Susceptibility of young and adult rat kidneys to impacts of mosquito coil fumes

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ABSTRACT
The present study aimed to investigate the effect of exposure to mosquito coil fumes (6 hr/day, 6 days/week, for 4 weeks) on the biochemical and histological structure of kidney in young and adult male rats. Atomic analysis of the coil was estimated to indicate the main constituent elements of the coil. Results showed a non-significant change in body weight gain and somatic indices, except for the heart somatic index that increased significantly in both ages compared to control groups. Biochemical analysis showed a significant increase in serum levels of creatinine, urea, uric acid, neutrophil gelatinase-associated lipocalin (NGAL), cystatin C. However, serum total protein content was reduced significantly. Urine analysis showed a marked increase in creatinine, urea, total protein, microalbuminuria, and NGAL, along with a significant reduction at glomerular filtration rate (GFR). Moreover, serum and urine Na⁺ and K⁺ levels showed no obvious changes in both ages compared to control groups, except for serum sodium in the young group. Histopathological examination confirmed the destructive effect of coil fumes on tubules and glomeruli of exposed kidneys, which was obvious in young than adult rats. Thus, inhalation of coil fumes may provide a serious risk on the kidney health of exposed human beings.

Introduction
Mosquito nuisance is a widespread problem, especially in rural areas. Usage of mosquito coils is popular in low-income countries, especially those exposed to malaria in Asia, Africa, and South America due to cheapness and availability [1]. Recently, this type of insecticides is commonly used in several Egyptian villages, for pest control.

At an earlier time, the main active ingredient of these coils was extracted from dried pyrethrum (Chrysanthemum cinerariafolium) flowers [2]. But nowadays, coils are synthetically produced from keto alcoholic esters of pyrethrum (pyrethroid esters) including; d-allethrin, prallethrin, transfluthrin, meperfluthrin, and others [2,3]. Those are strongly lipophilic esters, able to paralyze insect organs through the peripheral and central nervous system [3].

Although mosquito coils were reported to be safer for human beings than other insecticides, such as DDT, they are not risk-free. Several studies referred that the health implications of inhaling fumes from one burned one mosquito coil is equivalent to burning 75–137 cigarettes in released particulate matter and 51 cigarettes in emitted formaldehyde [2,4].

Coils are usually produced on biomass as a base material (e.g. coconut powder and sawdust) to be well designed for smoldering, where
during burning, several volatile organic compounds, heavy metals, and persistent organic pollutants were emitted inducing severe impacts through inhalation [5,6]. During overnight sleeping, children and adult inhabitants are exposed to inhale these components. Epidemiological studies showed asthma and persistent wheeze in long-term exposure of children [7,8].

Recent studies showed impairment of biochemical parameters and histopathological changes in lung, liver, and brain of exposed animals to mosquito coil fumes, including adult rats [9,10] and rabbits [11].

In proper, the kidney is highly vulnerable to the effects of environmental toxins, where it represents an important target for hazards to maintain body homeostasis and manage wastes [12]. Experimental studies showed the bad impacts of exposure to mosquito coil fumes for longer periods on renal structure and function in adult male rats [9].

Biomarkers such as creatinine, urea, uric acid were routinely used for evaluating kidney function in both serum and urine samples. However, these markers are sometimes fraught with imprecision [13]. Moreover, microalbuminuria has recently been used as a sensitive biomarker of early damage in the kidney, but this investigation is also insufficiently sensitive to detect the earliest alterations in kidney function [14]. Recently, neutrophil gelatinase-associated lipocalin (NGAL) and cystatin C (CysC) are recently used to sensitively reflect renal impairment [15], especially tubular and glomerulus injuries, respectively [16].

The effect of exposure to mosquito coil fumes on the kidney of different ages is not clear with the average time of exposure and new biomarkers are needed for more illustration. Accordingly, the present study aimed to assess the hazards of mosquito coil exposure on renal injury in young and adult albino rats using biochemical and histopathological studies.

**Material and methods**

**Experimental material**

Mosquito coils used in the present study were purchased from retail groceries located at Aga villages, Mansoura, Dakahlia, Egypt. They were of Laojun brand (JinjiangLaojun Chemical Co., Ltd, China). This brand was fixed throughout the study to avoid varieties of commercial products. Coils were black colored, each weighed 24–32 g that valid to alight for 7–10 hours. The active ingredient of this type of mosquito coils was declared to be meperfluthrin (0.05%), as one of the pyrethroid esters [10].

