Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.5, No.1, 2015

Hepatotoxicity of Rambo and Cork Mosquito-Coil Smoke and the Protective Effects of G. Latifolium Leaf Extract

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Abstract
The protective effects of *G. latifolium* aqueous leaf extract against mosquito coil (Cork and Rambo) smoke-induced hepatotoxicity in albino rats were investigated using a total of 33 albino rats, weighing between 200–250 g. The rats were randomly grouped into five main groups: A, B, C, D, E and F. Group A was used as the control and it contained 3 rats only; groups B and C contained 6 rats each and were exposed to Cork and Rambo mosquito coil smokes only, respectively, for 31 days. Groups D and E contained 9 rats each and were each subdivided into three subgroups (namely D1,D2, and D3; and, E1, E2,& E3), each containing 3 rats. The subgroups were exposed to their respective mosquito coil smokes and simultaneously treated with different concentrations of aqueous extracts of *G. latifolium* leaf for 31 days. The results showed the presence of phytochemicals like alkaloids, flavonoids, tannins, saponins and vitamin C. Exposure to Rambo and cork insecticides smoke caused significant ($P<0.05$) elevation of AST, ALT and ALP, creatinine and bilirubin levels and reduction of Hb, % PCV, serum albumin and total protein. However, co-treatment *with G. latifolium* leaf extract significantly ($p<0.05$) ameliorated the observed effects. Histopathological analysis of the liver of exposed rats showed impairment of the hepatic architecture resulting in necrosis and hemorrhages while in rats co-treated with *G. latifolium* extract, these effects were significantly ($p<0.05$) reduced. Thus, *G. latifolium* extract can protect against Cork and Rambo smokes-induced hepatotoxicity. Comparatively, exposure to Cork smoke had more devastating effects than Rambo smoke.
Keywords: Cork and Rambo insecticide smoke, liver toxicity, *G. latifolium* protection and phytochemicals.

Introduction

The endemic nature of malaria infestation in Nigeria and the economic implication of being infected have left many families with no choice other than to look for any way possible to avoid it. Malaria, caused by bites of plasmodium parasite-infected mosquitoes, is responsible for almost one million deaths per death (*Garba et al.,* 2007). One of the commonest and cheapest methods used by many families to avoid mosquito bites is the use of mosquito coil smoke to drive away mosquito from their sleeping rooms, especially, at nights. Mosquito coils are slow burning devices which emit smoke containing one or more insecticides. Each coil burns for several hours and are used in close proximity to persons requiring protection against mosquitoes bites (Garba *et al.,* 2007). There has been a growing concern among the public regarding the routine and prolonged use of mosquito coils (*Anvita et al.,* 2006). It poses a serious public health hazards and ecological challenges because of the effects associated with their use, especially innocuous and chronic inhalation of the fumes (*Taiwo et al.,* 2008). The main active ingredients in mosquito coils are pyrethroids, which are effective against many genera of mosquito, including *Aedes, Anopheles* and *Mansonia* (*Krieger et al.*, *2003*). They also contain chemicals such as organophosphorus, organochlorine and carbamate (*McEween and Stephenson,* 1979). It has been reported that burning one mosquito coil would release the same amount of particulate matters as burning 75-137 cigarettes and emission of formaldehyde as high as that released from burning 51 cigarettes (*Liu et al.,* 2003). These chemicals have the potential to produce harmful effects on airways *(Brashier et al.,* 2010; *USEPA*, 1999a).
This study has therefore been designed to examine the biochemical, haematological and histological effects of inhaling mosquito-coil smoke by albino rats and the protective effect of *Gongronema latifolium* (Utazi) extracts. *Gongronema latifolium* (*Asclepiadiaceae*) is a wild climber widely distributed in the South Eastern States of Nigeria. The leaves have been found very efficacious as an antidiarrhoea, antioxidant and antitussive (*Sofowora,* 2006 and *Iwu*,1993*).*

Material and Methods

Source of mosquito coils and G. latifollium plant: Zee Cork and Rambo insecticide coils were purchased from a retail outlet at Abakpa Market, Abakaliki, Ebonyi State and they contained 0.2 % and 0.4 % Allethrin, respectively. Fresh *G. latifolium* leaves were purchased from the local farmers in the same market and transported to the Department of Biochemistry laboratory, Ebonyi State University. The leaves were sun dried and ground into powder and stored in freezer until exposure and analysis.

