European Journal of Biology and Medical Science Research
Vol.8, No.3, pp.16-27, June 2020
Published by ECRTD- UK
Print ISSN: ISSN 2053-406X, Online ISSN: ISSN 2053-4078

HISTOMORPHOLOGICAL ALTERATIONS OF THE ALVEOLI CELLS OF ADULT WISTAR RATS FOLLOWING SECOND DEGREE EXPOSURE TO SMOKED LEAF OF NICOTIANA TABACCUM USING ADULT WISTAR RATS

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ABSTRACT: *Introduction: The present study was aimed at investigating the effects of second degree exposure of Nicotiana Tabaccum leaf smoke on the alveoli cell in the lungs using adult wistar rats. Experimental procedure: Thirty-six wistar rats were used for the study. The experiment's duration was four weeks.The rats in subgroups A were exposed to smoke from 0.5g of sterile cotton wool, while those in subgroups B, C and D were exposed to 0.2g/kg bw, 0.4g/kg bw and 0.8g/kg bw of Nicotiana tabaccum dry leaf smoke respectively, mixed with 0.5g of sterile cotton wool. Results: lungs showed lymphocytic infiltrations, alveolar degeneration and mild pneumonitis in the alveolar septa.The results showed that the doses of 0.4 and 0.8g/kg bw of the extract significantly increased the percentage of lymphocytic infiltrations in the alveolar septa (P<0.05) from 1.0±30.5 at the end of week 1 (1st stage) to 0.8±65.6 at the end of week 4 (3rd stage), while the lymphocytic infiltration ssignificantly increased (P<0.05) from 107.5± 2.00 at week 1 to 347.5±43.1 (ml-1) at the week Conclusion:Nicotiana tabaccum smoke could adversely affect the cell of the alveolar septum of adult male wistar rats.*

KEYWORDS: Alveolar, Nicotiana tabaccum, lymphocytic infiltrations

INTRODUCTION

According to World Health Organization (WHO), tobacco use is described as the single most important preventable risk to human health in the developed countries and cause of premature death worldwide **(**Ukoha, U., Uchechukwu, D. and Stephen, M.; 2012**).**

Tobacco products are categorized into smoked (combustible) and smokeless tobacco. Smoking tobacco include cigarette, roll-your own, cigars and pipes while smokeless tobacco comes in two different forms which are tobacco snuff and chewing tobacco (Aduema, W**.** *et al*; 2012). Tobacco is consumed in every part of the world, most especially in the developing countries with a world population of about 2.4 billion consuming tobacco, either in the form of snuff, chewing or smoking (Aghaji M.;(2008). Tobacco use leads most commonly to diseases affecting the heart and lungs, with smoking being a major risk factor for heart attacks, strokes, chronic obstructive pulmonary disease, emphysema and cancer (Nichter, M. and Cartwright, E. (1991)**.**

LITERATURE/THEORITICAL UNDERPINNING

During smoking of cigarette, cigars, pipes and other tobacco products, in addition to the mainstream smoke drawn and inhaled by smokers, a stream of smoke is released between puffs into the air from the burning cone. Once released, this stream (also known as the side stream smoke.) is mixed with exhaled mainstream smoke as well as the air in an indoor environment to form the second hand smoke to which both smokers and non-smokers are exposed. This second hand smoke contains a variable proportion of exhaled mainstream smoke ranging from 1 to 43%. (Baker R.R. and Proctor C.J.;1990). Nicotine is the principal alkaloid contained in tobacco and it is believed to be the primary reason for cigarette smoking in many people particularly as they derive satisfaction and pleasant sensation from inhaling it (Benowitz, N. L. and Jacob, P.;1984)**.** Nicotine is addictive in human because a portion of nicotine molecule is similar to acetylcholine, an important brain neurotransmitter (Brody A.L *et al***,** 2006) and is widely consumed through cigarette smoking and tobacco chewing in 30 – 40% of the world population (Huang J et al, 2014)**.** In adults, second-hand smoke causes serious cardiovascular and respiratory diseases, including coronary heart disease and lung cancer. In infants, it raises the risk of sudden infant death syndrome. In pregnant women, it causes pregnancy complications and low birth weight. Second-hand smoke causes more than 1.2 million premature deaths per year.65 000 children die each year from illnesses attributable to second-hand smoke (Huang J et al, 2014).

Even though the addictive properties of nicotine are attributed to its ability to increase synaptic neurotransmission in the central nervous system, particularly of dopamine from the mesolimbic

dopaminergic neurons (Dani JA, and De Biasi M.; 2001).,it is capable of increasing synthesis and release of neurotransmitters and hormones, induction of oxidative stress, activation of transcription and the catecholamine-synthesizing enzyme tyrosine hydroxylase, as well as prevention of apoptosis (Ashakumary L. and Vijayammal P.;1997). The predominant effects of nicotine in humans include increased release of catecholamines into the blood stream that increases pulse rate and blood pressures, the release of plasma free fatty acid and the mobilization of blood glucose (Benowitz, N. L. and Jacob, P; 1984)

METHODOLOGY

Animal management and grouping

Thirty-six male wistar rats weighing between 220 to 250g were used for the study. The animals were kept in a plastic cage with iron nettings placed in a well ventilated standard housing conditions, twelve hours light and twelve hours darkness. The animals were acclimatized for two weeks before the commencement of the experiment. During this period, the animals were observed to ensure that they were disease free. They were fed with rat chows and given water ad libitum, and an ambient temperature range of 25 - 27 maintained at 50% humidity. The animals were weighed with an electronic weighing balance prior to the commencement and termination of the experiment.

