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Evidence of health complications caused by mosquito coil smoke inhalation in mouse model

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ABSTRACT: Direct exposure to mosquito coil smoke (MCS) is a regular event in the entire life of most tropical country peoples, although it has countable significant health implications. However, noxious effect of mosquito coil smoke exposure on living being remains elusive. Herein, to address the mosquito coil smoke implication on health, Swiss albino mice were directly used to detect the health indicator alteration upon MCS administration as experimental model. In total 45 albino mice were grouped and exposed with MCS in two different time period viz, short time for 20 days and long time for 40 days. At the end of exposed time period, blood samples were collected and various health indicators analyzed. We revealed that biochemical indicators like serum aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT), bilirubin, urea, glucose, cholesterol, and triacylglycerol (TG) as well as hematological indicators like red blood cell (RBC), hemoglobin (Hb) and leukocyte (white blood cell) were significantly augmented in the exposed mice as compared to controls. In contrast, serum levels of total protein and albumin were remarkably decreased while serum creatinine and globulin level remained relatively unchanged. Furthermore, the microscopic analysis showed that the histopathological lesions were present on the lung tissue of exposed mice resulting, the alveolar septa and bronchiolar epithelial thickening, emphysema, and atelectasis of lung tissue as well as increased alveolar macrophages. Despite of, liver histopathology of the exposed mice showed same findings with a little deviation. Collectively, our findings propose that inhaling mosquito coil smoke induce significant health hazards on mice model followed by modification of vital health indicators and histological tissue-alignment alterations of lung and liver tissues.

KEYWORDS: Mosquito coil smoke, hematological indicators, histopathological lesions, emphysema.

INTRODUCTION

Mosquitoes are a group of very familiar insects that grow and pass their early stages of lives in a variety of aquatic habitats: permanent and transient. Permanent habitats are river, ponds, lakes etc. and transient habitats include hollow trees, bromeliad tanks etc. [1]. Several lines of evidence established that mosquitoes carried out as main vectors of some catastrophic mammalian and animal diseases such as malaria, dengue, filariasis, chikungunya and West Nile virus, and which have made us to approve several methods to regulate their reproduction around our habitats. Among the insecticides that are used to regulate mosquito population, four major types- namely sprayers, mosquito coils, liquid vaporizers and sprayer units have the annual worldwide consumption of billions of units [2]. Because of their cheapness and readily availability, mosquito coils are the preferred anti-mosquito materials in many developing countries like Bangladesh. Mosquito coils are burned, which emit smoke containing one or more

insecticides as a regular practice in indoors to repel mosquito in maximum households of those countries [3]. The most familiar effective ingredients are various pyrethrins, elucidating for about 0.3 - 0.4% of the coil's mass [4], which are very potent against maximum genera of mosquitoes counting Aedes as well as Anopheles. [5]. Toxic substances such as aldehydes, cyanide, sulphates, cyanohydrins and polyaromatic hydrocarbons (PAH) such as acenaphthene, phenanthrene, benzo(a)pyrene etc. are generated from the combustion of the remnants (organic fillers, binders, dyes etc.) of mosquito coil [6]. Evidence shows that the effect of burning 75 to 137 cigarettes is equivalent to the effect of burning a single mosquito coil on health. Despite of it's also noticed single MC emitting formaldehyde as equivalent to 51 cigarettes [7]. Insecticides evaporate with smoke prevent mosquitos entering the room [4]. That's why, mosquito coils are widely used overnight in living rooms where augmented exposure may occur and people are often inhaled to those toxic chemicals [8]. Epidemiologic studies show that, children, who are exposing a long term of mosquito coil, have respiratory problems like asthma and persistent wheeze [9]. In addition, it has been reported that coil smoke has mutagenic effect that cause chromosomal aberrations in metaphases and exposed rats and mice have a significantly higher incidence of chromosomal mutation [10]. Toxicological studies in rat models by using mosquito coils have reveled central declination of the epithelial metaplasia and morphologic dissimilarities of the alveolar macrophages [11]. However, the most menacing point is that a large number of unconscious people use mosquito coil as a daily basis event almost all over the year in their closed sitting rooms and bedrooms to get rid of mosquito and mosquito borne diseases. Therefore, this study was conducted to evaluate the toxic effect of mosquito coil smoke on human health indirectly using mice model.

