S) lined by endothelium (arrow head) and kupffer cells (H & E ×400); 2) Quail treatment with AS liver tissue section (75 mg of kg·bwt^{-1}) showing desquamation of the epithelial cells (arrows) of the bile ductless and focal aggregation of the lymphocytes around the branches of the hepatic artery (O) (H & E ×400); 3) Quail treatment liver tissue section (75 mg of AS kg·bwt^{-1} + 50 mg HEPA kg·bwt^{-1}), showing a number of hepatocytes undergo fatty changes (v), focal aggregation of the lymphocytes (arrow) and multiple necrotic changes (H & E ×400); 4) Quail treatment liver tissue section (75 mg of AS kg·bwt^{-1} + 100 mg HEPA kg·bwt^{-1}), focal aggregation of the lymphocytes (arrow) (H & E ×400); 5) Quail treatment liver tissue section (75 mg of AS kg·bwt^{-1} + 200 mg HEPA kg·bwt^{-1}), showing a slight desquamation (H & E ×400).

Table 2. Effects of different levels of HEPA on some growth parameters of male Japanese quail expose to AS.

Growth parameters	Controls		Doses of HEPA (mg/kg b.w)		
	G1 (n = 8)	G2 (n = 8)	50 (n = 8)	100 (n = 8)	200 (n = 8)
Total feed consumption (g)	2542.50	2308.71	2201.87	2349.25	2467.00
Initial body (g)	113.83 ± 4.45	113.83 ± 4.95	113.00 ± 3.35	113.17 ± 4.53	114.17 ± 2.48
Final body (g)	228.17 ± 2.85^{a}	187.17 ± 16.15^{d}	200.83 ± 10.36^{c}	211.50 ± 13.38^{bc}	219.00 ± 7.92^{ab}
Body gain (g)	110.64 ± 14.29^{a}	71.00 ± 14.95^{c}	87.83 ± 10.96^{b}	98.33 ± 17.00^{ab}	103.33 ± 8.89^{ab}
Relative liver weight (%)	1.19 ± 0.12^{b}	1.45 ± 0.19^{a}	1.54 ± 0.27^{a}	1.13 ± 0.15^{b}	1.15 ± 0.20^{b}

n = number of animal, ^{a,b,c,d}Means bearing different letters in a row differ significantly at $p < 0.05$, Group 1: 10 ml/kg of distilled water only negative control group; Group 2: intoxicated birds receiving 75 mg of AS/kg b.w only positive control group.

Table 3. Effects of different levels of HEPA on some oxidatives stress parameters of male Japanese quail.

Oxidative stress parameters in the liver	Controls		Doses of HEPA (mg/kg b.w)		
	G1 (n = 8)	G2 (n = 8)	50 (n = 8)	100 (n = 8)	200 (n = 8)
Liver protein (mg/ml)	9.70 ± 0.43^{a}	6.68 ± 0.47^{c}	6.92 ± 0.52^{c}	6.96 ± 0.17^{c}	8.65 ± 0.71^{b}
MDA (nmole/mg tissues)	12.19 ± 1.94^{d}	22.17 ± 0.41^{a}	18.20 ± 1.21^{c}	20.18 ± 0.99^{b}	12.19 ± 0.90^{d}
SOD (UI/mg tissues)	23.22 ± 1.11^{a}	12.47 ± 0.87^{d}	12.72 ± 0.56^{cd}	14.20 ± 2.37^{bc}	15.12 ± 1.05^{b}
CAT (UI/mg tissues)	7.02 ± 0.32^{a}	5.25 ± 0.12^{c}	5.52 ± 0.39^{c}	6.11 ± 0.55^{b}	6.04 ± 0.1^{b}
POD (μM/mg tissues)	19.37 ± 0.402^{a}	13.28 ± 0.33^{d}	14.21 ± 0.31^{c}	17.00 ± 0.49^{b}	13.67 ± 0.32^{d}

n = number of animal, ^{a,b,c,d}Means bearing different letters in a row differ significantly at $p < 0.05$, Group 1: 10 ml/kg of distilled water only negative control group; Group 2: intoxicated birds receiving 75 mg of AS/kg b.w only positive control group.

Table 4. Effects of different levels of HEPA on some blood parameters of male Japanese quail expose to AS (means ± SE).

