

Lipid Profile and Liver Histochemistry in Animal Models Exposed to Cigarette Smoke

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Abstract: Cigarette smoke is known to be an important predisposing factor to many diseased conditions, such as cardiovascular diseases, liver disease, atherosclerosis and other metabolic disorders. The aim of this study was to examine the effects of exposure to smoke from burnt cotton wool and cigarette on plasma lipids, liver biochemistry and histology, in adult Wistar rats. The animals were divided into three groups of Control A: exposed to fresh atmospheric air; Group B: exposed to cotton wool smoke; and, Group C, exposed to cigarette smoke; and the experiment lasted for 35 days. The animals exposed to cigarette smoke and cotton wool smoke showed higher values of low density lipoprotein (LDL), and lower values of high density lipoprotein (HDL) compared to the control. The observation of the micro architecture and enzymes of the liver tissue revealed reduction in the number and size of liver cells, numerous fibrous tissues, elevated liver transaminases and reduction in endogenous anti-oxidants, with evidence of fatty degeneration, in animals exposed to cigarette smoke compared to those exposed to cotton wool smoke and fresh atmospheric air. Cigarette smoke caused accumulation of lipids in the liver cells, with evidence of on-going necrosis and fibrosis, which indicated the presence of non-alcoholic fatty liver disease.

Keywords: Cigarette smoke, enzymes, histology, lipids, liver.

INTRODUCTION

Cigarette smoking is a preventable predisposing factor to many clinical conditions [1]. It is associated with increased risk of cardiovascular and metabolic diseases such as alteration in the levels of plasma lipoproteins and accumulation of lipids in the liver [2]. Many chemically reactive molecular species are present in cigarette smoke, including reactive oxygen species and radicals which are toxic to the cell and various cellular processes [1]. Cigarette smoke increases the expression of several enzymes, such as cytochromes and other enzymes involved in drug metabolism, especially in the liver [3-5], thereby causing varying degrees of hepatotoxicity. Nicotine, a major constituent of cigarette smoke, is mainly metabolised in the liver, and induces lesions characterised by steatosis and focal or confluent necrosis, and varying degrees of fibrosis [6].

Tobacco smoking alters lipid metabolism through increase in lipolysis, insulin resistance and tissue toxicity [7], leading to increased levels of plasma lipoproteins. Hyperlipidemia is a metabolic risk factor which plays a significant role in the process of

atherosclerosis, characterized by the formation of atheromatous plaques in the intima of large and medium-sized arteries [8], 2009). However, with cessation of smoking, there is the likelihood of some degree of restoration of lipid metabolism [7].

Gupta and colleagues evaluated the acute effects of cigarette smoking on serum lipids in apparently healthy individuals [8], and observed no statistically significant difference in the levels of triglycerides (TG), very low density lipoprotein-cholesterol (VLDL), and high density lipoprotein-cholesterol (HDL) between smokers and non-smokers; although serum cholesterol and low density lipoprotein cholesterol (LDL) levels were slightly higher in smokers than non-smokers, the differences were also not statistically significant. Other studies, however, have reported significant differences in plasma lipoprotein levels between cigarette smokers and non-smokers [9].

Long-term exposure to cigarette smoke causes permanent inflammation and an imbalance in lipid profile [10]. This stimulates the accumulation of lipid in liver cells (hepatocytes), leading to the development of non-alcoholic fatty liver disease [2]. Fatty degeneration is one of the most common pathological changes in the liver due in most cases to excessive intake of alcohol. However, non-alcoholic fatty liver disease has been recognised, and cigarette smoking is a culprit.

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Nicotine increases the circulatory pool of atherogenic LDL through accelerated transfer of lipids from HDL and impaired clearance of LDL from plasma compartment [11], leading to increased deposition of LDL in the arterial wall [12]. In addition, free radicals present in tobacco smoke trigger and augment lipid peroxidation, which causes low-density lipoprotein (LDL) oxidation and atherosclerosis [13, 14]. The higher concentrations of LDL, VLDL, TG and lower concentration of HDL correlate positively with the development of severe and premature atherogenesis [15]. Although carbon monoxide is also a component of cigarette smoke, studies suggested that most of the effects of cigarette smoke are due more to the actions of nicotine than carbon monoxide [16].