Research Design

A total of 33 albino rats, weighing between 200–250 g were used for the experiments. They were obtained from the Animal House of the Department of Biochemistry, University of Nigeria, Nsukka and transported to the Animal House of the Department of Biochemistry, Ebonyi State University, Abakaliki, where they were acclimatized for a period of two weeks under standard laboratory condition (12 h light and 12 h darkness, temperature at 23±1^{0}C). All the rats were allowed access to water and dry ration. The rats were randomly grouped into six main groups: A, B, C, D, E and F. Group A was used as the control and it contained 3 rats only; groups B and C contained 6 rats each and were exposed to Rambo and Cork mosquito coil smoke, respectively, for 31 days. Groups D and E contained 9 rats each and were each subdivided into three subgroups (namely D1,D2, & D3; and E1, E2,& E3), each containing 3 rats. The subgroups were exposed to their respective mosquito coil smokes and simultaneously treated with different concentrations of aqueous extracts of *G. latifolium* leaf for 31 days. The rats were housed in wooden cages (90cm x 60cm x 60cm) with ¼ of the upper part covered with 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 coil smoke. At the end of the exposure period (31days), the animals were sacrificed and their blood samples collected each, into EDTA and heparin tubes for haemoglobin (Hb) and packed cell volume (PCV) determination respectively. The heart and liver of the sacrificed rats were excised from the entire animals using a forceps and a clinical blade. These organs, which were carefully collected, were fixed inside tubes containing 10 % formalin for histological analysis (*Garba et al.*, 2007).

Preparation of Leaf extract

Exactly 250 g of dry samples of *Gongronema latifolium* leaves were ground and soaked for 8 h in 1000 ml of distilled water. This was allowed to stand and settle. The mixture was filtered and the extract was sun dried for 9 h (*Ugochukwu et al.,* 2003). A stock solution of the plant extract was prepared by dissolving 10 g of each of the extracts (*Gongronema latifolum)* in 100 ml of normal saline and orally administered to the rats according to their weights at a concentration of 50 mg/kg.

Determination of phytochemicals and proximate compositions

These were determined using standard methods based on Crude fibre by yang 2002, protein by Harbone (1973), moisture by AOAC (1999) ash and carbohydrate by Onwuka (2005), while phytochemicals such as tannin and alkaloids by Harbone (1998), saponin and flavonoids were by *Sofowora* (2006).

Biochemical parameters Determination

Total protein was determined using Biuret spectrometric method as described by *Talib* and *Khurana* (1999) using serum protein kit method, albumin, bilirubin by Bradford (1976) creatinine, Hb, and PCV were by Renee (1992) using creatinine kit reagents, Aspartate aminotrasferase(AST), ALT and ALP by the method described by *Talib,* (1999).

Data Analysis: statistical analysis was done using analysis of Variance (ANOVA), Means were compared for significance using Duncan's Multiple Range test ($p<0.05$) (Sokal and Rhoji, 19689).

Results

The phytochemical and proximate analysis of *G. latifolium* aqueous extracts revealed that the plant contains alkaloids, flavonoids, tannins, saponins, vitamin C, carbohydrate, protein, fibres, moisture and ash (Table 1).

The result showed a significant ($P<0.05$) elevation of AST, ALT and ALP activities on exposure to different brands of mosquito coil smoke, with cork smoke exerting the highest effect. AST, ALT and ALP activities in rats exposed to cork were 75.82±0.20 u/l, 85.02 ± 0.03 u/l and 78.12 ± 0.05 u/l, respectively, while those of Rambo were 62.38 ± 0.02 u/l, 68.25 ± 0.05 u/l and 70.65±0.05 u/l, compared to the control values which were 31. 67 ± 0.05 u/, 40.88 ± 0.18 u/l, respectively. Generally, these results show varied effects of the mosquito coil smokes on the serum enzymes analysed

Effects on haematological parameters showed significant ($P<0.05$) reduction of Hb, PCV, total protein and serum albumin. The effect was higher with cork smoke than Rambo smoke. All values were significantly ($P<0.05$) lower than the control values.

Also exposure to both cork and Rambo smoke significantly crashed the total protein concentration compared to the control, and effect was higher in rats exposed to cork smoke than Rambo smoke. Serum creatinine level significantly ($p<0.05$) increased. Creatinine values due to cork and Rambo exposures were 22.75±0.27 mg/dl, and 18.22±0.22 mg/dl respectively, which were significantly ($p<0.05$) higher than the control. Bilirubin level increased significantly ($p<0.05$) with values of 30.83±0.21 mg/dland 28.05±0.18 mg/dl on exposure to cork and Rambo respectively, compared to the control value of 7.52±0.29 mg/dl.

In rats co-treated with *G. latifolium* on exposure to mosquito-coil smoke, the adverse consequences observed in rats exposed to mosquito coil smoke only were significantly ($p<0.05$) inhibited compared to the negative control. However, in spite of the treatment, the levels were still significantly ($p<0.05$) different from the levels observed in the positive control group.

