IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 14, Issue 11 Ver.X (Nov. 2015), PP 12-21
www.iosrjournals.org

Protective Effect of Gongronema Latifolium Leaf Extract on Rambo And Raid Mosquito Coil Smoke-Induced Liver Toxicity in Albino Rats.

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Abstract: *A total of 30 albino rats were used for the experiment and were grouped into A, B and C. Group A rats represented the control group while groups B and C were exposed to different brands of mosquito coil (rambo and raid) 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 animals were exposed to Rambo and Raid mosquito coil smoke respectively and co-treated with aqueous extract of Gongronema latifolium while the sub-group 2 animals were exposed to the respective mosquito coil smoke only (to serve as the negative control). The effects of 8 hour daily inhalation of Rambo and Raid mosquito coil smoke respectively were investigated in albino rats. The activities of liver enzymes, (AST, ALT, ALP) and renal function parameters such as total bilirubin, serum creatinins, total protein, Albumin and blood indices such as Hb and PCV were used to assess the potential effects of inhalation of these mosquito coil smokes on the liver. The result revealed that AST, ALT, ALP, total protein, albumin, creatinine and total bilirubin, were more significantly elevated due to inhalation of Raid mosquito coil smoke by albino rats than Rambo mosquito coil smoke, while Hb and PCV levels were more significantly elevated in Rambo mosquito coil exposed rats than the rats exposed to Raid mosquito coil smoke, which showed significant difference from the control. The levels of all these parameters in albino rats due to inhalation of Rambo and Raid mosquito coil smoke respectively were significantly different from the levels observed in the rats co-treated with aqueous extract of Gongronema latifolium. More so, some pathological changes were observed in the liver of the rats exposed to Rambo and Raid mosquito coil smokes such as hepatic necrosis, haemorrhages, hepatic injuries that could lead to necrosis of the hepatocytes. It was observed that the co-treatment with aqueous extract of G. latifolium reduced the damaging effects of smoke from these mosquito coils.*

Keywords: *Rambo, raid, mosquito and malaria.*

I. Introduction

Burning mosquito coils indoors to repel mosquito is a common practice in many household in tropical countries. The evaluation and assessment of the inhalation toxicity of smoke emitted from mosquito coils appears to be particular challenging due to the complex nature of the type of exposure atmosphere [1]. There has been growing concern among the public regarding the routine and prolonged use of mosquito coil [2]. Of particular concern is the general abuse, wanton over-use of these mosquito coils and insecticides spray in the control of mosquito, cockroaches and houseflies in most urban and rural dwellings, grain stores, animal, poultry houses and in public places such as fish, meat and vegetable markets. These therefore pose a serious public health and ecological challenges because of the effects dissociated with their use, especially innocuous and chronic inhalation of the fumes of the insecticides [3,4, 5 and 6]. The major active ingredients of the mosquito coil are pyrethium accounting for about 0.3-0.4% of coil mass [7, 8 , 9 and 10]. Most mosquito coils sold in Ghana and Nigeria contain 0.1-0.3% allethrim [11, 12 and 13].

Mosquito coils release various aromatic compounds such as benzopyrenes and benzoflouroethane, which have the potential to produce harmful effects on airways [14, 15 and 16] and consist of an insecticidal/repellent organic filters capable of burning with smoudering binders and additives such as synergist, dyes, and fundicides

[17, 18 and 19]. Also mosquito coils contain some combustible materials such as sub-micron particles coated with heavy metals, alethrim and wide range of organic vapour, such as phenol, o-cresol, benzene and toluene [20 and 22]. The gas phase of mosquito coil smoke contains carbonyl compounds (Formaldehyde, acetaldehyde and polycyclic aromatic hydrocarbons) generated through incomplete combustion of biomas with properties that can produce strong irritating effects on the upper respiratory tract [5].

II. 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^{o}C) and were allowed access to water and dry ration. The rats were randomly group into three (3), 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 (1 and 2) with six (6) 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,Transfluthrin,Beta-cyfluthrin

Preparation and Administration of Plant Sample

About 250g of fresh G.latifolium leaves purchased at Abakpa market, Abakaliki were ground to 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 the extract allowed to dry 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 .

III. Blood Sample Collection And Biochemical Assay.

At the end of the exposure period (31 days) to mosquito coil smoke, blood 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 [13].

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^{o}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.

