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Hepatotoxic Activities of *Nicotiana Tabacum* In Albino Wistar Rats.

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ABSTRACT

The intake of *Nicotiana tabacum* is on the increase globally. Biochemical and histological analysis were conducted to determine the effects of *Nicotiana tabacum* on the liver enzymes of albino Wistar rats. Twenty rats, divided into four groups (A, B, C and D) of equal rats were fed with rat chow and water. Groups B, C and D were treated with 4mg/kg, 6mg/kg and 8mg/kg of *Nicotiana tabacum* respectively. After twenty eight (28) days of oral administration of *Nicotiana tabacum*, the animals were sacrificed, blood collected for liver enzyme (AST, ALP and ALT) assay and liver was harvested for histological analysis. One way analysis of variance was used to compare means and a level of $p<0.05$ was considered significant. Results showed a significant progressive increase in body weight ($p<0.05$) of control, 4mg/kg and 6mg/kg treated groups when initial and final body weights were compared. There was also a dose dependent increase in ALT, ALP and AST levels of the rats treated with *Nicotiana tabacum*, with significance ($p<0.05$) only when 8mg/kg *Nicotiana tabacum* treated rats were compared with control. Increase in the dosage of *Nicotiana tabacum* showed increased inflammation of the liver cells and blood vessels. These findings therefore, suggest a compromise in liver of rats administered with *Nicotiana tabacum*.

Keywords: Liver, *Nicotiana tabacum*, Aspartate aminotransferase, Alkaline Phosphatase

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INTRODUCTION

Cigarette smoking is a globally recognized health hazard, yet despite worldwide antismoking campaigns, some people continue to consume cigarettes on a regular basis (Langgassner, 1999). The World Health Organization estimates that the use of tobacco caused 5.4 million deaths in 2004 and 100 million deaths over the course of the 20th century (WHO, 2008). Similarly, the United States Center for Disease Control and Prevention describes tobacco use as "the single most important preventable risk to human health in developed countries and an important cause of premature death worldwide" (Ukoha et al., 2012).

Tobacco botanically known as *Nicotiana tabacum* is a perennial herbaceous plant and it is the most commonly grown of all plants in the *Nicotiana* genus. Its leaves are commercially grown in many countries and it grows to heights between 1 to 2 metre to be processed into tobacco products (Ren and Timko, 2001). Tobacco is known and used throughout all quarters of the globe in two major forms: the smoked and the smokeless.

Nicotiana tabacum contains nicotine and other phytochemical constituents such as potent tobacco specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone), 4-methyl-nitrosamino)-4-(3-pyridyl)-butanal (NNA), and N-nitrosonornicotine, heavy metals (Cadmium (Cd), mercury (Hg) etc) and 23 polycyclic aromatic hydrocarbons which has been implicated in tobacco associated cancers and diseases (Stepanov et al., 2010; Addo et al., 2011).

In the present study we investigated the effect of *Nicotiana tabacum* (tobacco snuff) on some Liver enzymes and histology.

MATERIALS AND METHODS

Animals

Twenty (20) albino Wistar rats weighing between 150-190 g were procured from the animal house of Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria. The rats were fed with standard rat chow and clean tap water *ad libitum*. They were exposed to 12-hour light and 12-hour darkness and allowed to acclimatize for a period of seven days prior to experimental use.

Nicotiana tabacum or Smokeless Tobacco (Snuff)

Commercially prepared tobacco snuff was bought from Abraka main market. The snuff was put in a plastic container and stored in a cool place. The concentrations of tobacco snuff used were calculated as 40%, 60% and 80% of *Nicotiana tabacum* $LD_{50.}$

Ethical Approval

The standard rules and regulations of use of animal for research purpose was strictly adhered to as approved by the Research and Ethics Committee, Faculty of Basic Medical Sciences, Delta State University, Abraka.

Animal Grouping and Experimental Protocol

The animals were randomly assigned into four (4) groups (A, B, C and D) of five (5) rats each. All animals were fed with rat chow and clean water. Groups B, C and D were treated orally with 4mg/kg, 6mg/kg and 8mg/kg of *Nicotiana tabacum* respectively while Group A was the control.

