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Research Article

Exposure to Mosquito Coil Smoke Delays Healing of Acetic Acid Induced Gastric Ulcer in Male Wistar Rats

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Summary: Mosquito Coil Smoke (MCS is used in several Nigerian homes and some part of the world with reported grave consequences to the respiratory tract majorly. There is paucity of information on its outcome of its exposure on the gastrointestinal tract being a related organ to the respiratory tract. Forty-five male Wistar rats (123.1 ± 7.3g) were used in this study, they were grouped into 3 (n=15/group; 5 each day of experiment). Rats in the first group served as control (no MCS). The second and third groups were exposed to MCS for 8-10 h daily for 2 (2p) and 6 (6p) weeks respectively, in a well-ventilated room of 38.3m3 in size each day. After the period of exposure, chronic gastric ulcer was induced by intraluminal application of 50% acetic acid. The animals were sacrificed on days 0 (no ulcer induction), 3 and 10 post ulceration for complete blood count and ulcer scores. Stomach was excised for histology and biochemical assays, homogenized gastric tissues were analyzed by spectrophotometry for malondialdehyde (MDA), catalase and nitric oxide (NO) estimations. Data were expressed as mean ± SEM. Gross ulcer area (mm3) increased significantly on days 3 and 10 in 6p (167.3±16.03; 65.20±3.93) and 2p (152.7±6.20; 68.70±3.45) compared to control (93.26±2.80; 34.82 ±1.84) respectively. Lymphocytes count (%) decreased significantly on day 3 in 2p (60.60 ±1.97) compared to control (70.60 ± 0.87), Neutrophil count (%) in 6p (36.40 ±1.08: 30.20 ±1.46) increased significantly compared with control (25.60±0.80; 26.00 ±1.58) on day 3 and 10 respectively. MDA concentration in 6p and 2p increased significantly compared to control on day 3. Nitric oxide decreased significantly in 6p and 2p on day 3and 10 compared to control. Mosquito coil fumes proofs toxic to the stomach and more to inflamed rats stomach by delaying healing of gastric ulcer through reduction in NO and raised oxidative stress markers..

Keywords*: Mosquito coil smoke, gastric ulcer, ulcer healing, biochemical assay, oxidative stress*

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INTRODUCTION

The World Health Organization (WHO) predicted about 219 million cases of malaria worldwide in 2017, with expected 435,000 deaths from malaria in 2017 alone (WHO, 2018). The worst hit nations are majorly in Africa and affects mostly under under-5 years' individuals.

Mosquitoes are well known for causing disease in both animal and even human. They have been implicated in ailments such as malaria, West Nile virus, Dengue virus. The knowledge of its hazards has made many adopts multiple methods to reduce its breeding population in the environment. One of the several methods used in achieving this is the use of insecticides products which has increased in hundreds of thousand times (Krieger *et al* 2013).

Man is regularly exposed to environmental toxicants that could damage the biomolecules processes and thereby harm cells and tissues and invariably affects health at various levels. Certain lethal ecological toxicants such as pesticides and insecticides have bounty biological effects, being toxic not only to target organism but also to humans in several ways. The effect of usage of pesticides and insecticides in the ecosystem and has touched every section of life. (Kamsin 1997; Patil *et al*., 2003).

Mosquito coils are used locally because of its relative low cost and convenience (Milla *et al*, 2001). The mosquito coils are burnt indoors and outside the buildings in a manner suggestive of the common practice. Mosquito Coil fume (MCS) is insecticides repellant, and burns with fumes (Krieger *et al* 2003). Pyrethroids are the most abundant dynamic component of mosquito coils. Pyrethroids are active in destroying arthropods (Krieger *et al*., 2003). Studies have described that MCS has potential to alter genes in humans, such genes like tumour suppressor genes which could promote the proliferation of malignant mass within the organ of interest (Keshava *et al*., 1999).

Meanwhile, epidemiologic studies have described substantial exposure to MCS as hazardous and can

promote asthmatic symptoms in children (Aziziz and Henry, 1991, Fagbule and Ekanem 1994: Koo and HO, 1994). Rats expressed focal declination of the tracheal epithelial lining, metaplasia of the cells and mechanical alteration to alveolar macrophages following toxic exposure to MCS, (Liu and Sun, 1988: Liu and wong, 1988).

Despite reports of potential adverse health effects from exposure to MCS, several countries still indulge in the use of mosquito coils regularly to prevent mosquito bites (Liu *et al*., 2003). This is largely so because it is readily available, acceptable and affordable. Previous studies show effects of MCS emissions on irritating and carcinogenic compounds and other pollutants on the respiratory tract, nervous system, metabolic organ such as kidney, liver, immune system, memory and reproductive functions (Liu *et al*., 1987; Azizi *et al*., 1991; Fagbule *et al*., 1994; Cheesebrough 1998; Panda 1999; Abdel-Rahman *et al*., 2001; Okine *et al*., 2004; Garba *et al*., 2007; Wolansky *et al*., 2007;). Erstwhile, study in humans reported the possibility of wave-pattern deposits of particulate matter from the mucocilliary organs of the respiratory tract into the gastrointestinal tract (Moller *et al*., 2004). However, there is dearth of information on the effect of MCS on the gut inflammation and healing processes of gastric ulcer thereby making research in this area pertinent in view of the communications between the respiratory tract and the gut anatomy.

MATERIALS AND METHODS

Drugs and Chemicals

Mosquito coil: Double Rabbit' mosquito coil produced by Gongoni Company limited) were procured and used in this study. It contained pyrethroids (d-trans-allethrin; 0.2 %w/w and insert ingredient 99.8%ww) and was purchased in a retailed store at Bodija in Ibadan. Each mosquito coil has diameter of about 12 cm; 85cm long when straightened and weighs about 15g.

