Journal of Agriculture and Ecology Research International
4(1): 1-9, 2015; Article no.JAERI.2015.046
ISSN: 2394-1073

SCIENCEDOMAIN *international*
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Effect of Carbendazim Exposure and Vitamin E Supplementation in African Giant Rats

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Authors' contributions

This work was carried out in collaboration between both authors. Author AOO designed the study, wrote the protocol and wrote the first draft of the manuscript. Author TAJ managed the literature searches, analyses of the study performed the structural equation modelling and discuss the conclusion. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAERI/2015/16715
Editor(s):
(1) Anonymous.
Reviewers:
(1) Myrene Roselyn D'souza, Mount Carmel College, Bangalore, India.
(2) Anonymous, Pakistan.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=1186&id=37&aid=9112

Original Research Article

Received 11th February 2015
Accepted 7th April 2015
Published 4th May 2015

ABSTRACT

Aim: Carbendazim is a broad spectrum benzimidazole and metabolite of benomyl used to control farm pests. Unregulated use of pesticides inadvertently affects the ecosystem and loss of biodiversity with precipitous decline of some wildlife species. We investigated the toxicity of carbendazim in African giant rat AGR on exposure and the ameliorative effect of tocopherol (vitamin E).
Methodology: The AGR were randomly divided into four experimental groups of 4 animals each. Group A was exposed to carbendazim only; B- carbendazim + vitamin; C- vitamin only and D- blank (control). Assessment was done clinically, microhaematocrit (erythrogram) and hemocytometric (Leucogram) methods. Cholinesterase (AchE) and markers of oxidative stress were quantified, and tissue changes examined microscopically.
Results: There was decrease in body weight of the AGRs, abortion after 23 days of exposure to carbendazim and significant changes in the erythrogram ($p<0.05$). Malondialdehyde MDA increased significantly ($p<0.05$) in group A. Vitamin E supplementation reduced MDA level

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significantly ($p<0.05$). Also, remarkable decrease in acetylcholinesterases in the pesticide intoxicated rats ($p<0.05$). The liver showed diffuse hepatocellular degeneration in carbendazim exposed group but which was comparatively reduced in the Vitamin E "supplementated" AGR.
Conclusion: Strict measures are needed to monitor intensive agricultural practices in the use of chemicals for pest control.

Keywords: African giant rat; antioxidant; carbendazim; pesticides; toxicity.

1. INTRODUCTION

In many parts of the world dramatic changes in farming practices have occurred. Not only have farms grown larger but there is also an increase in specialization in agriculture with the efficient use of costly farm machine, fertilizers, and irrigation and soil improvement techniques [1]. The resultant system of intensive agriculture has less use for crop rotation which is known to be a useful way of controlling pest, in view of this, there is an increase in the use of pesticides thereby increasing the release of chemical toxicant into the environment which could cause devastating health effects such as endocrine disruption, carcinogenicity, mutagenic effect and also cause ecosystem degradation by contamination along food chain and eventually loss of biodiversity [2,3].

Wild animals have been used as a resource throughout the course of human existence [4]. Wildlife resources play conspicuous role in the rural as well as national economics of tropical Africa [5]. The populations of numerous wildlife species have undergone a precipitous decline in the past century, resulting in significant reduction for many and extinction or near - extinction of others [6]. Many of these problems have been attributed directly to habitat loss and over exploitation resulting majorly from increasing human activities.