IV. Results of histopathological analysis

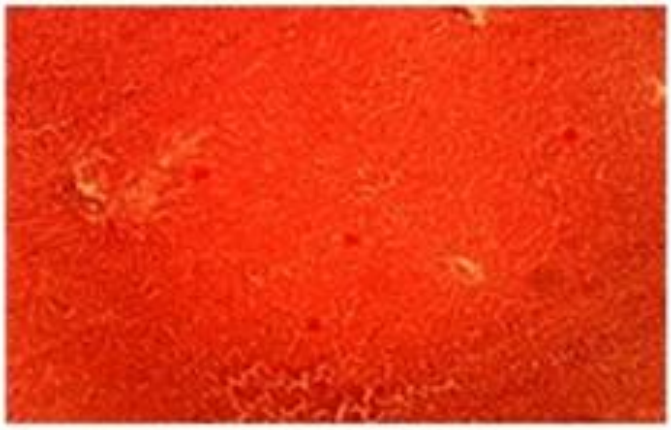

Fig. 1. Photomicrograph of a liver section of the control animal showing the normal liver architecture with normal hepatocytes,the cynusoids and hepatic vessels well preserved(A) and (B) (H& E;X400)

Fig 2.Photomicrograph of a liver section of an albino rat showing the effect of exposure to raid mosquito coil smoke for 31 days without co-treatment with extract Arrows , showing extensive distortion of hepatocytes and injury leading to focal areas of necrosis of the hepatocytes (H&E;X 400).

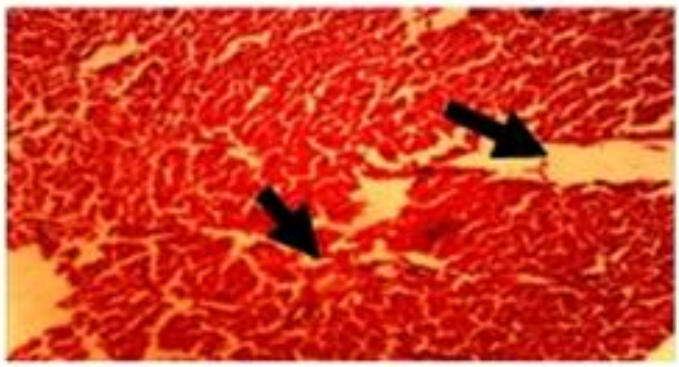

Fig.3..Photomicrograph of a liver section of an albino rat showing the effect of exposure to rambo mosquito coil smoke after 31 days without co-treatment with extracts (A)Impairment of normal organization of hepatic architecture with the presence of hepatic necrosis. (B) distorted hepatic vessels with the presence of hepatic haemorrhages (H & E; X 400).

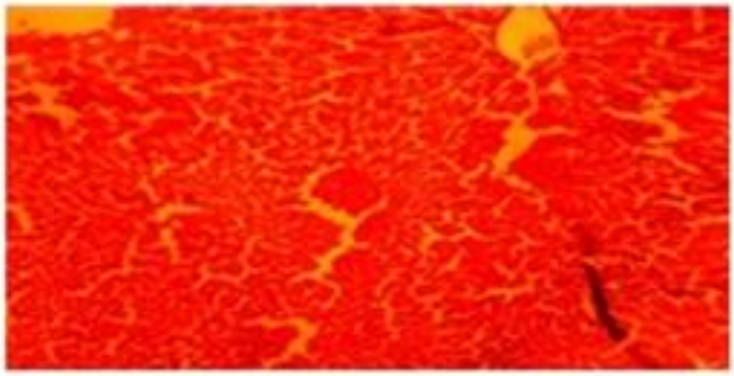

Fig .4.. Photomicrograph of a liver section of albino rat showing the effect of treatment with G.latifolium extract after exposure to raid mosquito coil smoke for 31 days (A)improved liver architecture with scarttered hepatic vessels (B) degeneration of the hepatic vessels that could lead to necrosis of hepatocytes(H&E;X400)

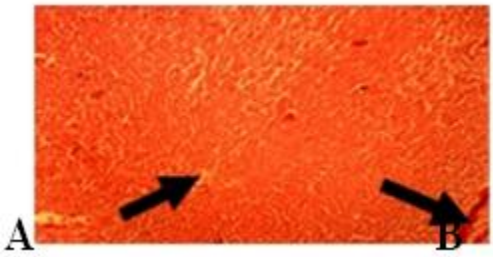

Fig. 5. .Photomicrograph of a liver section of albino rat treated with G. latifolium extract after exposure to Rambo mosquito coil smoke for 31 days showing (A) mild change in liver architecture and minimal focal areas of necrosis and(B) Mild haemorrhages (H& E;X400)