The other chemicals / Reagents: used for this study were gift from the Department of Physiology University of Ibadan. Ketamine was purchased from retail Pharmaceutical Company in Ibadan, Nigeria.

Animal Model and grouping

Animals and Husbandry: This study was carried out in the animal house of Physiology Department College of Medicine, University of Ibadan, Nigeria for a period of 2 -6 weeks. Forty-five (45) adult male Wistar rats (123.1±7.3g) were used. Rats were purchased from the Central Animal House of College of Medicine where they got acclimatized for two weeks. The rats were kept in plastic cages at room temperature of 25 ± 2^0C. They had free access to drinking water and standard laboratory rodent pellet diet (Vital Feeds PLC, Ibadan Nigeria). Ethical approval was sort and obtained from Animal Care and Use Research Ethical Committee (ACUREC), University of Ibadan, Reference number – UI- ACUREC/18/0088, and in accordance with the Guidelines of the National Institute of Health - (NIH, 1985).

Animal Grouping: The experiments were conducted in two rooms (3.8 x 3.6 x 3.0 m), measuring the total surface area of 71.76 m^3 with adequate cross ventilation. Each room was apart in a distance of about 100 meters from another to prevent the fumes from crossing to the control groups. Forty-five rats were randomized into 3 groups (n=15, per group), control, 2p and 6p. The groups 2p and 6p were exposed to MCS for 2 weeks and 6 weeks respectively for 8-12 h daily while control was not exposed to MCS. Five animals each were sacrificed from each group on day 0 (prior to gastric ulcer induction), and another set of 5 animals on days 3 and 10 (post gastric ulcer induction).

At the end of experimental periods, whole blood and gastric tissues were collected for hematological analysis, biochemical variables and histology, accordingly.

Determination of Haematological Variables: Blood samples were collected via retro-orbital route through heparinized hematocrit tube. This was let into an ethylene-diamine-tetra-acetic acid (EDTA) bottle. Consequently, haematological analysis was performed using methods of Dacie and Lewis (1984).

Gastric Ulcer Induction: Gastric ulcer induction was by the method described by Tsukimi and Okabe, (2005) with slight modification. Briefly, the abdomen was opened under ketamine-xylazine cocktail anaesthesia and the stomach was exposed. The anterior and posterior walls of the stomach were clamped together with a pair of 9 mm diameter eye forceps. A 50%acetic acid solution of 0.2 ml was injected slowly into the clamped portion through the anterior end of the stomach via a 23-gauge needle. The acid solution was withdrawn after 60 seconds and the abdomen was closed in two layers with 4-0 nylon suture.

Histological Study: The stomach, kidney, and liver of the sacrificed rats were excised and preserved in 10% formalin solution prior to section of about 5µm of fixed tissue from paraffin wax. Tissues were fixed using Heamatoxylin and Eosin (H&E) stain. The

stained sections were assessed for possible pathologic changes and presence of inflammatory cells.

Determination of Reactive Oxygen Markers and Antioxidant Assays

Protein concentration: Protein concentrations of the samples were determined by the Biuret method as described by Gornal *et al*., (1949).

Lipid peroxidation: Lipid peroxidation was determined from formation of thiobarbituric acid reactive substances (TBARS) by the procedure described by Varshney and Kale (1990). Malondialdehyde was determined as described previously by Adeniyi *et al*., (2014).

Catalase activity: Catalase activity was assessed by the method of Sinha (1972). The method described the role of dichromate in acetic acid when heated in the presence of H_2O_2 leading to the formation of unsteady perchromic acid.

Superoxide Dismutase (SOD) activity: The determination of SOD activity was by the method of Misra and Fridovich (1972).

Reduced Glutathione: The method of Beutler *et al* (1963) was used to assay the quantity of reduced glutathione (GSH) in the homogenized gastric tissues.

Nitric oxide: Nitric oxide was assayed by the method described by Ignarro *et al*., (1987). It relies on a diazotization response reported by Griess, (1879).

Statistical analysis

The results were expressed as mean ± standard error of mean (SEM). Data were analysed using GraphPad Prism software version 7 (Graph Pad Software, San Diego California, USA). One-way analysis of variance (ANOVA) was used to compare means, followed by Newman-Keuls Multiple comparison test. Data was considered significant at $P<0.05$.

RESULTS

Effect of exposure of Mosquito Coil Fumes on percentage mean body Weight (%) changes of Animals.: There was no significant difference in the effect of mosquito coil fumes on percentage change in body weight from the beginning of two weeks exposure to MCS to the sixth week of exposure compared with control.

Effect of Mosquito Coil Fumes on Relative Organ Weight (100g/100) (Liver, Kidney, Lungs): There was no significant change in relative weight of organs of test groups compared to control groups.

Effect of Mosquito Coil Fumes on Relative Stomach weights (100 g/100) of Stomach: The relative stomach weight (100g/100) was significantly increased, $p<0.05$; in the 6p group (1.21±0.13 g/100g) on day 10 post ulceration compared to the 2p (0.85±0.03 g/100g) and control (0.84±0.07 g/100g).

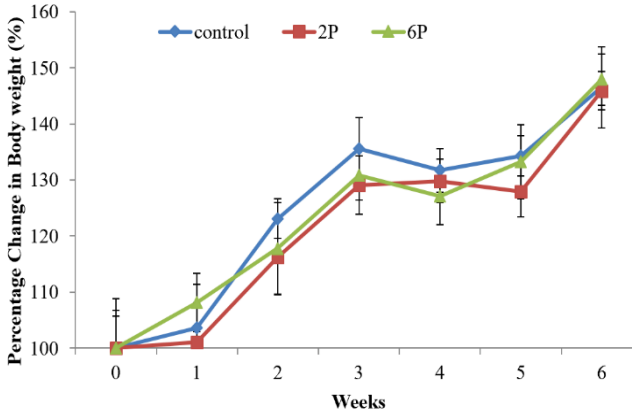


Figure 1:
Effect of exposure of mosquito coil fumes on percentage Mean body weight changes (%) of animals.

