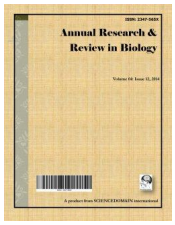

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Histological and Biochemical Effects of Cigarette Smoke on the Liver of Wistar Rats

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Authors' contributions

Authors SIO and AAN designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors TPPC and AC managed the analyses of the study. Authors AOO, JCA and UFO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This work aims at determining the effect of cigarette smoke on the liver of albino Wistar rats, to evaluate the histological, as well as the biochemical changes in the adult albino rat's liver and to elucidate the relationship between exposure period and effect.
Study Design: An experimental study which lasted for 4 weeks was conducted at the Animal House of The College of Health Sciences, Nnamdi Azikiwe University, Nnewi campus.
Methodology: Twenty four (24) adult albino Wistar rats were divided into four groups (A, B, C and D) each consisting of six rats. The average weight of each group was taken. Group A (normal control) had no exposure to cigarette smoke throughout the period of experiment. Groups B, C and D were exposed to cigarette smoke for two, three and four weeks respectively. The duration of exposure for each cigarette is 10±4 minutes and one hour interval was left between burning of each cigarette. After the experiment, blood samples were collected through direct cardiac punctures and delivered into plain test tubes for biochemical assay, and the animals painlessly sacrificed under

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chloroform anesthesia. The liver was excised, fixed in 10% formal saline for 48 hours and processed using paraffin wax processing techniques.
Results: Rats in groups B, C and D showed varying degrees of loss of appetite, moderate irritability and breathing difficulties with significant decrease in body weights after second and third weeks (*P*=.001). Significant increase in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities between the control and test groups was observed (*P*=0.000). Histologicaly, the liver of rats in group C shows marked necrosis and fatty deposition.
Conclusion: The study reveals adverse effect of passive cigarette smoke on the liver morphology and biochemistry of the animal model. The need for similar study in humans is advocated.

Keywords: Cigarette smoke; albino wistar rats; Liver, aspartate aminotransferase; alanine aminotransferase; necrosis.

1. INTRODUCTION

Smoking causes a variety of adverse effects on organs that have no direct contact with the smoke itself, such as the liver. It induces three major adverse effects on the liver: direct or indirect toxic effects, immunological effects and oncogenic effects. Smoking yields chemical substances with cytotoxic potentials which increase necro-inflammation and fibrosis. In addition, smoking increases the production of pro-inflammatory cytokines (IL-1, IL-6 and TNF-α) that would be involved in liver cell injury. It contributes to the development of secondary polycythemia and in turn to increased red cell mass and turnover, which might be a contributing factor to secondary iron overload disease, promoting oxidative stress of hepatocytes. Increased red cell mass and turnover is associated with increased purine catabolism which promotes excessive production of uric acid [1]. Wannamethee and Shaper [2] stated that cigarette smoking was significantly associated with increased levels of gamma-glutamyl transferase (GGT) and alkaline phosphatase (ALP) and was inversely associated with increased aspartate aminotransferase (AST) after adjustment for alcohol intake, body mass index and physical activity. Hence there is a need to find out the relationship between elevated liver enzymes and liver tissue changes. Hepatologists have traditionally paid scant attention to the deleterious effects of cigarette smoke. This reflects the fact that smoking *per se* does not appear to cause liver injury and therefore is not considered a causative agent for chronic liver diseases. However, there is increasing evidence that cigarette smoke may negatively impact the incidence, severity, and clinical course of many types of chronic liver diseases [3]. Cigarette smoke exposure, whether passive or active, carries a high disease burden Worldwide [4]. In a recent comprehensive assessment of mortality attributable to modifiable risk factors in the United States adult population, tobacco use was held responsible for 467,000 deaths (approximately one out of five) in 2005 [5]. According to Mamdouh et al. [6], Second-hand cigarette smoke exposure can result in degenerative changes in hepatocytes, cellular infiltration, periportal fibrosis, and congestion of both central and portal veins. In their study, they observed that “Adult albino rats exposed to cigarette smoke for two weeks showed variable grades of degeneration of hepatic parenchyma and by the end of three weeks, the liver showed marked degeneration and localized areas of necrosis. Most of the hepatocytes in the areas of necrosis showed pyknosis and karyorrhexis. Despite the large body of evidence documenting the effects of passive smoking or active smoking on the heart and lungs, reports investigating the impact of cigarette smoking on pathogenesis of liver injury are scant.

Lorenzo et al, provided evidence that CS causes oxidative stress and apoptosis in the liver [7,8]. Accordingly, two recent studies in patients with biliary cirrhosis identified tobacco use as an independent predictor of advanced fibrosis at presentation [9]. In contrast to hepatitis C, the impact of tobacco use in fibrosis progression remains controversial [10] and available materials would suggest that the cigarette smoking may aggravate necro-inflammation, thereby contributing to accelerated fibrogenosis [11].