Sample Collection and Analysis

The animals were anaesthetized with diethyl ether and about 5 ml of blood samples were collected into sterile containers without anticoagulant for liver enzymes assay. The liver was isolated, blotted dry and weighed and placed in 10% formalin for histopathological analysis.

Estimation of serum liver enzymes

The serum enzymes alanine aminotransferase (ALT), aspartate amino transferase (AST) and alkaline phosphatase (ALP) were determined, using Agape Diagnostic kits, India.

Determination of serum aspartate aminotransferase

This was estimated by method as described by Bergmeyer and Walefeld (1978). 1000µl of the reagent was added to 100 µl of the samples and then mixed and incubated at 370 C for 1minute. The change in absorbance of the sample was measured per minute spectrophotometerically at the wavelength of 590nm as follows:

AST activity (U/L) = ▲AB/min×1768

Determination of serum alanine aminotransferase

This was estimated by method as described by, Bergmeyer and Walefeld (1978). 1000µl of the reagent was added to 100 µl of the samples and then mixed and incubated at 370 C for 1minute. The change in absorbance of the sample was measured per minute as follows: ALT activity (U/L) = ▲AB/min×1768.

Determination of serum alkaline phosphatase

This was estimated by method as described by, Bowers and McComb (1966); 0.5mL of the reagent was added to 0.05mL (50 µl) of the samples and then mixed and incubated at 370 C for 10 minute. The change in absorbance of the sample was measured per minute spectrophotometerically at wavelength of 590nm as follows:

Absorbance of sample/Absorbance standard × Value of standard (U/L).

Histological Analysis

Histopathological examination were done using the method of Humason (1962). The liver tissues were fixed in 10% neutral formalin, dehydrated embedded in paraffin, sectioned and stained with hematoxylin and eosin.

Data analysis

All data were expressed as mean± SEM. One way analysis of variance was used to test for difference among the all the groups. Duncan's multiple range test was used to test for significant differences among the means. A $p< 0.05$ was considered statistically significant.

RESULTS

In this study, the effect of smokeless tobacco on Alkaline Phosphatase, Alanine aminotransferase and Aspartate aminotransferase in adult albino Wistar rats was accessed. Twenty rats were used for the study and the data was subsequently subjected to statistical analysis.

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Effect of *Nicotiana tabacum* on Body Weight

In this experiment, the effect of *Nicotiana tabacum* (NT) on body weight was determined. *Nicotiana tabacum* caused a dose dependent increase in the body weight of the animals. This increase in the body weight was significant ($p<0.05$) when the initial body weight was compared with the final body weight of the rats except for the rats treated with 8mg/kg *Nicotiana tabacum*.

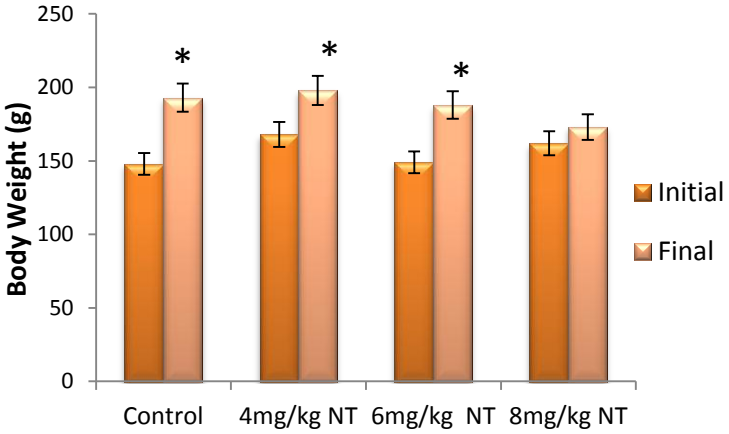

Figure 4.1 Effect of *Nicotiana tabacum* on Body Weight *(n=6); * p < 0.05 compared with initial body weight*

Effect of *Nicotiana tabacum* on Alkaline Phosphatase

In this aspect of the study, the effect of *Nicotiana tabacum* (NT) on Alkaline Phosphatase on albino Wistar was examined. *Nicotiana tabacum* increased the Alkaline Phosphatase in a dose dependent manner with the highest dose (8mg/kg) statistically significant ($p<0.05$) when compared with the control.