Discussion

On exposure of the rats to mosquito coil smokes, the following clinical signs were observed: head shaking, scratching of nostrils, sneezing and ruffled fur. These responses may be attributed to the inhalation of some irritants released from the coil smoke, such as aldehydes, polycyclic aromatic hydrocarbons (*Liu et al,* 2003). *Taiwo et al* (2008), reported depression, muscular tremor, head shaking and scratching of nostrils as physical symptoms of exposure to mosquito coil smoke.

Our results on the effects of exposure of rats to mosquito coil smoke showed significant ($p< 0.05$) increase in serum levels of AST, ALT, ALP, bilirubin and creatinine, followed by a significant ($p < 0.05$) decrease in serum total protein and albumin (Figures 6 and 8). Our results are in tandem with the results of *Abubakar* and *Hassan* (2007), who reported a significant increase in ALT, ALP and AST activities due to exposure to different brands of mosquito coil (wam, rambo and cork) smokes for 14 days. Similarly, *Foldtron et al.,* (1988) and Abu-El zahab et al., (1993) showed that serum enzymes were elevated in serum of rats exposed to pyrethroid insecticide (fenvalarate) and mixed pyrethroids (tetramethrin and sumithrin), respectively.

A study by *Woodman* ., (1980) indicated that the increase in serum enzymes activities often seen following liver damage does not indicate the inability of the liver to synthesize the enzymes, but rather a loss of material from the damaged hepatocytes, and this increase leads to a rise in enzyme activities in the sera of these animals suggesting a liver tissue damage. Also, *Martin et al.,* (1983) noted that liver tissues which are known for their high content of transaminases, lose their enzymes in case of liver cell damage. Our results also agree with the results obtained by *Ahmed et al* ., (1989) who reported a decrease in serum albumin and total protein in insecticides treated-animals.

However, contrary to our result on serum albumin, Hassan (2007) reported an increase in serum albumin and serum total protein levels which he attributed to loss of plasma fluid into the tissue due to inflammation resulting from exposure to irritants released from the coil smoke, such as aldehyde, pyrethrin and sulphates. These chemicals can induce inflammatory responses capable of causing damage to the liver cells which are the sites of protein synthesis, leading to the release of plasma protein thereby causing a arise in plasma protein (*Liu and Wong.*,1989).

The observed decrease in the level of serum albumin and total protein in our work could be due to impaired protein synthesis and losses due to haemorrhage or excessive protein catabolism. This agrees with the report of *Okine et al .,*(2004) who stated that the reduction in serum albumin in mosquito coil-inhaled rats could be due to a decrease in the protein biosynthetic activity of the liver, which affects transport of substances synthesized by the liver.

Our results also showed elevation of bilirubin level. Elevated bilirubin is one of the biochemical indices used to assess hepatotoxicity. *Ramnic* (2006) explained that elevated level of bilirubin are found in liver diseases (hepatitis, cirrhosis),excessive haemolysis/destruction of RBC. The 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 (*Stephen et al* .,(1997). The observed effects were more pronounced in the rats exposed to cork mosquito coil smoke. This agrees with the work of *Garba and Adelaiye* (2007), who reported similar results.

Results of the of biochemical analysis are confirmed by the histopathological analysis, which revealed impairment of normal organization of hepatic architecture indicated by proliferation of hepatocytes, necrosis, haemorrhages and widespread vacuolar degeneration due to inhalation of both cork and Rambo coil smokes. Previous reports have shown the presence of cytoplasmic vacuolation of the hepatocytes and leucocytes infiltration, congestion of blood vessels, haemorrhage, necrosis and inflammatory leucocytes as marked signs of hepatic tissue impairment due to intoxication with pyrethroid insecticides *Abu El-Zahab et al .,*(1993). According to *Garba* and *Adelaiye* (2007), exposure of albino rats to mosquito coil (Cork, Swam and Rambo) smokes for 21 and 28 days were presented with haemorrhagic spot, necrosis, widespread fibrosis and interstitial mononuclear cellular infiltration in liver section of rats, and the severity of the pathological effects was dependent on the duration of exposure.

However, co-treatment of the rats with various concentrations of *G. latifolium* leaf extract significantly ($P<0.05$) ameliorated the observed toxic effects in the liver of the rats exposed to mosquito coil smokes only. It was observed that exposure to the mosquito coil smokes and simultaneous administration with *G. latifolium extract* prevented elevation of the AST, ALT and ALP levels contrary to the observed effect in those exposed to the mosquito coil smokes without treatment. Thus the levels in the treated animals were significantly ($p<0.05$) lower than values found in the negative control rats (those exposed to smokes without treatment with extract). This shows the protective effects of *G. latifolium* extracts against an increase in serum enzymes due mosquito

coil smoke inhalation. Thus the leaf contain some antioxidants which act as free radical scavenger and hence exerts a protective effect against insecticides exposure (*Alhazza*,1998). It maintains capillary integrity through the production of an intercellular cement substance and this function promotes the healing of wounds and haemorrhages (*Jacob*, 1999). *Alhazza* and *Bashandy* (1998) noted that the influence of antioxidant (vitamin C) against the effect of mosquito coil insecticide containing permethrin.