African giant rat AGR belongs to the family Nesomyidea, they are also referred to as pouched rat because of their large cheek pouches [7]. They are one of the wild rodent of the genus Cricetomys growing up to 0.9 meters including their tail which makes up half of their body length [8]. They are found inhabiting different places in Africa in the savanna around the edges of forest and in mountain up to 3500 m above sea level. The AGR is a solitary animal, with nocturnal habits and prefers to live in burrows. It feeds on large varieties of plant and animal foods, such as tubers, grains and nuts [9]. The continued globalization, human population growth and associated landscape changes due to habitat loss and other human induced landscape changes further enhances the interface between wildlife and human thereby facilitating additional disease emergence [10]. There are many sources of environmental toxicity, organic or inorganic like the use of pesticides which include insecticides, fungicides, rodenticides and herbicides [11]. Benzimidazole fungicides are broad spectrum fungicides, used for pre and post harvest treatment and for control of a wide range of pathogens [12].They readily penetrate plants through the roots and leaves and can directly enter natural water by drainage. Most of these compounds persist in the environment after application some even remain for years. Carbendazim is a widely used broad spectrum benzimidazole fungicides and a metabolite of benomyl. It is used to control plant disease in cereals and fruit [13]. Studies have shown that high doses of carbendazim cause infertility and destroy the testicles of laboratory animals [14]. It is of major concern due to its suspected hormone disrupting effect and action by inhibiting the development of spindle formation at mitosis [12].

The process of oxidation damages cell membranes and other structures including cellular protein, lipids and DNA. However, reactive oxygen species formation can increase in environmental stress [15,16]. Oxidation can be accelerated by stress, sunlight, pollution and other factors [17]. Anti-oxidants inhibit the oxidation of other molecules or neutralizes the effect of free radicals. Tocopherol, a class of organic chemical compounds is fat soluble anti-oxidant and also noted to have many other functions in the body [18,19]. Five tocopherols are identified by their prefixes as alpha, beta, gamma, delta and epsilon. Alpha tocopherol is an important lipid soluble anti oxidant in the glutathione peroxide pathway and it protects cell membrane from oxidation by reacting with radicals produced in the lipid peroxidation chain reaction.

In assessing the general health condition of animals, biomedical changes, histological and

hematological changes can be used to determine the health condition of a particular organism [20,21]. In this study, the effect of carbendazim exposure and the ameliorative effect of tocopherol (vitamin E) were assessed on African giant rat.

2. MATERIALS AND METHODS

2.1 Study Animals

The African giant rats (*Cricetomys gambianus*) were purchased from a local market in Ibadan, Oyo state, Nigeria. A total of sixteen animals were purchased. The animals were housed in the Experimental animal house of the Faculty of Veterinary medicine, University of Ibadan and stabilized for 2 weeks. During stabilization, vitalite was added in their water for the first three days in order to reduce the effect of stress, after which they were dewormed using albendazole (shanuzole®). The animals were fed with compounded concentrate (Crude protein 14%, Fat 7%, Crude fibre 10%, Calcium 1%, Available phosphorus 0.35%, Metabolisable energy 2550 kcal/kg) and clean water (20 cl) was supplied ad-libitum. The experiment protocol complied with the ethical guidelines of the University of Ibadan.

2.2 Source of Pesticide

The pesticide used was forcelet, a preventive, and synthetic agricultural fungicide which contains 50% carbendazim, belonging to the toxicity class IV.

2.3 Antioxidant (vitamin E)

Vitamin E of the brand name Eviol, di- alpha-tocopheryl acetate manufactured in Greece by GAP SA was used. Each tablet contains 100 mg of tocopherol. Dosage used was 25 mg/kg of the animal for 14 days (pre-treatment).

After stabilization, the animals were weighed and were then divided into four experimental groups (A to D) comprising four animals each. Two groups were pretreated with vitamin. Thus, group A was given carbendazim only; B- carbendazim + vitamin; C- vitamin only and D- blank (control).

2.4 Sampling

After four weeks of exposure all the rats were weighed using sensitive weighing scale (AMW-PN-201C) and blood samples were collected for analysis accordingly.

The rats were sedated with 0.2 ml Ketamine Chloride administered intra-peritoneally. 2 ml of blood was collected from peri-orbital sinus into lithium heparinised bottles for hematology and blank bottles for serum needed for antioxidant assay. This was aliquot into Eppendorf tubes using Pasteur pipette and stored at -20°C until analyzed). Afterwards, six rats were necropsied (2 from each group) for gross and microscopic tissue examination.

