The Protective Effect of Gongronema latifolium Leaf Extract Against Hepatotoxicity of Rambo and Baygon Mosquito Coil Smoke Respectively, in Albino Rats

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Abstract: A total of 30 albino rats used for the experiment were grouped into A, B and C. Group A rats served as the control group while groups B and C were exposed to different brands of mosquito coil (Rambo and Baygon) smoke respectively for 31 days. Each of the experimental groups (B and C) contained 12 rats and sub-divided into two (1 and 2) with six rats in each sub group. Sub-group 1 rats were exposed to rambo and baygon mosquito coil smoke respectively and co-treated with aqueous extract of Gongronema latifolium while the sub-group 2 rats were exposed to the respective mosquito coil smoke rambo, baygon only (to serve as the negative control). The effects of 8 hour daily inhalation of rao and baygon mosquito coil smoke respectively for 31 days were investigated in albino rats. The activities of specific enzymes AST, ALT, ALP and renal function parameters such as total bilirubin, creatinine, total protein, albumin and blood indices, Hb and PCV were used to assess the potential effects of inhalation of these mosquito coil smokes on the liver. The result showed that AST, ALT, ALP, total bilirubin and creatinine were more significantly elevated due to inhalation of baygon mosquito coil smoke by albino rats than rambo mosquito coil smoke, while total protein, albumin, Hb and PCV were more significantly elevated due to inhalation of rambo mosquito coil smoke by rats than baygon mosquito coil smoke. The levels of these parameters in albino rats were significantly elevated due to inhalation of rambo and baygon mosquito coil smoke respectively and differed significantly from the control. There were also significant differences in the levels of the above parameters in the rats exposed to the rambo and baygon mosquito coil smoke respectively more than the rats exposed to the respective mosquito coil smoke and co-treated with aqueous extract of Gongronema latifolium. Besides, obvious pathological changes such as increased proliferation of hepatocytes leading to hepatic transformation from normal to abnormal lesion, distortion of hepatic architecture, hepatic necrosis and haemorrhages were observed in the liver of the rats expose to Rambo and baygon mosquito coil smoke respectively and these conditions were ameliorated due to co-treatment with aqueous extract of Gongronema latifolium.

Key words: Rambo • Baygon • Mosquito Coils and Gongronema latifolium

INTRODUCTION

Exposure to the smoke of mosquito coil can pose significant acute and chronic health risk. For example, burning one mosquito coil would release the same amount of particulate matters as burning 75-137 cigarettes and the emission of formaldehyde from burning one coil can be as high as that released from burning 51 cigarettes [1]. These chemicals have the potential to produce harmful effects on air ways [2, 3].

The toxicity of these pyrethroid-based mosquito coil insecticides to mammals has received much attention in the recent years. Animals exposed to these insecticides exhibited changes in their physiological activities besides other pathological effects [4]. During the course of exposure period the smoke exposed-rats show clinical signs suggestive of acute upper respiratory tract sensory irritation [5]. Exposure to coil smoke could result to the inflammatory processes such as irritation of various organs by toxic chemicals from coil smoke and the
reduced activity in the exposed rats could as well be due to toxic chemicals in the smoke [6]. Increased sensitivity occurs following repeated exposure to cyanide which is found in pyrethroid such as â-cyfluthrin [7]. Baron and Finegold [8] also explained that cyanide which is released from mosquito coil is known to cause reduced metabolism and consequently results in reduced energy output which may explain the observed body weakness.

The sneezing that results after some days of exposure could be the result of irritants released from the coil smoke such as aldehydes, sulphates and polycyclic aromatic hydrocarbons such as acenaphthrene, penanthrene benzo(a) pyrene etc [9]. Pollutant concentration resulting from mosquito coil could substantially exceed health-based air quality standard compounds including carcinogens and suspected carcinogens in the coil smoke from mosquito coil obtained from Malaysia [10].

Epidemiological studies have shown that long term exposure to mosquito coil smoke can induce asthma and persistent wheeze in children [11]. Exposure to the smoke of mosquito coil made from these pyrethroids similar to the tested ones can pose significant acute and chronic health risks, not different from those from cigarette smokes [12].

Chronic exposure of human, animal and untargeted, but useful insects and other organisms to low doses of synthetic pyrethroids consequently results to morbidities and reproductive problems [13-17].

