



DURATIONAL EXPOSURE-DEPENDENT EFFECT OF CARBAMATE TREATED NET ON HEPATIC AND RENAL FUNCTIONS IN WISTAR RATS.

ESSIEN, M. N., EBAN, L. K., BASSEY, N. O., UKPANUKPONG, R. U. AND AGUNWA, A. O.

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ABSTRACT

Assessment of the duration exposure-dependent effect of carbamate treated net on hepatic and renal functions of albino Wistar rats after 30 and 60 days was carried out. Serum ALT, AST and ALP levels were determined to assess liver function while serum creatinine and urea levels were measured for kidney function. Eighteen (18) male albino Wistar rats weighing 138-146g were divided into 3 groups of 6 rats each. Group 1 served as control, group 2 animals were exposed to carbamate treated net for 30 days and group 3 animals exposed for 60 days. The results showed that the levels of serum AST and ALT increased in all the experimental groups exposed when compared to the control group. ALT increased significantly ($p < 0.05$) in the rats exposed for 60 days (203.83 ± 0.307) while AST increased highest in experimental groups exposed for 30 days (203 ± 1.613) and 60 days (362 ± 0.365) respectively when compared to the control (150.5 ± 0.34). ALP increased significantly ($p < 0.05$) only in the group exposed for 30 days (17.67 ± 0.21), but decreased in the group exposed for 60 days when compared to the control group. Serum creatinine increased insignificantly ($p > 0.05$) in the group exposed for 30 days but decreased in the group exposed for 60 days while serum urea level in the group exposed for 30 days remain unchanged but decreased after 60 days when compared to the control group. Statistically, there was a significant increase ($p < 0.05$) in body weight and organ weight of the animals exposed for 30 and 60 days. Therefore, this present study demonstrates that exposure to carbamate treated net may alter the integrity and function of liver thereby causing hepatotoxicity while the exposure of rats to carbamate treated net may not pose any significant nephrotoxicity in rats.

Keywords: Carbamate, Serum Enzymes, Serum Creatinine and Urea Level

INTRODUCTION

Pesticides have now become an integral part of our modern life and are used to protect agricultural lands, stored food produce, control harmful organisms as well as to eradicate pests transmitting infectious diseases. It has been estimated globally that almost \$38 billion are spent on pesticides every year (Jokanovic, 2008). The Food and Agricultural Organization (FAO) has defined pesticide as, any substance or mixture of substances intended for preventing, destroying or controlling any pest, including vectors of human or animal disease, unwanted species of plants or animals, causing harm during production, processing, storage, transport, marketing of agricultural commodities that may be administered to animals for the control of insects, arachnids or other pests in or on other bodies.

Pesticides include substances used as plant growth regulator, defoliant, desiccant, or agent for thinning fruit or preventing the premature fall of fruits. Also used as substances applied to crops either before or after harvest to protect the commodity from deterioration during storage and transport. The term pesticide is a family name that includes: herbicides, insecticides, nematocide, molluscicide, piscicide, avicide, rodenticide, bactericide, insect repellent, animal repellent, antimicrobial and fungicides (Randall, 2014). The most common of these are herbicides which account for approximately 80% of all pesticides use. Pesticides have been used by humans to protect their crops since before 2000BC (Jurewicz and Hanke, 2008).

The 19th century saw more natural pesticides: Pyrethrum which is derived from chrysanthemums and rotenone, found in roots of some tropical vegetables (Miller, 2002).

Essien, M. N., Department of Biochemistry, Faculty of Basic Medical Sciences, University of Calabar, Calabar Nigeria.

Eban, L. K., Pharmacology Department, Faculty of Basic Medical Sciences, University of Calabar, Calabar, Nigeria.

Bassey, N. O., Department of Biochemistry, Faculty of Basic Medical Sciences, University of Calabar, Calabar, Nigeria.

Ukpanukpong, R. U., Department of Biochemistry, Faculty of Basic Medical Sciences, University of Calabar, Calabar, Nigeria.

Agunwa, A. O., Department of Biochemistry, Faculty of Basic Medical Sciences, University of Calabar, Calabar, Nigeria.

Swiss chemist Paul Miller discovered that Dichlorodiphenyltrichloroethane (DDT) was a very effective organochlorine insecticide such as the popular DDT, but they were replaced by organophosphates and Carbamates in 1975. Since then, pyrethrin compounds have become the dominant insecticides (Ritter, 2009). The use of pesticide has increased 50-fold since 1950 and 2.3 million tonnes of industrial pesticides are now used each year (Miller, 2004). There are primary and secondary benefits for pesticide use. Primary benefits are direct gains from the use of pesticides and secondary benefits are effects that are long-term (Copper and Dodson, 2007). Primary benefits include controlling pests and plant disease vector to improve crop yields, crop livestock quality and controlling human livestock disease vectors to save human and animal lives and reduce the spread of diseases. Pesticides may cause acute and delayed health effects in people who are exposed. Pesticide exposure can cause a variety of adverse health effects ranging from simple irritation of the skin and eyes to more severe effects such as affecting the nervous system. The WHO and UN Environment Programme estimate that 3 million agricultural workers in the developing world experience severe poisoning from pesticides, and about 18,000 of whom die each year (Miller, 2004).

