

Anti-peroxidative and Biochemical Protective Activity of *Khaya Senegalensis* Stem Bark Extract on Rats Fed Pesticide-infused feed

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Abstract

Anti-peroxidative and biochemical protective activity of aqueous extract of *Khaya senegalensis* stem-bark on rats fed pesticide-infused feed was investigated. Animals were fed with cypermethrin-infused feed at a dose of 300mgkg⁻¹ feed for 42 days. Different groups of animals were co-treated daily with 50mgkg⁻¹, 100mgkg⁻¹ and 200mgkg⁻¹ body weight (bwt) of plant extract orally. The extract control group received 200mgkg⁻¹ bwt plant extract while the pesticide control animals were fed with cypermethrin-infused feed. Serum biochemical parameters, lipid peroxidation marker; malondialdehyde (MDA), and antioxidant enzymes; catalase (CAT) and superoxide dismutase (SOD) activities were measured. Serum levels of ALP, AST, ALT, Urea, and MDA were elevated in the pesticide control animals as well as decreased activities of CAT and SOD as compared to co-treated, normal, and extract control rats. Our investigation showed that daily oral dose of aqueous extract of *Khaya senegalensis* stem-bark administered along pesticide-infused feed consumption produced significant ($p < 0.05$) protection in a dose-dependent increase. The study shows that pesticide (cypermethrin) induces lipid peroxidation and also alters biochemical parameters. Aqueous extract of *Khaya senegalensis* stem-bark may contain biologically active components that play a protective role against pesticide-mediate alteration of biochemical parameters and also exhibit anti-peroxidative activity in rats.

Keywords: Anti-peroxidative, Biochemical parameters, Pesticide, *Khaya senegalensis*, rats.

1.0 Introduction

Oxidation reactions occur when life essential oxygen combusts within the human body and produces byproducts referred to as oxygen free radicals which steal electrons from other molecules, like lipids causing lipid peroxidation [1]. An overload of free radicals has been linked to certain diseases, including heart disease, liver disease and some cancers. There has been an increased interest in finding natural antioxidants from plants because they attack free radicals, retard the progress of many chronic diseases and also retard the lipid oxidative [2].

The toxicity of many pesticides is associated with the production of free radicals which are implicated in the pathophysiology of many diseases [3]. This might be followed by observation of increasing amount of free radical damaged products particularly marker of lipid peroxidation in the body fluid [4, 5]. Under normal condition, excessive formation of free radical and concomitant damaged at cellular and tissues concentration is control by cellular antioxidant defense. However, where there is increase in the reactive oxygen species or a large decrease in the reactive capacity of the cellular antioxidant defense, severe oxidative stress may results which can cause cell death while intense stresses may cause tissues injuries. Literature have shown that the mechanism of pesticides action is via changes in the cellular oxidative stress leading to generation of free radicals and alteration in antioxidant molecules, the radical scavenging enzymes, and lipid peroxidation [6].

The plant, *Khaya senegalensis* (Desr) A. Juss otherwise known as dry zone Mahogany belongs to the family *Meliaceae*. 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. It is a medicinal plant widely distributed in the savannah region of Nigeria [7]. According to Marius *et al* [8] *Khaya senegalensis* extract showed significant antioxidant activity *in vitro*. Atawodi *et al* [9] have shown that extract of *Khaya senegalensis* materials contain polyphenolic-rich compounds. It is in the light of these findings that the present study was set out to investigate whether aqueous extract of *Khaya senegalensis* stem-bark could have protective effects against pesticide-mediate lipid-peroxidation and biochemical's alteration in rats.

2.1 Materials

2.1.1 Chemicals and reagents

All chemicals and reagents used in this study were of analytical grade and were purchased from Sigma Aldrich, USA and Randox Laboratory, UK.

2.1.2 Experimental animals

Female wistar albino rats, weighing about 180-220 g 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 libitum*. The rats were allowed to acclimatize in this condition for two weeks before commencement of the experiment.