2.5 Hematologic, Cholinesterase, Oxidative assays

The Packed cell volume PCV, Hemoglobin concentration, Red cell count was estimated by the microhaematocrit method, Leucocyte and Platelet counts were determined using the haemocytometric method as described by Schalm [22].

Cholinesterase (AchE) was quantified as Butyryl Cholinesterase activity using an *In vitro* diagnostic kit (Fortress Diagnostics, BXC0801), following manual procedure. In brief, 1000 µl of the working reagent and 20 µl of the sample were pipetted into the test tube, mixed and initial absorbance read after 90 seconds incubation at the assay temperature. Similarly, 20 µl of the sample was incubated with 1000 µl of buffer and 200 µl of substrate before reading absorbance activity. Wavelength was set at 405 nm, 37°C and 1cm cuvette light path.

Activity= ΔAbs/min X 55000 [serum start]
Activity=ΔAbs/min X 65800 [substrate start]

The determination of Malondialdehyde (MDA) was carried out using the thiobarbituric acid (TBA) test as described by Vidyasagar et al. (2004). The concentration of MDA was read from standard calibration curve in nanomoles of MDA per ml of serum.

Concentration of MDA= absorbance at 540 nm/1.56x10 5 $m^{-1}cm^{-1}$

Superoxide Dismutase (SOD) activity was estimated using the method of Misra & Fridovich [23]. In brief, 1 ml of serum was diluted in 9 ml of distilled water. Aliquot of 0.2 ml was added to 2.5 ml of 0.05 M phosphate buffer at pH 10.2 to equilibrate in the spectrophotometer. The reaction was started by the addition of freshly prepared 0.3 ml of adrenaline. Reference cuvette contained 2.5 ml of phosphate buffer, 0.3 ml of substrate (adrenaline) and 0.2 ml of water. The

increase in absorbance was monitored every 30 second for 150 seconds and expressed in Unit/mg protein.

%Inhibition=

increase in absorbance for substrate X 100/increase in absorbance of blank

Catalase (CAT) activity was estimated using method of Sinha [24]. Briefly, dichromate was reduced to chromic acetate when heated in the presence of hydrogen peroxide (H_2O_2). Chromic acetate produced was measured colorimetrically at 570 nm. Result was expressed in µmol/mg protein.

2.6 Pathology

Samples from the liver, kidney and brain were processed routinely for histopathology. Slides were evaluated using the Olympus light microscope (CX21) attached to a digital computerized camera (AmScope, MU900).

2.7 Statistical Analysis

The data were presented in Mean±SEM, subjected to one-way ANOVA, and followed by Bonferoni test for multiple comparisons using SPSS 16. P values <0.05 were considered significant.

3. RESULTS

There were no behavioral changes observed in the animals, but there was a decrease in body weight of the animals in the group A and B. There were no changes in the external morphological features of the animals. During the period of exposure, there was delivery of a malformed feotus by one of the animals in group A after 23 days of exposure to carbendazim.

The hematological parameters of the animals after four weeks of exposures shows the values of the packed cell volume, hemoglobin count, red blood cell count, white blood cell count, the platelet count, the absolute values of the lymphocytes, the neutrophils, eosinophils and the mean cell volume (MCV) and the mean cell hemoglobin concentration (MCHC) among the groups (Table 1).

There was significant differences in the packed cell volume, the hemoglobin concentration and the red blood cell counts ($p<0.05$).

The increases in Malondialdehyde (MDA) was significant ($p<0.05$) in the pesticide intoxicated rats compared to control. Vitamin E supplementation reduced MDA level significantly ($p<0.05$).

There was a sharp remarkable decrease in acetylcholinesterase levels in the pesticide intoxicated rats ($p<0.05$). Vitamin E supplementation normalized the AchE levels comparable to that in control (Table 2).

Pesticide intoxication reduced superoxide dismutase (SOD) activity in the exposed rats ($p<0.05$). Supplementation with Vitamin E later increased SOD activity in the rats ($p<0.05$). Carbendazim also reduced catalase activity.