Also, in line with the carcinogenic properties of tobacco in several organs, a number of studies indicate that cigarette smoking is associated with an increased incidence of hepatocellular carcinoma in cirrhotic patients [12]. Many people believe that they are safe from the risk of second-hand smoke as long as they are not the ones smoking or do not allow smoking in their

homes or other areas they frequent whereas considerable evidences indicate that exposure to environmental tobacco (ETS) or "passive smoking" is harmful to the health of non-smokers [13]. Passive smoke or environmental tobacco smoke (ETS) is a mixture of exhaled mainstream smoke and side stream smoke, as well as contaminants that diffuse through the cigarette paper and mouth end of the cigarette between puffs.

This work aims at determining the effect of cigarette smoke on the liver of albino Wistar rats, by evaluating the histological, as well as the biochemical changes in the adult albino rat's liver after exposure to cigarette smoke. It is also aimed at elucidating the relationship between exposure period and effect.

2. MATERIALS AND METHODS

2.1 Experimental Design and Conduct

A total of 24 adult albino Wistar rats of varied weight and sexes were obtained from the Faculty of Pharmacy and Pharmaceutical Studies, Nnamdi Azikiwe University, Agulu and transported to the Animal House of Faculty of Health Science and Technology, Nnamdi Azikiwe University, Nnewi campus in a well ventilated steel cage and allowed for one week to acclimatize. The animals were housed under standard laboratory conditions in a controlled room with 12 hours light; 12 hours dark cycle (12 hours: 12 hours dark – light cycle) at room temperature of 23±2°C and 55±3% humidity. The animals were fed with chow (manufactured by Grand feeds Mills Ltd Jos, Platue State), and water *ad libitum* throughout the study period. Animals were divided into four groups (A, B, C and D) each consisting of six rats and the average weight of each group was taken. Group A (normal control) had no exposure to cigarette smoke throughout the period of experiment. Groups B, C and D were exposed to cigarette smoke (Benson and Hedges brand, five sticks of cigarette/day, each stick containing 1.0 mg of nicotine, 100 mg of tar, and 10 mg of carbon monoxide) at the bottom of polypropylene box by slow suction [14] for two, three and four weeks respectively. The duration of exposure for each cigarette is 10±4 minutes and one hour interval was left between burning of each cigarette. After exposure, half of group D was sacrificed, while the other half was left for two weeks without further exposure to evaluate the effect of arrest of exposure to cigarette smoke and whether or not partial or complete recovery occurred. The exposure was performed in a closed room.

2.2 Sample Acquisition and Processing

Rat's general condition were noticed and recorded. The rats of each group were weighed every week throughout the experiment. After the experiment, the animals were bled through direct cardiac punctures and blood collected was delivered into plain test tubes. Blood samples were allowed to clot for 20 minutes and centrifuged at 4,000 rpm for 10 minutes. Sera were collected using micropipettes and analyzed for liver function test using Reitman- Frankel method [15]. The animals were painlessly sacrificed under chloroform anesthesia. A midline abdominal incision was made anteriorly, the liver was excised, fixed in 10% formal saline for 48 hours and processed using appropriate paraffin wax processing techniques of dehydration, clearing and infiltration. They were embedded, sectioned at 5 micromes, satined using the Haematoxylin and Eosin Staining technique, mounted in a reseinous mountant [16] and taken for microscopic examination and photomicrography using a coloured film with an Olympus photomicroscope.

2.3 Statistical Analysis

Results are expressed as mean and standard error of mean (mean±SEM); which presents the average value obtained from three different animals per cigarette smoke exposed group. Data collected were analyzed using SPSS windows version 15.0 software. The level of statistical significance of the difference between control and cigarette smoke exposed groups was determined using unpaired Student's *t* test. While for the main effects on treatment groups, One-Way Analysis of Variance (ANOVA) was used for comparisons of multiple group means. Statistical differences were determined at the 5% level ($P<0.05$).

