

The Effect of Chronic Consumption of *Nicotiana tabacum* (snuff) on the Spleen of Adult Male Wistar Rats

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ABSTRACT

Nicotiana tabacum (snuff) is a widely consumed plant all over the world. It is the scientific name of tobacco plant. Its effect on the oral cavity, liver and kidney has been widely studied. This study was undertaken to assess the effect of its chronic consumption on the spleen. Twenty wistar rats weighing between 170-202g were used for the study. They were divided into four groups, A, B, C and D of 5 rats each. Group A, the control, received normal feed, water and groups B, C and D received normal feed, water and snuff through oral route at different doses of 0.4, 0.6 and 0.8mls respectively of snuff for six weeks. The rats were sacrificed at the end of six weeks by cervical dislocation and the spleen was dissected out and preserved in 10% neutral formal saline. It was processed using routine histological procedures and stained with haematoxylin and eosin (H & E) stains. While the control group showed normal cytoarchitecture of the spleen, groups B and C showed varying degrees of hyperplasia of the splenic nodules. Group D displayed distortion of splenic architecture and degeneration of the lymphocytes. These results indicate that the chronic consumption of the *N. tabacum* may alter the function of the spleen thereby resulting in altered immune function.

Key words: Effect, *Nicotiana tabacum*, spleen, adult male, wistar rats.

Nicotiana tabacum is the most widely grown commercial non food plant in the world. Despite the efforts aimed at curtailing or stopping its use, its consumption has, nevertheless increased steadily. Tobacco products are consumed in the form of snuff, chewing, smoking, sucking of the flowers, eating or cooked green or injecting infusion of its leaves. There is wide consumption of tobacco and its products throughout the world with its attendant mortality. An annual 5 million deaths is attributed to tobacco smoking; it is the second leading cause of mortality among adults worldwide (Aghaji,2008, Uwakwe and Modebe, 2008, NIH 2009)

Snuff contains nicotine as an active ingredient and the most toxic substance known. It is second only to caffeine as the most widely used central nervous system stimulant and is second to alcohol as the most abused drug. In combination of tars and carbon monoxide found in cigarette smoke, nicotine represents a serious risk factor for lungs and cardiovascular system, various cancers, as well as serious illnesses (Williams, 2000) Nicotine has also unpredictable effect of both stimulant and depressant phases of its action on autonomic ganglia, neuromuscular junction, central nervous system and different receptor sites (IARC 1986) Many studies on

the effect of tobacco consumption by active and passive smoking have been documented. Smoking and some of the health hazards include bronchogenic carcinoma, coronary artery disease, gastro intestinal disorders and malignant neoplasm.

Despite the wide consumption of tobacco by smoking, inhalation of dust and chewing in on our society, much is yet to be known about its effect on different organs, and tissues. This study was, therefore, conducted to determine the effect of *N. tabacum* on the spleen.

MATERIALS AND METHODS

Breeding of Animals

Twenty male wistar rats procured from Department of Agriculture, University of Nigeria, Nsukka were taken to acclimatize for two weeks in the animal house of the Department of Human Anatomy, Faculty of Basic Medical Sciences, Anambra State University, Uli. The animals were kept in a big cage, compartmentalized into four for the various groups. The dimensions of this cage was 225cmx46cmx40cm. The cage had a stainless gauze on top for ventilation. The rats were fed on growers marsh obtained from Agrofeed Mills (Nigeria)Limited and water was provided 'ad libitum' throughout the period of experiment.

Ethical approval was sought and received from the College of Medicine, Anambra State Uli Ethical Committee on the use of animals for experimental investigations and on the need to observe completely the rules guiding the employment of animals for scientific investigations. The study was carried out in accordance with the National Institute of Health guide for the care and use of laboratory animals. The rules guiding good laboratory practices were adhered to (Adeniyi et al, 2010).

Preparation of *Nicotiana tabacum* (snuff)

The tobacco *Nicotiana tabacum* (snuff) used in this study was bought from a tobacco dealer at Maxwell Nigeria Limited, Enugu, Enugu State, Nigeria. The powdered form of the tobacco (snuff) was put into a plastic container and stored in a cool place. 200 mg of the snuff was dissolved in 20 mls of distilled water to form a solution. LD50 of 1ml(10mg/ml) was obtained and sublethal doses of 0.4, 0.6 and 0.8ml were chosen and administered orally to the animals in groups B,C and D respectively.

Animal Treatment

The animals were randomly divided into four(4) groups of five rats each. The groups were labeled A,B,C and D. 200mg of tobacco was measured out with electric balance(HAUS, made in USA) and dissolved in 20ml of distilled water obtained using 20ml syringe. The animals in the control group A received 1ml of distilled water for six weeks. The treatment group B,C,D received doses of 0.4, 0.6 and 0.8ml per kilogram bodyweight respectively through an oral route with the aid of orogastric tube also for six weeks. At the end

of six weeks, the animals both control and treatment groups were sacrificed by cervical dislocation and the spleen dissected out and fixed in 10% formal saline for 48 hrs.

