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Determination of toxic effects of commercial and local mosquito repellents in *Oryctolagus cuniculus* (New Zealand white)

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Abstract

Mosquito coils are slow-burning products which release smoke containing one or more insecticides. It burns for several hours in a confined place in order to provide protection against mosquitoes which spread diseases like malaria. The present study examined the *in vivo* effect of inhaling mosquito coil and two locally made mosquito repellents on liver function and haematology parameters of adult male rabbits. A total of 16 rabbits were divided into four groups i.e. groups A, B, C and D. Groups A, B and C were exposed to mosquito coil, rice husk and oranges peels respectively for 8 hours daily. Group D was not exposed and served as a negative control. The rabbits were exposed for four weeks. On day 29 blood samples were collected from the ear pinna of rabbits for haematology and liver function tests. It was observed that both haematological and biochemical results showed varying indices from that of the negative control. However only the group exposed to orange peels (group C) presented a statistically significant difference at $P< 0.05$ in lowering the blood glucose levels. Elevated levels of alanine aminotransferase were seen with statistical significance at $P < 0.05$ between the group exposed to rice husk and the control and also between the rice husk and mosquito coil groups respectively. The results showed increased levels of alanine aminotransferase which could indicate acute liver problems in the rice husk treated group. It is also worthy of note that the glucose levels were lowered in the group treated with orange peels. However there was evidence of toxicity in all treated groups.

Keywords: Haematology, Liver function, Mosquito repellent, Oranges peels, Rice husk, Toxicity

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Introduction

Insect repelling products such as mosquito incense, liquid vaporizers, creams and aerosol sprays are used to prevent mosquito bites so as to prevent diseases like malaria, *filariasis*, and dengue. Traditional use of plant-based repellents has been in practice for decades in an attempt to protect against mosquitoes. Ethnobotanical information on the use of plants as repellents is a useful tool for the development of new products (Maia and Moore, 2011).In Sokoto North Western Nigeria, people use charcoal to burn the available dry form of the local repellents.

Pyrethrum powder is the active components in mosquito coils and is very popular among the low income earners of Asia, Africa and South America (Weili *et al*., 2003). Toxic effect of burning one mosquito coil is the same as burning 75 to 137 cigarettes (Chen *et al*., 2008).

Urea and creatinine levels in rats exposed to mosquito smoke were shown to be high (Garba *et al*., 2007a) followed by a significant increase in white blood cell count (Garba *et al.,* 2007b). Das *et al*. 1994 demonstrated smoke from mosquito coil causes mutagenicity and chromosomal aberrations in

metaphases in exposed rats and mice. Azizi and Henry (1991) reported that long term exposure to mosquito coil smoke has been shown by some workers to cause asthma and persistent sneezing in children.

Appearance of resistant parasites and mosquito strains have paved way to search for new products (Nwane *et al*., 2009) Orange peels (*Citrus sinensis*) are mostly thrown into the garbage after which they eventually find their way into piles of wasted products. Interestingly the chemical contents of the peels have pharmacological activities (Muhtadi *et al*., 2015). Orange is a rich source of vitamin C, phenolic compounds, pectin and flavonoids such as hesperidin, narirutin, eriocitrin, and naringin. The use of the peels as mosquito repellent is mostly seen among the low income families. However this product has not been upgraded to commercial production, rather the peels are gathered and dried for local use as repellents.

Ash from rice husk contains high amount of silica, some alkalis and trace elements. Silicosis can occur after inhaling silica dust which damage lung tissues leading to scarring and chronic lung disease (Colinet, 2010).This study was carried out to evaluate and compare the toxic effects of two locally used mosquito repellents (Dry orange peels and Dry rice husk) and a commercial coil (Wavetide brand company), containing 0.05% merperfluthrin as active ingredient.

Materials and Methods

A total of sixteen (16) adult male New Zealand White rabbits divided into four groups of four rabbits each, were used for this study. The rabbits were purchased from Sokoto Central Market, Sokoto Nigeria. The rabbits were kept in the research laboratory of the Department of Veterinary Pharmacology and Toxicology for two weeks to acclimatize to their new environment, and baseline physiological parameters were observed daily to rule out any ailment that may render them unfit for the study.

Commercial mosquito coil (Wavetide brand company, China) which was very much available in market was used in the study. The brand contained 0.05% merperfluthrin. Rice husk were gotten from local rice processors in Sokoto town while the orange peels were accessed from an orange vendor at the Sokoto bulk market. The animals were kept at room temperature and fed with wheat brand and vegetables. Feed and water were given *ad libitum* throughout the experiment.

The rabbits were divided into groups A, B, C and D. They were kept in different boxes of four rabbits each. The boxes were covered all round with a wire mesh and a window provided on top of the box to allow for ventilation. Each group was kept in a different room. The rabbits in group A, B and C were exposed to mosquito coil, rice husk and orange peels respectively for four weeks while those in group D served as control and were not exposed to any insect repellent.

They were exposed for eight hours daily by allowing the repellents to burn slowly on top of a mesh wire under which hot charcoal is provided from 9 pm to 5 am throughout the study period in an attempt to mimic average period of time that the repellents are used by humans at night. Small clay bowls were used to burn the charcoal .The rabbits in each group were observed for any clinical signs associated with the exposure to the different fumes.