3. RESULTS

The result on the body weight at start, shows a significant difference in the weight of the different groups before the commencement of the experiment ($P=0.001<0.05$), and this significant difference was retained throughout the experiment (Table 1). For the control group (A), all animals (6 rats) were in a good general

Table 1. The mean values of body weight±standard deviation, (in grams) of experimental groups and control: showing significant difference in the weight between the experimental groups and the control group (group A). Significant change (P < 0.05)

Duration	Group A	Group B	Group C	Group D	F-Value	P-Value
AT START	225.00±24.29	178.33±16.02	198.33±9.83	220.00±21.91	7.756	0.001
1ST WEEK	236.67±20.66	191.00±12.45	206.67±5.16	231.67±20.41	9.715	0
2ND WEEK	243.00±19.56	Sacrificed	206.00±4.18	230.83±20.60	11.373	0
3RD WEEK	254.17±22.17	Sacrificed	Sacrificed	227.78±20.62	7.694	0.008

condition and showed a normal behavior, activity, and eating pattern. There was an increase in weight across all groups after the first week (Fig. 1). There was also, a gradual and progressive significant increase in body weight (P<0.05) in respect to duration. This could be attributed to the fact that the effect of cigarette smoke on body weight takes some time to manifest. Rats in groups B, C and D showed varying degrees of loss of appetite, moderate irritability and breathing difficulties. There was significant decrease in body weight after second and third weeks. Liver sections of rats group C showed dilated and normal central vein while those of group D showed marked necrosis of hepatocytes (Fig. 2).

4. DISCUSSION

In this study the effects of cigarette smoke exposure were tried on adult albino rats. The decrease in body weight of the experimental animals, in this study, might be related to a diminished food consumption caused by developed anorexia [17] or due to increased lipoprotein lipase activity in adipose tissue, which provided a counter-regulatory role in weight gain [18]. The liver seem to be appropriate organ to give a true reflection to the extent of toxicity caused by cigarette smoke exposure as shown in this study, cigarette smoke exposure resulted in degradation of the hepatocytes, expanded portal tracts with chronic infiltrating inflammatory cells and dilatation of central veins. These changes were observed first at the periphery of the hepatic lobules then extended to involve the whole lobule. The previous alterations were time dependent; these changes were apparent in a high aggravated form with group (D). This may be due to the presence of many other toxic materials, and also nicotine produce. Walter and Israel [19] explained the dilatation and congestion of portal veins by the portal hypertension caused by obstruction at sinusoidal level as a result of degenerating ballooning hepatocytes. The presence of infiltrating lymphocytes, mainly in the portal areas in addition to the peripheral parts of the lobules, might be explained as a defense reaction of the lobule in response to the toxicity of inhaled cigarette smoke, being metabolized in the liver [20]. Also, an increase in the activity of AST (Table 2) and ALT (Table 3) in cigarette smoke exposed rats may indicate tissue damage due to loss of functional integrity of cell membrane [21]. Nicotine is oxidized primarily into its metabolite cotinine in the liver, which generates free radicals in tissues and induces oxidative tissue injury [22]. Thus the damage to the tissues in cigarette smoke exposed animals may be due to excessive generation of radicals.

Table 2. Changes in the activities of AST in sera (values are mean±SD from rats of each group). F-Value=32.534 and P-Value=0.000

Group	N	Mean±S.D
A	4	11.88±1.797
B	4	14.13±2.220
C	4	23.63±4.956
D	4	26.24±5.548

Table 3. Changes in the activities of ALT in sera (values are mean ± SD from rats of each group). F-Value=31.027and P-Value=0.000