Pesticides use is widespread in Latin America and about \$3 billion is spent each year in the region (Laborde *et al.*, 2015). It has been recorded that pesticide poisonings have been increasing each year for the past two decades, it is indicated by studies that organophosphate and carbamate insecticides are the most frequent source of pesticide poisoning (Laborde *et al.*, 2015). Pesticide raises a number of environmental concerns, over 98% of Sprayed insecticide and 95% of herbicides reach a destination other than their target species, including non-target species, air, water, and soil (Miller, 2004). Pesticides are one of the causes of water pollution, and some are persistent organic pollutants and contribute to soil and flower (pollen, nectar) contamination. In addition, pesticide use reduces biodiversity, contributes to pollinator decline, destroys habitat especially for birds (Palmer *et al.*, 2007) and threatens endangered species (Miller, 2004).

Carbamate insecticides are derivatives of carbamic acids and the first carbamate insecticide, carbaryl was introduced in 1956 (Reiner, 2001). They inhibit the acetylcholinesterase enzyme and cause over stimulation of nervous system. Carbaryl (1-naphthyl N-methyl carbonate), broad spectrum carbamate insecticide is extensively used worldwide for more than 120 different crops and ornamental plants (Akinola and Ukpanukpong, 2015). Because of very low mammalian toxicity together with the short half-life in the environment, carbaryl are the most popular insecticide and effectively acts against 160 harmful insects (Akinola and Ukpanukpong, 2015). Carbamates are common insecticides that inhibit cholinesterase activity causing acute muscarinic manifestations such as diarrhea, emesis, bronchorrhea, bronchospasm, salivation, urination, and some nicotinic symptoms including muscle fasciculations and weakness. Neuropathy can develop to days to weeks after exposure. Diagnosis is clinical and sometimes with a trial of atropine, measurement of RBC acetylcholinesterase level or both

(Gerald and Rika, 2018). A number of enzymes, physiological system and organs of mammalian systems including hepatic and renal organs are influenced by pesticides.

MATERIALS AND METHODS

Materials and Apparatus

Equipments, chemicals and Reagents:

Materials used in laboratory include dissecting set, cage, glass wares such as syringes (2mls, 5mls) test tubes, pipettes and micro-pipette slides were from Singer Company Nigeria Ltd. Centrifuge, Water bath, Spectrophotometer, Hematocrit, Refrigerator and microscope were from Olympus, Japan while triple beam weighing balance, specimen jars, and beakers were from Ohaus, USA. All diagnostic reagents chloroform, buffer solution, distilled water and kits for liver serum enzymes, creatinine and urea were of analytical grade

Experimental animals: Eighteen (18) male albino Wistar rats weighing 138-146grams were obtained from Department of Biochemistry Experimental Research Animal House of the University of Calabar, Calabar. They were kept in standard laboratory cages at room temperature in the animal facility of the Department of Biochemistry, University of Calabar. Acclimatization of the animals to the environmental conditions was allowed for two (2) weeks prior to the commencement of the experimental carbamate exposure procedures. The animals were fed with a standard laboratory diet (pellet feed) and tap water.

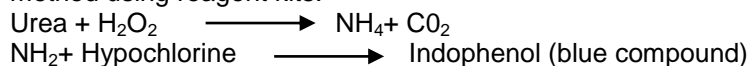
Exposure of animals to carbamate treated net: The animals were divided into 3 groups consisting of six (6) animals each. Group 1 served as the control while group 2 and 3 were the experimental groups respectively. The cage housing the animals were placed in a constructed wooden exposure chamber covered with carbamate treated net. Prior to the commencement of the exposure, animals in the control group were anaesthetized with chloroform and sacrificed. The blood samples were collected with sterile syringe (5ml) into plain sample bottle. The liver, kidneys and heart were obtained and their weight recorded. The animals in the experimental group 2 and 3 were exposed for a duration of 30 and 60 days respectively. The animals were exposed to the carbamate for 9hrs \ day. At the end of each exposure day, the animals were removed from the exposure chamber and transferred to carbamate vapor-free section of the animal house.

Collection and preparation of samples for analysis:

Blood samples for bioassay were obtained from rats by cardiac puncture using sterile syringes (5 ml) under chloroform vapour anaesthesia syringes comes after 24 hours of termination of carbamate exposure. Blood was collected into well labeled plain screw-cap sample bottles for biochemical analysis. The blood sample was allowed to clot and then centrifuged at 10,000 rpm for ten minutes using a haematocrit centrifuge. Semi-automatic pipettes were used to aspirate serum into labeled specimen tubes. The serum was stored in a refrigerator until when required for analysis. The liver and kidneys were carefully removed and their weights recorded.