The results of the phytochemical analysis showedthat *G. latifolium* leaf has high contents of ascorbic acids besides the phytochemical/antioxidant (saponin, alkaloids, tannins and favonoids). *Mensa et al.,* (2008) also reported high content of vitamin C besides the presence of alkaloids and flavanoids in *G. latifolium*. The remarkable protection of *G. latifolium* extract against hepatotoxicity may be attributed to the synergistic actions of antioxidants (saponins, alkaloids, flavonoids cardiac glycosides and sterotriterpenes) in the plant leaf extract (*Essien et al.,* 2007).

Alkaloids are reported to exhibit antioxidant activity and are effective scavengers of superoxide anions (*Ramanthan et al.,* 1989). The presence of flavonoids has been reported to have antioxidative effects (*Middleton*, 1996). The antioxidant activity of flavonoids which ultimately maintain the haeme iron in its ferrous state vis-à-vis the ferric state that is associated with production of defective methaemoglobin seen to enhance erythropoiesis. It has been reported that flavonoids inhibit peroxidation of polyunsaturated fatty acids in cell membranes and inhibit the formation of superoxide ions and hydroxyl radicals which are too strong peroxidation agents (*Facino et al.*,1998) and this antioxidant activity may protect both the haematopoietic committed blood cells from the attack of the reactive free radicals in the body while saponins are known to have hypocholesterolmic activity which may aid in lessening the metabolic burden on the liver. This action is assumed to be a direct effect of the extract on the haematopoietic system.

Our results also showed that co-treatment with *G .latifolium* significantly ($p<0.05$) protected the rats against reduction in total protein, PCV and Hb level and elevated serum creatinine and total bilirubin levels compared with the negative control (those exposed without treatment). The result show that the extract may enhance the population of red blood cells produced from the bone marrow increase the oxygen-carrying capacity of the whole blood. Our results also showed that Hb and PCV levels were significantly improved as a result treatment with *G. latifolium*.

CONCLUSION

In conclusion our results have shown that mosquito coil smoke inhalation induced many biochemical changes in exposed albino rats. Such changes include elevation of liver enzymes and various other biochemical changes such as changes in serum albumin, creatinine, total protein, bilirubin and changes in haematological parameters (haemoglobin and packed cell volume) and cause some degenerative changes in the hepatic architectures of the exposed albino rats and hence can pose significant health risk similar to that of cigarette smokes (*Liu et al.*, 2003). However, treatment with G. latifolium leaf extract ameliorates the toxicity in exposed rats possibly due to it high antioxidant content such as vitamin C, alkaloids, flavonoids and saponins; the potency of *G. latifolium* can be ascribed to the synergistic actions of these antioxidants. It is our candid suggestion that the society should reduce the degree of exposure to mosquito coil smokes.

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Table 1: Proximate composition and phytochemicals of *G. latifolium*

Extract	Vit.C (mg/g)	%Carbohydrate	%Protein	%Moisture	%Fibre	%Ash	Saponins (mg/g)	Tannins (mg/g)	Alkaloid (mg/g)	Flavonoids (mg/g)
G. latifolium	175.0±0.30	30.9±2.0	13.7±4.0	20.50±0.20	17.30±1.5	18.00±0.5	0.81±0.31	0.065±0.20	0.030±0.01	0.063±0.05

This table shows the percentage phytochemical compositions of *G. latifolium* extract.

Table 2: Comparative hepatotoxic effects of Two mosquito coil smokes (Rambo and cork) and the protective effects of *G. latifolium*

Treatments	AST(u/L)	ALT(u/L)	ALP(u/L)	Albumin (g/dl)	Total protein (g/dl)	Total Bilirubin (g/dl)	Conj Bilirubin (g/dl)	HB (g	PCV(g/dl)
Control	31.67±0.13	40.88±0.15	32.84±0.06	5.1±0.05	9.74±0.24	7.52±0.29	8.26±0.11	12.22±0.03	36.48±0.12
Cork	75.82±0.20	85.02±0.05	78.12±0.05	2.05±0.03	7.88±0.02	35.19±0.84	24.51±0.27	5.14±0.32	15.40±0.95
Rambo	62.30±0.05	68.25±0.05	70.65±0.05	8.57±0.03	2.83±0.01	28.05±0.18	18.22±0.22	8.62±0.56	25.84±1.67
G. latifolium	47.05±0.03	47.64±0.09	51.15±0.08	4.09±0.06	12.87±0.03	25.58±0.5	16.45±0.10	10.48±0.09	30.92±0.42

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