Toxicological studies using mosquito coil in rats showed focal deciliation of the tracheal epithelium, metaplasia of the epitheloid cells and morphologic alteration of the alveolar macrophage [18, 19].

Biochemical analysis of the smoke indicates that such gases as carbon monoxide, isoprene and benzene are emitted and the aerosial particulates are mutagenic [20].

MATERIALS AND METHODS

Experimental Design: A total of thirty (30) albino rats weighing between 200-250g used for the experiment were obtained from the animal house, department of Biochemistry, University of Nigeria, Nsukka. The animals were maintained under standard laboratory conditions, (12 hrs light and 12hrs darkness, temperature at 23± 1°C) and were allowed access to water and dry ration. The rats were ran domly group into three, A, B and C). Group A animals were used as the control while groups B and C animals were exposed to different brands of mosquito coil (rambo Cork) smoke respectively, 8 hrs. for 31 days Each experimental group contained 12 rats, sub-divided into two [21 and 22] with six rats in each sub-group. Sub-group 1 rats were exposed 8hrs for 31 days to the respectively mosquito coil smoke and co-treated with aqueous extract of Gongronema latifolium while sub-group 2 rats were exposed 8hrs for 31 days to the respectively mosquito coil smoke alone (negative control). The rats were caged in wooden cages (90cm x 60cm x 60cm) with ¼ of the upper part covered with a wire gauze to provide good aeration. The control group animals were kept in a room of similar ventilation, but without mosquito coil smoke for the period of the experiment. The rats in each group were observed for any clinical signs associated with the exposure to the active ingredients from the coil smoke.

Mosquito Coil: The brands of mosquito coil used were rambo and cork mosquito coils purchased from a retail outlet at Abakpa market, Abakaliki, Ebonyi State. The brand commercially purchased for the experiment contained Imiprothrin transfaluthrin,Beta-cyfluthrin.

Preparation and Administration of Plant Sample: About 250g of fresh G. latifolium leaf was purchased at Abakpa market, Abakaliki were ground into powdered form at the Department of Biochemistry, Ebonyi State University and soaked for 8hours in 1000ml of distilled water and allowed to stand and settle. This was filtered and dried for 9 hours. A stock solution of the plant extract was prepared by dissolving 10g of the extract in 100ml of normal saline. This was administered to the sub-group 1 rats through oral administration according to their weights at a concentration of 50mg/kg.

Blood Sample Collection and Biochemical Assay: At the end of the exposure period (31 days ) to mosquito coil smoke, blood sample was collected by transection of the jugular vein using anticoagulants (EDTA and Heparin) tubes for Hb and PCV analysis respectively. The plasma/serum was obtained by centrifuging the PCV sample at 450rpm for 10 mins. The serum collected and the Hb samples were immediately taken to the Department of Chemical Pathology, Ebonyi State University Teaching Hospital for Hb and PCV determination, while Total protein, Albumin, Total bilirubin and Creatinine were determined Spectrophotometrically using Randox laboratory Kits at the Biochemistry department laboratory. Also liver enzymes, AST, ALT and ALP were determined at the same laboratory using Randox laboratory kits and spectrophotometer [11].
**Histological Analysis:** The livers of the rats were dissected out and fixed in 10% formaldehyde for 24 hours. After fixation the tissues were dehydrated through ascending grades of alcohol (75%, 90% and 95%) at interval of two hours.

This was followed by clearing in xylene for 30mins. Then the tissues were embedded in paraffin wax melted at 55°C. The embedded tissues were sectioned at 4μm using a rotary microtome and these sections were mounted on clean slides using DPX as the mountant. The mounted sections were stained with Haematoxylin and Eosin. Light microscope examination of the sections was then carried out.

**Result of Histological Analysis:**

Plate 1: Photomicrograph of a liver section of the control showing (A) normal liver architecture with normal hepatocytes and the canals of Hering (Plate 4).

Plate 2: Photomicrograph of a liver section of an albino rat showing the effect of exposure to baygon mosquito coil smoke for 31 days without treatment showing (A) increased proliferation of hepatocytes leading to hepatic transformation from normal to abnormal cell lesion with distortion of hepatic architecture (H&E;X400).