Group	N	Mean±S.D
A	4	9.38±1.888
B	4	15.38±3.750
C	4	25.75±6.538
D	4	39.74±9.546

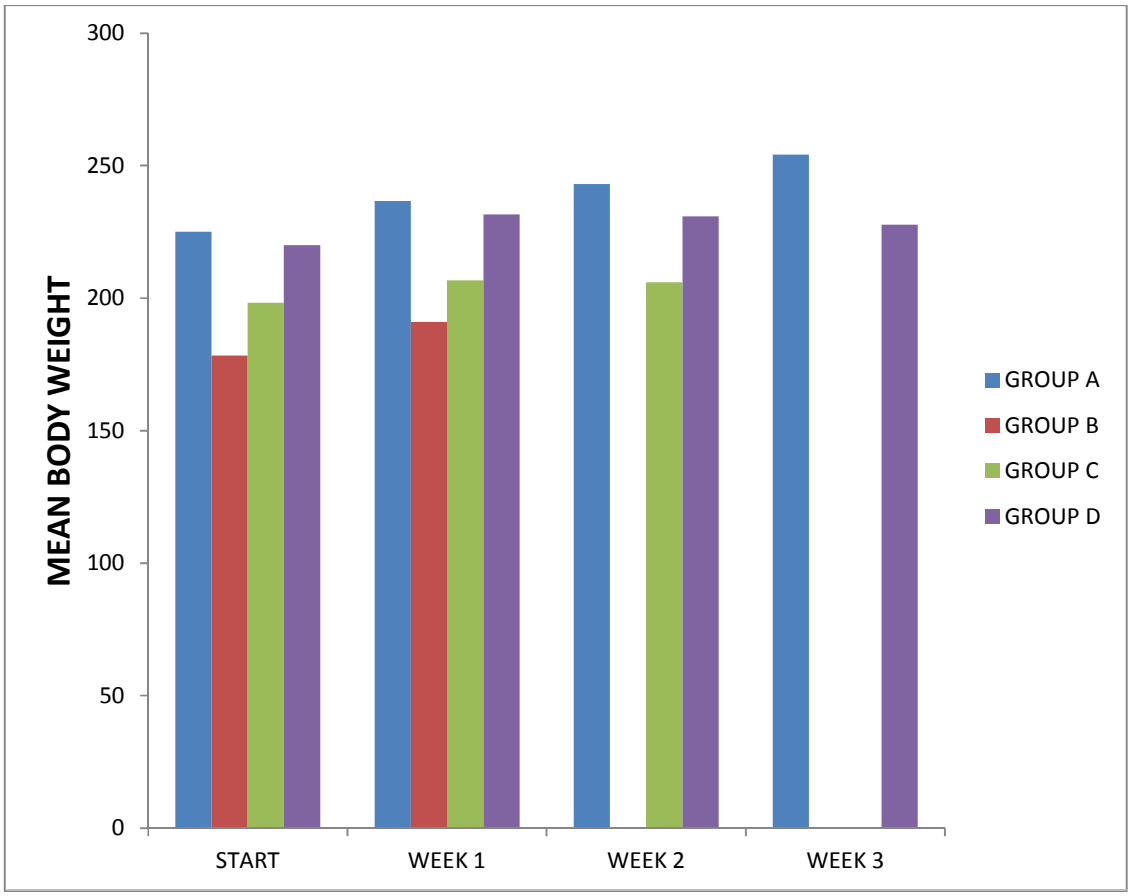

Fig. 1. The mean body weight of cigarette and nicotine groups in relation to control group during the experiment

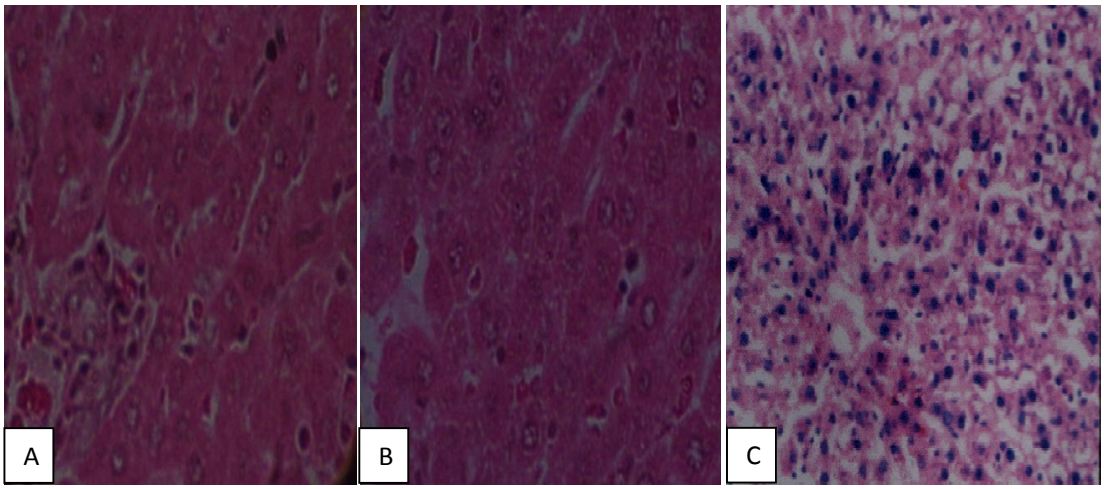

Fig. 2. A photomicrograph of the liver; (A) Control rat showing normal hepatocytes and central veins (H & E X400), (B) Group C showing dilated and normal central vein (H & E X400), (C) Group D showing marked necrosis of hepatocytes (H&E X400)

5. CONCLUSION

The occurrence of hydropic and fatty degenerations of liver cells at the end of the second week denotes the rapid onset of liver affection after inhalation of cigarette smoke. In this experimental study, the marked morphological and biochemical changes in the liver have confirmed that the contents of inhaled cigarette smoke including nicotine and other hazardous compounds, are very dangerous to the liver. Although a moderate level of liver damage was observed, congestion was the most prominent histopathological change recorded in this. A longer duration o exposure may cause more harm. Cigarette smoke is dangerous and should be avoided as much as possible. Biochemical analysis of samples from human subjects, with relevant information on their biodata and smoking pattern, as well as duration, will help to deduce more information regarding the effect of cigarette smoke.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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