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Effects of tobacco smoking on morphology of spleen of mice

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ABSTRACT

The study on effects of tobacco smoking on morphology of spleen of mice was carried out to investigate the harmful effect of of tobacco smoking on morphology of spleen of mice. A total of fifteen (15) White Swiss Albino (*Mus muculus*) male mice (collected from ICDDR, B) at 42 days were used for this experiment and grouped (each of which has 5 mice) as the control group (C), the cigarette fume treated group (CF) and the cigarette root treated group (CR). Control group was under normal feed (standard mice pellets purchased from ICDDR, B) and water; for the fume treated group (CF), fume of 5 cigarettes was given using a special fumigation box for 5 minutes 3 times daily at one hour interval with normal feed and water & for the root treated group (CR), five cigarette roots were mixed with half liter of water and supplied *ad libitumly* with normal feed. The total experimental tenure was 4 weeks. After 4 weeks all the mice were killed and spleen was collect, preserved and processed and stained (H & E) for histo-pathological investigation. Gross morphological study was done by eye estimation, balance and slide caliper. Shape and color change were higher but decreased size of spleen was found in treated groups. Necrosis and vacuoles in treated groups, but hemorrhage, erosion of capsule of fume group and thinner trabecule in root exposed were found. These indicate smoking can cause a great harm to the spleen.

INTRODUCTION

Cigarette smoke contains over 4000 chemicals, many of which have toxic, carcinogenic, and other effects on biological systems (Smith et al., 2000). Still, over 1.1 billion people continue to smoke, representing one-sixth of the world's population (Jha et al., 2002).Tobacco caused 100 million deaths in the 20th century. If current trends continue, it may cause one billion deaths in the 21st century. If unchecked, tobacco-related deaths will increase to more than eight million per year by 2030 (Peto et al., 2001). More than 80% of those deaths will be in low- and middle-income countries (WHO 2013). Exposure to cigarette smoke has been recognized as the significant contributor to mortality (Wells, 1994). Smoking is the leading cause of preventable morbidity and mortality in the developed world, causing over 4.8 million deaths per year globally (Ezzati et al., 2000).

Although these chemicals' direct effects on host cells are important, many of smoking's deleterious effects are proposed to be caused, at least in part, by its effect on the immune system (Sopori et al., 2002). It is postulated that this increased susceptibility reflects cigarette smoke-induced impairment of the immune system (Holt and Keast, 1977). Cigarette smoke (SM) affects a wide range of immunological functions in humans and experimental animals, including both the humoral and cell-mediated immune responses (Sopori et al., 1994; Sopori and Kozak, 1998). Cigarette smoke impacts immune cells including alveolar Macrophages (Twigg et al., 1994, McCrea et al., 1994, Ohta et al., 1998, Ouyang et al., 2000 and bronchial epithelial (Laan et al., 2004), natural killer (Newman et al., 1991, Lu et al., 2007),

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dendritic (Robbins et al., 2008, Vassallo et al., 2005) and T and B cells (Kalra et al., 2000, Savageet al., 1991, van et al., 2006). In addition, smokers demonstrate elevated autoantibody levels (Korpilahde et al., 2004, Freemer et al., 2006). The aim of this study is identify the effect of smoking on morphology of spleen of mice which is helpful to compare with human spleen.

MATERIALS AND METHODS

The experiment on tobacco smoking on the morphology of spleen in mice was performed in the laboratory of the department of Anatomy & Histology, Bangladesh Agricultural University from July 2012 to December 2013.

Experimental animals

Fifteen (15) white Swiss Albino male mice (*Mus muculus*) at 7 days old were purchased from the Animal Resource Center, ICDDR,B, Mohakhali, Dhaka. Before being used in the experiment, mice were adapted for 35 days in order to acclimatize in the environment. All groups were housed in compartmentalizes rectangular metallic cage (9x11x7 cube inches) wrapped with wire mesh. The collected mice had no developmental disorders, detectable genital diseases or any other diseases that may cause any problem in the experiment or affect the result of the experiment.

Rearing and care

The mice were cared at animal care room, department of Anatomy and Histology, Bangladesh Agricultural University, in proper hygienic conditions, with experimental & normal feeding (standard mice pellets from ICDDR,B) *ad libitum*. During the experimental period uniformity of the management practices was maintained as much as possible. The ventilation of the rearing house of mice was sufficient as a standard one. The room temperature was 28 ± 2^0c and relative humidity 70-80% with natural day light.

Experimental design

Fifteen (15) white albino male mice at 42 days were randomly selected for the experiment and were divided into following groups, Control group (C) - normal feeding & watering *ad libitum*, Cigarette fume group (CF) - fume of 5 cigarettes three times daily at one hour interval for 5 minutes and normal feeding & watering *ad libitum*, Cigarette root group (CR) - root of 5 cigarettes mixed with half liter of water and supplied *ad libitum* with normal feeding. The experimental tenure was 4 weeks.