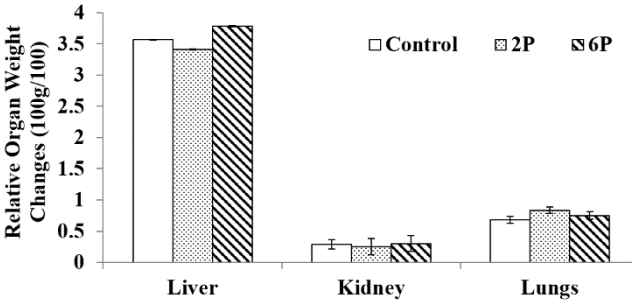


Figure 2:
Effect of mosquito Coil Fumes on relative organ weights.

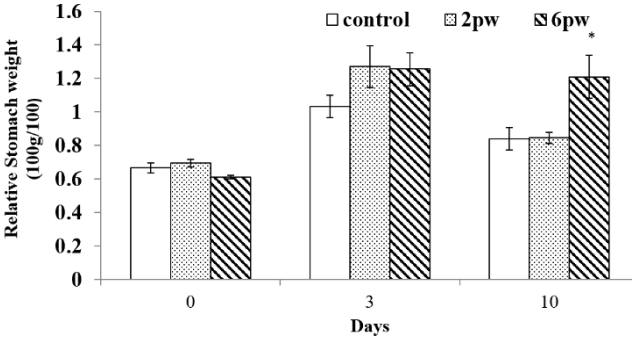


Figure 3:
Effect of mosquito coil fumes on relative stomach weight
***** $p<0.05$ when compared to control and 2pw

Effects of Mosquito Coil Fumes on Ulcer Area, Parietal and Mucous cell count: Different experimental days produced varying ulcer patterns as well as changes in the mucous and parietal cells. On the initial day following ulcer induction (Day 0), 6P group produced significant mucous cell count and parietal count (55.33 ± 4.57; 25.33 ±1.40) compared to

control (48.67 ± 6.94; 18.67 ± 1.39), respectively (Table 1). On day 3 following induction of ulcer, the ulcer area, mucous cell and parietal cell count changed significantly in both exposed groups; 2P (152.7±6.20, 39.33± 7.54, 3.33± 0.30) and 6P (167.3±16.03, 2.44±5.73, 25.33±1.40) compared to control (93.26±2.80, 69.33± 4.66, 26.78 ± 6.73), respectively (Table 2). In a similar pattern, two MCS groups sustained the increased in ulcer area by day 10 of ulcer induction. Groups 2P (68.70 ± 3.45) and 6P (65.20 ± 3.93) ulcer area increased significantly compared to control (34.82 ± 1.84). The parietal cell count decreased statistically in 2P (22.00 ± 5.28) and6P (23.11 ± 2.04) compared to control (12.44 ± 1.47), respectively. The mucous cell count on day 10decreased in 2P (39.33± 7.54) compared to the control (58.89± 7.89), (Table 3)

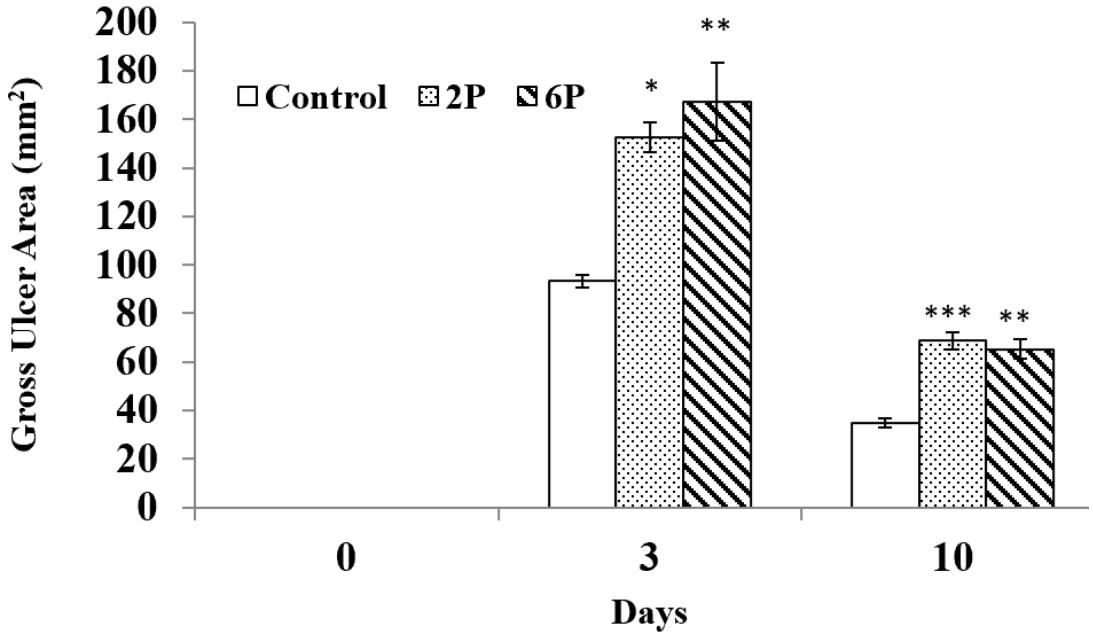
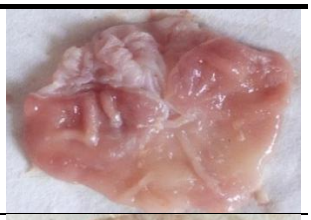
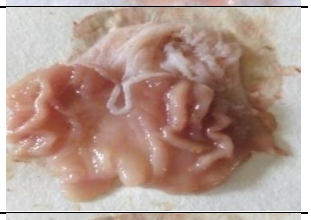
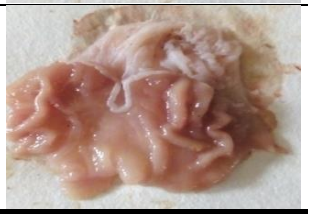


