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Research Article

Cytoprotection by Extract *of Khaya senegalensis* Stem Bark on Rats Fed Pesticide-Infused Feed

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ABSTRACT

Cytoprotection by aqueous extract of *Khaya Senegalensis* stem bark on rats fed pesticide-infused feed was investigated. Induction of tissues injuries was done by feeding rats with cypermethrin-infused feed at a dose of 300mgkg^{-1} feed for 6 weeks. Different groups of animals were co- and post-treated with 200mgkg^{-1} body weight (bwt) of plant extract orally. The extract control group received 200mgkg^{-1} bwt extract while the pesticide control animals were fed with cypermethrin-infused feed. Investigation showed that daily oral dose of aqueous extract of *Khaya senegalensis* stem-bark administered produced significant ($p<0.05$) protection by decreasing the severity of cypermethrin-mediate tissues architectural damage. However, treatment with same extract dose failed to reverse alteration induced prior to its (extract) administration. This study, therefore confirmed that cypermethrin-induced tissues deformation as part of its mechanism of it toxic action in the body, and this can be protected by extract of *Khaya senegalensis* stem-bark.

Keywords: Cytoprotection, *Khaya senegalensis*-extract, Rat-tissues, Pesticides-infused feed.

INTRODUCTION

The widespread use of pesticide in public health and agriculture programs has caused severe environmental pollution and health hazards including cases of severe acute and chronic human poisoning. These toxic chemicals have become an integral part of the ecosystem, although many of them are extremely toxic to mammals and other non-target creatures.

The term "pesticide" is a general name of compound that are used to kill pests. They include compounds labelled as insecticide (organophosphates, organochlorines, and pyrethroids), rodenticides (anticoagulants), fungicides (captan), and fumigants (methybromide) (Ellehorn *et al*., 1997). Several studies have indicated that pesticides induce oxidative stress. Traces of these are evident in several organs, tissues, and cells such as liver, brain, kidney, and erythrocytes (Gupta *et al*., 1999; Kale *et al*., 1999; Arun *et al*., 2009). The induction of oxidative stress by pesticides is attributed by alteration of antioxidant system via lipid peroxidation (Uner *et al*., 2001).

Cypermethrin is a synthetic pyrethroid insecticide used to control many pest species in agriculture, animal breeding and the household. It is widely used to control insect pests particularly leaf and fruits eating *lepidoptera, coleopteran;* cattle ecto parasites, sheep scab, and lice. It is also used against disease vector insects in limited areas. Cypermethrin is a mixture of eight different isomers, each of which may have its own chemical and biological properties. Cypermethrin is light stable and is available as emulsifiable concentrate or wettable powder (EXTOXNET, 1996). It is chemically named as: Alpha-cyano-3-(phenoxyphenyl)-methyl-cis, trans-3-(2, 2-dichlorovinyl)-2, 2-dimethyl cyclopropane carboxylate (Kidd *et al*., 1991), with a molecular formula: $C_{22}H_{19}C_{l2}NO_3$.

Several studies have indicated that cypermethrin toxicity is associated with free radical generation leading to the induction of oxidative stress (Abdollahi *et al*., 2004; Sharma *et al*., 2005). Toxicity of cypermethrin have been ascribed to induced cellular damage in tissue such as; liver, kidney and the brain as part of its mechanism of toxic action in the body (Ferah *et al*., 2005; Altug *et al*., 2006; Inayat *et al*., 2007).

Khaya senegalensis (Desr) A. Juss otherwise known as dry zone Mahogany belongs to the family *Meliaceace*. The plant is well known in European and West African countries. In Nigeria, it is called "*Madachi*" in Hausa, "*Ojonwo*" in Yoruba, and "*Ono*" in Igbo. The plant is highly used in trado-medicinal practice, and has been reported to have some pharmacological properties. Marius *et al* (2007) and Atawodi *et al* (2009) have confirmed that *Khaya senegalensis* extract possess some antioxidant properties, and their findings has trigger the search for antidote from plant against pesticide-induced tissue alterations.

MATERIALS AND METHODS

Materials

Chemicals: Cypermethrin (Best) [African Agrochemical Ltd, Kano Nigeria], Eosin and Heamatoxylin (Philip Harris Biological Ltd, Weston-super Mare), Microheamatocrit centrifuge (Gelman Hawksley Ltd, England) among others were used in the study.

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Table 1: Percentage body weight gain following oral administration of aqueous extract of *Khaya senegalensis* stem bark in Cypermethrin-intoxicated rats (Mean± SEM).