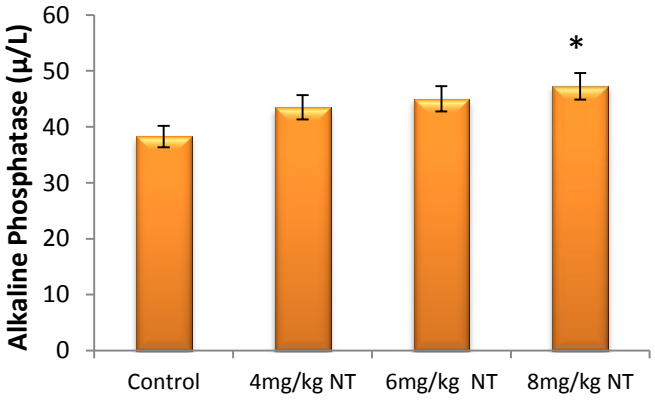

Figure 4.2 Effect of *Nicotiana tabacum* on the Alkaline Phosphatase *(n=5); *p < 0.05 compared with control group*

Effect of *Nicotiana tabacum* on Alanine Aminotransferase

In the figure below, the changes in alanine aminotransferase due to the effect of *Nicotiana tabacum* (NT) was determined. The *Nicotiana tabacum* increased the liver enzyme as the dose increased. The group fed with 6mg/kg and 8mg/ml *Nicotiana tabacum* showed a significant ($p<0.05$) increase when compared with control.

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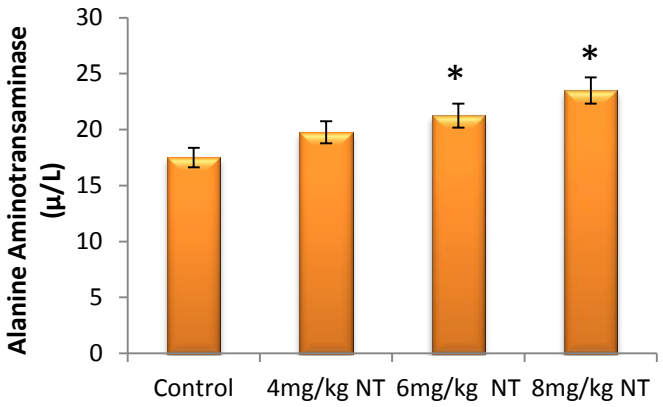

Figure 4.3 Effect of Smokeless tobacco on the Alanine Aminotransferase *(n=5); *p < 0.05 compared with control group*

Effect of *Nicotiana tabacum* on Aspartate Aminotransferase

In fig 4.4, effect of *Nicotiana tabacum* on Aspartate Aminotransferase of albino Wistar rat was ascertained. The smokeless tobacco increased the aspartate aminotransferase enzyme as the dose of the smokeless tobacco increased. *Nicotiana tabacum* increased the Aspartate aminotransferase in a dose dependent manner with the highest dose significantly different (p<0.05) when compared with the control.

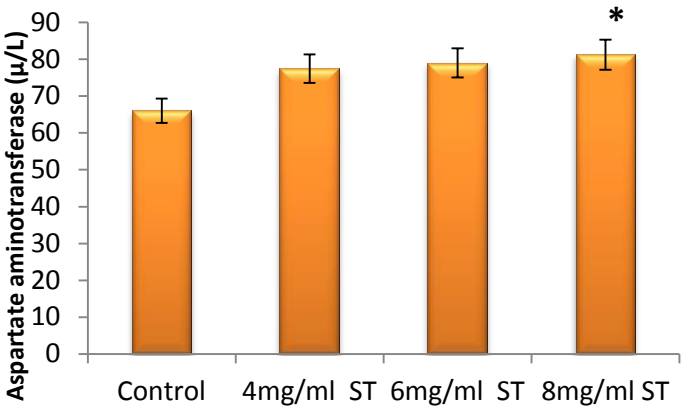

Fig 4.4 Effect of Smokeless tobacco on the Aspartate Aminotransferase *(n=5); *p < 0.05 compared with control group*