Plate 3: Photomicrograph of a liver section of albino rat showing the effect of exposure to Rambo mosquito coil smoke for 31 days without treatment with extract (A) impairment of normal organization of hepatic architecture with the presence of hepatic necrosis (B) Distorted hepatic vessels with the presence of hepatic haemorrhages.

Plate 4: Photomicrograph of a liver section of albino rat treated with *G.latifolium* extract after exposure to baygon mosquito coil smoke for 31 days showing (A) minimal changes in liver architecture and mild rearrangement of hepatic vessels (B) mild focal areas of hepatic haemorrhages (H&E;X400).

Plate 5: Photomicrograph of a liver section of albino rat treated with *G.latifolium* extract after exposure to Rambo mosquito coil smoke for 31 dayshowing (A) mild changes in liver architecture and minimal focal areas of necrosis and (B) mild hepatic haemorrhages.
Result of Biochemical Analysis:

Liver Enzymes

Fig. 1: Effects of Rambo and baygon mosquito coil smoke respectively on serum enzymes. Exposure to these brands of mosquito coil smoke respectively for 31 days caused significant (p < 0.05) increase in serum enzymes (AST, ALT, ALP) levels in albino rats with exposure to baygon mosquito coil smoke showing a higher effect compared with the control.

Renal function parameters

Fig. 2: Haematological effects of exposure to Rambo and baygon mosquito coil smoke respectively on Hb and% PCV levels in albino rats for 31 days. The effect caused significant (p < 0.05) reduction in Hb and% PCV levels in the exposed rats compared with the control and baygon mosquito coil exerted a higher effect compared with the control.

Haematological parameters

Fig. 3: Effects of exposure to rambo and baygon mosquito coil smoke respectively on renal function parameters (serum albumin, total protein, total bilirubin and creatinine) in rats for 31 days. The effect caused a significant (p<0.05) increase in total bilirubin and creatinine levels, with baygon mosquito coil smoke showing a higher effect. Also the exposure caused a reduction in serum albumin with rambo mosquito coil slightly higher and a slight increase in total protein level compared with the control.

Fig. 4: The protective effect of G.latifolium extract against reduced Hb and% PCV levels is shown in Fig. 4. Co-administration of the extract caused significant (P<0.05) increase in haematological parameters (Hb, % PCV) which indicates a protective effect against damaging effect of baygon mosquito coil in albino rats.
Fig. 5: This shows the protective effect of *G. latifolium* extract on liver enzyme activity in baygon mosquito coil smoke-exposed rats for 31 days. The figure shows a significant (P< 0.05) reduction in liver enzymes (AST, ALT, ALP) after co-administration of the extract which shows a protective effect of *G. latifolium* extract against elevated serum enzyme levels due to exposure to baygon mosquito coil smoke.

Fig. 6: The effect of co-administration of *G. latifolium* extract on renal function parameters after exposure to baygon mosquito coil smoke is shown in Fig. 5. The extract caused a significant (P< 0.05) decrease in elevated creatinine and bilirubin levels caused by exposure to cork mosquito coil smoke. The decreased in serum albumin and total protein levels were also significantly (P< 0.05) improved which shows the protective effect of the extract.

Fig. 7: The protective effect of *G. latifolium* extract against reduced haemoglobin and packed cell volume levels due to exposure to Rambo mosquito coil smoke is demonstrated in Fig. 6. The Hb and PCV levels were significantly (P<0.05) improved after co-administration of the extract and this also significantly (P<0.05) differ from the control.

Fig. 8: This shows the protective effect of *G. latifolium* extract on liver enzyme activity in Rambo mosquito coil smoke-exposed rats. The figure shows a significant (P< 0.05) reduction in liver enzymes (AST, ALT, ALP) after co-administration of the extract which indicates the protective effect of *G. latifolium* extract against exposure to Rambo mosquito coil smoke.
DISCUSSION