Histological Changes

After fixing the tissue of both control and experimental rats, the tissues were stained with haematoxylin and eosin. The sections of 5 microns were produced with Berg Rotary Microtome. The sections were mounted and examined with the light microscope and the photomicrograph of each side was taken for histopathologic analysis.

Statistical Analysis

Data were expressed as the mean and Standard Error of Mean (SEM). One way Analysis of Variance (ANOVA) was applied for data analysis. Level of significance was considered at $p < 0.05$.

RESULTS

Gross Observations

There were no significant morphological changes observed between the treatment groups B, C, D and control group till the completion of the experimental procedure. Table 1 shows that there was a significant increase in the weight of the rats between the beginning and the completion of the experiment in the various groups ($p < 0.05$). It was observed that after the daily administration of snuff (*Nicotiana tabacum*), the animals appeared to exhibit restless behaviour, pupillary dilatation, tremors and increased respiration.

Table 1 - The weekly mean \pm SEM of body weight in snuff fed rats and control group

Groups	1 st Week	2 nd Week	3 rd Week	4 th Week	5 th Week	6 th Week
A	170.0 \pm 22.35	175 \pm 14.14	190.0 \pm 7.35	205.0 \pm 9.10	207.0 \pm 9.40	208.0 \pm 10.95
B	202.0 \pm 17.89	190.0 \pm 13.73	195.0 \pm 17.75	200.0 \pm 15.60	202.0 \pm 16.70	225.0 18.44
C	184.0 \pm 16.73	194.0 \pm 15.73	200.0 \pm 15.03	222.0 \pm 16.06	224.0 \pm 18.75	223.0 20.50
D	175.0 \pm 10.01	180.0 \pm 25.01	185.0 \pm 35.33	190.0 \pm 45.06	200.0 \pm 19.60	250.0 15.25

Histological Changes

The spleen of the control group (A) showed normal histological features such as prominent white pulp comprising the splenic nodules (SN) or malpighian corpuscles and red pulp scattered all over the splenic cell. Each splenic nodule consists of an arteriole surrounded by lymphocytes (see plate 1). Group B which received 0.4ml of snuff also showed normal histological features as in the control but had slight hyperplasia of the splenic nodules (see plate 2). Plate 3 shows the histological features of group C which received 0.6ml of snuff. There was moderate hyperplasia of the splenic nodules. Those in group D that received 0.8ml of snuff showed distortion of the splenic architecture and degeneration of the lymphocytes (plate 4).

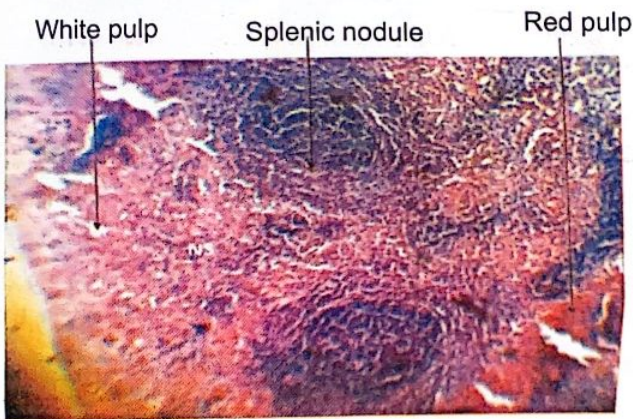


Figure 1: Photomicrograph showing the effect on the spleen section of group A (control) showing normal spleen, made up of the prominent white pulp comprising the splenic nodules (SN) or malpighian Corpuscles and red pulp scattering all over the splenic cell. Each splenic nodule consists of a central artery surrounded by the lymphocyte.

The histological section of the spleen in group B showed slight hyperplasia of the splenic nodule (Plate 2).

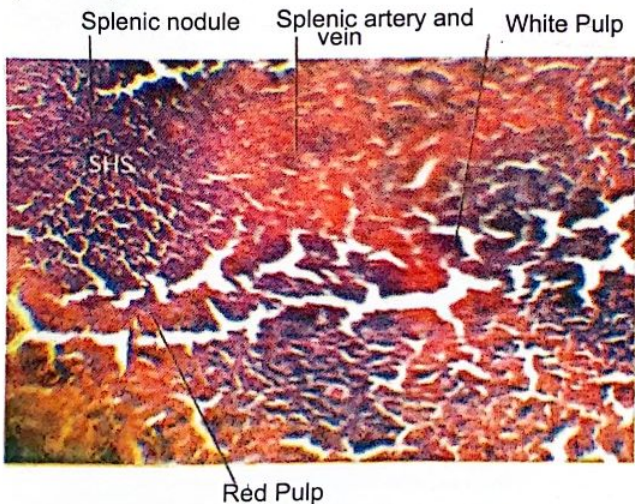


Figure 2: Photomicrograph showing the effect on the spleen section of group B that received 0.4mls of snuff, histological slight hyperplasia of the splenic nodules.

