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Histopathological and Biochemical Changes in the Rats Kidney Following Exposure to a Pyrethroid Based Mosquito Coil

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Abstract: This study was carried out to investigate the effect of inhaling mosquito coil smoke on the histomorphology and biochemistry of the rats' kidney. A total of thirty (30) adult albino rats of the Wister strain were used, they were randomly divided into six groups of five rats each. Group I served as control (no exposure to mosquito coil smoke). While rats in groups II-V were exposed to mosquito coil smoke for 12 h, 7, 14, 21 and 28 days respectively. At the end of each experimental period, blood was obtained from each rat for the determination of serum levels of Na+, k+, Cl- , HCO_3^- , Urea and Creatinine. The rats were then sacrificed and the kidneys obtained were processed for routine histological analysis. Biochemical analysis of blood serum showed a significant increase ($P<0.05$) in the levels of urea and creatinine in rats exposed to the mosquito coil smoke but serum levels of Na+, k+, Cl- and HCO_3^- were not affected significantly. Histopathological assessments of the kidney tissues of the rats exposed to mosquito coil smoke were severe multifocal congestion, cystic dilatation in the medulla; proteinaceous casts within ducts, interstitial mononuclear cellular infiltration and widespread fibrosis .The severity of the lesions were dependent on the duration of exposure. These findings suggest that inhaling mosquito coil smoke may cause nephrotoxicity in rats. But further investigation to study the mechanism of its toxicity and the reversibility / irreversibility of the pathological effects is recommended in further studies.

Keywords: Mosquito coil, urea, creatinine, nephrotoxicity and pyrethroid

INTRODUCTION

Mosquito coils are slow-burning devices which emit smoke containing one or more insecticides, each coil burns for several hours and are used in close proximity to persons requiring protection against mosquitoes in order to prevent malaria, a disease of wide distribution. This is paramount because the burden caused by malaria is considerable amounting to 300-400 million clinical cases per year- 80% of which occur in Africa and are responsible for almost one million deaths per year[1]. The scourge of malaria had led parents and their children in low income communities to adopt the use of mosquito coils to control the mosquito populations around residential areas because they are cheap and readily available[2]. The availability and the relative cheapness of this insecticide have made mosquito coils very popular in Asia, South America and Africa including Nigeria. The annual worldwide consumption of the four major types of residential insecticides products – aerosols, mosquito coils, liquid vaporisers and vaporising units are in the billions of units[3].

Mosquito coils consist of an insecticide /repellent, organic fillers capable of burning with smouldering,binder and additives such as synergists, dyes and fungicides[3]. The most common active ingredients in mosquito coils are various pyrethroids that are effective against many genera of mosquitoes including Aedes, Anopheles and Mansonia[3].

Mosquito coils are often used overnight in sleeping quarters where elevated exposure may occur and children and their parents are often exposed to chemically complex mosquito coil smoke containing small particles (1µm), metal fumes and vapours[4] that may reach the alveolar region of the lung[5]. Researchers have also found that the gas phase of mosquito coil smoke contain carbonyl compounds (formaldehyde and acetaldehyde) with properties that can produce strong irritating effects on the upper respiratory tract [6]. Epidemiologic studies have shown that long term exposure to mosquito coil can induce asthma and persistent wheeze in children[7,8,9].

Toxicological studies using mosquito coils in rats have shown focal deciliation of the tracheal epithelium, metaplasia of epithelial cells and morphologic alteration

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of the alveolar macrophages[4,10]. In our earlier study we were able to demonstrate that inhaling mosquito coil smoke challenges the immune system in experimental rats because the coil was shown to have caused a significant increase in WBC count, basophil and lymphocyte percentages with severe congestion of venous sinusoids, hyperplasia and or regression of the red and white pulps[11] .

Because human exposure to the vapors emanating from mosquito coil is on a daily basis in most low income residential areas because the coils are used in sitting rooms and bedrooms, this study was therefore designed to examine the effect of inhaling mosquito coil smoke on the histomorphological and biochemical integrity of the kidney in Wister albino rats.

MATERIALS AND METHODS

Animal and Husbandry: This study was carried out in the Departments of Human Anatomy and Human Physiology, University of Maiduguri, Nigeria between March and October, 2005.