Here, we found that serum aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT), cholesterol; triacylglycerol (TG) and glucose level were significantly augmented in mice models. Along with this red blood cell (RBC), hemoglobin (Hb), and leukocyte (white blood cell) levels were also significantly accelerated, whereas total protein and albumin levels in serum were remarkably decreased. Despite of it, serum creatinine and globulin level was remained relatively unchanged. Thus, the harmful effects of Mosquito coil elucidated on mice model in this study.

MATERIALS AND METHODS

Experimental animals

Swiss albino male mice were selected as experimental animal to carry out this study. Mice, weighing about 20-25 g were collected from the "Animal Resource Division" of ICCDR'B, Mohakhali, Dhaka, Bangladesh. The experiment was conducted in a room of size 17.5 m3 (3.5m × 2.5m × 2m). A total of forty five (45) male mice weighing about 20-25 g were used. Two weeks acclimation period of mice were followed and personally investigated by color tattoo indication and weighed. The mice were maintained in cages at room temperature of 22 ± 3°C, corresponding moisture 50-60% with a ½ day light: ½ day dark cycle manner. They had free access to drinking water and provided ideal laboratory diet. Along with the ideal laboratory animal ethical guidelines, this study was maintained at the laboratory of animal house, department of Biochemistry & Molecular Biology, University of Rajshahi, Bangladesh.

Test compounds

Mosquito coils were collected locally from several outlets situated within Rajshahi, Bangladesh. The brand mosquito coils commercially purchased for the experiment consist of pyrethroids (d-trans-allethrin). The used non brand mosquito coil was measured 13 cm diameter and 30 g weight.

Treatment schedule

The total 45 Swiss albino male mice were grouped into three groups in random manner and each group consists of fifteen mice. Group I counted as control and without inhalation to mosquito coil smoke; Group II was exposed with mosquito coil smoke for short time for 20 days, while Group III was exposed with mosquito coil smoke for long time for 40 days time period. The study was accomplished by igniting one mosquito coil per day for 8 hours (8 p.m. to 4 a.m.).

Blood sample collection for serum biochemistry and hematological study

After the completion of treatment schedule, mice of all groups were anaesthetized by using highest dose of pentobarbital anesthesia (90 mg/kg) injection in peritoneal region and then weighed and sacrificed. The blood was collected into cold heparinized tube from mice abdominal aorta and placed the tubes at 4°C. Then, the blood samples were centrifuged at 8000 rpm, at 4°C

for 15 minutes and the blood plasma were separated within 30 minutes of blood collection. After that, the serum was separated into another eppendorf tubes by micropipette. Finally the collected serum was kept at -80°C for serum biochemistry results analysis. For hematological study, blood was peaked from each group of mice of indicated time period by tail puncture.

H&E staining of mice liver and lung tissue

The liver and lung were collected from each of three mices of every experimental group of indicated time period after dissected. These two organs were rinsed in 1X PBS for 10 min two times to discard any blood and debris affix on the outer surface of organs. Then sliced tissues were occupied with 10% formalin for 3 days. Then the tissues were dried out and placed in ethanol in ascending order as well as fixed with paraffin and cleaned in xylene. Before performing with H&E staining, the tissue slide was de-paraffinized and rehydrated. Tissues slide was placed in a rack and performed de-paraffinization with xylene 2 min 3 times. Then slide was washed with ethanol 100% (2X3 min), 90% (2X3 min) and step wise 80%, 70%, and finally rehydrated 30 min with cold water, subsequently the slide was washed with 1X PBS for 10 min two times. Finally for staining, slides were stained with haematoxylin and eosin. Then tissue containing glass slides were cleaned, dried and mounting with a drop of Canada balsam with the help of cover slip. And then the prepared slide was observed under microscope.

Statistical analysis

Statistical analysis and tabulated presentation of all data were performed using the Statistical Package software for Social Sciences, SPSS (version 21.0). In this study, the data are presented as the mean values and standard deviation (SD) of 3 independent experiments. Statistical analysis of significance was based on Student's t-test, in which *p< 0.05 and **p< 0.01.