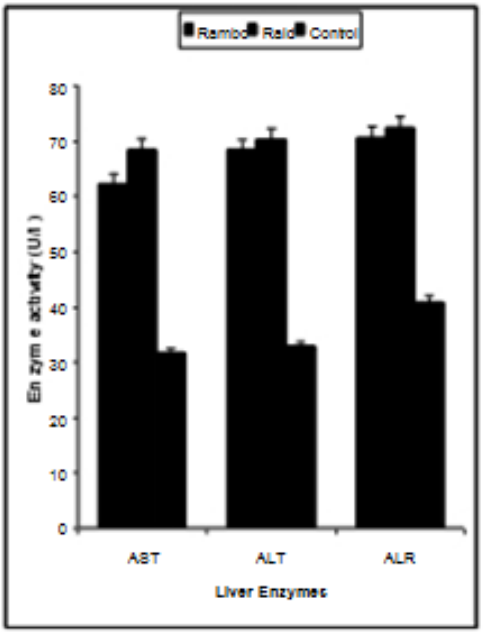

Fig.6.exposure to these brands of mosquito coil smoke respectively for 31 days caused significant ($P<0.05$) increase in serum enzymes (AST, ALT, ALP) levels in albino rats with exposure to raid mosquito coil smoke showing a higher effect

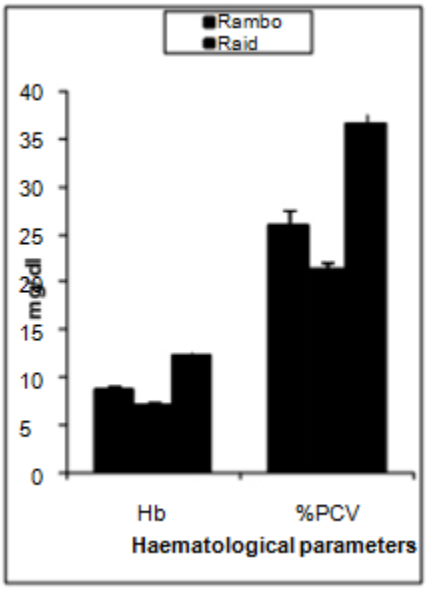

Fig.. 8. Haematological effect of exposure to rambo and raid mosquito coil smoke respectively on Hb and % PCV levels in albino rats for 31 days . The effect caused a significant (P< 0.05) reduction in Hb and % PCV levels in the exposed rats compared with the control with raid showing a higher effect.

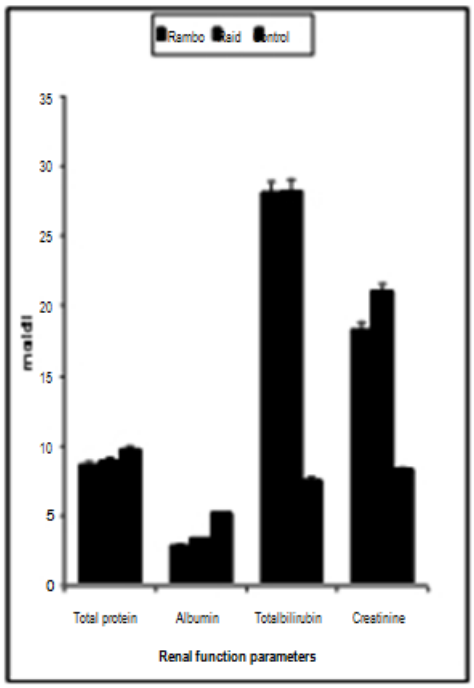

Fig.7. Effect of exposure to rambo and raid mosquito coil smoke respectively on renal function parameters (total protein, albumin, total bilirubin and creatinine) in albino rats for 31 days .This effect caused a significant (P< 0.05) increase in total bilirubin and creatinine levels with raid showing a higher effect. Also the exposure caused a reduction in serum total protein and albumin levels with Rambo mosquito coil smoke showing a higher effect