SERUM BIOCHEMICAL ANALYSIS

Determination of Serum Urea Level: The concentration of urea in serum was estimated by the end point colorimetric method using reagent kits.

**Calculation**

$$\text{Urea concentration} + \frac{\text{A sample}}{\text{A standard}} \times \text{standard concentration}$$

Where;

$$\begin{array}{l} \text{A sample} = \text{Absorbance of sample} \\ \text{A standard} = \text{Absorbance of standard} \end{array}$$

Determination of serum creatinine level: Serum creatinine level was determined by Jaffe's reaction as described by Cook (1971).

Calculation

Changes in absorbance of the sample and standard as well as the concentration of creatinine in serum were calculated as follows:

$$\begin{array}{l} \Delta \text{A sample or a standard} = \text{A standard} = A_2 - A_1 \\ \text{Creatinine concentration in serum} = \frac{\text{A sample}}{\text{A standard}} \times \text{standard concentration} \end{array}$$

Where $\Delta \text{A sample}$ = change in absorbance of the sample

Determination of Alanine Amino Transaminase

(ALT): Serum Activity of ALT was determined using Agappe Kit based on Reitman and Frankel (1957). Alanine transaminase catalyzes the transfer of amino group from glutamic acid to pyruvate to form α -ketoglutarate. Activity of ALT is measured by monitoring the concentration of pyruvate hydrazine formed with 2,4-dinitrophenylhydrazine at 546nm. 250ul of serum was added to 50ul of reagent. After mixing, tubes were incubated for 30 minutes at 37^oC. The change in absorbance was recorded against blank at 340nm.

Determination of Aspartate Amino Transaminase Activity Using Agappe Kit Based on Reitman and Frankel (1957):

Alanine transaminase catalyzes the transfer of amino group from glutamic acid to oxaloacetate to form α -ketoglutarate and aspartic acid activity of AST is measured by monitoring the concentration of oxaloacetate hydrazine formed with 2,4-dinitrophenylhydrazine. 250ul of serum was added to 50ul of reagent. After mixing, tubes were incubated for 20 minutes at room temperature. The change in absorbance was recorded against blank at 340nm. Distilled water was used as blank.

Determination of alkaline phosphatase activity:

Alkaline phosphatase activity was assayed according to the method described by Bassey *et al.* 1946 and modified by Wright and Plummer 1974.

Statistical Analysis: Quantitative data were analyzed using one-way analysis of variance ANOVA followed by post hoc (Duncan test) for significant values. Social Science Application Software SPSS version 20 was used for statistical analysis and the charts were plotted using Microsoft excel application software. Data are expressed as mean \pm SEM level of probability tested at ($p < 0.05$).

RESULT

Duration Exposure-Dependent Effect on Alanine Transaminase (ALT): As seen in and figure 1, there was a statistically significant difference ($p < 0.05$) among the groups when compared to the control except in the group of rats exposed to carbamate treated net for 30 days. The increase in the group of rats exposed to carbamate treated net for 60 days was statistically different from the control group and had the highest mean ALT concentration. Therefore, exposure to carbamate treated net was observed to cause an increase in all the experimental groups and the increase was significant ($p < 0.05$) in the rats exposed to carbamate treated net for 60 days.

Duration Exposure-Dependent Effect on Alkaline Phosphatase (ALP):

There was a significant increase ($p < 0.05$) in all the experimental groups when compared to the control group except in the group of rats exposed to carbamate treated net for 60 days. The increase in the group of rats exposed to carbamate treated net for 30 days was statistically different from the control group and had the highest mean ALP concentration. Therefore, exposure to carbamate treated net was observed to cause an increase in serum ALP level but an insignificant decrease ($p < 0.05$) in the group of rats exposed for 60 days when compared to the control group.

Duration Exposure-Dependent Effect on Aspartate Transaminase (AST):

The result of the duration exposure-dependent effect of carbamate treated net on serum AST level in table 2 and figure 3 shows a statistically significant increase ($p < 0.05$) in all the experimental groups when compared to the control group. Statistically, the serum AST level of rats exposed for 60 days had the highest mean AST concentration and was different from all the groups. Therefore, exposure to carbamate treated net was observed to cause a significant increase ($p < 0.05$) in serum AST levels of rats in all the experimental groups.

Duration Exposure-Dependent Effect on Creatinine:

The result of the duration exposure-dependent effect of carbamate treated net on serum creatinine level is shown in table 2 and figure 5. It was observed that there

was an insignificant increase ($p < 0.05$) in serum creatinine level of rats exposed for 30 days when compared to the control group and a decrease in the serum creatinine level of rats exposed for 60 days.

