# Toxicological Effects of Cigarette Smoke on Some Biochemical Parameters of Alloxan-Induced Diabetic Rats

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***Abstract***

***It is believed that while normal people may suffer complications of active and passive cigarette smoking, diabetes patients may suffer more. This study therefore aimed at investigating the toxicological effects of cigarette smoke on some biochemical parameters of alloxan-induced diabetic rats. Adult male Wistar rats (n = 8/group) were divided into three groups, 2 experimental groups group 1 was diabetic and exposed to cigarette smoke while group 2 was diabetic but unexposed and one control (group 3). Animals were sacrificed after 2 weeks of exposure to cigarette smoke and the blood glucose, total protein, total (cholesterol, vitamin C and malondialdehyde concentrations were determined. Diabetic rats exposed to cigarette smoke (group 1) showed significant increases (p < 0.05) in the blood glucose and MDA concentrations and significant decreases (p < 0.05) in the total protein, total cholesterol and vitamin C concentrations compared to groups 2 and 3. From the results, it could be deduced that exposure to cigarette smoke may increase diabetic complications.***

**Key words:** Cigarette smoke, glucose, diabetes, lipid peroxidation

## Introduction

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia often accompanied by glycosuria (Celik *et al.,* 2002; ADA, 2005). Diabetes mellitus maybe caucused/exacerbated by certain chemicals or compounds which elicit oxidative stress (Traverso *et al.,* 1999; Ogugua, 2000). Hence oxidative stress and diabetes have been weighed side by side. Recent experimental findings suggest that over production of the reactive oxygen species (ROS) of diabetes mellitus can increase its complications (Meister, 1994). Cigarette smoke has been identified to have 2 major phases, a tar phase and a gas phase and both phases are rich in oxygen centre. Carbon-centered and nitrogen-centered free radicals as well as non-radical oxidants (Church and Pryor, 1991).

The free radicals generated during cigarette smoking have been suggested to increase oxidative stress in normal people and the complications of diabetics (Traghen, 1997). This work therefore aimed at evaluating the toxicological effect of cigarette smoke on some biochemical parameters of alloxan-induced diabetic rats.

## Materials and Methods

**Animals:** Twenty four Wistar adult albino rats were obtained from the Department of Physiology, Faculty of Veterinary Medicine University of Nigeria, Nsukka and housed in the Animal House of the Department of Biochemistry, University of Nigeria, Nsukka, Nigeria. The rats were held in stainless wire-rat-cages and were fed *ad libitum* with 25% crude protein commercial chicks' mash diet (Top Feed, Nigeria, Ltd). The rats were divided into 3 groups of eight (8) rats each after acclimatization for period of 7 days. The experimental groups (1 and 3) were induced with diabetes but group 1 was further exposed to cigarette smoke for 2 weeks. Group 3 served as the control (non-diabetic). All rats were sacrificed on the 15$^{th}$ day.

**Induction of diabetes:** Rats were fasted for 12 h before diabetes was induced by slow intraperitoneal injection of 150mg/kg alloxan (Sigma, St. Louis, MO. USA). Alloxan was prepared fresh few minutes before administration by dissolving in normal saline (0.9%). The diabetic state was confirmed on the 8th day by blood glucose determination (Creatz Felat and Soling, 1961).

**Blood sample collection:** Blood samples were collected through ocular puncture into a sterilized anticoagulant free test tube. Blood from the micro-capillary tube was dropped on the one touch glucometer (Lifescan, USA) and the blood glucose concentration read directly from the machine. The blood samples were centrifuged at 3000 rpm for 5 min and the serum was collected and used for the biochemical assays.

**Biochemical assays:** Total protein and total cholesterols concentration were determined using Randox test kits (Quimica Clinical). Vitamin C concentration was determined using the method of Good Hart and Shills (1973) while MDA concentration was determined using the method of Das *et al.* (1990).

**Statistical analysis:** Data entry and analysis were done using SPSS version — 17.0 and values were represented as meant ±SD. The difference between groups were compared for statistical significance using sample t-test with the level of significance set at $p<0.05$

## Results and discussion

From the results, group 1 (diabetic and exposed to cigarette smoke) showed a significant increase ($p<0.05$) in the MDA and glucose concentrations

while the total cholesterol, total protein and vitamin C concentrations significantly decreased ($p<0.05$) compared to the other groups .

**Table 1: The results of the biochemical parameters**

| Groups | Blood glucose (mg/dl) | Total protein (g/dl) | Total Cholesterol (mm/d) | Vit C (mg/dl) | MDA (% TBABD) |
|---|---|---|---|---|---|
| Group 1 (diabetic exposed to cigarette smoke) | 245.0 ± 0.94 | 6.4 ± 0.56 | 3.04 ± 0.11 | 2.7 ± 0.61 | 203.9 ± 16.3 |
| Groups 2 (diabetic untreated) | 149.33 ± 1.52 | 6.8 ± 0.42 | 3.67 ± 0.23 | 3.02 ± 0.13 | 160.5 ± 5.6 |
| Group 3 (control) non-diabetic | 88.5 ± 2.12 | 8.23 ± 0.78 | 3.83 ± 0.21 | 4.34 ± 0.4 | 77.1 ± 1.31 |

Although several researches have been carried out to address the rampant nature and complications of diabetics, diabetes still remains a major health problem (ADA, 2005). The highest blood glucose concentration from the results was obtained in group 1 (diabetic and exposed to cigarette smoke) compared to the groups (2 and 3). This work agrees with the findings of Ko and Cockram (2005) who suggested that cigarette smoke is a risk factor for insulin resistance, a precursor of diabetes. Other researchers have suggested that smoking can induce changes in the blood vessel which impair insulin sensitivity by reducing the flow of blood in the muscle tissues (Targher, 1997; Houston, 2006). The high blood glucose observed in group 2 compared to groups may be due to the effect of alloxan which destroyed the B-cells of the pancreases (Cooke and Plotnick, 2008). The MDA concentration which is a marker of lipid peroxidation significantly increased ($p<0.05$) in group 1 compared to groups 2 and 3. West (2000) had similar findings and suggested the increase to be due to increased production of ROS and sharp reduction in antioxidant defenses leading to increased oxidative stress. The MDA concentration was higher in group 2 compared to group 3 which agrees with the findings of Slatter (1999) who reported higher lipid peroxidation in diabetic patients than in normal people. The decrease in total protein observed in group 1, diabetic and exposed to cigarette smoke is in accordance with the findings of Mulhuauser (1996) who reported that smoking increased the risk of albuminaria in both types of diabetes due to oxidative damages by free radicals on proteins. Kasap *et al.* (2007) reported that cigarette smoke generates free radicals and interferes in cholesterol synthesis. This probably accounts for the decreased total cholesterol in group 1 compared to the other groups. Vitamin C concentration significantly decreased ($p<0.05$) in group 1 compared to groups 2 and 3. This decrease may be due to the role of vitamin C in fighting free radicals generated in cigarette smoking diabetics (Shigeoka, 2002). From these findings, it could be ascertained that diabetic complications are greatly increased by cigarette smoking.

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