**Elemental analysis of mosquito coil**

A small piece of mosquito coil was grinded by mortar, where element constituents were detected by using a scanning electron microscope unite (JEOL JSM-6510LV, USA) provided with Energy-dispersive X-ray spectroscopy (EDAX) technique for detecting atomic composition, Japan allowed at faculty of Agriculture, Mansoura University.

**Experimental animals**

Male Wistar albino rats (n = 40) of two ages; about four weeks young weighed (80–85 g) and nine weeks adult weighed (225–235 g) were purchased from the Egyptian Institute for Serological and Vaccine Production, Helwan, Egypt.

The animals were chosen to mimic the young human age (10–12) years and adults about (35–40) years. Animals were left to be acclimated for one week in a housing room with free access to water and food ad libitum during the whole time of the experiment. The restrictions of the Animal Care Committee of Mansoura University, Egypt was followed in animal handling and care over the experimental period.
Design of exposure

Inhalation of ablaze coil fumes was achieved in a limited ventilated space with a closed door mimicking the house room. A stainless-steel chamber (1.5x 0.9 × 2.1) m³ was used. For permitting airflow inside the chamber, two opening holes of 5 cm diameter in all sides are made, except the upper and lower sides. In the middle of the chamber ablaze mosquito coil was placed on a metal wire for support during the exposure period.

Research plan

After the acclimation period, each age group was classified, as 10 rats in each to control young (CNY) and adult (CNA) groups away from coil fumes or any source of pollution. Exposed young (EXY) and adult (EXA) groups that transferred to the exposure chamber to wholly inhale fumes of alight mosquito coil for 6 hours/day (8 am to 2 pm), 6 days/week for 4 weeks, during January 2018. The recorded temperature and humidity during this period were 20 ± 3°C and 45 ± 5%, respectively.

Bodyweight changes and somatic indices

Means of body weight were assessed at the end of each week for each group, then % of body weight gain at the end of the experimental period was calculated using the following equation,

\[
\text{Body weight increase\%} = \left( \frac{\text{Final weight}}{\text{zero weight}} \right) \times 100\%
\]

At the end of the experiment, somatic indices of organs (kidney, liver, heart, lung) were determined using the equation;

\[
\text{Somatic index (SI)\%} = \left( \frac{\text{Total organ weight}}{\text{total body weight}} \right) \times 100\%
\]

Collection of urine and blood samples

At the end of the last exposure day, each animal of every group was kept separately in a clean metabolic cage for collecting urine overnight. In the morning, urine was collected, and animals were sacrificed under anesthesia. Each blood and urine samples were centrifuged at 855 x g for 15 min., where supernatants of urine and serum were kept at −20°C till analysis.

Biochemical studies

Serum and urine samples of each group were assessed for the levels of creatinine, urea, total protein, electrolytes [sodium (Na⁺), and potassium (K⁺)] in the presence and absence of coil fumes exposure. All parameters were assessed according to the enclosed methods of Spinreact Kit, Ctra Santa Coloma, Spain. NGAL expression was detected using ELISA Kit of BioPorto Diagnostics, Danmark according to manufactures’ protocol. Moreover, the detection of serum uric acid was conducted according to the protocol of Spinreact Kit, Ctra Santa Coloma, Spain. While serum cystatin C and urine microalbuminuria were assessed according to the enclosed protocols of ELISA kit; MyBiosource, Inc., San Diego, USA.

The glomerular filtration rate (GFR) was estimated from the equation provided by Pottel et al. [17]:

\[
\text{GFR} = \frac{(\text{urine creatinine} \times \text{urine volume})}{\text{serum creatinine ml/min}}
\]

Histological studies

From sacrificed rats, the right kidneys were excised, kept in 10% formol saline, dehydrated with alcohol, cleared with xylene, then embedded in paraffin wax and 5 μM sections were stained by hematoxyline and eosin (H&E) for microscopic examination.

Statistics

Data were analyzed statistically using a graph Pad Prism software program (v 5.04 Graph Pad Software Inc., La Jolla, CA, USA) in which, one-way analysis of variance (ANOVA) test, followed by Tukey test were applied. Obtained results
were expressed as mean ± standard deviation (SD), where values were considered statistically significant at \( p < 0.05 \).