The results showed that exposure of albino rats to Rambo and Baygon mosquito coil smoke respectively showed significant (P<0.05) increase in levels of serum liver enzymes (AST, ALT, ALP), creatinine and bilirubin, followed by a significant (P<0.05) decrease in serum total protein albumin levels, Hb and% PCV. Abubakar and Hasson [4], observed a significant (P<0.05) increase in AST, ALT and ALP due to exposure to different brands of mosquito coil (Wam, Rambo and Cork) smokes, respectively, for 14 days. Similarly, Foldtron et al. [10] and Abu-El-zahab et al. [5], observed a rise in serum enzyme in rats exposed to pyrethroid insecticides such as fenvalarate and mixed pyrethroids (tetramethrin and sumithrin) respectively. Woodman [22], in his work indicated that the increase in serum AST, ALT and ALP activities often seen following liver damage does not indicate the inability of the liver to synthesize the enzymes, but instead, a loss of material from the damaged hepatocytes due to exposure to mosquito coil. Abubakar and Hasson [4] reported that this increase leads to a rise in enzyme activities in the sera of these animals suggesting a liver tissue damage. Also, [16] observed that liver tissues lose their enzymes in case of liver cell damage and these liver tissues are known for high content of transaminases.

The results also showed a significant (P<0.05) decrease in serum albumin and total protein. This result agrees with Imamura et al. [13] who reported a decrease in serum albumin and total protein in insecticides treated-animals. This is contrary to the work of Abubakar and Hassan [4] who reported an increase in serum albumin and total protein levels. According to Abubakar and Hassan [4], the increase could be as a result of loss of plasma fluid into the tissue due to inflammation which could result from the exposure to irritants released from the coil smoke. Liu and Wong [15], suggested that irritant such as aldehyde, sulphates and pyrethriod which can induce inflammatory responses that can cause damage to the liver cells which is the sites of protein synthesis and this leads to the release of plasma protein thereby causing a rise in plasma protein. A decrease in the level of serum albumin and total protein could be due to impaired protein synthesis and losses as a result of haemorrhage or excessive protein catabolism. Okine et al. [17], observed that the lower level of serum albumin in mosquito coil-inhaled rats could decrease the protein biosynthetic activity of the liver and affect the transport of substances such as lipids synthesized by the liver.

One of the biochemical indices used to assess hepatotoxicity was, elevated bilirubin in the mosquito coil-exposed rats. Ramnic [19] explained that these elevated bilirubin levels are found as a result of liver diseases (hepatitis and cirrhosis), excessive haemolysis and destruction of the red blood cells.

According to Stephen et al. [21], bilirubin level depends not only on the amount of haemoglobin broken down, but also on the ability of the liver to excrete the increased amount of bilirubin present in it.

The effects of mosquito coil smoke in albino rats were more pronounced in the rats exposed to Baygon mosquito coil smoke, where the liver enzyme activities were significantly higher than the effects due to exposure to Rambo mosquito coil smoke, although exposure to both coil showed significant (P<0.05) difference from the control.

Garba and Adelaiye [11], in their work, reported a high AST and ALT activities as a result of exposure to Rambo mosquito coil smoke for 21 days. It was also observed that exposure to Rambo and Baygon mosquito
coil smoke respectively induced many histopathological changes in the liver. The most marked signs of hepatic impairment observed were impairment of normal hepatic architecture, necrosis and proliferation of hepatocytes leading to hepatic transformation from normal to abnormal cell lesion.

Sakr and Hanafy (2002) reported the presence of cytoplasmic vacuolation of the hepatocytes and leucocytes infiltration as marked symptoms of hepatic tissues impairment due to intoxication with pyrethroid insecticides. Abu-El-zahab et al. [5], observed the presence of congested blood vessels, haemorrhage, necrosis and inflammatory leucocytes in rats exposed to pyrethroid-based mosquito coil smoke.

Abou-Zaid [6], in his work observed necrosis, blood vessel congestion and leucocytic infiltration in the liver of new born mice exposed to mosquito coil smoke for 15 days.

Further, Garba and Adelaiye [11] noted that exposure of albino rats to mosquito coil (Cork, swam and Rambo) smoke respectively for 21 and 28 days presented changes such as haemorrhagic spot, necrosis, widespread fibrosis and interstitial mononuclear cellular infiltration in liver sections of albino rats.

According to Garba and Adelaiye [11], the severity of the pathological effects was dependent on the duration of exposure.

**CONCLUSION**

In conclusion, the study of the protective effect of Gongronema latifolium against hepatotoxicity of rambo and baygon mosquito coil has revealed an insight on the medicinal importance of the plant extract. Also the study showed the effectiveness of the extract as a protective material against mosquito coil effects on the liver.

**REFERENCES**