Experimental feeding procedures

The cigarettes were chosen for this purpose is gold leaf (British American tobacco, Bangladesh), Abul biri (Abul biri factory, Ltd. Bangladesh) with filter as a cigarette with average smoke production.

The side stream smoke was generated by burning cigarettes gold leaf in a smoking chamber. The cigarettes were lightened in a box and smoke was produced then the mice were placed in this box for five minutes three times daily at one hour interval up to 4 weeks. The chamber atmosphere was monitored for carbon monoxide. Root of cigarette was collected from local market. After collection of the root then five roots were soaked in half liter of water for overnight and the root soaked water was supplied to the cigarette root (CR) group *ad libitumly* up to 4 weeks.

Sample collection

After 4 weeks all the experimental animals including control animals were killed. Then the spleen was collected by the help of sharp knife. The average length, width, weight, color and shape of the collected organs of control and experimental mice were measured. Then the collected samples were preserved in fixatives.

Tissue processing and staining

Immediately after collecting the specimen (spleen) was fixed in the Bouin's solution for 72 hours. Then the fixed tissues (spleen) was properly trimmed with 1.5x1cm size and stained with H and E with a standard histopathological technique (Luna LG, 1968).The gross and histological study was performed in the Anatomy and Histology Laboratory of Bangladesh Agricultural University, Mymenshing, Bangladesh. The gross study was done by visual observation and detail histological

study was completed using high power light microscopy (X10, X20, X 40, X 60, and X100).

Statistical analyses

After study period we were collect their gross anatomical data and were analyzed using IBM® SPSS® Statistics (version 21) software and reveal the results in tabular form.

Photography and illustration

Necessary photography was done during gross and histological investigation for better illustration of the result. The gross anatomical pictures were taken directly from organs and the histological pictures were taken from light microscope. The Olympus-BX-51 microscope was used and necessary illustration was carried out by Adobe Photoshop.

RESULTS AND DISCUSSION

Effects of smoking on spleen

Gross anatomical changes

Effect of smoking on gross structure of the spleen in different groups of mice is shown in plate 1 and (Figure 1, 2, 3, 4 and plate 1). The colour changes were measured in a scale of 10 and alteration of structural shape of the spleen were measured in a scale of 4, where the higher value indicates the higher degree of deterioration. The colour change was significantly higher in treated group, fume (CF=4.250±0.310^{a}) and root (CR=6.004±0.175^{b}) than control group (C=0.25±0.147^{c}). In comparison with fume (CF) and root (CF) exposed animals, the result shows that the colour change was significantly (p<0.01) higher in root exposed (CR) than fume (CF) exposed animal (Figure 1). The colour change of ffume (CF) and root (CR) group indicate congestion (red arrow). Shape changes is insignificant in case group, fume (CF=2.231±0.054^{b}) and root (CR=0.59±0.109^{a}) and control group (C=0.12±0.043^{c}) (Figure 2). The length of the spleen is significantly (p<0.01) lower in case, fume (CF=15.50±0.393^{a}mm) and root (CR=12.00±0.756^{b}) than control group (C=15.50±0.316^{a} mm) whereas lowest value in fume (CF) treated group respectively (Figure 3). The wide of the spleen of control animal (C=5.000±0.141^{a}mm) is significantly (p<0.01) higher than fume (CF=4.998±0.368^{a}) and root (CR=4.500±0.387^{b}) treated group whereas the width is slightly higher in root exposed (CR=4.500±0.387^{b}mm) animals than fume (CF) treated animals respectively (Figure 3). The weight of the spleen of the case group, fume (CF=0.138±0.003^{b}gm) and root (CR=0.218±0.005^{b}) is significantly (p<0.01) lower than control group (C=0.232±0.009^{a}gm). In comparison with fume and root exposed, the result shown that the weight was significantly (p<0.01) higher in root exposed (CF) group than fume exposed (CF) group (Figure 4).

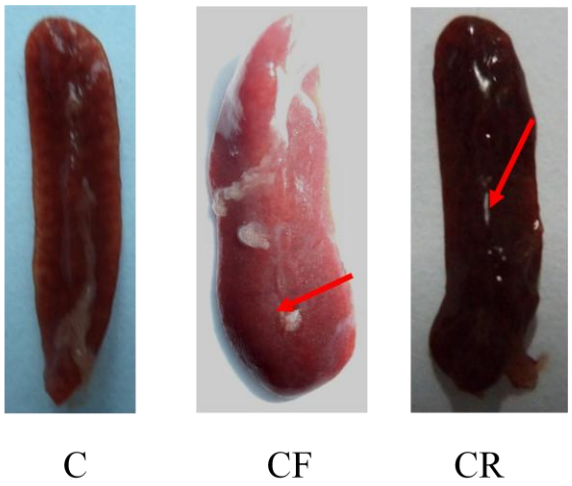


Plate 1
Spleen of mice. The control group(C) shows no gross anatomical change. Fume (CF) and root (CR) group showing congestion (red arrow).