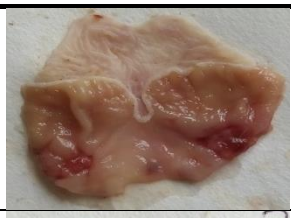
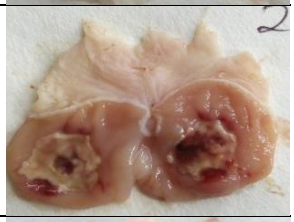
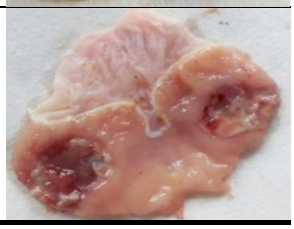
Figure 4:
Effect of mosquito coil fumes on ulcer area
** p<0.05 significant increase in ulcer score in two weeks group on day 3 compared to control.*
*** p<0.01 significant increase in ulcer score in six weeks group on day 3 and 10 compared to control.*
**** p<0.001 significant increase in ulcer score in two weeks group on day10 compared to control.*

Table 1:
Effect of mosquito coil fumes on gastric ulcer area on day 0

Group	Ulcer Area (mm^2)	Mucous cell count	Parietal cell count	Gross stomach Tissue
Control	0.00	48.67± 6.94	18.67 ± 1.39	
2p	0.00	45.44±4.00^{NS}	23.00± 1.84^{NS}	
6p	0.00	55.33±4.57*	25.33±1.40*	

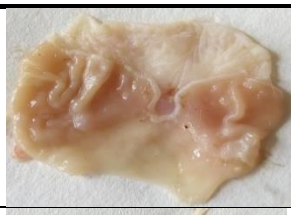
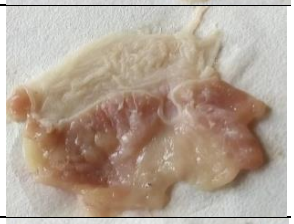
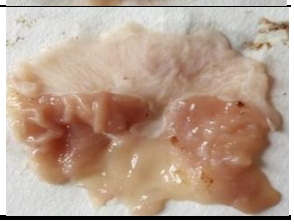
***significant at p<0.05 compared to control, NS – Not significant compared to control*

Table 2:
Effect of mosquito coil fumes on induced gastric Ulcer Area on day 3

Group	Ulcer Area (mm^2)	Mucous cell count	Parietal cell count	Gross stomach Tissue
Control	93.26±2.80	69.33± 4.66	26.78 ± 6.73	
2p	152.7±6.20**	2.21± 0.71***	3.33± 0.30***	
6p	167.3±16.03**	2.44±5.73***	3.06 ± 5.29***	

***significant at p<0.01 compared to control, ***significant at p<0.001 compared to control, NS – Not significant compared to control*

Table 3:
Effect of mosquito coil fumes on induced gastric ulcer area on day 10

Group	Ulcer Area (mm^2)	Mucous cell count	Parietal cell count	Gross stomach Tissue
Control	34.82±1.84	58.89± 7.89	12.44 ± 1.47	
2p	68.70±3.45**	39.33± 7.54*	22.00± 5.28**	
6p	65.20±3.93**	46.89±6.29NS	23.11 ± 2.04**	

**significant at p<0.05 compared to control, **significant at p<0.01 compared to control, NS – Not significant c.f control*

Effect of Mosquito coil fumes on Haematological Parameters: As shown in Table 4, Lymphocytes count was significantly decreased, p<0.05 in the 2p group (60.60±1.97) on day 3 post ulceration compared to control (70.60±0.87). Neutrophils count was significantly increased, p<0.05 in the 2p group (36.60±2.02) on day 3 post ulceration compared to control (25.60±0.81). There was no significant change in the heamoglobin concentration, erythrocyte count and platelets count in all the groups across the different experimental days (Table 5).

Table 4:
Effect of Mosquito coil fumes on Blood Leucocyte Count and Differential WBC

White blood cell and differential	Day 0			Day 3			Day 10		
	Control	**2P**	**6P**	**Control**	**2P**	**6P**	**Control**	**2P**	**6P**
Leucocytes (10^3/µL)	5.06 ±0.83	3.95 ±0.25	5.75 ±0.29	6.67 ±1.15	4.00 ±0.22	6.42 ±0.73	9.61 ±0.63	6.79 ±1.17	5.65 ±0.79
Monocytes (%)	1.50 ±0.50	1.60 ±0.25	2.00 ±0.32	1.80 ±0.37	2.20 ±0.37	1.40 ±0.25	1.20 ±0.20	2.00 ±0.32	1.40 ±0.25
Neutrophils (%)	28.50 ±1.19	25.60 ±1.17	30.60 ±2.48	25.60 ±0.81	36.60 ±2.02*	36.40 ±1.08*	26.00 ±1.58	25.80 ±3.68	30.20 ±1.46*
Lymphocytes (%)	68.00 ±1.47	70.80 ±0.97	66.00 ±2.30	70.60 ±0.87	60.60 ±1.97*	64.40 ±0.81*	72.20 ±1.69	70.40 ±3.70	68.00 ±1.61
Eosinophils (%)	2.00 ±0.41	1.00 ±0.45	1.40 ±0.40	2.20 ±0.37	0.60 ±0.25	1.80 ±0.37	0.60 ±0.25	1.80 ±0.58	0.40 ±0.25