Cigarette smoke lowers the level of endogenous antioxidants [17]. The elevation in serum levels of liver enzymes could be used in assessing the degree of liver injury. According to [18], cigarette smoke increases the levels of some liver enzymes, such as alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), which are capable of inducing alterations in membrane permeability properties of the liver. ALT and AST are commonly used markers of hepatocellular injuries. Serum elevation of ALP could occur due to blockage of bile ducts or impairment of bile production in the liver [19].

Reduction in the level of glutathione, an endogenous antioxidant enzyme, favours the hepatotoxic effect of cigarette smoking [20, 21]. The mechanism of liver injury occurring from cigarette smoking is probably by enhancing lipid peroxidation [20]. In order to determine the extent of adverse effects of tobacco smoke on drug metabolism, biochemical markers such as cotinine (a metabolite of nicotine) can be measured in the plasma of smokers [22]. The current study was aimed at determining the effects of cigarette smoke on the plasma lipids and liver histochemistry of Wistar rats.

MATERIALS AND METHODS

Experimental Animals

A total of twelve adult male Wistar rats were used. They were housed in the Animal House of the Anatomy Department, University of Ilorin. They were fed on rat pellets and water *ad libitum* and allowed to acclimatise for 7 days. The animals which weighed on the average 160 g were randomly divided into 3 groups of 4 animals per group.

Treatment of Animals

Group A animals were exposed to normal fresh atmospheric air (Control Group); Group B animals were allowed to inhale smoke from cotton wool, while Group C animals were exposed to smoke from a stick of Rothmans® cigarette each. The amount of tobacco in each stick of cigarette was 0.738 g, containing 1 mg of nicotine. Exposure of the Wistar rats to smoke was carried out in a Smoking Chamber at 1900 h daily for 35 days.

Blood and Tissue Samples

The animals were sacrificed by cervical dislocation 24 hrs after the last administration. Blood samples were collected into lithium heparinised bottles for biochemical studies. The liver was excised and part of it was weighed, placed in 0.25 M sucrose solution and homogenized. The homogenate was centrifuged at 5000 rpm for 5 min using a centrifuge (Gallenkomp, England). Part of the liver was fixed in formol saline and processed for histological examinations.

Biochemical Studies

The blood samples and supernatants collected were used in determining the activities of some liver enzymes and anti-oxidant enzymes. Using biochemical kits from Randox Laboratories Limited (Antrim, UK), the enzyme activity of superoxide dismutase was assessed according to the method of Mistry and Fridovich [23], and the activity of Glutathione peroxidase in liver homogenates was also determined by adopting the method of Paglia and Valentine [24]. The following lipid parameters were assessed using appropriate biochemical kits: cholesterol, triglycerides, low density lipoprotein, high density lipoprotein, and calcium. The serum activities of alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase were also similarly assessed, using the Randox kits.

Histopathological Examinations

Tissue specimens were taken from the liver of each of the 3 groups of rats and fixed in 10% formal saline solution for 24 hours. Trimming was done on the fixed tissue specimens and washed in tap water for 12 hours. Serial alcohols (methyl, ethyl and absolute) were used for dehydration of the tissue samples. Tissue specimens were cleared in xylene and embedded in paraffin. The paraffin blocks were sectioned at 5 micron thickness by rotary microtome. The obtained tissue sections were collected on glass slides and stained

Table 1: Plasma Lipids

Groups	N	Parameters				
		HDL (mmol/l)	LDL (mmol/l)	TGA (mmol/l)	Cholesterol (mmol/l)	Calcium (mmol/l)
A	4	1.350±0.14	2.100±0.16	1.400±0.12	3.600±0.29	3.200±0.19
B	4	1.125±0.15	2.300±1.16	1.275±1.13	3.750±0.18	2.453±0.09
C	4	1.133±0.09	2.275±0.45	1.175±0.13	3.575±0.43	2.278±0.13

Data presented as Mean±SEM; HDL: high density lipoprotein; LDL: low density lipoprotein; TGA: triglycerides

using Haematoxylin and Eosin stains, and Gordon and Sweet's stains [25].