Blood parameters	Controls		Doses of HEPA (mg/kg b.w)		
	G1 (n = 8)	G2 (n = 8)	50 (n = 8)	100 (n = 8)	200 (n = 8)
Total proteins (g/dl)	4.41 ± 0.23^{a}	2.26 ± 0.11^{d}	2.6 ± 0.07^{c}	3.19 ± 0.11^{b}	2.55 ± 0.15^{c}
Total cholesterol (mg/dl)	231.76 ± 22.16^{a}	102.84 ± 9.63^{d}	122.17 ± 3.67^{c}	148.88 ± 3.47^{b}	130.85 ± 2.19^{c}
AST (IU/l)	162.94 ± 2.47^{c}	215.74 ± 7.88^{a}	185.19 ± 3.61^{b}	168.17 ± 7.57^{c}	179.64 ± 5.36^{b}
ALT (IU/l)	25.80 ± 2.13^{c}	40.61 ± 1.21^{a}	39.93 ± 1.23^{a}	39.41 ± 1.88^{a}	32.03 ± 3.80^{b}
Urea (mg/dl)	10.70 ± 0.82^{c}	16.46 ± 1.02^{a}	15.85 ± 1.01^{a}	13.60 ± 0.85^{b}	14.10 ± 2.18^{b}
Creatinine (mg/dl)	0.68 ± 0.10^{c}	1.30 ± 0.93^{a}	1.08 ± 0.14^{b}	0.74 ± 0.14^{c}	0.97 ± 0.12^{b}

n = number of animal, ^{a,b,c,d}Means bearing different letters in a row differ significantly at $p < 0.05$, Group 1: 10 ml/kg of distilled water only negative control group; Group 2: intoxicated birds receiving 75 mg of AS/kg b.w only positive control group.

4. Discussion

The present study revealed that the oral daily administration of AS at the dose of 75 mg/kg bw generated depression, anorexia, diarrhea and dizziness. Similar results were observed by Prakash *et al.* [39] 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 [40]. A significant decrease in body weight, body weight gain and liver protein was observed in the AS treated groups. This decrease might be associated firstly to the toxic symptoms, such as cholinergic signs and secondly to the decreased feed consumption. This hypophagia may be related to the effects of the active principles (AS) on the central structures involved in the control of feed intake. It could then be suggested that AS may have inhibited this center thus decreasing the feed intake and consequently the body weight gain observed in the work. The reduction of body weight, body weight gain and liver protein could be attributed to systemic toxicity in Japanese quail. Correlation between decreased AChE activity and increased MDA concentration has been previously reported [41].

This study has shown that AS increased the concentration of MDA in liver organ. MDA is a byproduct of lipidperoxidation resulting from interaction of oxygen radicals with polyunsaturated fatty acids residues in membrane phospholipids that damages important biomolecular Naudi *et al.* [42]. Oxidative damages have been reported to be a key factor in the subcellular damage resulting from pesticide exposure [43]. Thus the high MDA contents in the liver in the AS group are indications of the level of lipoperoxidative changes, reflecting alteration in the structural and, consequently functional status of the organs. Furthermore, increase in lipid peroxidation might have resulted from failure of internal antioxidant system of the body to counteract the ROS being generated [44] as a result of exposure to AS and its ability to penetrate the blood-brain barrier [45]. The increase in MDA concentration in the liver indicates the participation of free radical-induced damage to the organ and this may be responsible for the decrease in the concentration of SOD, CAT and POD, observed in the AS treated group. The lipoperoxidative damage of the liver of the AS group may have altered its structural integrity and functional status consequently affecting the synthesis of these enzymes.

After 60 days, the quail developed significant hepatic damage, with changes in serum levels of ALT and AST, as well as altered concentrations of urea and creatinine, indicative of hepatic and renal damage. These alterations could have been related to lower levels of SOD, CAT and POD, observed in the AS treated group and increased lipoperoxidation. The liver is the main organ involved in the bio-

transformation of xenobiotics, and is therefore the site of multiple oxidative reactions, with free radical formation [46]. Increase in the levels of serum aminotransferases is known to reflect the severity of liver injury [47]. The leakage of large quantities of enzymes into the blood stream is associated with massive centrilobular necrosis, ballooning degeneration and cellular infiltration of the liver. Serum AST level is related to the function of the hepatic cell and increase in serum level of ALT is due to increased synthesis of this enzyme [48]. The increase in the transaminases is a clear indication of cellular leakage and loss of functional integrity of the membrane resulting from liver damage [49]. The underlying mechanism by which this insecticide exerts their negative effects may be attributed to the production of ROS.