Table 5:
Effect of Mosquito coil fumes on Platelets and Red Blood Cell Variables

	Day 0			Day 3			Day 10		
	Control	**2P**	**6 P**	**Control**	**2P**	**6PW**	**Control**	**2P**	**6P**
Erythrocyes ($x10^6$/µL)	6.70 ±0.23	7.14 ±0.32	6.58 ±0.21	6.90 ±0.21	6.90 ±0.20	6.37 ±0.20	7.08 ±0.69	6.74 ±0.62	6.52 ±0.33
Platelets ($x10^3$/µL)	147.80 ±21.06	129.40 ±14.42	128.60 ±11.06	157.20 ±36.63	123.00 ±6.56	122.60 ±15.07	192.20 ±12.58	163.20 ±7.34	154.80 ±15.16
Heamoglobin (g/dL)	13.15 ±0.32	14.40 ±0.52	13.24 ±0.41	14.00 ±0.14	13.70 ±0.27	12.52 ±0.30	14.48 ±1.15	13.84 ±1.34	13.08 ±0.55
PCV (%)	40.50 ±0.96	43.80 ±1.63	41.20 ±1.66	41.60 ±0.81	41.40 ±1.03	37.20 ±0.66	43.40 ±3.78	40.80 ±3.89	38.80 ±1.53

No significant difference in all comparable

Table 6:
Effect of mosquito coil fumes on activities of tissue endogenous antioxidant enzymes, nitric oxide and reactive oxygen radicals

Biochemical Analysis	Day 0			Day 3			Day 10		
	Control	**2P**	**6 P**	**Control**	**2P**	**6P**	**Control**	**2P**	**6P**
Catalase(µmol/min/mg protein	12.74 ±1.21	14.42 ±1.16	14.91 ±1.54	10.13 ±1.13	10.68 ±2.17	16.00 ±2.51	12.36 ±2.80	15.54 ±1.71	9.30 ±1.80
Glutathione (GSH)	1.54 ±0.07	1.37 ±0.08	1.69 ±0.09	1.58 ±0.136	1.27 ±0.10	1.43 ±0.12	1.39 ±0.128	1.56 ±0.082	1.40 ±0.150
MDA(ng/mg protein)	2.12 ±0.20	2.12 ±0.16	2.49 ±0.27	2.18 ±0.26	2.77 ±0.12*	3.57 ±0.64*	1.04 ±0.10	1.59 ±0.19#	2.34 ±0.33#
NO (µM/mg tissue)	5.78 ±0.24	4.44 ±0.52&	4.81 ±0.08	5.24 ±0.27	2.69 ±0.35ß	2.04 ±0.45ß	5.59 ±0.11	4.25 ±0.19α	3.57 ±0.14α
Protein (mg/g tissue)	5.23 ±0.59	4.20 ±0.19	4.62 ±0.38	4.46 ±0.15	4.12 ±0.17	4.19 ±0.16	4.39 ±0.35	5.09 ±0.43	4.34 ±0.19
SOD(µmol/mg protein)	0.24 ±0.03	0.43 ±0.04**	0.49 ±0.05**	0.70 ±0.04	0.52 ±0.04ß	0.30 ±0.04ß	0.56 ±0.02	0.46 ±0.06 α	0.35 ±0.02 α

** p<0.05 significant increase in 6P group (day 3) and 2P group compare to control,*
α p<0.05 significant decrease in 6P group (day 10) and 2P group compare to control.
 # p<0.05 significant increase in 2P (day 10) and 6P group compared to control.
ß p<0.05 significant decrease in 2P and 6P group (day 3) compared to control.
*** p<0.01 significant increase in superoxide dismutase activity compared to control.*

Effects of mosquito coil fumes on the histology of the liver, kidney and stomach: Changes in the microscopic appearances of the liver, kidney and stomach of control rats and those exposed to mosquito coil fumes are shown in Plates 1 – 7.

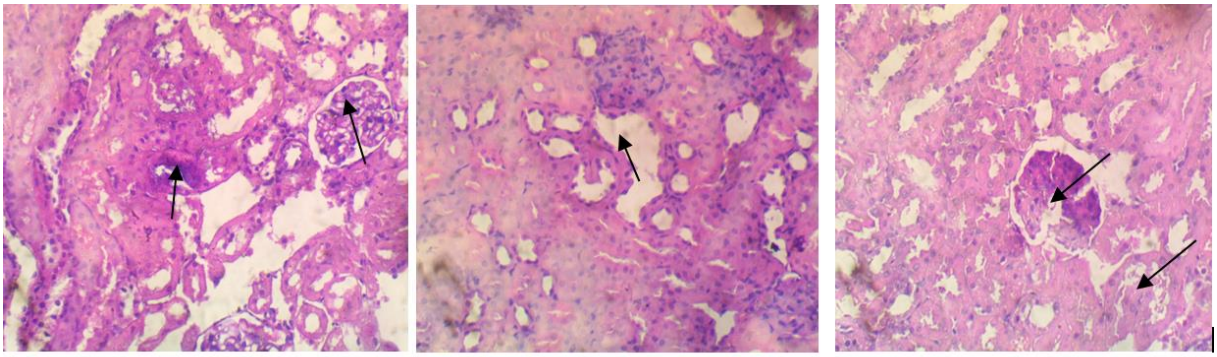


Plate 1:
Representative photomicrograph of the kidney (H&E) X400
Control= the glomeruli appear normal. There are a few foci of mild sloughing off of tubular epithelial cells (arrows). No remarkable vascular changes**.** 2p= the glomeruli appear normal. There are a few foci of tubules lined by flattened epithelial cells (arrow). Vascular changes are mildly congested. 6p= There are multiple foci (arrows) of degeneration of tubular epithelial cells.