Statistical analyses of data

The biochemical data obtained were expressed as means ± SEM, and analysed using the Student's t-test. The level of significance was taken at p values < 0.05.

RESULTS

Biochemical Analyses

Compared to the control group A, HDL levels were reduced in the experimental animals, while the levels of LDL increased (Table 1). These reduction and increase, which were statistically not significant ($p>0.05$) were however more in the group exposed to smoke from burnt cotton wool.

The activities of liver transaminases (ALT, AST) increased in the treatment groups exposed to cotton wool smoke and cigarette smoke, with a more increase in the latter, compared to the control. However, the levels of serum ALP, SOD and liver glutathione peroxidase decreased progressively in the treatment groups, and exposure to cigarette smoke resulted in more reduction in these enzyme activities (Table 2). These differences were, nevertheless, not statistically significant ($p>0.05$).

Histopathological Observation

The liver of the Control Group showed a normal micro architecture (Plates 1, 4). The hepatocytes

appeared normal, and the lobules and bile canaliculi were well outlined. There were no fatty degenerations present, no haemorrhagic reaction observed, and no evidence of neutrophilic infiltration. The animals exposed to cotton wool smoke (Plates 2, 5) showed areas of neutrophilic infiltration, hepatocytes with many vacuolar spaces, slight distortion of the bile canalicular network and hypochromic staining of the liver tissue. Animals exposed to cigarette smoke had degeneration of the liver parenchyma and disruption of the canalicular network (Plates 3, 6). There were pigments present and the capsules were positive for reticulin. The nuclei were reduced in size, with more vacuolar spaces in the cytoplasm and areas of necrosis, as well as neutrophilic infiltrates and numerous reticulin fibres. Abscesses of eosinophilic bodies were present, preventing proper staining of the tissue. Fatty degeneration was also evident.



Plate 1: Photomicrograph of a section through the liver of control rat showing normal morphology of the central vein (CV), hepatocytes (mono-, and binucleated) (H), sinusoids (S) and bile canaliculi (BC); H&E x150.

Table 2: Results of Enzyme Analysis

Groups	N	Enzymes				
		ALP (IU/L)	ALT (IU/L)	AST (IU/L)	SOD (IU/L)	GPx (IU/L)
A	4	89.75±16.92	36.25±06.60	111.50±15.68	320±18.91	16650
B	4	84.50±07.93	36.50±04.84	110.25±14.59	271±12.25	14750
C	4	67.00±13.14	37.75±01.44	123.75±10.57	260±27.51	14200

Data presented as Mean±SEM; ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; SOD: superoxide dismutase; GPx: glutathione peroxidase

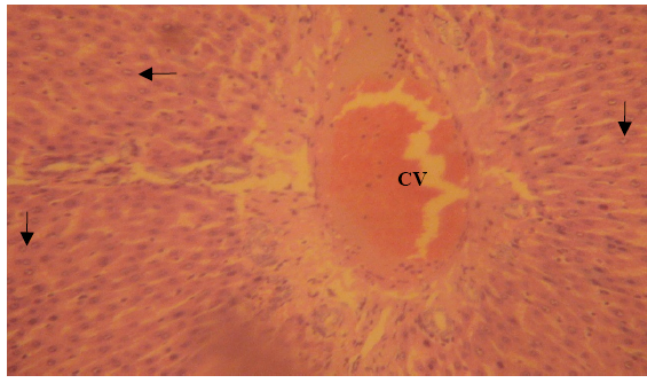


Plate 2: Photomicrograph of a section through the liver of animals exposed to cotton wool smoke showing central vein (CV) with a wider diameter; slight distortion of the bile canalicular network (appearing more horizontal) and many hepatocytes with nuclear spaces (arrows); H&E x150.