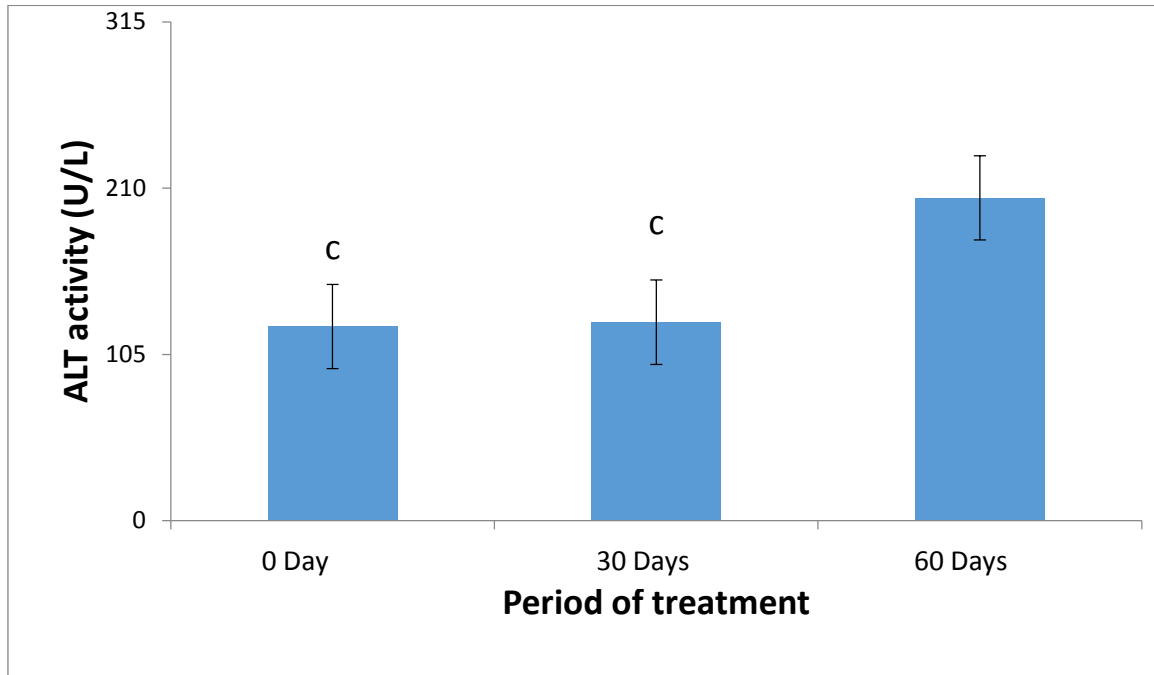


Fig 1: Serum Alanine Transaminase (ALT) Activity (U/L). Values are expressed as mean \pm SEM, n=6. $a = p < 0.05$ vs c, $b = p < 0.05$ vs c, $a = p > 0.05$ vs b.