**Results**

**Element constituents of coils**

EDAX analysis showed the main elements of mosquito coil with their relative percent, as illustrated in (Table 1). Data showed that carbon and oxygen showed the highest ratio to the other elements that included, Na, Mg, K, S, Cl, P, Ca, Si, and some metals; Al, Fe, Cu, Zn, and Mn.

**Effect of mosquito coil fumes on body weight changes and somatic indices**

By comparing the mean body weight of each group through the experimental period, results showed a non-significant decrease in both young and adult exposed groups compared to their respective control groups. Moreover, % of bodyweight increase recorded 236.90% and 122.03% in young and adult exposed groups compared to 254.93% and 125.11% of control groups, respectively. However, somatic indices of kidney, liver, and lung exhibited unpronounced increase with mosquito coil exposure in both ages, but heart somatic index (HeSI%) elevated significantly in both exposed groups compared to their respective control groups, where EXY and EXA recorded 0.568%, 0.467% compared to CNY and CNA (0.463% and 0.370%), respectively (Table 2).

**Effect of mosquito coil fumes on biochemical parameters**

**Serum samples**

Serum creatinine, urea, and uric acid were elevated with exposure to mosquito coil fumes compared to control groups. This increase was more pronounced with the young group than adults, where % of the increase in these parameters were 61.36%, 62.5%, 93.23% in the young exposed group, but in adult exposed one, they recorded 38.46%, 52%, and 74.24%, respectively, where the increase in serum creatinine in adult was non-significant. In contrast, serum total protein decreased significantly by −33.44% in the young exposed group and −31.32% in adult exposed ones compared to their control groups (Table 3).

On the other hand, levels of serum NGAL and cystatin C increased significantly in both ages exposed to fumes recording (116.67%, 80.70%) and (144.2%, 121.85%) for both parameters in the young and adult exposed groups, respectively. Moreover, the detected serum electrolytes (Na⁺ and K⁺) in both exposed groups were not significantly changed (Table 3).

**Urine samples**

By urine analysis, results showed significantly increased levels of creatinine, urea, urine protein, and microalbuminuria in both exposed groups, except with creatinine levels in the exposed adult group. These parameters increased in urine by 15.67%, 37.93%, 87.38%, and 21.26%, respectively, in the young exposed group. Moreover, in the adult exposed group, the % of the increase was 2.33%, 29.23%, 44.29%, and 20.86%, respectively. However, GFR reduced significantly in young and adult exposed groups (−30.15% and −38.10%). Contradictory, NGAL levels exhibited a more pronounced increase in the young exposed group (116.95%) than the adult exposed

| Element | Weight | Atomic |
|---------|--------|--------|
| C       | 60.62  | 68.9   |
| O       | 44.15  | 39.14  |
| Na      | 1.76   | 1.55   |
| Mg      | 1.49   | 1.25   |
| K       | 2.48   | 1.29   |
| S       | 0.56   | 0.35   |
| Cl      | 0.74   | 0.43   |
| Mn      | 0.36   | 0.13   |
Table 2. Mean body weight and somatic indices of studied groups.

| Groups Weeks | CNY | EXY | CNA | EXA |
|--------------|-----|-----|-----|-----|
| 0 week       | 81.2 ± 9.47 | 84 ± 11.5 | 231 ± 16.4 | 227 ± 16.3 |
| 1st week     | 114 ± 12.8 | 115 ± 11.3 | 250 ± 19.1 | 240 ± 17.5 |
| 2nd week     | 155 ± 17.2 | 150 ± 17.7 | 264 ± 19.3 | 250 ± 20.7 |
| 3rd week     | 188 ± 24.5 | 186 ± 17.3 | 275 ± 22.1 | 268 ± 23.0 |
| 4th week     | 207 ± 28.7 | 199 ± 25.9 | 289 ± 47.8 | 277 ± 29.9 |
| % weight increase | 254.93% | 236.90% | 125.11% | 122.03% |
| KSI%         | 0.830 ± 0.037 | 0.880 ± 0.054 | 0.590 ± 0.079 | 0.673 ± 0.028 |
| HSI%         | 3.43 ± 0.229 | 3.49 ± 0.162 | 2.42 ± 0.201 | 2.46 ± 0.352 |
| HeSI%        | 0.463 ± 0.023 | 0.568*±0.075 | 0.370 ± 0.054 | 0.467*±0.022 |
| LSI%         | 0.752 ± 0.132 | 0.778 ± 0.110 | 0.638 ± 0.077 | 0.697 ± 0.027 |