2.2 Methodology

2.2.1 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 100 g/1000 ml distilled water at 25 °C. The mixture were left for 24 hrs and then filtered with Whatman no.1 filter paper. The filtrate was evaporated to dryness on water bath at 70 °C, and the concentrate was collected and used for the study.

2.2.2 Induction of oxidative stress in rats

Cypermethrin-infused feed were prepared following the method done by Altug *et al* [10]. About 3 ml of cypermethrin insecticide (300 mg) was diluted with 2 liters of water, and was used to mixed 1 kg feed. The mixed cypermethrin-infused feeds were molded, sun-dried before given to the animals in the pesticide control and co-treated groups. The cypermethrin dose (300 mg) was selected based on literature reports on dietary studies with rats on toxicity of cypermethrin by Ferah *et al* [11], and the test concentration was calculated from the percentage of the active ingredient from the commercial formulation of cypermethrin (Best[®]) insecticide (10% cypermethrin).

2.2.3 Animal grouping/treatment

Animals were randomly grouped into six groups of six rats each and were treated as follows;

- I. Normal control (untreated)
- II. Pesticide control (fed with cypermethrin-infused feed)
- III. Extract control (received 200mgkg⁻¹ bwt extract)
- IV. Co-treated A(Cypermethrin-infused feed + 50mgkg⁻¹ bwt extract)
- V. Co-treated B(Cypermethrin-infused feed + 100mgkg⁻¹ bwt extract)
- VI. Co-treated C (Cypermethrin-infused feed + 200mgkg⁻¹ bwt extract)

Extract was administered orally using oral gastric tube. The experiment lasted for 42 days and animals were sacrificed by humane decapitation, blood sample were collected and serum separated and then used for the estimation of biochemical parameters.

2.2.4 Estimation of biochemical parameters

Hepatoprotective effect of *Khaya senegalensis* stem-bark extract was assessed by measuring serum level of Aspartate and Alanine Aminotransferase (AST and ALT) [12], Alkaline phosphatase (ALP) [13], Serum albumin [14], Total protein [15], serum total/direct bilirubin [16] while nephroprotective effect of the extract was assessed by determining serum urea level [17] and creatinine [18] methods respectively. Antioxidant action of the extract was assessed by measuring lipid peroxidation biomarker such as serum malondialdehyde (MDA) by Okhawa [19] method while catalase (CAT) was determined by Aebi [20] method and superoxide dismutase (SOD) activity was estimated by Martin [21] method.

2.3 Data analysis

The results were pooled and expressed as mean \pm SEM of five rats in each group. Means were compared by one way analysis of variance (ANOVA) followed by Duncan's multiple range test [22] and significant difference was accepted at $p < 0.05$.

3.0 Results and Discussion

The mean daily feed and water intake values are presented in Table 3.1. No death occurred in any of the experimental groups throughout the experiment. There were initial decrease in feed consumption by the animals fed with cypermethrin-infused feed, but were returned to normal after 1-2 weeks. Prolonged and indiscriminate uses of pesticides have been reported to cause both acute and chronic toxicity to non-target species including human being [4]. Declined in feed consumption observed at the beginning of the experiment could be due to some behavioral changes induced by cypermethrin in the feed.

The values for oxidative stress marker and antioxidant enzymes were presented in Table 3.2. Mean values of MDA for the Pesticide control animals showed a significant ($p < 0.05$) increased while a decrease in CAT and SOD activities were observed as compared to the mean values of co-treated groups. Moreover, a great decreased in MDA level was observed in rats co-treated with 200mgkg^{-1} bwt extract and it was not significantly different ($p > 0.05$) from the mean value of normal control animals. From the pathomechanism of cypermethrin, it was found that tissues injuries induced by cypermethrin involve depletion of endogenous antioxidant enzymes [23]. Thus, reasonable cellular protection agents against cypermethrin toxicity may have at least some antioxidant properties to scavenge the intracellular reactive oxygen species. In this regard, cellular protection ability of aqueous extract of *Khaya senegalensis* stem-bark might have significantly reduces depletion of the endogenous antioxidant reverse as part of its therapeutic action. Evidence from scientific studies have indicated that *Khaya senegalensis* extract contain significant amount of phenolic compounds [24]. So, increase in CAT and SOD activities and a decrease in MDA level in treated rats may suggests the plant-extract free radical scavenging action as well as confirming its antioxidant activities.