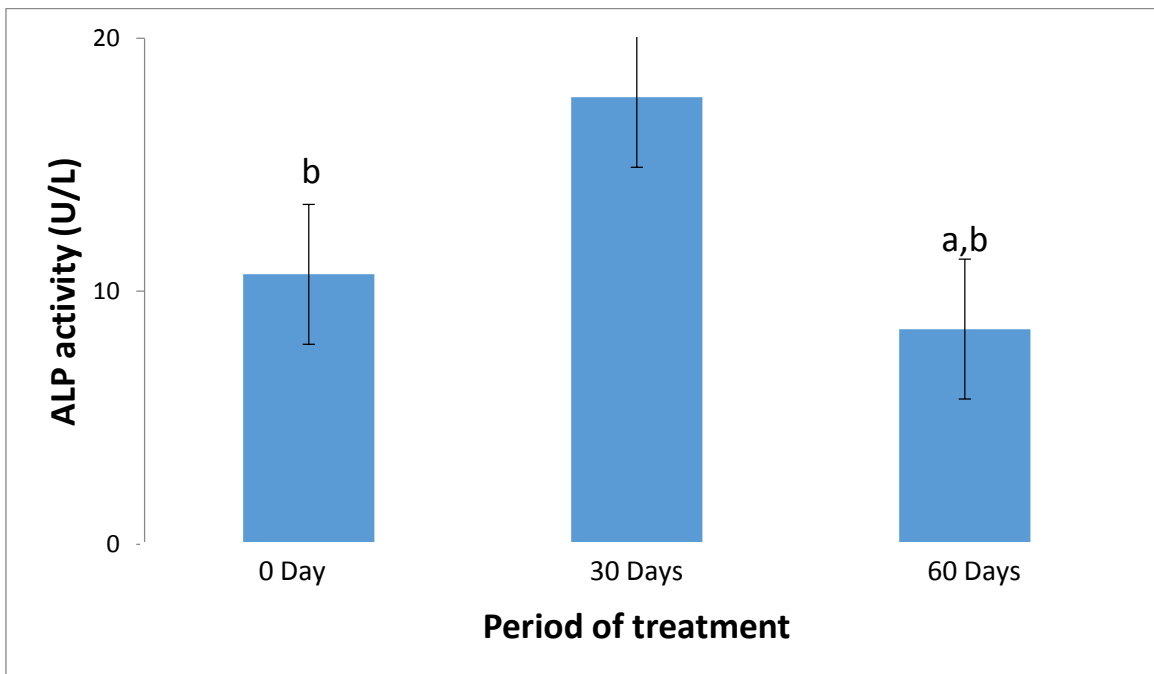
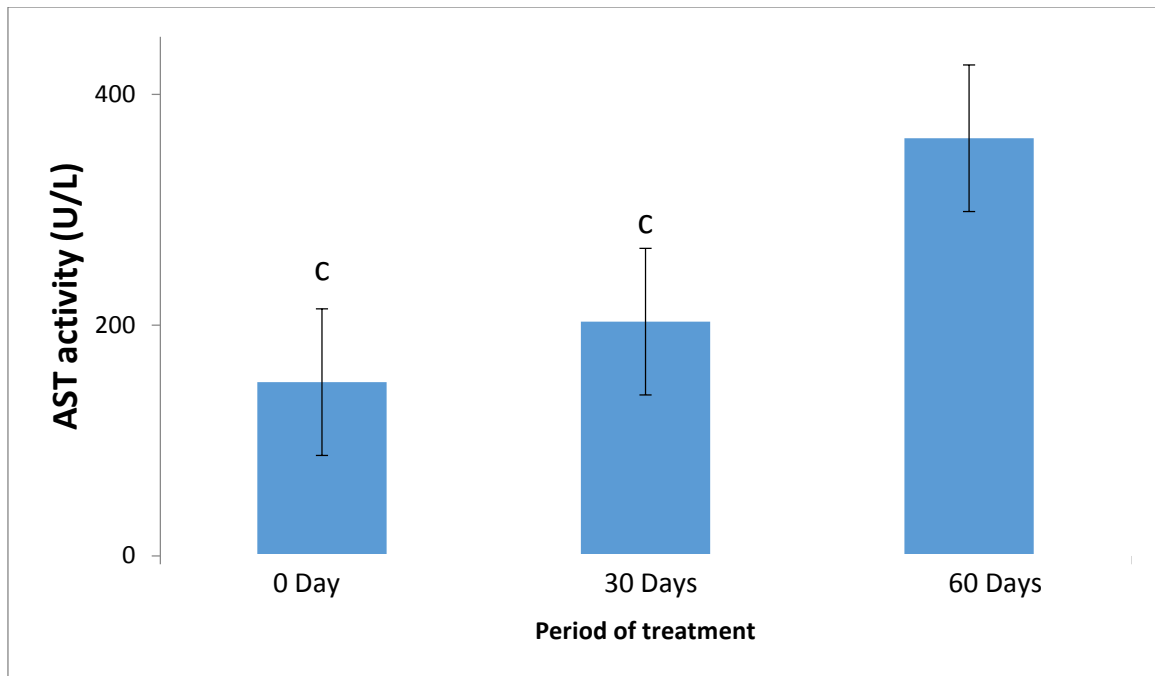
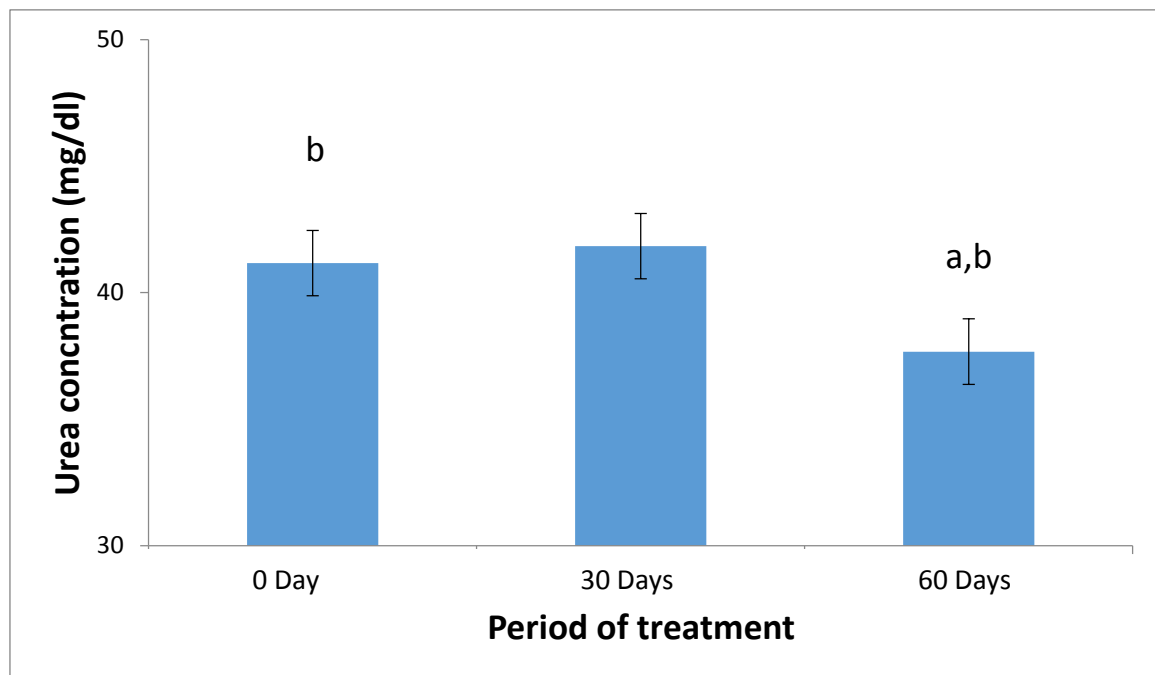