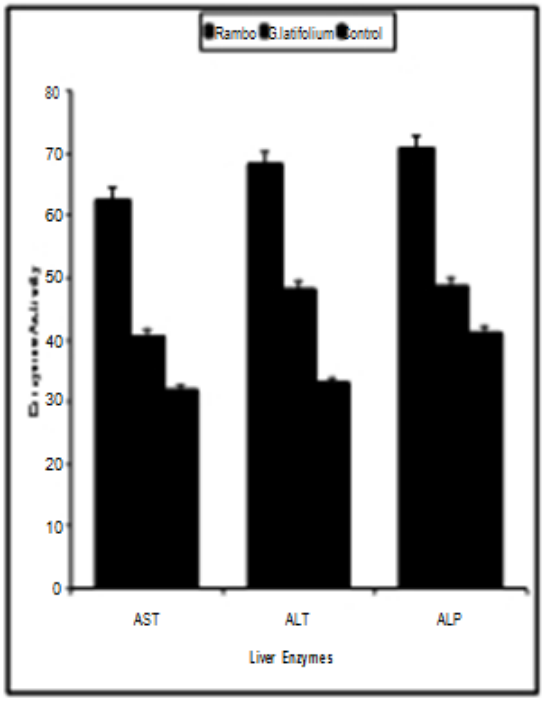

Fig. 9 .This shows the protective effect of G.latifolium extract on liver enzyme activity in Rambo mosquito coil smoke-exposed rats. The figure shows a significant ($P< 0.05$) reduction in liver enzymes (AST,ALT,ALP) after co-administration of the extract which indicates the protective effect of G.latifolium extract against exposure to Rambo mosquito coil smoke.

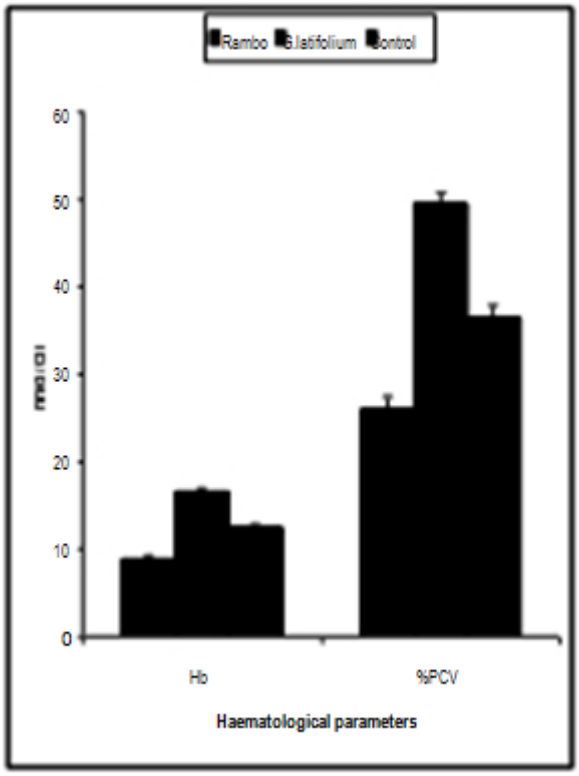

Fig..11. The protective effect of G.latifolium extract against reduced haemoglobin and packed cell volume levels due to exposure to Rambo mosquito coil smoke is demonstrated in fig.6. The Hb and PCV levels were significantly ($P<0.05$) improved after co-administration of the extract and this also significantly($P<0.05$) differ from the control.

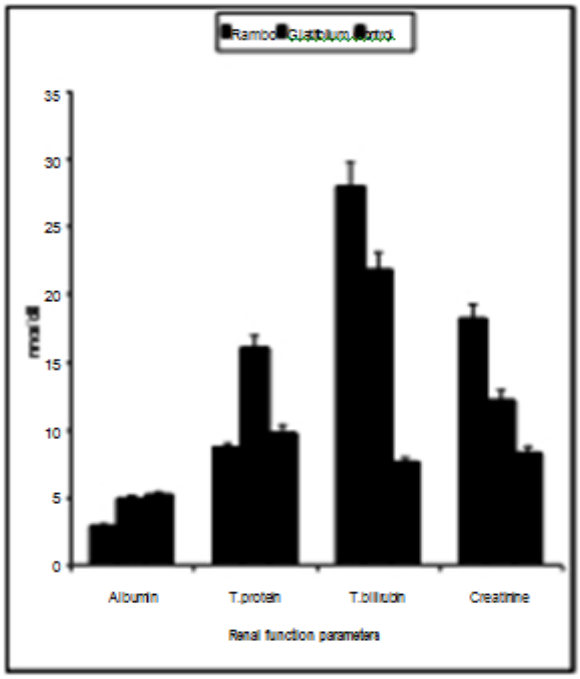

Fig.10.The effect of co-administration of G. latifolium extract on renal function parameters after exposure to Rambo mosquito coil smoke is demonstrated in fig.5.