A total of thirty (30) adult albino rats of the Wister strain (210g±20) of both sexes were used. They were purchased from the animal facility centre of the National Veterinary Research Institute Vom, Plateau State, Nigeria. Following an acclimation period of 2 weeks, the rats were individually identified by color tattoo and weighed. The rats were kept in plastic cages at room temperature of 32 ±4°C and <30% relative humidity with a 12 hour light/dark cycle. They had access to drinking water and standard laboratory diet (Sanders SEEPC feed PLC, Jos, Nigeria) *ad libitum*.

Test Article: Mosquito coils were purchased from various retail outlets located within Maiduguri, Nigeria. The brand commercially purchased for the experiment contained pyrethroids (d- trans - allethrin) 0.2 %w/w and inert ingredient 99.8% w/w. The mosquito coil used measured 12cm diameter, 85cm length and 14.0g weight

Experimental Design: The experiments were conducted in two (A and B) undisturbed rooms of size 26.2m^3 (3.0 x 3.5 x 2.5) with cross ventilation. The rats were randomly divided into six groups of five rats per group. Group I served as control and were not subjected to mosquito coil smoke inhalation; Group II were used for the 12 hour acute mosquito coil smoke inhalation study while Groups III-VI were used for the subchronic mosquito coil smoke inhalation study.

Acute Mosquito Coil Smoke Inhalation Study: The rats in group II were exposed via whole body inhalation to the commercially available mosquito coil for 12 hours (8 pm - 8 am). At the end of the experimental period of 12 hours blood and kidney were obtained from each rat for biochemical and histological analysis respectively.

Subchronic Mosquito Coil Smoke Inhalation Study: The rats in group III, IV, V and VI were also exposed to the commercially available mosquito coil via whole body inhalation. The experiment was carried out by igniting one mosquito coil every day for 8 hours (10 pm to 6 am) for 7, 14, 21 and 28 days respectively. At the end of each experimental period, blood and kidney were obtained from each rat for biochemical and histological analysis respectively.

Blood Sample Collection and Biochemical Assay: Blood was obtained by transection of the jugular vein and a test tube held directly below the dripping blood. Plasma serum was obtained by centrifuging the blood sample at 12,000 rpm. The serum collected was immediately taken to the Department of Chemical Pathology, University of Maiduguri Teaching Hospital for the analysis of urea, creatinine and electrolytes (Na^+, K^+, Cl^-, HCO_3^-) using Randox laboratory kits. Urea concentration was measured using the diacetylmono-oxine method of Marchal[12], while the creatinine concentration was determined by the alkaline picrate method[13]. Serum sodium and potassium concentrations were determined using reagent titrimetrically while serum chloride concentration was determined using the mercuric nitrate method[14].

Histological Analysis: The rats were anaesthetised and the kidneys carefully dissected out, fixed in Bouins fluid, embedded in paraffin and sections cut at 5µm. Sections were stained with Haematoxylin and Eosin and mounted in Canada balsam. Light microscopic examination of the sections was then carried out.

Statistical Analysis: Data obtained from the study were expressed as the mean value ± standard error of mean. Differences among means of control and exposed groups were determined using Statistical Package for Social Scientist (SPSS 11.0) and GraphPad In Stat software. A probability level of less than 5% ($p< 0.05$) was considered significant

RESULTS AND DISCUSSIONS

Effect of Mosquito Coil Smoke Inhalation on Mean Body Weight: Mean body weight was reduced in all the groups exposed to the mosquito coil smoke when compared to the non exposed group though the reduction was not significant (Table 1).

Effect of Mosquito Coil Smoke Inhalation on Biochemical Parameters: The effects of mosquito coil smoke inhalation on biochemical parameters are presented in Table 2. Biochemical analysis of blood serum showed a significant increase ($P<0.05$) in the levels of urea and creatinine in rats exposed to the mosquito coil smoke but serum levels of Na+, k+, Cl- and HCO_3^- were not significantly affected (Table 2).