RESULTS

Effect of mosquito coil exposed to mice body weight

The harmful consequences of mosquito coil smoke inhalation on mean body weight of treated ant non-treated mice are recorded in Table 1. The percentage of mean body weight changes were reduced in both groups exposed to MCS for indicated time period as compared to control group. Although, short time exposed (group II) mice mean body weight slightly reduced but long time exposed mice significantly decreased their body weight as compared with both control and group II mice. So, it clearly indicates that long time mosquito coil inhalation may have negative health implications due to decreases body weight of exposed mice.

Table 1. Consequence of mosquito coil exposed on mice mean body weight

Groups	Introductory weight (gm)	Closing weight (gm)	Weight difference (gm)	% of weight change
Group I: Control	23.10±2.03	35.03±1.52	+11.93	51.65
Group II: Treatment 20 day	24.78±1.95	35.19±3.09	+10.41	42.01
Group III: Treatment 40 day	23.02±3.11	28.86±2.10	+4.84*	21.03

Effect of mosquito coil smoke inhalation on serum biochemistry and hematological indicators

The health implication effect of mosquito coil smoke inhalation on serum biochemistry and hematological indicators of treated ant non-treated mice are clearly tabulated in Table 2. The alteration of serum biochemistry and hematological indicators due to MCS inhalation are represented here as Mean ± SD. Biochemistry results of blood, indicate that significant alterations in MCS inhaled groups as compared with control groups in almost all health parameters such as liver function, kidney function and full blood count respectively. Comparing to controls liver function indicator of serums ALT, AST, ALP and bilirubin were significantly (*p<0.05) augmented in long term (treatment 40 days) coil smoke exposed mice, except serum ALP also significantly increased at short term treatment group (20 days). Otherwise, number of total protein levels and albumin content in serum were significantly (*p<0.05) decreased only at long time treated mice groups (40 days) but it was maintained the opposite trend at short time treatment mice groups. In case of serum globulin no major changes reported but changing trend was decreasing in both short and long time treatment period. In addition, kidney function indicator urea content in blood increased only in long time induced mice group. However, serum creatinine was maintained increasing trend without significant. Here, we observed that lipid profile and blood sugar parameters activity significantly (**p<0.01 for total cholesterol and *p<0.05) augmented in long time MCS inhaled mice group. Anyway, all indicators followed by increasing trends without significant in short time exposed groups. Farther more, we found that full blood

count (FBC) parameters (WBC, RBC and Hemoglobin) activity augmented dramatically in both short time and long-time inhaled mice groups as compared with control. Trends pattern for p values shows that the activity of serum ALT, AST, ALP, bilirubin, urea, T. cholesterol, TG and hematology indicators were augmented when the MCS inhalation duration were prolonged (40 days), on the other hand total protein, serum albumin and globulin concentration were abated as a regular changeable. These results suggest that blood biochemistry parameters were changed with MCS inhalation indicated time manner.

Table 2. Mosquito coil smoke inhalation effect on serum biochemistry and hematological indicators

Parameters	Group-I (Control)	Group-II (20 days)	Group-III (40 days)
Liver Function indicators			
S. ALT (U/L)	41.00 ± 5.79	44.20 ± 5.40	52.00 ± 8.06*
S. AST (U/L)	30.20 ± 5.17	33.20 ± 5.72	43.00 ± 7.61*
S. ALP (U/L)	87.40 ± 11.26	103.40 ± 10.64*	116.40 ± 12.84**
Bilirubin (mg/dl)	0.42 ± 0.10	0.59 ± 0.14	0.66 ± 0.14*
Total protein (g/dl)	5.51 ± 0.52	5.27 ± 0.83	4.61 ± 0.54*
S. Albumin (g/dl)	4.15 ± 0.36	4.04 ± 0.69	3.50 ± 0.47*
S. Globulin (g/dl)	1.36 ± 0.26	1.22 ± 0.24	1.11 ± 0.17
Kidney function indicators			
Creatinine(mg/dl)	0.68 ± 0.09	0.65 ± 0.11	0.83 ± 0.12
Urea (mg/dl)	28.00 ± 5.57	30.60 ± 4.82	37.40 ± 6.84*
Lipid profile and Blood sugar indicators			
T. Cholesterol (mg/dl)	137.80 ± 15.53	154.20 ± 12.91	175.80 ± 12.93**
TG (mg/dl)	89.40 ± 10.53	102.80 ± 9.78	107.60 ± 10.29*
Glucose (mg/dl)	92.60 ± 10.92	95.60 ± 09.48	111.20 ± 10.40*
Full blood count/Hematology indicators			
WBC (cells/ml)	$(10.40\pm1.82)\times10^6$	$(14.0\pm 1.87)\times10^6$*	$(13.80 \pm 2.68)\times10^6$*
RBC (cells/ml)	$(5.57 \pm 0.49)\times10^9$	$(5.57 \pm 0.47)\times10^9$	$(6.09 \pm 0.66)\times10^9$
Hemoglobin (g/dl)	10.74 ± 1.31	12.56 ± 0.83*	13.66 ± 1.11**