The extract caused a significant (P< 0.05) decreased in elevated creatinine and bilirubin levels caused by exposure to Rambo mosquito coil smoke .Also the decreased in serum albumin and total protein levels were significantly (P< 0.05) improved which shows the protective effect of the extract.

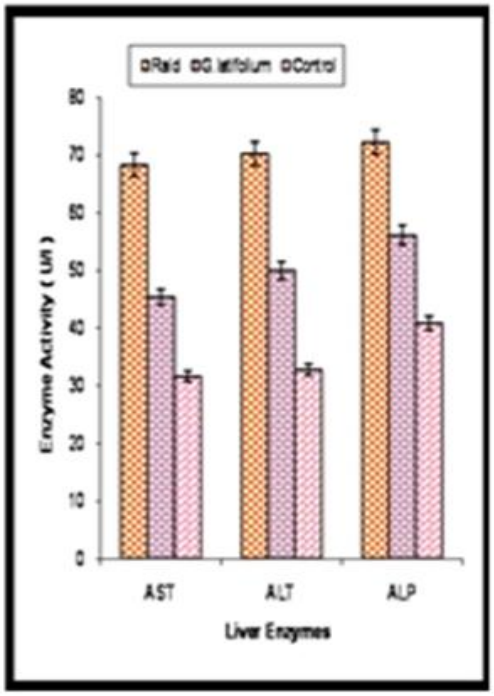

Fig 12.This shows the protective effect of G.latifolium extract on liver enzyme activity in raid mosquito coil smoke-exposed rats. The figure shows a significant (P< 0.05) reduction in liver enzymes (AST,ALT,ALP) after co-administration of the extract which indicates the protective effect of G.latifolium extract against exposure to Rambo mosquito coil smoke.

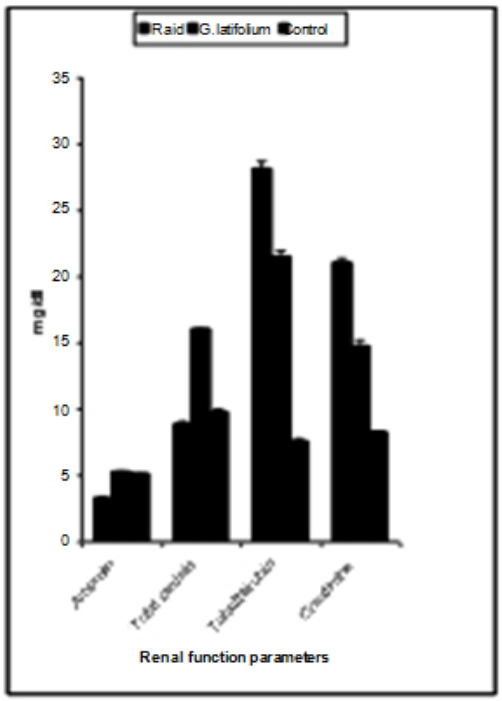

Fig 13.The effect of co-administration of G. latifolium extract on renal function parameters after exposure to raid mosquito coil smoke is demonstrated in fig.. The extract caused a significant (P< 0.05) decreased in elevated creatinine and bilirubin levels caused by exposure to Rambo mosquito coil smoke .Also the decreased in serum albumin and total protein levels were significantly (P< 0.05) improved which shows the protective effect of the extract.