BODY WEIGHTS	Normal Control	Extract Control	Pesticide Control	Co-treated group	Post-treated group
INITIAL WEIGHT(g)	197.38±1.36	208.88±1.75	214.5±1.86	220.38±1.08	216.75±1.35
FINAL WEIGHT(g)	234.25±0.85	240.71±1.39	220.5±1.30	242.85±1.01	253.75±1.80
Percentage GAINED	15.73±0.06^{a}	13.22±0.27^{ab}	2.72±0.43^{c}	9.25±0.07^{abc}	3.99±0.24^{c}

Values with different superscript(s) along the row are significantly different (p<0.05).

Table 2: Relative organ's weight following oral administration of aqueous extract of *Khaya senegalensis* stem bark in Cypermethrin-intoxicated rats (Mean± SEM).

ORGANS	Normal Control	Extract Control	Pesticide Control	Co-treated group	Post-treated group
Liver (x 10^{-2})	0.06^{a}	0.04^{a}	0.13^{a}	0.12^{a}	0.17^{a}
Kidney (x 10^{-3})	0.11^{b}	0.27^{b}	0.16^{b}	0.14^{b}	0.13^{b}
Spleen (x 10^{-3})	0.23^{c}	0.24^{c}	0.27^{a}	0.33^{c}	0.05^{c}

Values with different superscript along the row are significantly different (p<0.05).

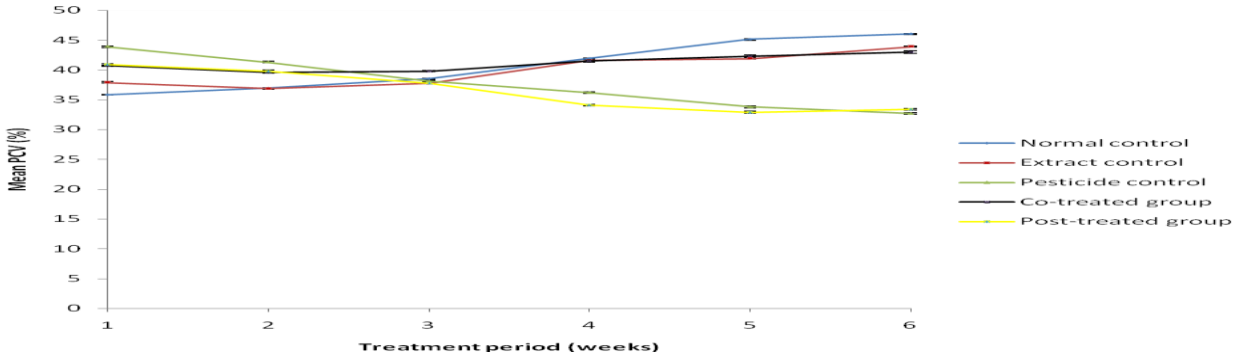


Fig.1: Profile of weekly PCV following administration of Aqueous Extract of Khaya senegalensis Stem Bark in Cypermethrin-intoxicated Rats.

Animals: Female wistar albino rats, weighing about 180-220g were purchased from the Department of Veterinary Physiology and Pharmacology, Ahmadu Bello University, Zaria. They were housed in clean cages and were fed on commercial animal feed (Pelleted growers feed) produced by Vital feed, Jos, Nigeria, and water was given to the animal *ad labium*. The rats were allowed to acclimatize in this condition for two weeks before commencement of the experiment.

Methodology

Plant sample collection, identification, and extraction: The stem-bark of *Khaya senegalensis* were collected in Samaru, Zaria in Kaduna state. It was identified at the Herbarium unit of the department of Biological Sciences, Ahmadu Bello University, Zaria. Specimen was deposited at the departmental Herbarium, voucher number (90081).

Extraction: After collection, the stem-bark were washed with tap water, air-dried at room temperature for one week and then pulverized to fine powdered using pestle and mortar. Aqueous suspension of the powdered sample were prepared by mixing 100g/1000ml distilled water at 25^{o}C. The mixture were left for 24hrs and then filtered with Whatman no.1 filter paper. The filtrate was evaporated to dryness on water bath at 70^{o}C, and the concentrate was collected and used for the study.

Animals exposure to cypermethrin: Animals were exposed to cypermethrin-toxicity via feeding, about 300mg cypermethrin according to literature report by Ferah *et al* (2005) on dietary studies with rats on toxicity of cypermethrin was used, therefore the test concentration was determined from the percentage of the active ingredient from the commercial formation of cypermethrin (BEST®) insecticide (10% Cypermethrin). In this regard, 3ml of cypermethrin insecticide were mixed with 2 liters of water before mixing it with 1kg feed. The cypermethrin-infused feed were molded, sun-dried before given to animals in the pesticide control, co-treated and post-treated groups respectively.