Results are presented as Mean ± SD, where N=5; and *p<0.05 was considered significant, **p<0.01 highly significant.

Effect of mosquito coil smoke inhalation on lung tissue histology

In control group (untreated), the cross section of lung tissue displayed normal histological structures and condensed organization with thin alveolar duct, alveolar sac, pulmonary artery and inter-alveolar septa (Figure 1a).

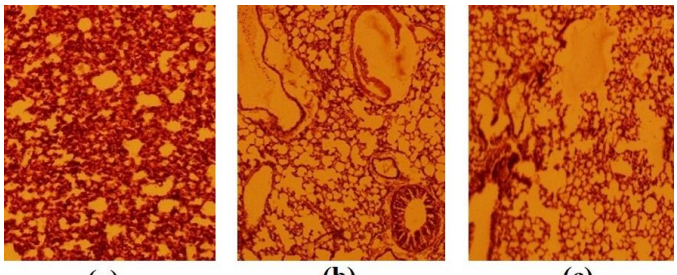

Figure 1. Microscopic views of lung tissue of experimental mice. (a) Lung tissue slide was prepared and visualized by microscope using H&E staining from Group-I (Control) mice. (b) and (c) Lung tissue slide of mosquito coil smoke exposed mice (Group-II for 20 days and Group-III for 40 days) were visualized by microscope using H&E staining. (Microscopic view, X400).

Conversely, mosquito coil smoke inhaled both mice groups histopathological lesions were noticed on the lung tissues including thickening of the alveolar septa, bronchiolar epithelial thickening, and emphysema, atelectasis of lung tissue and enlargement of alveolar macrophages (Figure 1b & c). This result shows that different extent of histo-tissue arrangemental alterations in a time dependent manner at inhaled mice group's as compared with control.

Effect of mosquito coil smoke inhalation on liver tissue histology

Noxious effect of mosquito coil smoke (MCS) on liver tissue in treated or non-treated mice groups are depicted in Figure 2. In control group (untreated), the cross section of liver tissue shows regular and well characterized hepatocytes and central vein with compact architectural configuration (Figure 2a). Differently, apoptotic and necrotic hepatocytes in the sinusoidal space and along with in the site of central vain were observed at the short-term inhaled mice groups of liver section (Figure 2b). Similarly, we found liver histopathology of the long term exposed mice group same degraded hepatocytes, expansion of central vein

and distention of sinusoid with a little deviation with treated group-II mice. Taken together, these results suggests that toxicological effects of mosquito coil smoke have a great implications on health condition of MCS inhaled mice groups.

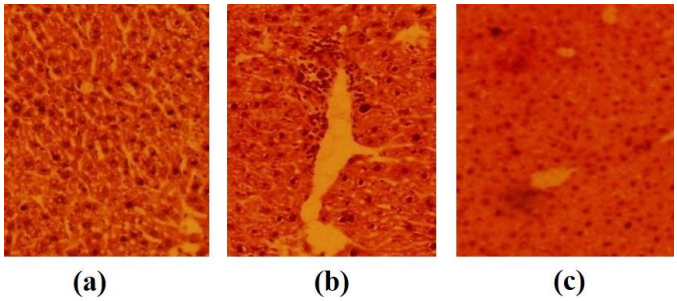

Figure 2. Microscopic views of liver tissue of experimental mice. (a) Liver tissue slide was prepared and visualized by microscope using H&E staining from Group-I (Control) mice. (b) and (c) Liver tissue slide of mosquito coil smoke exposed mice (Group-II for 20 days and Group-III for 40 days) were visualized by microscope using H & E staining. (Microscopic view, X400).