This study demonstrated that pre-treatment of quail with HEPA caused substantial decreases the clinical signs, behavioural alterations, AST and ALT levels at extract concentration of 200 $mg \cdot kg^{-1} \cdot bw$. Effective controls of AST level and ALT activity point towards an early improvement in the secretory mechanism of the hepatic cell [50]. The significant reduction in liver enzymes after pre-treatment with HEPA suggests that the extract is hepato-protective.

The histopathological studies in the liver of quail also showed that HEPA reduced the toxicity of AS and preserved the normal histological architecture of the liver tissue. Furthermore, HEPA treatment resulted in a decrease in the number of apoptotic cells. HEPA significantly suppressed lipid peroxidation, compensated deficits in the antioxidant defenses in liver tissue that resulted from AS administration. They suggested that the hepato-protective potential of HEPA in AS toxicity might be due to its antioxidant and anti-apoptotic properties, which could be useful for achieving optimum effects in AS-induced hepatotoxicity.

The decrease in the serum transaminases levels observed in current study provided supportive evidence that pre-treatment with HEPA reduced the severity of toxic injuries caused by AS administration. The reduction in the severity of necrosis and fatty infiltration observed in molecular architecture also showed that HEPA has hepato-protective activity against AS induced damage in these quails. The observed hepato-protection by HEPA suggests that the extract tends to prevent liver damage by preserving hepatocyte membranes thereby, suppressing the leakage of enzymes into the blood stream. The hepato-protective activity of HEPA is similar to the hepato-protective activity against CCl4 exhibited by *Acalypha racemosa* [51], *Vernonia amygdalina* [52] and *Rumex crispus* [53].

Elevation in the levels of end products of lipid peroxidation in the liver of quails treated with AS was observed. The increases in MDA and decrease protein levels in these quails livers suggest occurrence of lipid peroxidation. This observation concord to earlier reports that there is an increase in MDA in liver of rats treated with CCl4 which is attributed to enhanced lipid peroxidation, leading to tissue damage and failure of antioxidant defense mechanisms to prevent the

formation of excessive free radicals [54] [55]. Pre-treatment with HEPA decreased MDA concentrations and significantly increase protein levels. Thus, suggesting that the mechanism of hepato-protection of HEPA may be attributed to its antioxidant effect and free radical scavenging activity. Hence, eliminating deleterious effects of toxic metabolites from AS and inducing liver cell regeneration. It is possible that lipid peroxides generated by AS treatment may be scavenged by the extract resulting in depression of lipid peroxidation in the liver. The antioxidant and free radical scavenging activity of HEPA could be due to its constituent flavonoids and phenolic compounds Arukwe *et al.* [56]. Flavonoids are known to be antioxidants, free radical scavengers and anti-lipoperoxidants which cause hepato-protection [57] [58] [59]. The decreased enzymes activities of SOD, CAT and POD in AS-intoxicated quails agree with the findings of [60]. The decrease in enzymes activities in the liver observed in this study was probably in response to increased reactive oxygen species generation induced by AS administration. Similarly, CCl4 may cause oxidative stress and the consequent up-regulation of antioxidant enzymes to render cells more resistant to subsequent oxidative damage [61]. It is known that under oxidative stress some endogenous antioxidant protective factors such as SOD and CAT are activated in the defense against oxidative injury [62] [63].

In this study, pre-treatment with HEPA increased the activities of CAT, SOD and POD that were raised by AS-intoxication. The extract may have scavenged the free radicals generated thereby decreasing lipid peroxidation and oxidative stress in the quail. These results showed that HEPA possesses significant protective effects against AS-induced hepatotoxicity in quail and the hepato-protection appears to be dose dependent. The mechanism of the hepato-protection seems to involve the modulation of the antioxidant enzyme systems. These beneficial effects may be attributed to the individual or combined action of the phyto-constituents present in the extract such as polyphenols and flavonoids.

5. Conclusion

Based on the present study, it can be concluded that HEPA improve the hepatic alterations induced by AS intoxication. The antioxidant properties of these extracts support the bioactive roles of their protective effects on AS toxicity. Therefore, it is pertinent to further determine, isolate and purify the exact bioactive constituents with the potential hepato-protective property.

Acknowledgements

The authors are thankful to Dschang University, Cameroon for providing research facilities for undertaking this work.

Author Contribution Statement

Ngoumtsop Victor Herman: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed rea-

gents, materials, analysis tools or data; Wrote the paper.

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

Guiekep Nounamou Arthénice Jemima and Tchoffo Herve: Contributed reagents, materials, analysis tools or data; wrote the paper.

Mutwedu Bwana Valence: Contributed reagents, materials, analysis tools or data.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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