CNY: Control young, EXY: Exposed young, CNA: Control adult, EXA: Exposed adult groups
Values represented mean±SD, * significance relative to respective control group.
KSI, HSI, HeSI, LSI referred to kidney, hepatic, heart and lung somatic indices

Table 3. Biochemical analysis of serum parameters in studied groups.

| Group Parameter | CNY | EXY | CNA | EXA |
|-----------------|-----|-----|-----|-----|
| Creatinine (mg/dl) | 0.880 ± 0.05 | 1.42*±0.25 | 1.17 ± 0.16 | 1.62 ± 0.31 |
| Urea (mg/dl)     | 34.4 ± 5.56 | 55.9*±7.35 | 45.0 ± 5.07 | 68.4 *±6.30 |
| Uric acid (mg/dl)| 2.66 ± 0.89 | 5.14*±0.21 | 2.64 ± 0.83 | 4.60*±0.54 |
| Total protein (g/dl) | 9.45 ± 1.03 | 6.29*±1.14 | 10.6 ± 0.60 | 7.28*±1.27 |
| Cystatin-C (ng/ml) | 0.138 ± 0.06 | 0.337*±0.08 | 0.151 ± 0.02 | 0.335*±0.05 |
| NGAL (ng/ml)     | 0.126 ± 0.04 | 0.273*±0.03 | 0.171 ± 0.02 | 0.309*±0.07 |
| Na+ (mmol/l)     | 83.8 ± 12.3 | 82.6 ± 7.32 | 129 ± 25.0 | 134 ± 7.31 |
| K+ (mmol/l)      | 4.82 ± 0.72 | 4.56 ± 0.97 | 4.90 ± 0.47 | 4.77 ± 1.42 |

CNY: Control young, EXY: Exposed young, CNA: Control adult, EXA: Exposed adult groups
Values represented mean±SD, * significance relative to the respective control group.
% of change = (EX-CN)/CN x100.

The group that significantly increased by 76.69%. Excretion of urine electrolytes (Na+ and K+) were non-significantly reduced in both exposed groups, but the decrease in Na+ levels in young, as shown in (Table 4).

Histopathological findings

Microscopic examination of control kidney in both young (Figure 1(a,b)) and adult (Figure 2 A&B) showed typical configuration of renal aspects, which characterized by neat arrangement of the medulla and cortex with fully formed renal tubules, glomeruli, and blood vessels. However, exposure to mosquito coil fumes, resulted in histopathological alterations in the renal structure that were more pronounced in the young exposed group (Figure 1(c,d)) than in adult one (Figure 2(c,d)). This was represented by glomerulonephritis and several tubular necrotic patches, sever dilatation in blood vessels, with the appearance of several pyknotic nuclei. However, changes in adult kidneys appeared to be less than the
young exposed group compared to their respective control groups.

**Discussion**

Mosquito coils are insecticides primarily manufactured from the pyrethrum plant. However, with increasing demand for this type of pest control, due to its cheapness and availability, synthetic pyrethroid, mainly esters are produced [8]. One of these pyrethroid esters is meperfluthrin (the active ingredient of the mosquito coil used in this study). Few studies worked on coil brands contained meperfluthrin targeted its effects on liver function of adult rabbits [11] and toxicity of oral, dermal, and pulmonary application of mosquito coils on adult rats [10].

To evaluate the effect of coil fumes on kidneys of young rats and compare these effects to adult rats the change in body weight was first assessed as an indication of general body health. In this study, non-significant increase in the percentage of body weight was recorded either in young or adult exposed groups in comparison to their relative controls through the study period. This agreed with Garba et al. [1] and Ayorinde et al. [18] where adult male rats were exposed for 8 hours/day for 4 weeks. However, obtained data showed significant elevation in the heart somatic index (HeSI%) in young and adult exposed rats, while the change at other indices of body organs (HSI%, KSI%, and LSI%) were non-significant in both ages compared to their control groups. Ayorinde et al. [18] attributed this increase in HeSI% to increased blood flow velocity and blood vessel diameter due to hypoxic conditions result from inhalation of coil fumes.