Table 1: Effect of inhaling mosquito coil smoke on mean body weights of rats

GROUPS	EXPOSURE DURATION	INITIAL BODY WEIGHT (gm)	FINAL BODY WEIGHT (gm)	BODY WEIGHT DIFFERENCE (gm)	% WEIGHT CHANGE
I	0 HOURS	199.78± 0.56	204.50 ±1.19	4.72	2.30
II	12 HOURS	204.46± 3.11	201.14 ±2.51	- 3.32	1.65
II	7 DAYS	208.46±3.70	208.36± 3.69	- 0.10	0.05
IV	14 DAYS	205.06±4.48	204.66± 4.51	- 0.40	0.20
V	21 DAYS	207.68±3.96	204.52± 5.94	- 3.16	1.55
VI	28 DAYS	202.48±3.52	202.00 ±3.52	- 0.48	0.24

Results are presented as Means ± SEM. N= 5

Table 2: Effect of inhaling mosquito coil smoke on biochemical parameters.

Groups	Duration of Exposure	Na^+ Mmol/L	K^+ Mmol/L	Cl^- Mmol/L	HCO_3^- Mmol/L	Urea Mmol/l	Creatinine Mmol/L
I	0 HOURS	137.60±0.75	6.18±0.22	100.80±1.02	19.20±0.73	4.24 ± 0.12	86.80 ± 4.58
II	12 HOURS	137.60±0.75	6.60±0.28	100.00±0.63	18.80±0.73	5.88 ± 0.61	90.20 ± 2.92
III	7 DAYS	137.20±1.36	5.76±0.31	99.20±1.02	17.20±0.37	6.02 ± 0.44*	95.60 ± 3.46
IV	14 DAYS	140.40±0.75	6.12±0.09	102.4±01.17	17.40±0.40	6.32 ± 0.38*	98.00 ± 1.79
V	21 DAYS	137.20±1.02	6.10±0.25	99.20±1.02	18.40±0.24	6.43 ± 0.38*	112.20 ± 6.11**
VI	28 DAYS	137.33±0.09	6.19±0.35	100.20±2.02	18.50±0.14	6.89 ± 0.63**	117.20 ± 5.11**

Significance relative to control (Group I) *=P< 0.05, ** = P<0.01 N = 5 Results are presented as Means ± SEM. Na^+(Sodium); K^+ (Potassium); Cl^- (Chloride) and HCO_3^- (Bicarbonate)

Histopathologic Findings: No histological or macroscopic alterations were observed in the kidneys of the control rats, there was normal arrangement of the medulla and cortex with the Glomeruli and blood vessels neatly arranged (Fig .1).

The histological changes observed in the group exposed to mosquito coil smoke for 12 hours were severe multifocal congestion and tubular degeneration (Fig.2). Exposure of the rats to mosquito coil smoke for 7 days showed cystic dilatation of the medulla and necrosis of cells (Fig. 3) while exposure for 14 days presented with mononuclear cellular infiltration and proteinaceous casts within the ducts (Fig.4). Animals exposed to mosquito coil smoke for 21 days presented with mononuclear cellular infiltration, haemorrhagic spot and necrosis (Fig. 5). Widespread fibrosis and interstitial mononuclear cellular infiltration were observed in rats exposed to mosquito coil smoke for 28 days (Fig .6). It was observed that the severity of the pathological effects was dependent on the duration of exposure.

The chief function of the kidney is to process blood plasma and excrete urine. These functions are important because they play a vital role in the clearance and excretion of xenobiotics including drugs and drug-product, from the body. The estimation of the

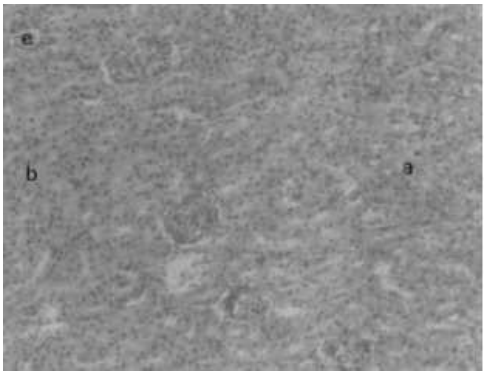

Fig. 1: Micrograph of the kidney of a control rat showing normal integrity of the cortex (a), medulla (b) and blood vessels (e) H and E stain. Mag. X 200

histological effect on the kidney tissues and the determination of some waste metabolic products excreted exclusively via the kidneys provide useful information about the health status of the kidneys; such metabolites include urea and creatinine[15]. Systemic electrolyte and water balance are regulated via the kidneys, thus the plasma electrolyte