In group C, the histological section showed moderate hyperplasia of the splenic nodule (see plate 3).

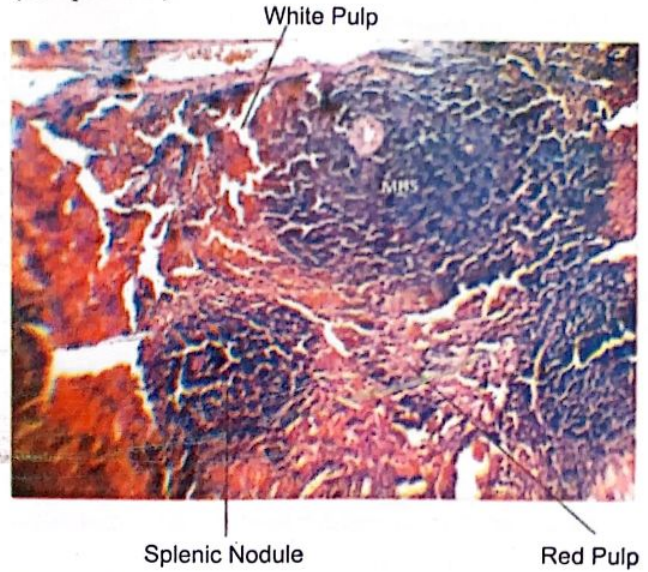


Figure 3: Photomicrographic showing effects on the spleen section of group C that received 0.6mls of snuff showed moderate hyperplasia of the splenic nodules (HSN)

Those in group D showed distortion of the splenic architecture and degeneration of the lymphocytes (Plate 4).

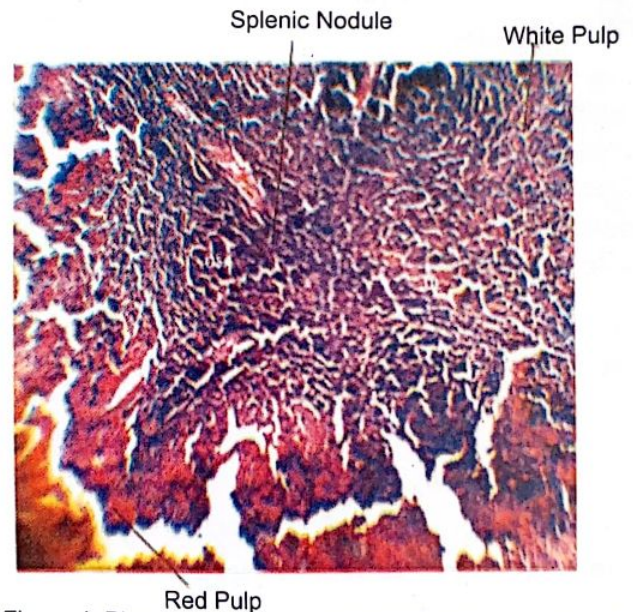


Figure 4: Photomicrographic showing effects on the spleen section of group D that received 0.8mls of snuff showed Distortion of the splenic architecture and degeneration of lymphocyte

DISCUSSION

The gross observations in the experimental animals in this study were restlessness, papillary dilatation and tremor. The effect of *Nicotiana tabacum* on the central nervous system has been widely documented. There is stimulation of the sympathetic ganglion and adrenal medulla resulting in the discharge of

catecholamines (Benowitz, 1982). The body weight of the experimental animals showed significant increase between the beginning and the completion of the study ($p < 0.05$). This is in contrast to report of Adeniyi et al, (2010) that showed significant decrease in the body weight. The reason for this increase in body weight could be as a result of the extended period of the experiment as against the shorter period by Adeniyi et al, 2010. The experimental animals fed with snuff showed the structure of the spleen and the effects of nicotine (see plates 2, 3 and 4). There is destruction and hyperplasia of the splenic nodule, degeneration of lymphocytes and this can as well cause hypersplenism which is the decrease in the number of red blood cells, white blood cells, and platelets in the blood, resulting from pooling of those cells by enlarged spleen (splenomegaly) and this can also lead to reduction in immune function of the spleen. There is no available literature on the effect of *Nicotiana tabacum* on the spleen and thus making proper comparison difficult.

CONCLUSION

The present study has shown that varying doses of *Nicotiana tabacum* with high nicotine content on adult male wistar rats causes destruction of the splenic cytoarchitecture and degeneration of the lymphocyte which may affect the immune function.

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