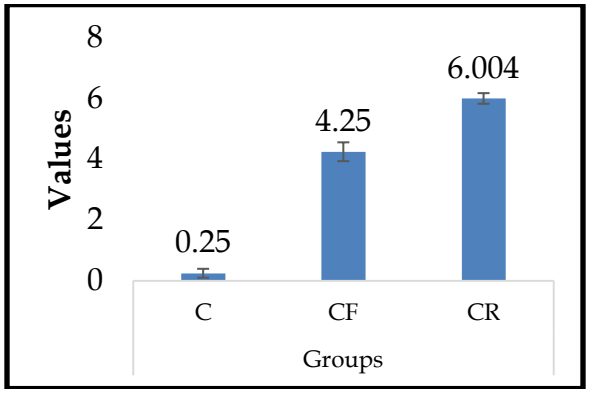


Figure 1
Effects of smoking on color of spleen.

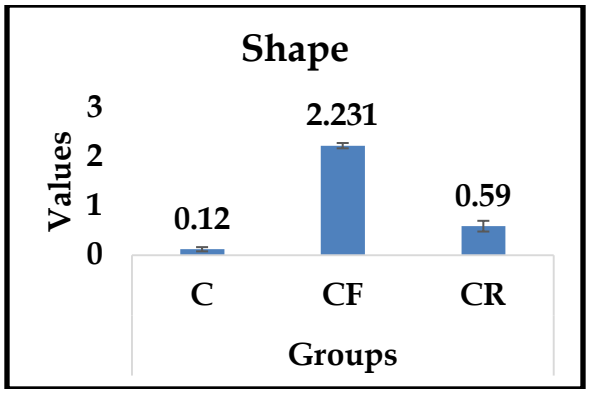


Figure 2
Effects of smoking on shape of spleen.

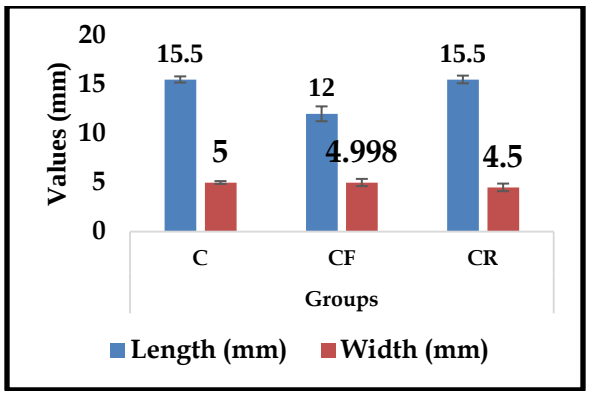


Figure 3
Effects of smoking on length and width of spleen.

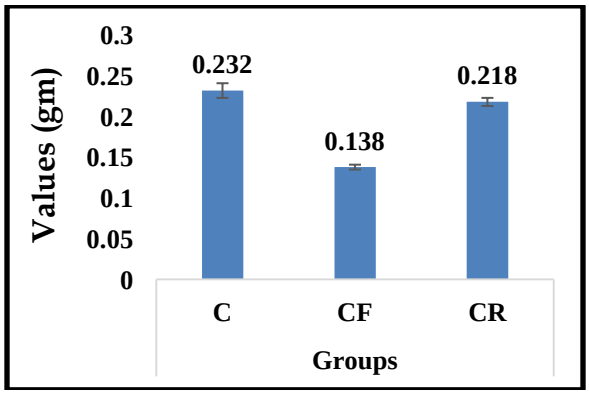


Figure 4
Effects of smoking on weight of spleen.

Histological changes

Spleen of the animal of control group (CF) showing there is no histological alteration (Plate 2).

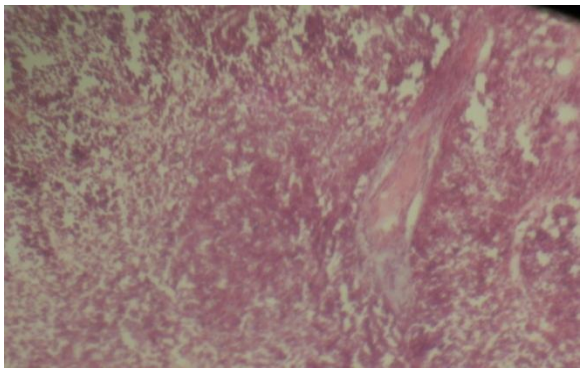
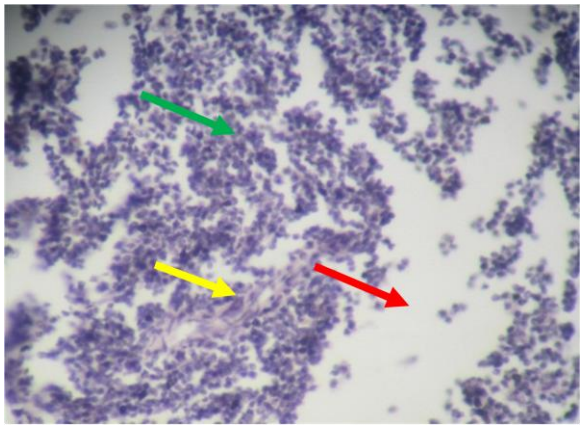