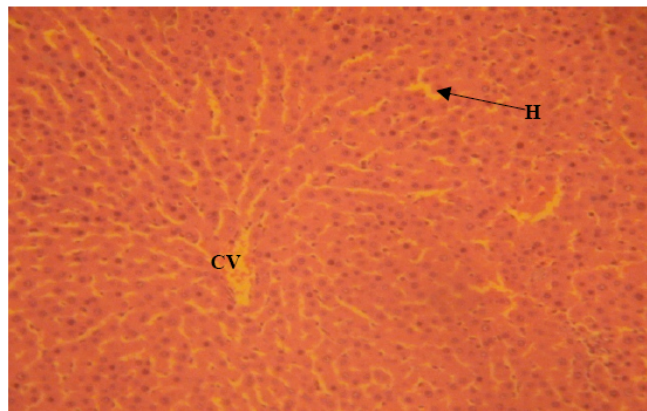


Plate 3: Photomicrograph of the liver of animals exposed to cigarette smoke showing degenerative changes and hypochromic staining; constricted central vein (CV); hepatocytes with smaller sized nuclei and nuclear spaces (H); H&E x150.

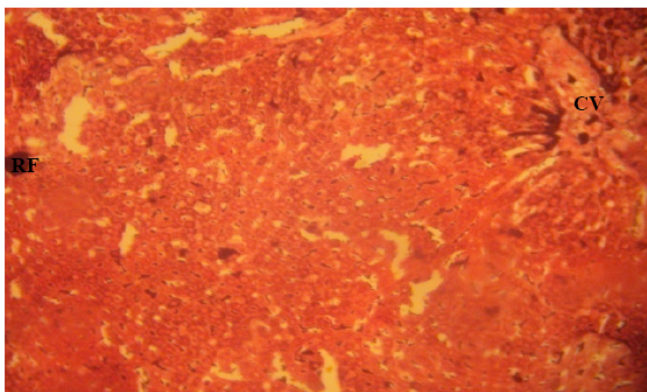


Plate 4: Photomicrograph of the liver of Control Group showing normal morphology of the central vein (CV) and reticulin fibres (RF). G&S x150.

DISCUSSION

There is a strong correlation between cigarette smoking and hyperlipidemia, a metabolic risk factor which plays a significant role in the process of atherosclerosis [26]. Various studies have shown that

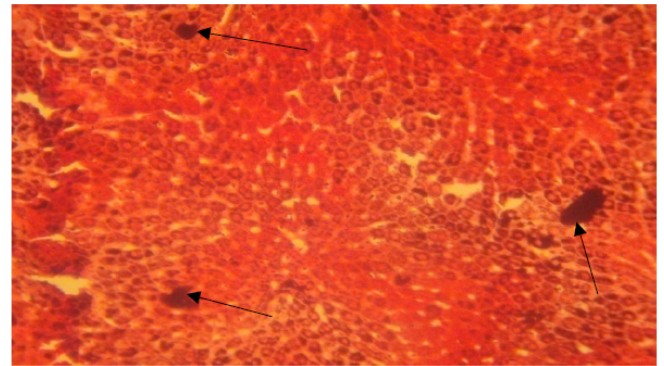


Plate 5: Photomicrograph of a section through the liver of animals exposed to cotton wool smoke showing hypochromic staining, few reticulin fibres (arrows), and numerous vacuolar spaces. G&S x150.

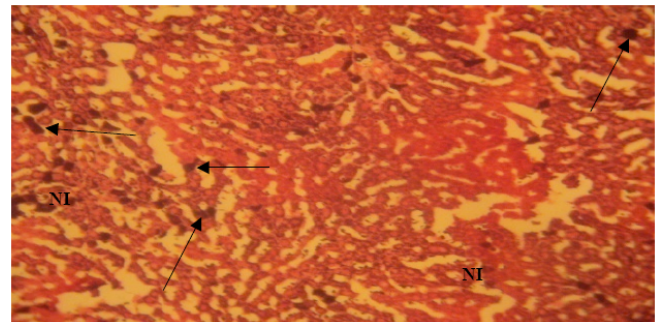


Plate 6: Photomicrograph of the liver of animals exposed to cigarette smoke showing disrupted architecture, neutrophilic infiltrates (NI) and numerous reticulin fibres (arrows). G&S x150.

tobacco smoke is associated with adverse plasma levels of lipoproteins, such that there is increased low density lipoprotein which has been implicated in atherogenesis, and decreased levels of high density lipoprotein [27]. Observations from the current study were also similar to previous works. However, the smoke of burnt cotton wool which contained basically carbon monoxide caused more decrease in HDL and more increase in LDL than the smoke of cigarette. Meanwhile, lower levels of triglycerides and total cholesterol were noticed in the experimental groups unlike what previous authors noted.