On the other hand, even the experiment lasted only for 4 weeks, a significant decrease in serum total protein and an increase in urine protein and microalbuminuria were observed in young and adult exposed groups. These data support the finding that longer exposure may lead to a significant decrease in body weight, as observed by Pauluhn and Mohr [19] who exposed animals for 13 weeks to coil fumes. In parallel, serum and urine creatinine, as well as urea levels showed significant elevation, especially in young exposed groups compared to adults. Taiwo Idowu et al. [9] attributed this to the affected protein biosynthesis in the liver and increased catabolism through repeated exposure to coil fumes containing components that may interfere with metabolic functions leading to metabolic derangement in liver cells [20]. The authors provided another explanation that serum total protein may be decreased.

### Table 4. Biochemical analysis of urine samples of studied groups.

| Group Parameter      | CNY     | EXY      | CNA      | EXA      |
|----------------------|---------|----------|----------|----------|
| **Creatinine**       | 734 ± 137 | 849*± 50.1 | 1074 ± 44.2 | 1099 ± 227 |
| (mg/day)             | 15.67%  | 16.08%   | 13.80%   | 16.8±2.06 |
| **Urea**             | 11.6 ± 2.29 | 16.08± 0.82 | 13.0 ± 0.82 | 29.23%   |
| (g/day)              | 37.03%  | 37.03%   | 37.03%   | 44.29%   |
| **Urine protein**    | 42.0 ± 7.00 | 78.7±11.7 | 79.7 ± 12.3 | 115.0±15.3 |
| (mg/day)             | 87.38%  | 87.38%   | 87.38%   | 20.86%   |
| **Microalbuminuria** | 10.0 ± 2.65 | 12.7 ± 3.79 | 16.3 ± 4.16 | 19.7 ± 6.11 |
| (mg/day)             | 21.26%  | 21.26%   | 21.26%   | 20.86%   |
| **GFR**              | 73.3 ± 9.29 | 51.2±6.93 | 53.8 ± 5.39 | 33.33±3.79 |
| (ml/min)             | 21.26%  | 21.26%   | 21.26%   | 20.86%   |
| **NGAL**             | 0.118 ± 0.03 | 0.256±0.05 | 0.163 ± 0.05 | 0.288±0.02 |
| (ng/ml)              | 116.95% | 116.95%  | 116.95%  | 76.69%   |
| **Na**               | 148 ± 14.8 | 88.3±3.51 | 141 ± 21.6 | 111 ± 9.71 |
| (mmol/day)           | 20.86%  | 21.26%   | 21.26%   | 20.86%   |
| **K**                | 100 ± 10.1 | 87 ±13.5  | 94.2 ± 11.6 | 93.4 ± 11.7 |
| (mmol/day)           | 20.86%  | 21.26%   | 21.26%   | 20.86%   |

CNY: Control young, EXY: Exposed young, CNA: Control adult, EXA: Exposed adult groups
Values represented mean±SD, * significance relative to respective control group.

% of change = (EX-CN)/CN x100
Figure 1. Photomicrographs of T.S. in the kidney of young control and exposed groups. The kidney of control young group showing typical renal architecture with intact renal tubules (RT) and glomeruli (G) with the appearance of a blood vessel (BV) (A X100 & B X400). The kidney of the young exposed group showed degenerated renal tubules (RT), segmented glomeruli (G), dilated blood vessels (BV), with the appearance of necrotic patches (stars) and pyknotic nuclei (arrowhead) (C100X & D X400).
Figure 2. Photomicrographs of T.S. in the kidney of adult control and exposed groups. The kidney of the adult exposed group showing degeneration in renal tubules (RT), some segmentation in glomerulus convoluted tubule (G), necrotic areas (stars), and few pyknotic nuclei (arrowhead).
damage. Another explanation was provided by Uthman et al. [11] who stated that the combustion of mosquito coils exerted their bad effects on organ histology through modification of tissue microsomal function leading to tissue or organ damage.

**Conclusion**

Relying on these foundations, it can be concluded that usage of mosquito coils represents an ecological challenge that showed harmful impacts on the kidney structure and function of either young or adult rats, while young rats were more vulnerable to mosquito coil fumes even at average periods of exposure. So, avoidance of inhalation of mosquito coil fumes especially in closed rooms is recommended even for short periods.

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