DISCUSSION

This research was planned to clarify the noxious effects of Mosquito coil inhalation on mice model. Not enough the body weight effect upon mosquito coil exposed was noticed. Declined mean body weight trend was evidenced in rat model upon MCS inhalation, although it was no significant but similar body weight measurement trend followed by our observations [2].

The experimental results (Table 2) reveal that the mosquito coil smoke elevated the level liver function enzymes in blood which indicates the hepatic damage or dysfunction. Similar result was found in a study reported by Abubakar *et al*. [12]. The increment of these enzyme levels is not due to elevated rate of biosynthesis, rather the crackage of these enzymes (Figure 2) from cytosolic damaged hepatic cells into the blood stream [13]. Elevated serum bilirubin explores the possibility of the impairment of the biliary excretory system has been previously confirmed by several studies [14].

The blood concentration of excretory constituents (urea and creatinine) is an important marker in assessing the functional capacity of the kidney [15]. The present study represents the higher serum urea level in mice which were directly inhaled mosquito coil smoke compared to control. This may be caused by the excessive catabolism of blood protein and tissue protein. But kidney dysfunction was not confirmed due to the lack of significant change in creatinine level. Increased level of serum urea is also related to the hepatotoxic effect of chlorine which is present as an inert ingredient of mosquito coil [16]. Reduced serum contents of total protein like albumin and globulin were also found in this study in coil smoke exposed mice. This may due to excess breakdown of these proteins or reduced protein biosynthetic activity of liver due to direct coil smoke exposure. Hematological study characterizes the elevated WBC and it may be the result of immune response against toxic smoke. Mosquito coil smoke is responsible for the elevation of Hb level [17]. This is due to physiological response of the body to low levels of oxygen intake while inhaling smoke.

No histological alteration was observed in the lungs and livers of the mice referred as control. Conversely, histopathological lesions were noticed on the lung tissue of coil smoke inhaled mice including fibrous thickening of the alveolar septa which is called pulmonary fibrosis, thickening of bronchiolar consequently results rigid lungs (Figure 1). As a result normal respiratory function is impeded. Another degenerative event named emphysema and atelectasis was observed in treatment group. Recruitment of polymorphonuclear leukocytes and monocytes in the respiratory tract, due to of epithelial injury caused by toxic substance of coil smoke, may be the reason of emphysema.

The deformation of normal liver tissue architecture, reduced intercellular space, inflammation and absence of the usual lobular arrangement became noticeable from the histological analysis of the liver of mosquito coil smoke exposed mice. These may be due to the exposure of various toxic substances dispersed through coil smoke.

CONCLUSIONS

Our work unveils that mosquito coil smoke exposure has adverse effects on lung, liver and kidney of Swiss albino mice. Biochemical assay revealed significant elevation of the liver function enzyme activity, serum level of bilirubin, urea, glucose, cholesterol, triglyceride and the reduction of the serum content of total protein like albumin and globulin level in the treatment groups comparing to the normal. The hematological analyses demonstrated the increment of the total WBC count and hemoglobin content in the smoke exposed groups. Microscopic alterations of lung and liver at tissue level were also found from the histological study. On the other hand, there was no significant change on serum creatinine level and total red blood cell (RBC) count. All these findings suggest that inhaling mosquito coil (local brand) smoke causes toxic effects on experimental mice models in time dependent manner. But further investigation is required to study the mechanism of its toxicity and the reversibility or irreversibility of these harmful effects on experimental model system.

AUTHOR CONTRIBUTIONS

This work was a collaboration among all of the authors. DKG performed all experiments. MFR proposed the original idea and reviewed the scientific contents described in the manuscript. All authors read and approved the final submitted version of the manuscript. MRK and MTH outlines and wrote the draft of the manuscript. MAR submitted the manuscript. RI reviewed the manuscript. All authors read and approved the final submitted version of the manuscript.

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CONFLICTS OF INTEREST

Authors declared that they have no conflict of interest.

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