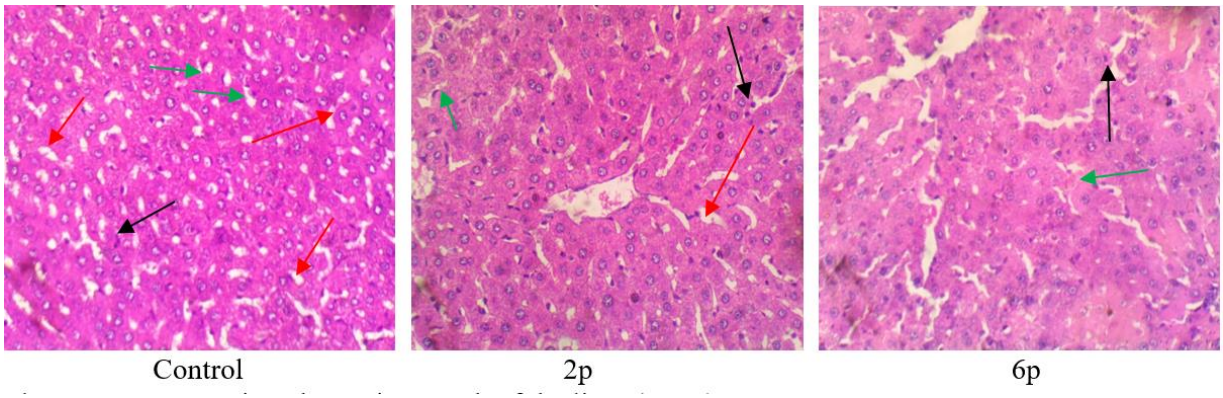


Plate 2:
Representative photomicrograph of the liver (H&E) x400
Control= There are a few foci of mild thinning of hepatic plates (cord atrophy) with resultant dilated sinusoids (red arrows). There are random foci of single-cell hepatocellular necrosis (black arrow). There is moderate Kupffer cell hyperplasia (green arrows). 2p= There are a few foci of mild thinning of hepatic plates (cord atrophy) with resultant dilated sinusoids (red arrow). There are random foci of single-cell hepatocellular necrosis (black arrow). There is moderate Kupffer cell hyperplasia (green arrow). There are no remarkable vascular changes. 6p= Hepatic plates are fairly closely-packed. There are random foci of single-cell hepatocellular necrosis (black arrow). There is moderate Kupffer cell hyperplasia (green arrow). There are no remarkable vascular changes.

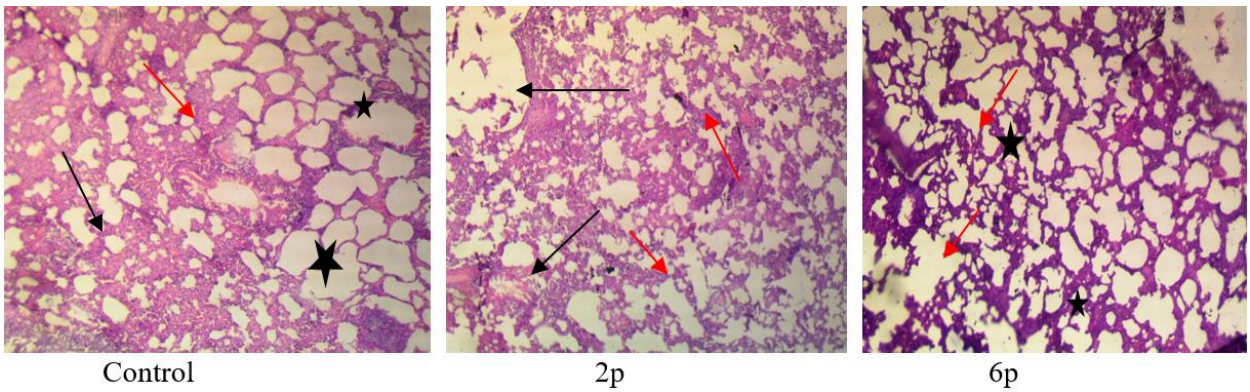


Plate 3:
Representative photomicrograph of the lungs (H&E) x400
Control= There are locally extensive foci of marked thickening of the alveolar interstitium (black arrows). [Compare left of photomicrograph with top-right]. Alveoli and bronchioles are clear (stars). There is moderate congestion of blood vessels (red arrow). 2p= the alveoli and bronchioles are clear and empty. There are a few foci of moderate thickening of the alveolar interstitium (black arrows). Alveolar interstitial capillaries are mildly congested (red arrows).
6p= There are locally extensive foci of marked thickening of the alveolar interstitium (black arrows). [Compare left of photomicrograph with top-right]. Alveoli and bronchioles are clear (stars). There is moderate congestion of blood vessels (red arrow).