Grossly, the vital organs appeared normal in the pesticide exposed and control groups except moderate pulmonary congestion. Histopathologically, there was severe diffuse hepatocellular swelling in carbendazim exposed group (Plate A & B). The severity of hepatocellular injury was reduced in the rats with vitamin E. Pulmonary congestion in rats exposed to carbendazim (Plate C) and normal lungs in control (Plate D). There was also neuronal necrosis and demyelination in neuropil of rats exposed to carbendazim (Plate E).

4. DISCUSSION

This study ascertained the fact that carbendazim exposure has effect on the values of hematological parameters of African giant rat. Carbendazim has been observed by various researchers to have a wide range of effect on variety of species ranging from invertebrates like annelids, mollusks, crusteceans, nematodes and vertebrates. Changes in the body weight agrees with the observation [14,25] that exposure of carbendazim to Wister rat for 30 days led to a decrease in body weight of the animals.

Carbendazim caused foetal abortion, this was also observed by Culik et al. [26], who reported carbendazim induced reproductive disorder in adult male, female and the foetus, his result showed that the foetus from pregnant rat exposed to carbendazim showed a lot of abnormalities from skeletal defect, visceral and soft skin and tissue malformation to dead foetus.

Table 1. The hematological parameters of AGR exposed and non-exposed to CBZ and Vit E

Group	PCV	HB	RBC	WBC	PLT	LYM	NEUT	MON	EOS	MCV	MCHC
A	34.0±1.0*	11.4±0.2*	5.02±0.32	6.05±0.20	1.03±0.03	4.37±0.25	1.47±0.47	0.12±0.1	0.1±0.0	50.2±20.0	33.5±0.4
B	29.75±4.33*	9.78±1.49*	4.74±0.76*	5.19±0.36	0.89±0.01	3.64±0.28	1.32±0.16	0.8±0.0	0.14±0.0	63.1±2.2	32.8±0.5
C	45.75±1.59	15.45±0.63	7.82±0.32	5.15±0.52	0.88±0.12	3.52±0.47	1.44±0.23	0.11±0.0	0.08±0.0	58.6±0.9	33.8±0.1
D	44.0±0.58*	14.73±0.29*	7.50±0.32*	6.18±1.61	2.64±1.38	4.03±0.98	1.96±0.64	0.11±0.0	0.08±0.1	58.7±0.5	33.5±0.3

**Values with superscript are significant at 0.05; Packed Cell Volume- PCV (%), Haemoglobin Concentration-Hb (g/dl), Red Blood Cell-RBC (*10^3/µL), White Blood Cell-WBC (*10^3/µL), Platelet Count-Plate (*10^5/ µL), Lymphocytes-Lympho, Neutrophils-Neutro, Monocytes-Mono, Eosinophils-Esino, Mean Cell Volume-MCV (fl), Mean Cell Haemoglobin Concentration-MCHC (pg)*

Table 2. The biochemical parameters of AGR exposed and non-exposed to CBZ and Vit E

Group	MDA	CAT	S0D	CHOLIN
A	24.7±0.19*	1.90±1.29*	2.60±0.6*	4.01±0.92*
B	19.95±15.46	2.16±0.84	2.68±0.11	6.10±2.0
C	11.96±20.02*	3.45±1.00	2.95±0.34	9.08±1.09
D	3.96±1.43*	3.36±0.25*	2.77±0.33*	9.71±6.46*

*AchE (CHOLIN)- Acetylcholinesteerase; MDA- Malonyl aldehyde; SOD- Superoxide dismutase; CAT- Catalase.. Values with different superscripts in row are statistically different and * are significant at 0.05*