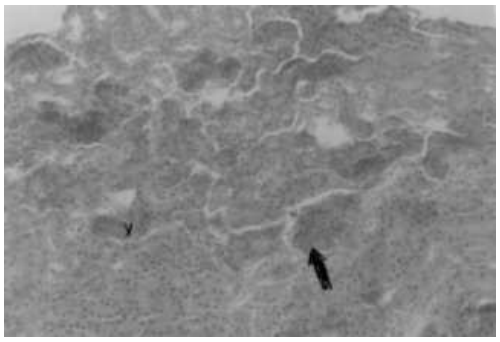

Fig. 2: Micrograph of the kidney of a rat exposed to mosquito coil smoke for 12 h showing severe multifocal congestion (arrow) and tubular degeneration (o) H and E stain. Mag. X 200.

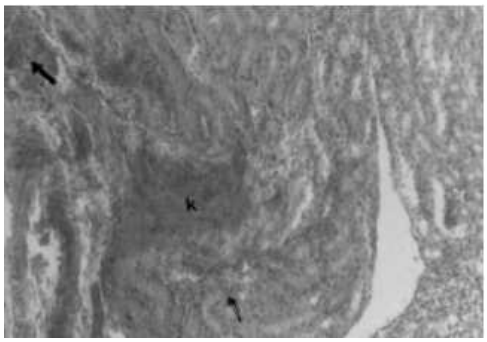

Fig. 3: Micrograph of the kidney of a rat exposed to mosquito coil smoke for 7 days showing cystic dilatation in the medulla (k) and necrosis (arrow) H and E stain. Mag. X 200.

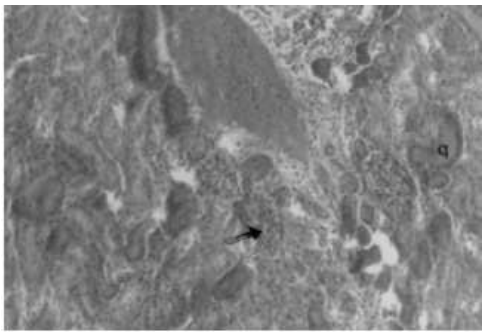

Fig. 4: Micrograph of the kidney of a rat exposed to mosquito coil smoke for 14 days showing proteinaceous casts in ducts (q) and mononuclear cellular infiltration H and E stain. Mag. X 200.

levels also provide vital information about the functional state of the kidneys[16]. Despite the various toxicological studies carried out on the toxic effects

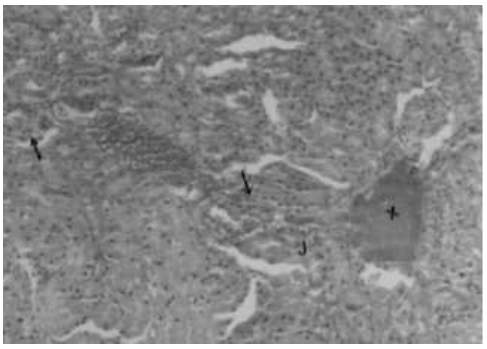

Fig. 5: Micrograph of the kidney of a rat exposed to mosquito coil smoke for 21 days showing mononuclear cellular infiltration (j) haemorrhagic spot (x) and necrosis (arrow) H and E stain. Mag. X 200.

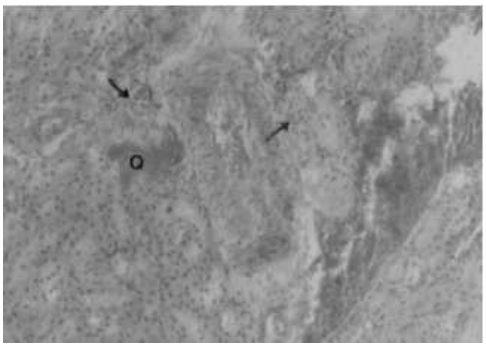

Fig. 6: Micrograph of the kidney of a rat exposed to mosquito coil smoke for 28 days showing mononuclear cellular infiltration (arrow) and fibrosis (Q) H and E stain. Mag. X 200.

associated with mosquito coil smoke inhalation , they still remain one of the most widely used insecticides in low income residential communities in Nigeria, because of their cheapness and availability[2].