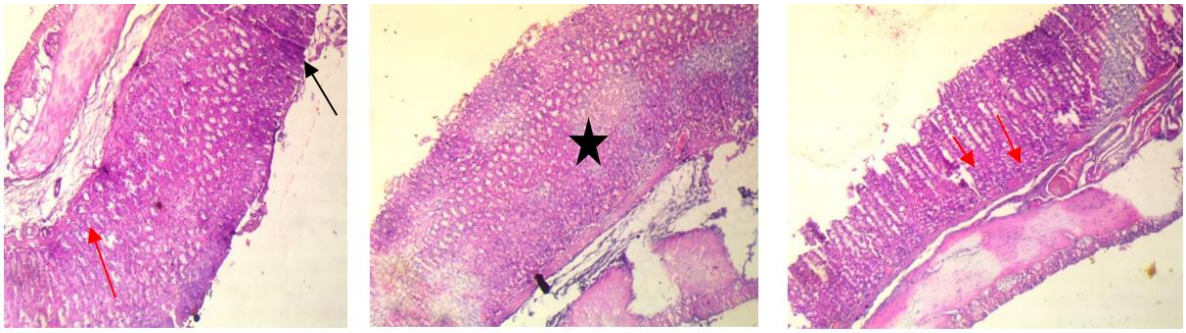


Plate 4: Representative photomicrograph of the stomach on day0 (H&E stain) x10
Control= There is mild loss of covering epithelium (black arrow) at a few foci. There are a few foci of loss of cells of the gastric glands (red arrow) at the base of the mucosa. Vascular changes are unremarkable.2p= There is mild loss of covering epithelium at a few foci. The gastric mucosa (star) appears normal. Vascular changes are unremarkable. 6p= There are a few foci of slight loss of the covering epithelium. The mucosa appears normal. However, there is moderate congestion of blood vessels (red arrows) in the mucosa and submucosa tunics.

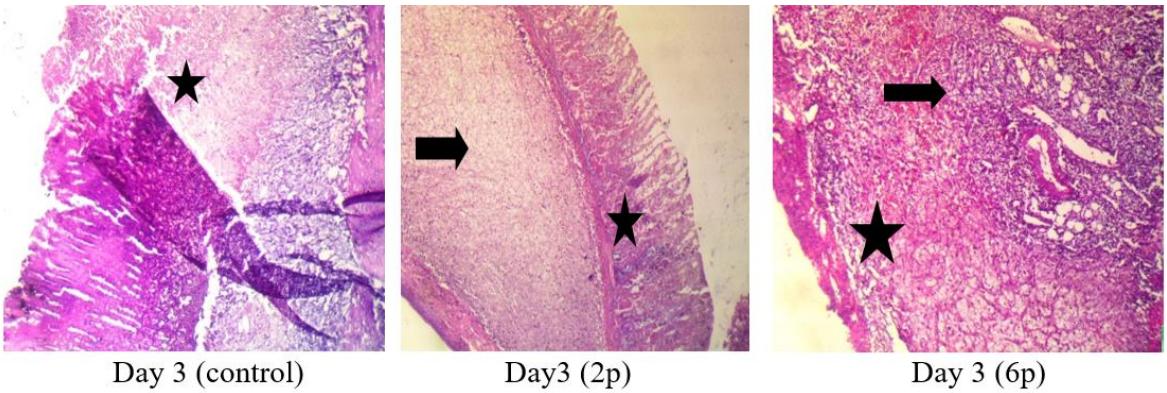


Plate 5: Representative photomicrograph of the stomach on day3 (H&E stain) x100
Control= There is a locally extensive focus of severe ulceration and necrosis of the affected mucosa (black arrow). Adjacent to the ulcerated focus, there are regenerating gastric glands that appear dilated and cystic. The submucosa is markedly expanded by oedema and mild accumulation of inflammatory cells. 2p= There is severe widespread ulceration and coagulative necrosis of the entire mucosa (star). There is expansion of the submucosa (arrow) with inflammatory oedema and mostly polymorphonuclear cells. 6p= There is severe widespread ulceration and necrosis of the entire mucosa with accumulation of inflammatory cells in the submucosa and muscularis (arrow). Inflammatory cells are mostly polymorphonuclear.

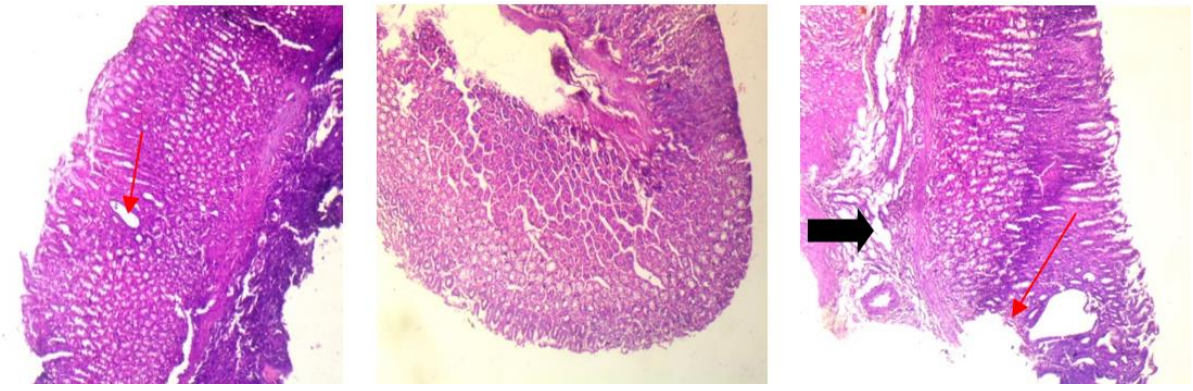


Plate 6:
Representative photomicrograph of the stomach on day 10 (H&E) x100
Control= the mucosa contains a few regenerating dilated glands (red arrow). The covering epithelium is intact. However, the submucosa is mildly expanded by accumulation of mononuclear inflammatory cells trapped in dense fibrous connective tissue. This finding might be suggestive of a healed ulcer. 2p= the covering epithelium is intact. The gastric mucosa, submucosa, muscularis and serosa appear normal. Vascular changes are unremarkable. 6p= There is a locally extensive focus of mild ulceration of the gastric mucosa. Adjacent to the ulcerated focus, there are regenerating gastric glands that appear dilated and cystic (red arrow). There is moderate accumulation of inflammatory cells in the submucosa. There is fibrosis (thick arrow) of the submucosa and muscularis. Inflammatory cells are polymorphonuclear.