The effect of aqueous extract on biochemical parameters are presented in Table 3.3. Serum biochemical parameters differs significantly ($p < 0.05$) between mean values in the pesticide and normal control group, extract control as well as the co-treated groups. However, there was no significant difference between the mean values of co-treated rats with 200mg/kg bwt extract and the Extract control. Serum renal indices were found to be elevated from animals in the pesticide control group and the co-treated that received 50mg/kg bwt extract. Cypermethrin have been reported to induce renal damage after dermal application at 15mgkg^{-1} and 30mgkg^{-1} bwt [25]. However is interesting to note that, the severity of cypermethrin-induced nephrotoxicity was reduced by the plant extract.

Hepatic indices of extract control rats differ significantly ($P < 0.05$) as compared to that of normal control and the co-treated animals with 200mg/kg bwt extract. The alteration in serum hepatic biochemical parameters was related to the intensity of cellular damage. Therefore, increase in serum ALT and AST levels along with decrease in serum albumin and total protein in the pesticide-fed animals may be the consequence of cypermethrin-induced pathological changes in the liver. Adverse effect of alpha-cypermethrin on liver has been reported by Manna *et al* [26]. In our finding, co-administration of aqueous extract of *Khaya senegalensis* stem-bark minimizes the incident of hepatotoxicity induced by cypermethrin in a dose-dependent increase.

Conclusion: We are able to confirm that cypermethrin can induce lipid peroxidation as part of the mechanism of its toxic action in the body, and aqueous extract of *Khaya senegalensis* stem-bark can prevent or slow down the oxidative insult induced by cypermethrin in rats.

Conflict of interest: The authors have declared no conflict of interest.

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Table 3.1 Effects of aqueous extract of *Khaya senegalensis* stem-bark on daily feed and water intake in cypermethrin-intoxicated rats (Mean \pm SEM)

Indices	Normal control	Extract control	Pesticide control	Co-treated A	Co-treated B	Co-treated C
Feed intake (g/100g/day)	7.40 $\pm 0.15^a$	6.42 $\pm 0.60^a$	6.21 $\pm 0.46^a$	7.60 $\pm 0.28^a$	6.87 $\pm 0.28^a$	6.03 $\pm 0.25^a$
Pesticide consumed (mg/100g/day)	-	-	1.86	2.28	2.06	1.8
Water intake (ml/100g/day)	6.87 $\pm 0.93^a$	6.87 $\pm 0.79^a$	6.89 $\pm 2.43^a$	6.28 $\pm 1.61^a$	6.37 $\pm 2.09^a$	5.75 $\pm 1.72^a$

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

Table 3.2 Effects of aqueous extract of *Khaya senegalensis* stem bark on antioxidant's enzyme and marker of lipid peroxidation induced by Cypermethrin in rats (Mean \pm SEM)

INDICES	Normal Control	Extract Control	Pesticide Control	Co-treated A	Co-treated B	Co-treated C
MDA (mmol/L)	1.31 $\pm 0.02^a$	1.45 $\pm 0.03^{ab}$	1.55 $\pm 0.06^b$	1.50 $\pm 0.15^b$	1.42 $\pm 0.04^{ab}$	1.34 $\pm 0.05^a$
CAT (U/mg protein)	6.33 $\pm 0.37^a$	5.63 $\pm 0.66^{ac}$	3.95 $\pm 0.08^b$	4.73 $\pm 0.19^{bc}$	5.08 $\pm 0.34^{bc}$	5.79 $\pm 0.22^a$
SOD (U/ml)	37.08 $\pm 2.43^a$	30.00 $\pm 1.65^{ac}$	11.56 $\pm 0.55^{bd}$	19.37 $\pm 0.57^{bcd}$	28.75 $\pm 0.80^{ace}$	32.50 $\pm 1.19^{ac}$