The body weight loss observed in this study though not significant agrees with other works of the same nature [17,18,19,20,21]. Biochemical analysis of serum obtained from the rats exposed to mosquito coil smoke showed significant increases in the serum levels of urea and creatinine. The blood concentration of excretory and electrolyte constituents is an important tool in assessing the functional capacity of the kidney because it demonstrate the presence or absence of active lesions in the kidney and the functional capacity of the different parts of the nephron[15]. However, urea, creatinine and electrolytes (Na^+, K^+, HCO_3^- and Cl^-) are the most sensitive biochemical markers employed in the diagnosis of renal damage because urea and creatinine are excreted through the kidney while the

electrolytes are reabsorbed and excreted in the tubules. So in cellular damage, there will be retention of urea and creatinine in the blood and non re-absorption and excretion of electrolytes by the tubules[2] as noticed in this study.

Urea and creatinine are waste products of protein metabolism that need to be excreted by the kidney, therefore marked increase in serum urea and creatinine as noticed in this study confirms an indication of functional damage to the kidney[15]. Urea level can be increased by many other factors such as dehydration, antidiuretic drugs and diet while creatinine is therefore more specific to the kidney since kidney damage is the only significant factor that increases serum creatinine level[23]. Therefore the significant increases ($p<0.05$) in urea and creatinine levels noticed in this study is a classical sign that the kidney was adversely affected by the exposure arising from inhaling mosquito coil smoke. Elevation in plasma levels of urea could also be attributed to increase in the activities of urea enzymes, ornithine carbomoyl transferase and arginase mostly associated in liver damage in many animal species, since the urea cycle is confined to the liver[24]. Elevated urea are also related with the hepatotoxic effect of chlorine present as an inert ingredient of mosquito coil[25] .This could most likely be responsible for the defective urea cycle and the inability of the liver to transform ammonia to urea leading to its build up in blood. Many factors may be responsible for the increment among which are excess breakdown of blood protein and increase in tissue protein catabolism. Similarly high urea may be associated with low blood volume. Damage to the kidney may result in reduced erythropoeitin production, resulting in high urea which may in turn be associated with low blood volume[26]. Thereby leading to an elevation in inflammatory cells types, this usually occurs during the inflammatory process[27]. Inflammation exposes the body organs to infections, leading to the release of high amount of white blood cells[27].

Histopathological lesions noticed in the present study which ranges from severe multifocal congestion, cystic dilatation in the medulla; proteinaceous casts within ducts, interstitial mononuclear cellular infiltration, haemorrhage, widespread fibrosis and necrosis can be related with the inert ingredients that are not listed on the labels of such products because analysis of the inert ingredients have shown the presence of substances such as propane, hydrotreated light petroleum distillates (hydrotreated kerosene) and hydrotreated heavy naptha (white spirits) and isobutane[28].

Propane and isobutane have been found to cause depression of the central nervous system and dizziness when inhaled while hydrotreated light petroleum distillates (hydrotreated kerosene) have been linked to skin tumors in laboratory mice[29]. Hydrotreated heavy naptha (white spirits) have been shown to have a damaging effect on kidneys and the nervous system[30]. Long-lasting and irreversible changes in brain cells have also been demonstrated in the offspring of animals exposed to white spirits and the damage to the brain was attributed to the inability to maintain normal calcium concentrations[31].

In a three-month feeding study with rats , pyrethrins have been shown to cause degeneration of renal tubules in doses equal or greater than 170 mg/kg[20] this agrees with the findings in this study.

These findings suggest that inhaling mosquito coil smoke may cause nephrotoxicity in rats. But further investigation to study the mechanism of its toxicity and the reversibility / irreversibility of the pathological effects is recommended in further studies.

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