The activities of liver enzymes are altered following insults to the organ, and these enzymes are used as markers to determine the type and the extent of such injuries. The current study revealed an increase in the activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), which are used as indicators of hepatocellular injuries. Necrosis, toxic and ischaemic injuries of the liver cells result in the leakage of these enzymes into the blood circulation [18], thereby increasing their levels in the body. There was probably no impairment in the production of bile, as the

serum level of alkaline phosphatase decreased, as against the increase levels that characterize blockage of bile ducts or impairment of bile production in the liver [19].

Studies by El-Zayadi showed that exposure to cigarette smoke reduced the levels of anti-oxidative enzymes [17], and as further observed by Watanabe et al and Barbaro et al [20, 21], the antioxidant activity of glutathione is reduced during exposure to cigarette smoke, facilitating more hepatotoxic damage to the organ. The endogenous antioxidant function of superoxide dismutase was compromised in this study, thereby predisposing the cells to the detrimental effects of various oxygen radicals and other chemically-induced oxidative stress that characterise tobacco smoking. The enzymes activities were much more reduced in animals exposed to smoke from cigarette than from burnt cotton wool. This shows that the degree of oxidative stress resulting from the other components of cigarette smoke, especially nicotine, was more than that caused exclusively by carbon monoxide, which is also a constituent of cigarette smoke.

The changes that occurred in the liver enzymes and endogenous anti-oxidants corroborated the histological observations of the liver that showed various degrees of alterations of the hepatocytes and canalicular network. There were reductions in nuclear sizes, numerous vacuolations and areas of necrosis, showing that the damage seen in this study was more of hepatocellular degeneration of liver parenchyma, for which the increased ALT and AST served as biomarkers. The cell membrane marker, ALP, was lower in the serum of animals exposed to smoke, than in the animals exposed to fresh air. The activity of ALP was lowest in the treatment group exposed to cigarette smoke, and this was confirmed through histological observations which showed no evidence of blockage of biliary or canalicular network, although there were some degrees of disruption. The cell membranes also increased in permeability to the transaminases, ALT and AST, resulting in their rising levels in circulation.

The liver of the treated animals revealed higher degrees of reticulin fibres than those of animals in the control group. It was however more numerous in the group exposed to cigarette smoke than those exposed to smoke from cotton wool. This could probably be responsible for the degree of fibrosis induced by nicotine, as noted by previous studies [6]. The presence of constricted vessels in the liver of cigarette-

exposed group may not be unconnected to the constrictive activity of nicotine, which was absent in the cotton wool-exposed group. Only the group exposed to cigarette smoke showed an evidence of accumulation of lipids in the hepatocytes, but without clear evidence of blockage of the ductile system.

Although the enhancement of lipid peroxidation is part of the mechanism responsible for the tissue damage seen in non-alcoholic fatty liver disease, this study probably established the process of necrosis and fibrosis as part of the mechanisms underlying liver injuries following exposure to cigarette smoke.

The evaluations of the micro architecture and enzymes of the liver tissue revealed reduction in the number and size of liver cells, numerous fibrous tissues, elevated liver transaminases and reduction in endogenous anti-oxidants, with evidence of fatty degeneration, in animals exposed to cigarette smoke compared to those exposed to cotton wool smoke and fresh atmospheric air.

CONCLUSION

Exposure to cigarette smoke causes accumulation of lipids in the hepatocytes, with various degrees of necrosis and fibrosis. The small sample size might be responsible for the statistically non-significant results of some of the biochemical parameters reported in this study. The use of a larger sample size, therefore, might be able to give statistically significant data.

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Received on 01-10-2011

Accepted on 12-11-2011

Published on 24-01-2012

DOI: <http://dx.doi.org/10.6000/1927-5129.2012.08.01.04>© 2012 Omotoso *et al.*; Licensee Lifescience Global.

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