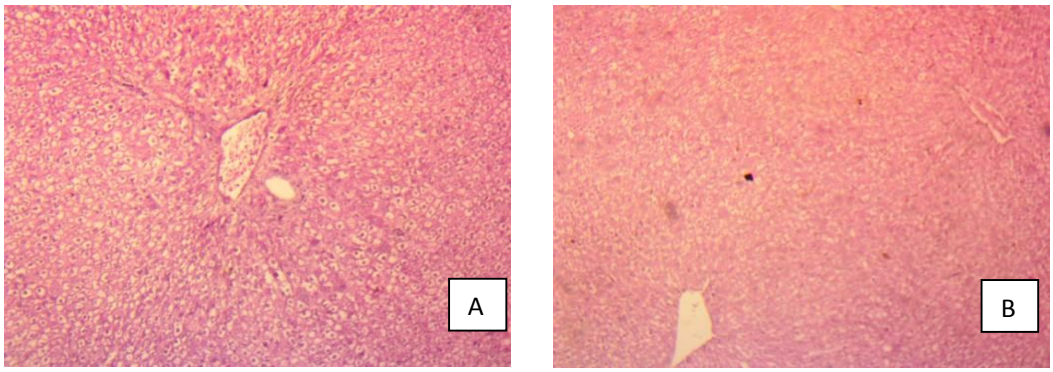

Plate A & B. Photomicrograph of the liver- A. Hepatocellular degeneration in carbendazim exposed rats. B. Normal hepatocytes in control. HE x100

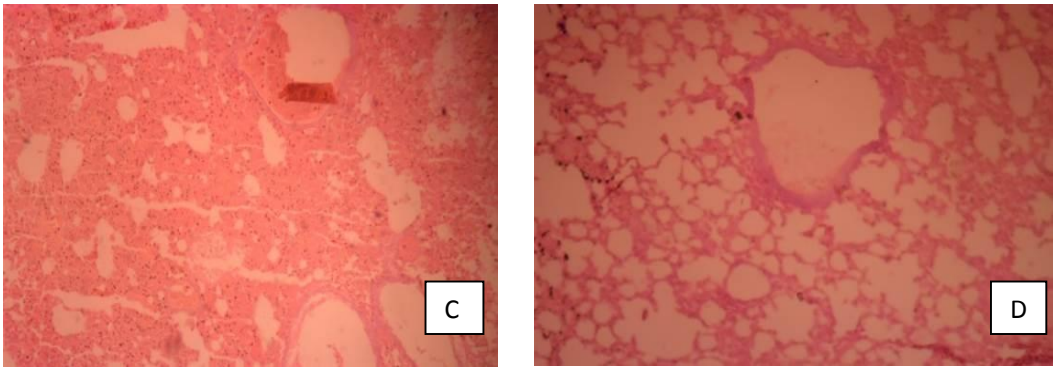

Plate C & D. Photomicrograph of the lungs- C. Pulmonary congestion in rats exposed to carbendazim. D. normal alveoli in control HE x100

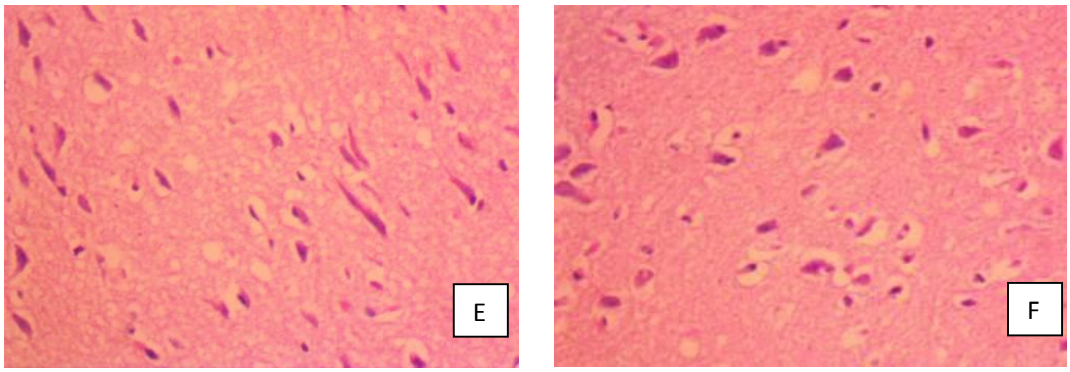

Plate E & F. Photomicrograph of the brain- A. neuronal necrosis and demyelination in rat exposed to carbendazim. B. Normal neurons in control. HE x100