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

Table 3.3 Serum biochemical changes following oral administration of aqueous extract of *Khaya senegalensis* stem-bark on rats fed cypermethrin-infused feed (Mean \pm SEM)

INDICES	Normal Control	Extract Control	Pesticide Control	Co-treated A	Co-treated B	Co-treated C
ALT(U/L)	5.57 \pm 0.51 ^a	7.53 \pm 0.33 ^{ab}	10.53 \pm 0.87 ^c	9.35 \pm 0.49 ^{bc}	9.13 \pm 0.63 ^{bc}	7.06 \pm 0.34 ^{ab}
AST(U/L)	17.73 \pm 1.07 ^a	23.53 \pm 1.03 ^{ab}	43.48 \pm 1.19 ^c	34.25 \pm 1.77 ^{bc}	29.63 \pm 0.82 ^b	19.18 \pm 1.28 ^{ab}
ALP(U/L)	97.73 \pm 1.75 ^a	128.63 \pm 8.57 ^{ac}	247.50 \pm 9.60 ^b	233.48 \pm 5.97 ^b	134.48 \pm 14.03 ^{ac}	130.35 \pm 9.56 ^{ac}
Albumin (g/L)	31.94 \pm 1.33 ^a	52.13 \pm 1.07 ^{ac}	26.04 \pm 1.32 ^b	27.00 \pm 1.68 ^{bc}	30.56 \pm 1.28 ^{ac}	32.53 \pm 1.19 ^{ac}
Total Protein (g/L)	58.00 \pm 2.10 ^a	52.13 \pm 2.59 ^{ac}	44.43 \pm 1.29 ^b	44.63 \pm 1.58 ^b	51.63 \pm 3.32 ^{ac}	50.75 \pm 3.65 ^{ac}
Total Bilirubin (μ mol/L)	5.37 \pm 0.35 ^b	8.51 \pm 0.34 ^c	12.44 \pm 0.96 ^a	8.78 \pm 0.18 ^c	7.13 \pm 0.39 ^{bc}	6.85 \pm 0.32 ^b
Direct Bilirubin (μ mol/L)	2.46 \pm 0.16 ^b	4.12 \pm 0.29 ^{ab}	5.29 \pm 0.35 ^a	4.49 \pm 0.23 ^{ab}	3.94 \pm 0.52 ^{ab}	2.26 \pm 0.25 ^b
Urea (mmol/L)	8.89 \pm 0.18 ^c	10.89 \pm 0.38 ^{ab}	11.67 \pm 0.18 ^b	11.64 \pm 0.47 ^b	10.75 \pm 0.32 ^{ab}	10.12 \pm 0.25 ^a
Creatine (μ mol/L)	75.41 \pm 2.03 ^b	95.64 \pm 2.64 ^{bc}	127.03 \pm 3.58 ^a	115.60 \pm 5.50 ^{ac}	99.98 \pm 2.81 ^{ab}	88.50 \pm 1.72 ^a

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

References:

- [1] RI Shobha, CU Rajeshwari, B Andallu. Anti-Peroxidative and Anti-Diabetic Activities of Aniseeds (*Pimpinella anisum* L.) and Identification of Bioactive Compounds. *American Journal of Phytomedicine and Clinical Therapeutics*, 2013; 1(5):516-527.
- [2] WA. Pryor. The antioxidant nutrients and disease prevention- What do we know and what do we need to find out? *Am J Clin Nutr* 1991; 53: S391-S393.
- [3] SV Lennon, SJ Martin, and TG Cotter. Dose-dependent induction of apoptosis in human tumour cell lines by widely diverging stimuli. *Cell Proliferation*, 1991; 24: 203-214.
- [4] W. N Aldridge. An assesment of the toxicological properties of pyrethroid and their neurotoxicity. *Critb Review Toxicology*, 1990. 21: 89-104.
- [5] B Giray, A Gurbay and F Hineal. Cypermethrin induced oxidative stress in rats brain and liver is prevented by Vitamin E or Allopurinol. *Toxicological Let*, 2001; 5 (118): 139-46.
- [6] Y Sharma, S Bashir, M Irshad, et al. Effects of Acute dimethoate administration on antioxidant status of liver and bria of experimental rats. *Toxicology*, 2005; 20 (6): 49-57.
- [7] D Zama, Z Meraihi, S Tebibel, et al. Chloryrifos-induced oxidative stress and tissue damage in liver, kidney brain and fetus in pregnant rats; the protective role of the butanolic extract of *Paronychia argentea*. *Journal of Pharmacology*, 2007; 39 (3): 145-150.
- [8] J.A Ibrahim, A. A Emmanuel, I.J Adela et al. Comparative studies on *Khaya senegalensis* A. Juss (Meliaceae) in Nigeria. *African Journal of Biotechnology*, 2006; 5 (11):1154-1160.
- [9] L Marius, D Jacques, and I. P Guissou. In vitro preliminary study of free radicals scavenging activities of extracts from *Khaya senegalensis* A Juss (Meliaceae). *Journal of Biological sci.*, 2007; 7 (4): 677-680.
- [10] SE Atawodi, JC Atawodi, Y Pala, et al. Assessment of polyphenol profiles and antioxidant activities of the leaves, stem and root barks of *Khaya senegalensis* (Desr). *A.Juss. Electronic Journal of Biology*, 2009; 3 (4): 80-84.
- [11] Y Altug, S Ferah, U Yigit, et al. The Pyrethroid Cypermethrin-induced Biochemical and Histological alteration in Rats Liver. *Journal of Health sciences*, 2006; 3: 774-780
- [12] S Ferah, KY Nefise-ulku, U Yigit et al. Neurotoxic effects of Cypermethrin in wistar rats; a hematological, biochemical and histological study. *Journal of Health Sciences*, 2005; 51 (3): 300-307.
- [13] S Reitman and SA Frankel. Colorimetric method for the determination of glutamic oxaloacetic and glutamic pyruvic transaminase. *Am Journal of Clinical Pathology*, 1957; 28: 56-63.
- [14] E J King and AR Armstrong. In vitro determination of alkaline phosphatase (ALP). *Canada Medical Association Journal*, 1934; 31: 376-9.
- [15] BT Doumax, WA Watson and HG Biggs. *Clinical chemistry. Acta*, 1971; 31:87
- [16] MM Bradford. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 1976; 72: 248-254.
- [17] L Jendrossik and P Grof. *Biochemistry*, 1938; 279: Pp 81.
- [18] MW Weatherburn. *Chemistry*, 1967; 39: Pp971
- [19] H Bartels, and M Bohmer. *Clinical chemistry. Acta*, 1992; 4 (37): 193
- [20] H Okhawa, N Ohishi, and K Yagi. Assay for lipid peroxidation in animals by thiobarbituric acid reaction. *Analytical Biochemistry*, 1999; 95:351-358.

- [21] H Aebi. Catalase in vitro; *Methods in Enzymology*, 1984; 105: 121–126.
- [22] JP Jr Martin and MS Dailey. Negative and positive assays of superoxide dismutase based on hematoxylin autoxidation. *Arch. Biochemistry Biophysics*, 1987; 28(5):329.
- [23] BD Duncan. Multiple range test for correlated and heteroscedastic means. *Biometrics*, 1957; 13: Pp 359–364
- [24] S Yilmaz, AT Ahmet, IP Karahan, et al. The effects of vitamin E and selenium on cypermethrin induced oxidative stress in rats. *Turkish Journal of Veterinary Animal science*, 2005; 22: 385-391.
- [25] Q Inayat, M Ilahi and J Khan. A morphometric and histological study of the kidney of mice after dermal application of cypermethrin. *Journal Paskistan Med. Ass*, 2007; 57: 587-591.
- [26] S Manna, D Bhattacharya DK Basak et al. Single oral dose toxicity study of alpha-cypermethrin in rats. 2004; 36 (1): 25-28.