Plate 2
Spleen of the animal of control group (C) showing there is no histological alteration (H and E stain).



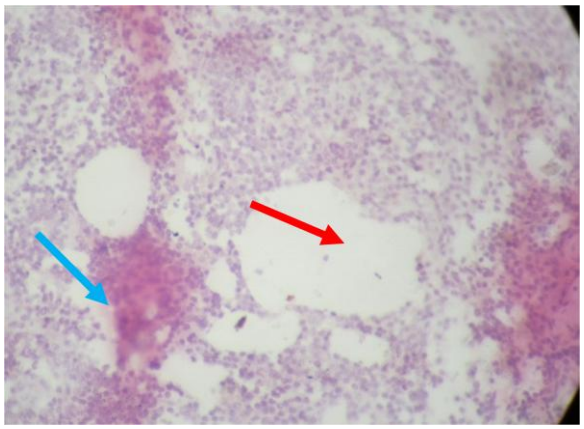


Plate 3
Spleen of the fume (upper plate) and root exposed (lower plate) animals showing disorganized histological structure. Necrosis and vacuoles (red arrow) was found in both treated fume (upper plate) and root (lower) exposed groups, but hemorrhage (blue arrow) was found in the fume (upper plate) group. Thinner trabecule (yellow)

and presence of lymphocytic infiltration (green arrow) root exposed (CR) group (H and E). (40X)

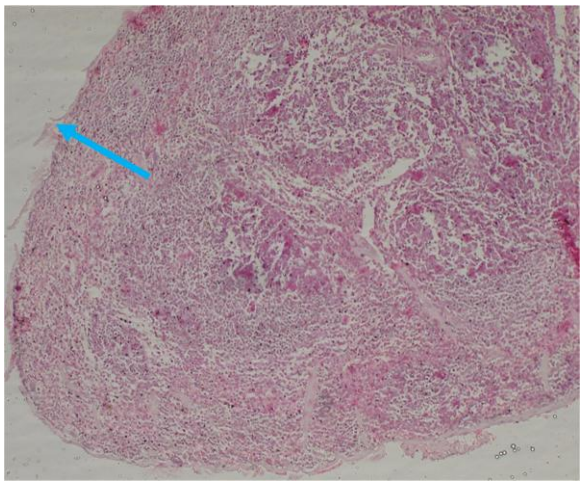


Plate 4
Spleen of fume group showing, erosion of capsule (blue arrow).

Spleen of the fume (CF) and root exposed (CR) animals showing disorganized histological structure. Necrosis and vacuoles (red arrow), were found in both treated (fume and root) exposed groups (plate 3), but hemorrhage (blue arrow) was found in the fume (CF) group. Thinner trabecule in root (yellow) exposed (CR) group (Plate 3). Erosion of capsule of fume treated group (blue arrow) was found (Plate 4).

Here, the important changes in the values were found regarding the colour and shape, and the changes were very significant and higher but length, width and weight was lower in all case groups, though the results are not similar to those of the finding of Hui et al., (2007) said that weight of spleen was increased. Adekomi et al., (2011) stated that the excised spleen appeared morphologically normal but not similar to the present study findings. The disagreement may due to variation in the use of experimental animals because they used rat as their experimental animals but in the present study mice was used. Another cause may be variation of experimental exposure time. The duration of Adekomi et al. study was only five days.

In the present study, the splenic morphological structures appeared less organized, making it difficult to identify the white pulps region, which was also stated by Diniz et al. *(*2013). Moreover, the quantitative morphometric evaluations revealed no differences in the total splenic area between control and treated group is similar to the result that obtained by the study of Diniz et al., (2013). Furthermore, no differences were found in the splenic capsule thickness in the white and red pulp areas of both experimental groups. (deleted these lines)

Necrosis, vacuole formation, hemorrhage and thinner trabecule were found in the treatment group. Those are closely related with the findings of Kanduc et al. (2002). The presence of inflammatory cells is supported by various researchers (Lai et al., 2006; Chalmers et al., 2001).

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