Animal grouping/treatment: Animal were allocated randomly into five groups of six rats each as follows;

Normal control (untreated)

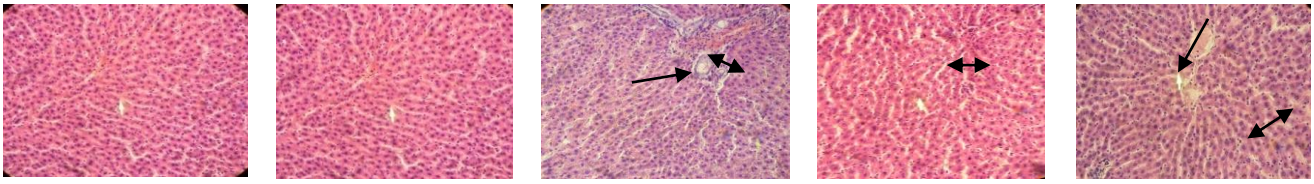
Extract control (receive 200mgkg^{-1} bwt extract only)

Pesticide control (fed with cypermethrin-infused feed)

Co-treated (Cypermethrin-infused feed + 200mgkg^{-1} bwt extract)

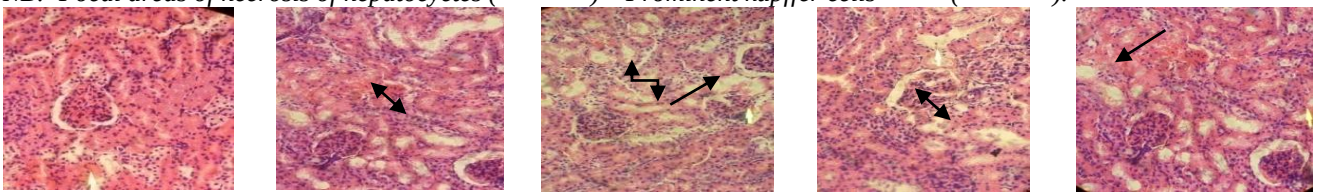
Post-treated (Cypermethrin-infused feed + 200mgkg^{-1} bwt extract for two weeks)

After 6 weeks of experiment, animals were sacrificed by humane decapitation. Liver, kidney and spleen were excised, rinsed in ice cold normal saline (NaCl), drained



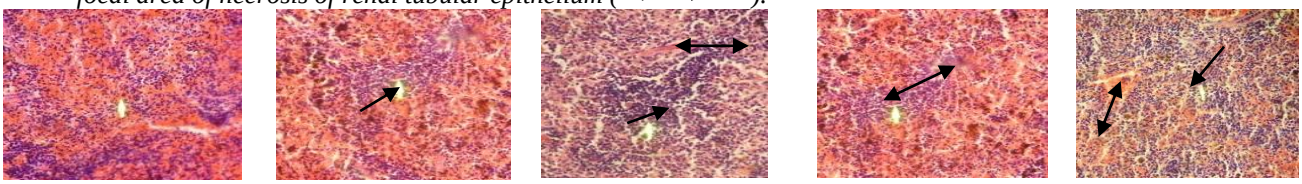
Normal Control *Extract control* *Pesticide control* *Co-treated* *Post-treated*

Plate 1. Photomicrographs of Liver Tissues of Experimental Rats (H & E Stain Original Mag. x 40).
NB: Focal areas of necrosis of hepatocyctes (⟶) Prominent kupffer cells (⟷).



Normal Control *Extract control* *Pesticide control* *Co-treated* *Post-treated*

Plate 11. Photomicrographs of kidney Tissues of Experimental Rats (H & E Stain Original Mag. X 40).
NB: Diffused necrosis of renal tubular epithelium (⟶), necrosis of glomeruli (⟷) and focal area of necrosis of renal tubular epithelium (⟷).



Normal control *Extract control* *Pesticide control* *Co-treated group* *Post-treat group*

Plate 111. Photomicrographs of Spleen Tissues of Experimental Rats (H & E Stain Original Mag. X 40).
NB: Depletion of lymphoid cells (⟷), and Haemosiderosis (⟶)

and then weighed before subjected to histological studies. Packed cell volume (PCV) was determined weekly by collecting blood from the tail vein of each rats using microhaematocrit method (Schalm *et al*., 1975).

Histological Evaluation: Histological examination was carried out to aid in assessing the effects of *Khaya senegalensis* extract on liver, kidney and spleen tissues preserved in 10% formalin by Heamatoxylin and Eosin staining method (Lillie, 1965; Lynch, 1969).

Data analysis: The results were pooled and expressed as mean ± SEM of five rats in each group. Means were compared by one way analysis of variance (ANOVA) followed by Duncan's multiple range test (Duncan, 1957) and significant difference was accepted at $p<0.05$.