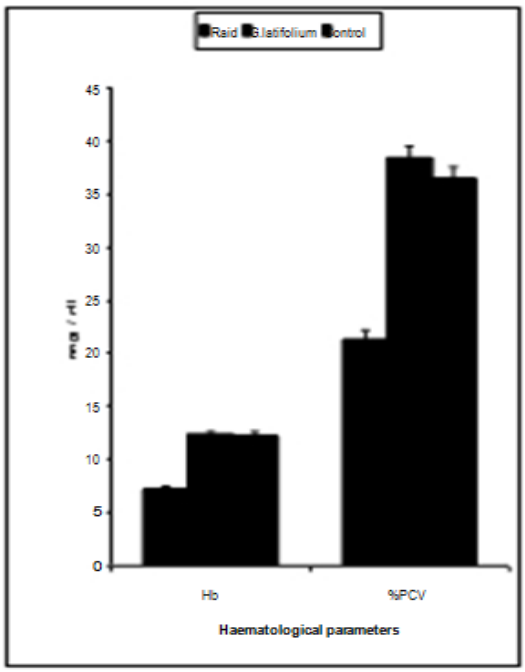

Fig 14. The protective effect of G.latifolium extract against reduced haemoglobin and packed cell volume levels due to exposure to raid mosquito coil smoke is demonstrated in fig.6. The Hb and PCV levels were significantly (P<0.05) improved after co-administration of the extract and this also significantly(P<0.05) differ from the control.

V. Discussion

Exposure of the albino rats to rambo and raid mosquito coil smoke respectively caused a significant ($P<0.05$) increase in the liver enzyme activities (AST, ALT and ALP) total bilirubin and creatinine with a decrease in the levels of total protein, serum albumin, Hb and PCV. According to Abubakar and Hassan (2007) [2] liver enzymes AST, ALT and ALP activities were significantly increased due to exposure to different grands of mosquito coil (Swam , Rambo and Cork) smoke respectively for 14 days. According to Foltron et al, (1988) [12] and Abu-Elzahab et al (1993) [6] increased level of serum enzymes were observed due to exposure to pyrethriod insecticides (Fenvalarate) and mixed pyrethriod (Tetramethrin and Sumithrin) respectively. A study by woodman (1980) [22] indicated that the increase in serum enzymes activities often seen due to liver damage does not indicate the inability of the liver to synthesize the enzymes but could be due to loss of materials from the damaged hepatocytes as a result of exposures to mosquito coil smoke and thus leads to high enzyme activities in the serum of animals indicating a liver damage. Martin and associates (1983) reported that the liver tissues which are known for their high contact of transminages, lose these enzymes in case of liver cell damage.

The results also agree with the results obtained by Imamura et al, (1983) [14], Ahmed et al, (1989) [7] and Badaway et al, (1992) [10] who reported a decrease in serum albumin and total protein in insecticides treated-animals. Also Abubakar and Hassan (2007) [2] noted that inflammation as a result of exposure to irritant released from the coil smoke, such as aldehyde, sulphates and-pyrethrim which can induce inflammatory responses capable of causing damage to the liver cells which are sites of protein synthesis can cause a rise in total protein and serum albumin levels. Liu and Wong (1989) [17] observed a decrease in the level of serum albumin and total protein which according to him could be due to impaired protein synthesis and losses as a result of haemorrhages or excessive protein catabolism. This agrees with the observation of Okine et al, (2004) [8] who observed that the lower level of serum albumin in the mosquito coil-inhaled rats could decrease the protein biosynthesis activity of the liver and affect the transport of distances as lipids synthesized by the liver.

Elevated bilirubin is one of the biochemical indices used to assess hepatoloxicity. Ramnic (2006) [19] , explained that high levels of bilirubin are found in disease conditions such as hepatitis, cirrhosis, excessive haemolysis/destruction of red blood cells Stephen et al (1997) [21] reported that 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.

The result of the study showed that the Rambo and Raid mosquito coil smoke respectively induced many histopathological changes in the liver of albino rats, and the obvious signs of hepatic impairment observed include, impairment of normal organization of hepatitis architecture. Haemorrhages, distortion of hepatocytes and necrosis of the hepathocytes- Sakr and Hanafy (2002) [20] observed the presence of cytoplasmic vacnolation of the hepatocytes and leukocytes infiltration as marked symptoms of hepatic tissue impairment due to intoxication with pyrethroid insecticides.

Abu El-Zahab et al, (1993) [6] reported the presence of congested blood vessels, haemorrhage, necrosis and inflammatory leucocytes in rats exposed to pyrethroid-based mosquito coil smoke as marked signs of hepatic impairment due to exposure of rats to mosquito coil smoke. Abou-Zaid (1995) [3], observed necrosis, congested blood vessels and leucocytic infiltra in the liver of new born mice exposed to the insecticides for 15 days. Graba and Adelaiye (2007) [13] observed that exposure of albino rats to cork, swam and Rambo mosquito coil smoke respectively for 21 and 28 days caused the presence of haemorrhagic spot, necrosis, widespread fibrosis and interstitial mononuclear cellular infiltration in liver sections of albino rats.

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