This study has ascertained the fact that carbendazim has been showed to have effect on the values of hematological parameter of Africa giant rat, specifically in having reductive effect in the packed cell volume, the hemoglobin concentration and red blood cell count. Although studies have shown that it reduces the white blood cell count, lymphocytes count and the neutrophils count but from this study the white blood cell count and the lymphocytes count did not seem to be affected by carbendazim exposure as the mean values obtained from the white blood cell count and the lymphocytes count for all the groups agrees with the values given by

Oyewale et al. [27]. Oxidative damaging effect of carbendazim on the reproductive ability of the affected animal has been confirmed by this work as was observed in the aborted foetus in one of the animals exposed to carbendazim, the effect of carbendazim on the platelet count can't be really ascertained as no values of the standard platelet count has been recorded for Africa giant rat. Carbendazim has been shown to have a reduction in the mean cell volume as the mean value obtained for the CBZ groups is lower than the values given by Oyewale et al. [27], Olayemi et al. [28] and Kelani & Durotoye [29], this condition can be said to be a microcytic anemia which could be due to the reduction in the red blood cell count. It was also observed that tocopherol itself as a supplement can enhance the quality of the blood parameter as seen in the increase in the blood packed cell volume, hemoglobin concentration and the red blood cell count for the Vit E group, but the effect of tocopherol as an effective antioxidant against carbendazim exposed animal is not ascertained in this work as the CBZ/Vit E group and Vit E group seems to have the lowest mean value of the packed cell volume, the hemoglobin concentration and the red blood cell count , this could be attributed either to low concentration of the tocopherol administered in the work which could not effectively inhibit the production of the reactive oxygen species or that the tocopherol concentration used for the work is said to be high therefore suppressing or inhibiting the production of reactive oxygen species needed for the normal functioning of the blood thereby leading to oxidative stress, this reason could also be explained for the packed cell volume and the red blood cell count. This work also disagrees with the report of Scholz & Schultes [30] that carbendazim causes leucopenia in animals as the values obtained for the CBZ group and the control group tend to be similar for almost all the types of white blood cells were higher in the CBZ groups than other groups .

5. CONCLUSION

In conclusion, this study has ascertained the toxic effect of carbendazim to Africa giant rat. Exposure of Africa giant rat to carbendazim, a systemic and acute fungicide inhibits the production of microtubules in the mitotic formation in the cell. It has an effect in the hematological profile of the animal, especially in the packed cell volume, the hemoglobin concentration and the red blood cell count in African giant rat. Furthermore, the antioxidant effect of tocopherol was evident on the erythrocyte of the blood component increasing the packed cell volume, the hemoglobin concentration and the red blood cell count of the of Africa giant rat but has little or no effect on the leucocytes components of the blood. The ameliorative effect of tocopherol on the giant rat exposed to carbendazim was effective probably as suppressing the production of the reactive oxygen species.

As more intensive form of agricultural practices tend to increase, leading to a more mechanized form of farming and the use of chemicals to reduce pest and increase yield, further studies should be carried out on the effect of carbendazim on the hematological parameter at higher concentration especially, the platelet and the leucocytes component and lymphocytes. Also studies should be furthered in the area of the ameliorative effect of tocopherol on Africa giant rat exposed to carbendazim to get a concentration that can give a balance between the production of reactive oxygen species and the body homeostatic balance.

ACKNOWLEDGEMENTS

We appreciate efforts of Ugo Angela .I. during experimentation of this work and B.O. Emikpe for technical support.

CONFLICTING INTERESTS

Authors have declared that no conflicting interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=1186&id=37&aid=9112