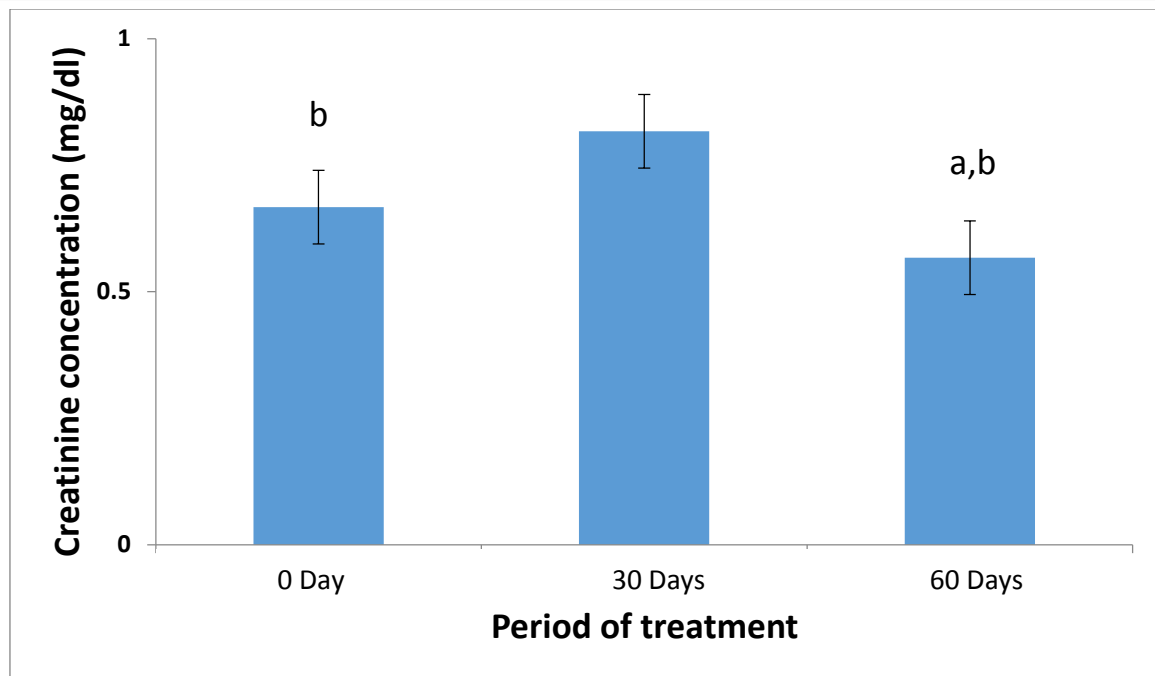
Figure. 2: Serum Alkaline Phosphatase (ALP) Activity (U/L). Values are expressed as mean \pm SEM, n=6. $c = p < 0.05$ vs a and b. $a = P > 0.05$ vs b



Values are expressed as mean \pm SEM, n=6. a= $p < 0.05$ vs c, b= $p < 0.05$ vs c.
Fig. 3: Serum Aspartate Transaminase (AST) Activity (U/L).



Values are expressed as mean \pm SEM, n=6. c= $p < 0.05$ vs a and b. a= $P > 0.05$ vs b
Fig. 4: Serum urea level (mg/dl).