DISCUSSION

We designed this study to explore and simulate daily and wanton use of MCS in residential areas using Wistar rat model. The potential health implication of MCS on the stomach of exposed normal gastric mucosa and experimentally induced gastric ulcer was investigated.

The body weight did not change considerably throughout the weeks of exposure to MCS. This is similar to the reports obtained in a previous study where there was no change in body weight compared to unexposed group (Parker *et al*,. 1984; EPA, 1986; Ishmael and Lithchfield, 1988; Schoenig, 1995). The lack of body weight changed observed could be due to the relative short period of exposure which did not reduce appetite for food in the exposed groups when matched with control.

However, there was a relative increased in kidney weight in the 6p group compared to the 2p group which could be attributable to the duration of exposure and suggesting that both forms of exposure could affect the kidney structurally and functionally. More so, with the reported congestion around the glomerular tuft in the histopathological evaluation of the kidneys of animals exposed for six weeks. This agrees with the report of Taiwo *et al*., (2008) which demonstrated glomerular and tubular and vascular damages in the kidneys of rats exposed to mosquito coil and various insecticidal spray fumes. That finding was further buttressed by report of Garba *et al* (2007) which demonstrated severe multifocal congestion, cystic dilation in medulla of kidney tissue exposed to pyrethroid based mosquito coil.

The increase in ulcer area of the 6p and 2P groups suggest a severe ulcer and a delayed healing processes on days 3 and 10 post ulceration respectively. This further reinforced the toxic effects of mosquito coil on stomach mucosa. The increased gross ulcer scores provide backing by the histopathology findings that showed delayed ulcer healing in the exposed groups relative to control. The reported severe ulceration and the subsequent delayed healing in the exposed groups could be associated to the increased lipid peroxidation within the mucosa that was indicated with the raised MDA values. Malondialdehyde results from peroxidation of polyunsaturated fatty acids and related esters within cell membranes and interpreted as oxidative damage to the tissues (Blandizzi *et al*., 2005).

Excessive production of reactive oxygen species (ROS) by the gastric mucosa has been established and it openly results in oxidative damage (Sun and Oberley, 1996; Ali and Harty, 2009). Oxidative stress promotes multiple factors that could activate ROS production or a weakening in antioxidant activities (Hidekazu, 2012).The gut is a major site of generation of ROS. Even with the huge defensive mechanisms from epithelium layers, pathogens can promote formation of inflammatory conditions through interactions of the epithelium, polymorphonuclear neutrophils (PMNs), as well as the macrophages that could promote inflammation and worsened by damaged oxidative cells (Bhattacharyya, 2014). Most popular gut diseases such as gastric ulcers, gut cancers, and inflammatory bowel disease (IBD) promotes oxidative stress (Bhattacharyya, 2014).

The reduced activities of Superoxide Dismutase (SOD) in the exposed group of animals especially on day 3 post-ulceration further highlights the deleterious effect of the fumes on the gastric mucosa and experimental gastric ulcer healing. Reduced SOD activity in the gut worsen gastric ulcer, while an increase in SOD promotes ulcer healing in patients (Naito *et al*., 1992). These responses presented the detrimental effects of ROS on tissue damage and the importance of antioxidant activity in promoting health. Likewise, the responses to nitric oxide from the gastric tissue which was low in the exposure groups compared to control in this study could be playing at least dual roles towards recovery from acetic acid injury. Nitric oxide (NO) is a signaling molecule that plays stimulates inflammation pathways. It provides anti-inflammatory roles normally (Sharma and Al-Omran, 2007). On the other hand, it could be considered a pro-inflammatory facilitator that induces inflammation due to excess production in stressful conditions. NO is synthesized and secreted into the endothelial cells by the help of nitric oxide synthase that convert arginine into citrulline producing NO in the process. Nitric oxide is understood to induce vasodilatation in blood vessels and stimulates immune responses (Sharma and Al-Omran, 2007). The vasodilation property of NO may also be the leading connection towards the faster healing and recovery reported in the control animals compared to the exposed groups.

The increased in leucocyte counts and a counterpart decrease in the lymphocytes in the exposed groups were reported in this study. These changes occurred most significantly on day 3 following ulceration which suggest the peak of ongoing inflammation. This increases neutrophil-lymphocyte ratio which has been reported to be elevated in peptic ulcer patients (Jafarzadeh*et al*., 2013). The quantitative and qualitative differentials in the recruitment of inflammatory cells also play a vital role in accelerating healing and recovery from injury. The histopathology findings in the stomach skewed to the already calculated degree of ulceration and the appropriate healing and recovery that ensued in the study. The persistent elevation of the inflammatory cells observed and the degree of necrotic tissues by day 10 in the exposed groups only added credence to the

toxic effect of the MCS on the stomach. This is not an uncommon finding in acetic acid induced ulcer.

In conclusion, it is apparent that exposure of rats to mosquito coil fumes in normal and experimental gastric ulcer is potentially harmful to the structure and possibly functions of the gastric lining. The delayed healing documented in this study is due to the generation of ROS and a reduced production of NO in the exposed groups. It is anticipated that additional work in this area would be channel to examine the effect of MCS on the human gastrointestinal functions, especially in patients with gastric ulcer.

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