After a period of four weeks, 3ml of blood sample was obtained from the ear vein of each rabbit from all groups for *in vivo* quantitative determination of Total Protein (TP), Albumin (ALB), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline Phosphatase (ALP), Direct Bilirubin (DB), Total Bilirubin (TB). Red blood cell count (RBC) and white blood cell count (WBC) and blood glucose (BG) values were also determined using automated blood cell counter (PCE 210 Tokyo). Liver function tests and glucose level determination were done with Randox Laboratories kit, UK which is based on enzyme detection. Using full

Data obtained from the study was expressed as mean ± standard error of the mean and analysed by ANOVA using Invivo Stat software version 3.0. Statistical significant differences between means at $P<0.05$ was considered significant.

Results and Discussion

Table 1 statistical significance was seen between the control group and the glucose levels of the group treated with orange peels to be lowest at $P<0.05$ while the group treated with rice husk had the highest ALT values with statistical significance compared to control at $P<0.05$. Table 2 the group treated with orange peels has the highest levels of direct bilirubin compared to all other groups at $P<0.05$. The WBC count in the RH treated group is the highest with statistical significance at $P<0.05$. All other observed parameters were not statistically significant. After 28 days exposure all the treated groups had varying levels of the parameters

Table 1: Serum levels of liver enzymes, blood glucose and albumin of rabbits exposed to different mosquito repellents

Groups	BG (mg/dL)	ALT (μ/L)	AST (μ/L)	ALP (μ/L)	ALB (g/dL)
A (MC)	102.50±7.82	67.51±17.31	20.87±3.71	208.72±32.85	3.12±0.02
B (RH)	92.00±7.82	124.87±14.90*	57.90±12.87	181.69±1859	3.12±0.02
C (OP)	79.25±7.82*	71.45±14.43	56.67±19.35	278.91±120.78	2.89±0.19
D (CN)	121.00±7.82	59.54±3.66	35.52±7.50	283.99±128.03	2.90±0.04

Key

MC= Mosquito coil, RH= Rice husk, OP= Orange peels, CN= Control group , Albumin (ALB), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline Phosphatase (ALP), and blood glucose (BG)

Table 2: Total Plasma proteins, bilirubins, red blood cells and white blood cells of rabbits exposed to mosquito repellents (Mean±SD)

Groups	TP (g/dL)	DB (mg/dL)	TB (mg/dL)	RBC (Cells/mm^3)	WBC (Cells/mm^3)
A (MC)	6.04±0.08	0.29±0.28	0.74±0.53	3.88±0.66	10.28±1.56
B (RH)	5.85±0.53	0.81±0.77	1.60±1.22	4.44±0.39	11.84±0.18*
C (OP)	6.34±0.37	1.40±0.42*	2.37±0.66	3.54±0.22	8.18±1.78
D (CN)	7.22±1.79	0.11±0.03	1.50±0.11	4.02±0.29	8.28±0.51

Key

MC= Mosquitoe coil, RH= Rice husk, OP= Orange peels, CN= Control group Total Protein (TP), Direct Bilirubin (DB), Total Bilirubin (TB).Red blood cell count (RBC) and white blood cell count (WBC)

evaluated which were obviously different from the control group. Differences observed in Table 1 showed that the level of glucose was statistically different between oranges peels treated (group C) and control (group D) ($P < 0.05$). There was also a statistically significant difference in the alanine amino transferase levels of group exposed to rice husk and that of the control group ($P < 0.05$).Plasma protein levels among the treatment groups was highest in orange peel group followed by mosquito coil group while the rice husk group was lowest. White blood cell count was also highest in the rice husk group and this may be correlated with a reflection of low total plasma protein values seen, thus plasma proteins are liver proteins which help to fight infections. Red blood cell count was lowest in the orange peel group which correlates with the fact that low blood glucose levels can cause destruction of red blood cells leading to increase in circulating bilirubin, thus highest level of direct bilirubin was seen in this group.

Results of the present study showed that among the exposed groups, the group which was exposed to Orange's peels showed the lowest mean value of blood glucose and white blood cells count. This may be due to the presence of vitamin C in the oranges peels which has been documented by Afkhami & Shojaoddiny (2007) to have blood glucose lowering effect. Table 2 shows the group treated with orange peels has the highest level of direct bilirubin compared to all other groups at $P<0.05$. The WBC count in the RH treated group is the highest and statistically significant at $P<0.05$. Statistical significance was seen between the control group and the glucose levels of the group treated with orange peels (lowest at $P<0.05$) while the group treated with rice husk had the highest ALT values with statistical significance compared to control at $P<0.05$. All other observed parameters were not statistically significant. From this study, the group exposed to Rice husk seemed to demonstrate the most toxic effects on both haematology and liver function test, this could be attributable to the fact that respirable silica dust from the Rice husk is capable of causing respiratory problems and or diseases e.g bronchitis, air way disease, fluid retention and heart failure as reported by Colinet in 2010. An increase in the level of ALT in the rice husk group compared to all other groups could be attributed to liver injury and other non-hepatic diseases like type 2 diabetes, metabolic and cardiovascular diseases (Wedemeyer *et al*. 2010).

However from the result of this study, we observed some haematological indices being affected by the

acute intoxications of the various repellents we gave although most were without statistical significance. This is in line with the findings of Ghani and Shahbaz (2014).

This research has shown that all the local and available mosquito repellents have different levels of toxicity on health and their use should be with caution to avoid liver and lung disease. Redefining the form of administration by avoiding the use of burning process that produces smoke which itself is toxic may probably reduce the toxic effect. A better approach could be by introducing the repellents in form of spray. Further research should also look into the different effects of the repellents on the various organs system by using histopathology techniques.

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