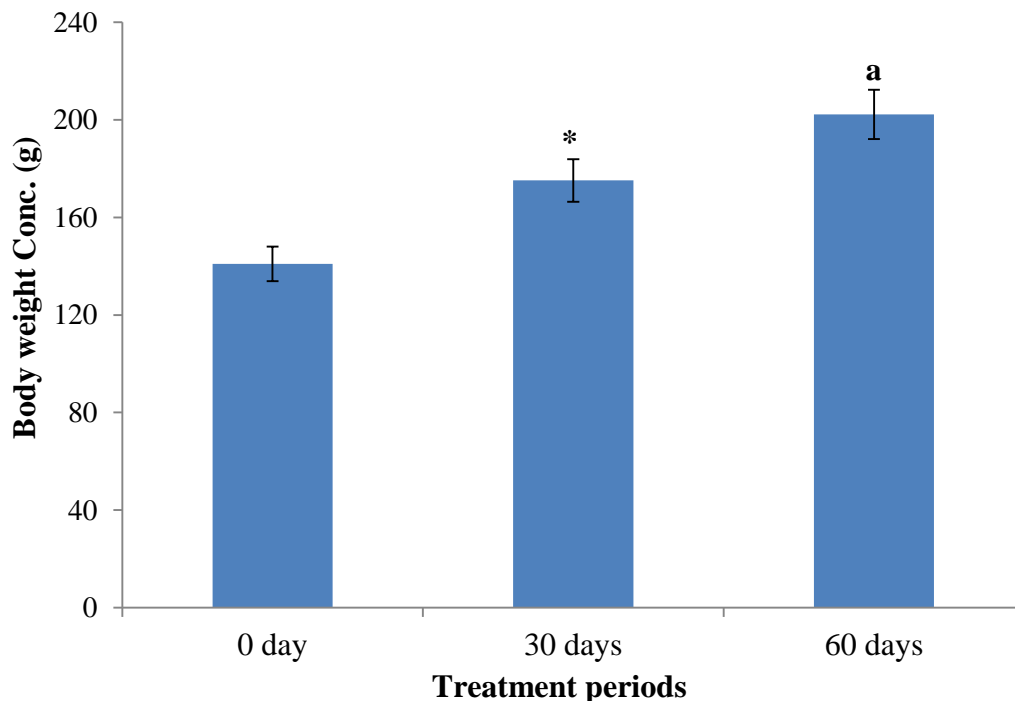


Values are expressed as mean \pm SEM, n=6. a= $p < 0.05$ vs b, c= $p < 0.05$ vs a and b.

Fig.5: Serum creatinine level (mg/dl).

4.7 Duration of exposure dependent effect on body weight indices

*Statistically significant at $P < 0.05$



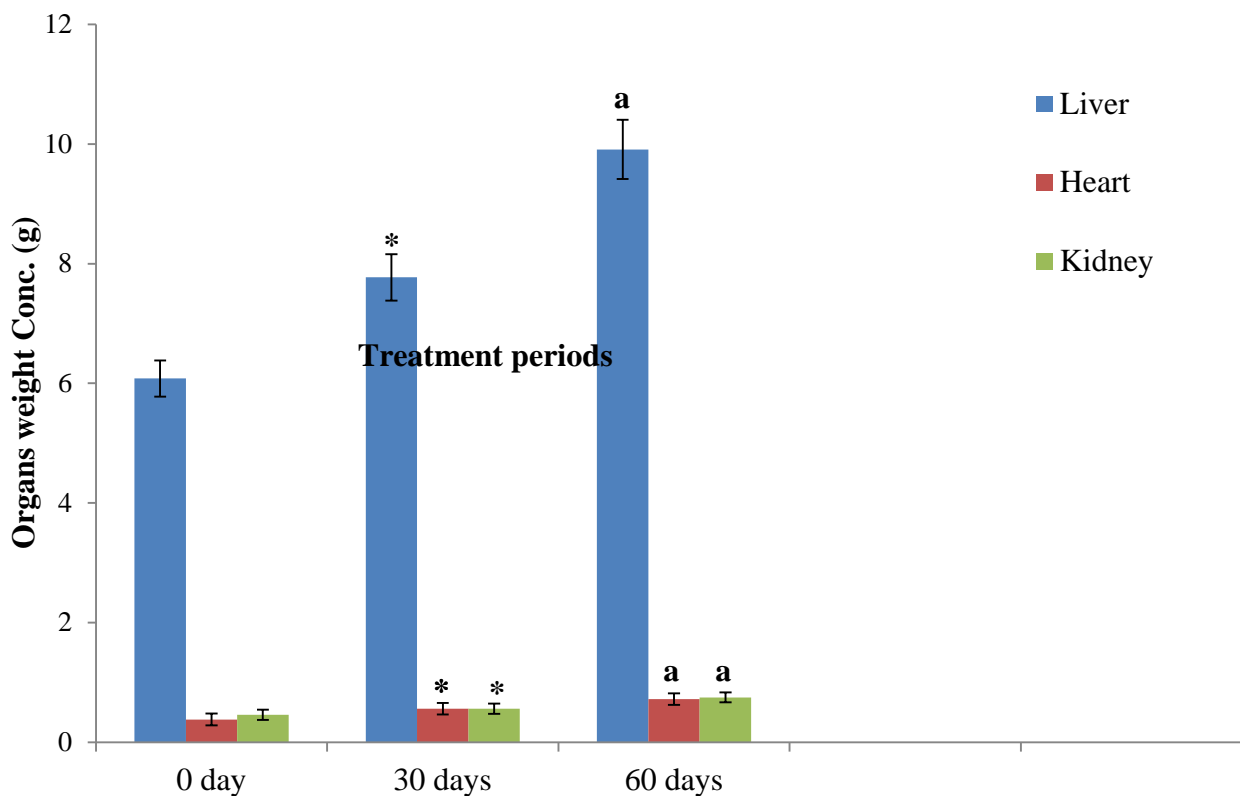
Values are expressed as mean \pm SEM, n=6. a= $p < 0.05$ vs b, c= $p < 0.05$ vs a and b.