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Microscopic Examination of the Liver

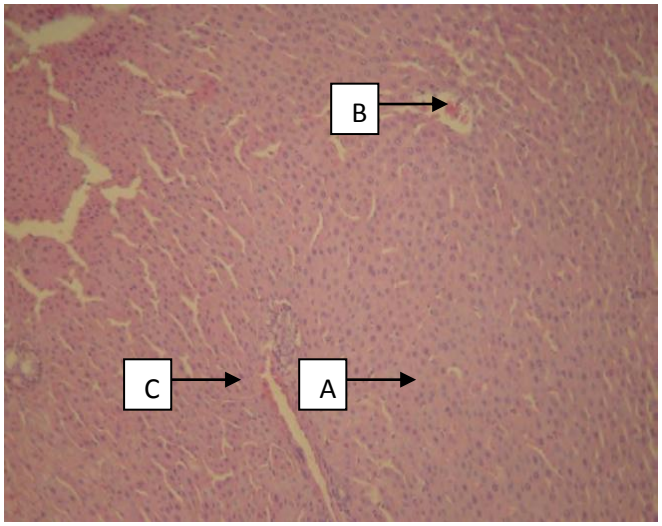

Plate 1: Microscopic representation of the liver of control rats. Normal liver with hepatocytes (A), central vein B, and portal triad (C) {x40 H & E}

In this experiment, the histoarchitecture of the liver show normal hepatocytes which were separated by sinusoids and normal central vein.

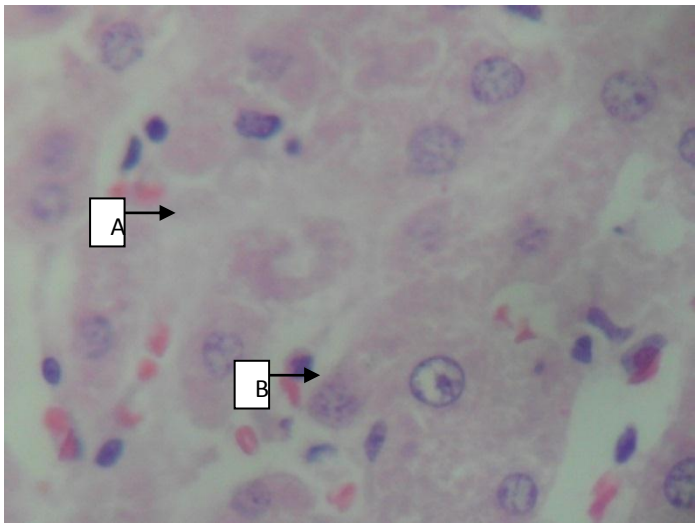

Plate II: Microscopic representation of the liver of rats administered with 4mg/kg *Nicotiana tabacum* (x40 H & E) showing mild sinusoidal (A) congestion and mild increase in the vascularization of the interlobular blood vessel (B).

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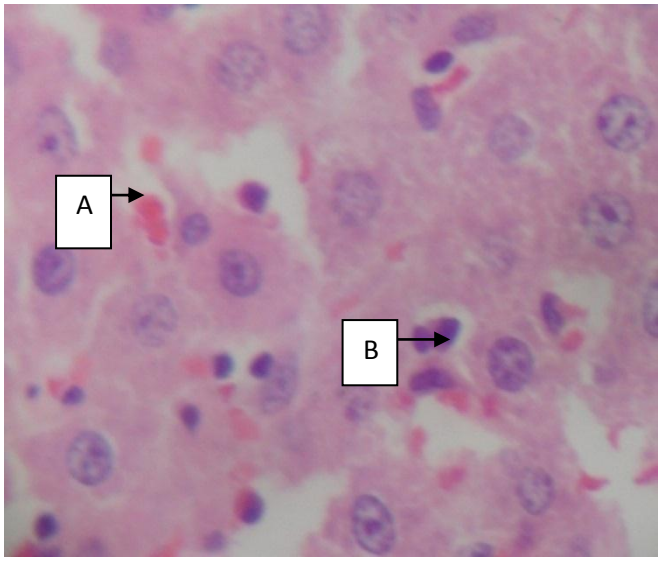

Plate III: Microscopic representation of the liver of rats administered with 6mg/kg *Nicotiana tabacum* (x40 H & E) showing further vascularization of interlobular blood vessels (A) and continuous congestion of sinusoids (B).