RESULTS

Percentage body weight gain: The percentage body weight gain following the oral administration of aqueous extract of *Khaya senegalensis* stem bark in cypermethrin-intoxicated rats showed least body weight gained (2.8%), by the Pesticides control rats and highest weight gained (15.7%) by the normal control rats as presented in Table 1.

Relative Organ's Weight: Table 2 shows the effects of oral administration of aqueous extract of *Khaya senegalensis* stem bark on relative organs weights in cypermethrin-exposed rats. The relative liver, kidney and spleen weights were not appreciably altered during the course of the study, which were not significantly difference ($P>0.05$) and without appreciable change in the relative weights of treated rats to control groups.

Packed Cell Volume (PCV): The profiles for mean PCV following the oral administration of aqueous extract of *Khaya senegalensis* stem bark in experimental rats are presented in figure 1. The PCV of animals from the Pesticide control decreased gradually throughout the experiment, while PCV of animals in the Extract control remained unchanged in the first and second week, thereafter, it rose in the third week and then continued throughout the experimental period. However, PCV of animals in the Normal control showed a gradual and steady increased during the course of the study. PCV of the Co-treated animals decreases at the initiation of the study but rose later and were maintained till the end of the study, while that of Post-treated rats' decreases up to the fourth week, and then remained steady following the administration of the plant extract.

Histopathological Evaluation: Liver tissues of all groups of the experimental animals were presented in plate 1. Focal areas of necrosis and prominent kupffer cells were seen in animals' tissues from the Pesticide control and the Post-treated rats. Prominent kupffer cells only were seen in the animals' tissues co-treated and no specific histopathological effects found in the animals tissues from the Extract control group.

Kidney tissues of all groups of experimental rats were presented in plate 11. Diffused necrosis of renal tubular epithelium in renal cortex with collapse of tubules and necrosis of the glomeruli was seen in the kidney tissues of animal from the Pesticide control and the Post-treated rats. However, focal areas of necrosis of renal tubular epithelium in cortical areas were seen in the kidney tissues of the Co-treated and the Extract control rats.

Changes in spleen tissues of all groups of experimental rats were presented in plate 111. Depletion of lymphoid cells with haemosiderosis was observed from animals in

all the Pesticide-treated groups. However, mild deformations were seen in the Co-treated and the Extract control animals.

DISCUSSION

Feeding studies with laboratory animals have showed adverse effects of cypermethrin; it reduces growth rate in rats (EXTOXNET, 1996). Therefore, in this study, while there were no significant changes in relative organs weight of rats in all cypermethrin-treated groups, a significant decrease in body weight was observed in the pesticide control animals. Aldana *et al* (2001) have reported that, intrapritroneal administration of 300mgkg^{-1} cypermethrin to rats for 7 days significantly reduces animals' body weight.

The decrease in PCV of rats following the consumption of cypermethrin-infused feed at a dose of 300mgkg^{-1} feed is an indication of anemia. Alterations in some hematological parameters of rats have been reported earlier with this agent (Ferah *et al*., 2005). Reduced rate of anemia in co-treated rats suggests possible protective effect of the extract against cypermethrin-induced anemia. This results agreed with the findings reported by Sanni *et al* (2005), that extract of *Khaya senegalensis* stem bark contain some important elements needed for blood production and these element may have contributed in prevention of anemia when the extract was given simultaneously with cypermethrin-infused feed, but failed to reverse the anemia induced.

In vivo studies of cypermethrin have shown that it causes necrosis, inflammation and cytoplasmic hypertrophy in hepatocytes, lymphocytic infiltration and congestion of vessels of kidney (El-tawil *et al*., 2001; Inayat *et al*., 2007). Necrosis of hepatocytes and prominent kupffer cells was observed in our study from the liver tissues of rats in the Pesticide control group. However, absences of hepatic necrosis in the co-treated rats suggest the ability of plant extract to protect the liver against the toxicity of cypermethrin.

Renal alterations such as; diffused necrosis of renal tubular epithelium in renal cortex with collapse of tubules, and necrosis of glomeruli were seen in tissues of pesticide control rats, where as focal areas of necrosis of renal tubular epithelium in renal cortex of animals from the extract control group was observed. This negative renal tissue alteration might be due to prolonged administration of the plant extract, similar case have been reported by Adebayo *et al* (2003) following continuous administration of ethanolic-extract of *Khaya senegalensis* over a prolonged period. It is interesting to note that, mild focal areas of necrosis of the renal tubular seen in renal tissues of animals in the co-treated suggests synergistic or antagonistic interaction effects of the extract and the pesticide. In sum, this could also explain the absence of lymphoid cell depletion in spleen tissue of co-treated rats.

CONCLUSION

In conclusion, we are able to report that cypermethrin induced cellular damage and can be ameliorated by the administration of *Khaya senegalensis* stem bark extract.

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