Fig. 6: Body weights levels of albino wistar rats exposed to carbamate treated net,

The result of the duration of exposure dependent effect of carbamate treated net on the body weight of albino Wistar rats is given in table 3 and fig. 6. It was observed that 30 days of exposure to carbamate resulted in a statistically significant increase in the body weight of rats in group 2 (175.15 ± 1.14) when compared to rats in the normal group (NC) (104.93 ± 1.09), and 60 days of

exposure to carbamate also resulted in a statistically significant increase in the body weight of rats in group 3 (202.29 ± 0.46) when compared to rats in the normal group (NC) at $p < 0.05$.

Duration of exposure dependent effect on organ weight indices.

* Statistically significant at $P < 0.05$ 

Values are expressed as mean \pm SEM, $n=6$. $a=p<0.05$ vs b, $c=p<0.05$ vs a and b.

Figure. 7.0: Organs weights indices levels of albino wistar rats exposed to carbamate treated net;

The result of the duration of exposure dependent effect of carbamate treated net on the liver weight of albino Wistar rats is given in table 4 and fig. 7. It was observed that 30 days of exposure to carbamate resulted in a statistically significant increase in the liver weight of rats in group 2 (7.77 ± 0.34) when compared to rats in the normal group (NC) (6.08 ± 0.26) and 60 days of exposure to carbamate also resulted in a statistically significant increase in the liver weight of rats in group 3 (9.91 ± 0.08) when compared to rats in the normal group (NC) at $p < 0.05$.

The result of the duration of exposure dependent effect of carbamate treated net on the weight of heart in albino Wistar rats is given in table 4. It was observed that 30 days of exposure to carbamate resulted in a statistically significant increase in the weight of the heart of rats in group 2 (7.77 ± 0.34) when compared to rats in the normal group (NC) (6.08 ± 0.26) and 60 days of exposure to carbamate also resulted in a statistically significant increase in the liver weight of rats in group 3 (9.91 ± 0.08) when compared to rats in the normal group (NC) at $p < 0.05$.

The result of the duration of exposure dependent effect of carbamate treated net on the weight of the kidney of albino Wistar rats is given in table 4. It was observed that 30 days of exposure to carbamate resulted in a statistically significant increase in the weight of kidney of rats in group 2 (7.77 ± 0.34) when compared to rats in the normal group (NC) (6.08 ± 0.26) and 60 days of exposure to carbamate also resulted in a statistically significant increase in the liver weight of rats in group 3 (9.91 ± 0.08) when compared to rats in the normal group (NC) at $p < 0.05$.

DISCUSSION

The duration dependent-exposure of carbamate treated net on the hepatic and renal functions of albino wistar rats was investigated in this study. Since the liver plays a critical role

in the biotransformation of certain foreign chemical substances and facilitates their elimination from the body, this makes it a prime target organ of any form of toxicity. Studies have shown that carbamate which is an insecticide contains chemicals and toxins that exerts health effects by inhibiting acetylcholinesterase and altering biochemical and physiological integrity and function of organs in humans. One of such organs is the liver which is highly susceptible to pesticide poisoning because it is the primary site of xenobiotic metabolism. Assessment of liver damage was determined by serum concentrations of ALT, AST and ALP. These liver transaminases AST and ALT provide information about the state and integrity of the liver. Although serum levels of AST and ALP are measured clinically as bio markers for liver health, an increase in ALT activity is more pronounced. When body tissues or an organ such as the liver is damaged, many enzymes usually found in the cytosol are released into the bloodstream causing the level of the enzymes to rise. The amount of these enzymes in the blood is directly related to the extent of damage to the liver. From this study, it was observed that there was a significant increase in the serum levels of ALT, ASP and AST in rats exposed to carbamate treated net for 30 and 60 days respectively when compared to the control group. This seemingly agrees with the long and short term toxicity studies on the influence of carbamate on the functioning of the liver (Samir *et al.*, 2000).

The kidney functions in the maintenance of homeostasis in the body by the reabsorption of important materials and excretion of waste products. Serum creatinine and urea were measured as parameters for kidney function in this study. The serum urea level of rats exposed for 30 days was observed to remain unchanged when compared to the normal control group while there was an insignificant increase ($p < 0.05$) in serum creatinine level of rats exposed for 30 days when compared to the control group. However,

these serum creatinine and urea levels decreased in rats exposed for 60 days. Therefore, exposure of the rats to carbamate treated net doesn't suggest nephrotoxicity as indicated by the decrease in serum levels of urea and creatinine. 50% kidney function must be lost before creatinine and urea levels in blood are raised (Ukpanukpong *et al.*, 2018). Therefore, serum level usually parallels severity of the disease (Vasudevan *et al.*, 2013).

Conclusion

Findings from this study demonstrate that long and short term exposure of albino wistar rats to carbamate treated net poses hepatotoxic threat but doesn't seem to impede kidney function. I, therefore, suggest stringent environmental laws and regulations be put in place in order to ensure protection of the ecosystem and human health, and to stem the threat of health hazards and pesticide poisoning. In addition, adequate precautionary measures should also be taken by those who use this product to avoid the risk of liver damage upon long or short term exposure.

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