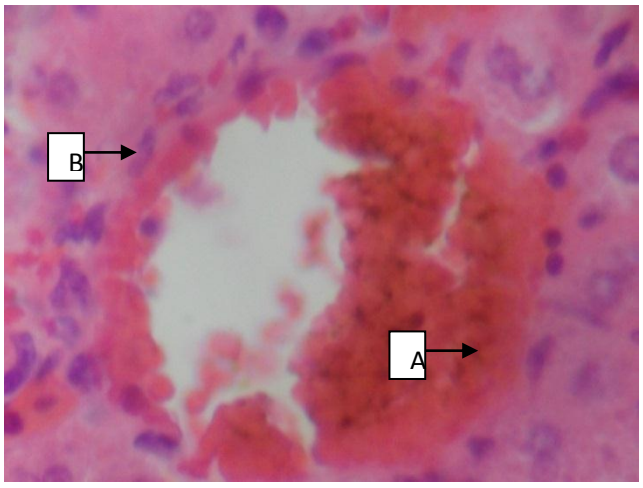

Plate IV: Microscopic representation of the liver of rats administered with 8mg/kg *Nicotiana tabacum* (x40 H & E) showing markedly increase in vascularization of liver (A) and congestion of sinusoids (B).

DISCUSSION

Despite a never-ending stream of research on the health hazards of smoking there are still obstacles to remove this "prestigious bad habit." The complexity of tobacco smoke leads to some confusion about the mechanism by which it causes hepatotoxicity.

Ordinarily, liver cell damage is characterized by a rise in plasma enzymes (AST, ALT, ALP etc). From our findings, AST concentrations were consistently higher than ALT levels which are expected since body cells contain more AST than ALT (Mayne, 1996). Usually, about 80% of AST is found in the mitochondria whereas ALT is a purely cytosolic enzyme. Therefore, AST appears in higher concentrations in a number of tissues (liver, kidneys, heart and pancreas) and is released slowly in comparison to ALT. But since ALT is localized primarily in the cytosol of hepatocytes, this enzyme is considered a more sensitive marker of hepatocellular damage than AST and within limits can provide a quantitative assessment of the degree of damage sustained by the liver (Al-Mamary et al., 2002).

This study showed that *Nicotiana tabaccum* significantly ($p<0.05$) increased the liver enzymes of albino Wistar rats. Based on the fact that Nicotine is known as the main alkaloid of *Nicotiana tabaccum*, accounting for over 90% of the total alkaloidal content (Bowman and Rand 1980), it is quite clear that this increase in liver enzyme is associated with the nicotine content as described by Yildz et al. (1999) that nicotine-induced free radicals which react with biomembranes causing oxidative destruction of polysaturated fatty acids and forming cytotoxic aldehydes by lipid peroxidation. The increase of the activities of AST, ALT, and ALP in plasma may be mainly due to the leakage of these enzymes from the liver cytosol into the blood stream (Navarro et al., 1993).

Studies suggest that nicotine cause liver injury in low levels leading to suppression of tissue repair, which permits injury to progress in an unchecked fashion. Moreover the inflammatory responses initiated by the injured cells provide an absolute ambience for further complications of the metabolites of nicotine for endogenous nitrosation (Nair et al., 1996).

Conclusion

In conclusion, this study showed clearly the detrimental effects of *Nicotiana tabaccum* The hepatotoxic property of *Nicotiana tabaccum* could be mostly due to the reactive oxidant generated as a result of the phytochemicals present in the plant. It would be recommended that further research should be done on the mechanism by which *Nicotiana tabaccum* exerts its effect on the hepatocytes.

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