Animals were divided into three groups (1, 2 and 3) with 12 rats in each group, and sub groups (A, B, C and D). Thus, the subdivision includes group (1A, 1B, 1C and 1D), (2A, 2B, 2C and 2D), and (3A, 3B, 3C and 3D). Group A served as the control, while groups B, C, and D were the experimental groups.

Animal treatment

The rats were exposed to varying doses of *Nicotiana tabaccum* dry leaf smoke mixed with constant dose(0.5g) of sterile cotton wool in a perforated plastic cage (2^{nd} degree exposure). The exposure was three times daily (9.00hours, 11.00hours and 13.00hours) for four (4) weeks. Each exposure lasted for 5 minutes (Adebayo AD *et al*, 2011). The exposure was done in three (3) stages with each stage for groups 1, 2 and 3 respectively as follows:

GROUP	SUBGROUP	Body weight (g)	No of rats	*Nicotiana tabaccum*	Sterile cotton (g)	Duration
Stage 1	1A	200- 250	3	**Nil**	**0.5**	**1 week**
Wk 1	1B	200- 250	3	**0.2**	**0.5**	
	1C	200- 250	3	**0.4**	**0.5**	
	1D	200- 250	3	**0.8**	**0.5**	
Stage 2	2A	200- 250	3	**Nil**	**0.5**	**2 weeks**
Wk 2	2B	200- 250	3	**0.2**	**0.5**	
	2C	200- 250	3	**0.4**	**0.5**	
	2D	200- 250	3	**0.8**	**0.5**	
Stage 3	3A	200- 250	3	**Nil**	**0.5**	**4 weeks**
Wk 4	3B	200- 250	3	**0.2**	**0.5**	
	3C	200- 250	3	**0.4**	**0.5**	
	3D	200- 250	3	**0.8**	**0.5**	

TABLE 1 showing distribution of animals grouping and treatment

All animals in stage 1(1A, 1B,1C and 1D) were sacrificed at the end of week 1, all animals in stage 2(2A, 2B, 2C and 2D) were sacrificed at the end of week 2 while all animals in stage 3(3A,3B, 3C and 3D) were sacrificed at the end of week 4.

Collection of lungs

At the end of each stage, the animals in both the control and experimental groups were sacrificed under ketamine and xylazine anesthesia. The lungs were carefully excised from the animal and fixed in a 10% formal saline for 48 hours after which routine paraffin method was used to process the lung tissue in the histology laboratory. The histological study on the lungs was done according to procedure described by **(**Drisbey B.D.; Rack J.H; 1970).It was embedded in paraffin wax and 5 µm sections were done using a rotary microtome, deparaffinised sections were routinely stained with haematoxylin and eosin (H&E) and assessed in a Nikon Eclipse E400, light microscope, for histological examination. Photomicrograph analysis and interpretation was carried out using Amscope 5.0 digital camera.

RESULTS/FINDINGS

Body weight index

The result on the body weight at start, shows a significant (P≤0.05), increase in the weight of the control groups in all the weeks (Tables 2a, b and c). There was significant decrease in the weight of animals in the experimental groups when compared with that of animals in the control group; and this decrease in weight was retained throughout weeks 2 (Table 1b) and week 4(Table 1c) of the experiment.

	1A	**1B**	**1C**	**1D**
Initial weight	234±5.0	240±0.7	224.±4.2	227.±3.5
Weight after wk 1	238.2±0.0	225.8±0.4	219.0±0.6	211.4±0.2
P-Value	0.4	0.9	0.9	0.8

Table 2a: Result of body weight of animals after week one (Stage one)

Showed no significant difference in weight loss across groups (P<0.05).

	2A	**B**	**2C**	**2D**
Initial weight	234±5.0	240±0.7	224.±4.2	222.±3.5
Weight after wk 1	245.2±0.0	229.8±0.4	215±0.1	192.±1.5
Weight after Wk 2	259.1±2.4	219.8±0.4	211±3.6	179.±1.5
P-Value	0.002	0.0005	0.412	0.0000

Table 1b: Result of body weight of animals after week two (Stage two)

Showed significant difference in weight loss (P<0.05). subgroup 2A P=0.002 (statistically significant) and subgroup 2B P = 0.005 (statistically significant)

	3A	3B	3C	3D
Initial weight	234±5.0	240±0.7	224.±4.2	245.1±2.8
Weight after wk 1	247.0±1.4	243.5±0.7	220.0±2.8	230.5±0.7
Weight after Wk 2	253.5±6.4	231.9±0.5	220.6±3.6	213.7± 5.2
Weight after Wk 4	255.1± 3.1	207.7±0.9	193.8±1.5	176.3±2.1
P-Value	0.05	0.0000	0.004	0.0000

Table 1c: Result of body weight of animals after week three (Stage three)

Showed significant difference in 3C (P≤ 0.05). P =0.004

Alveolar Histology

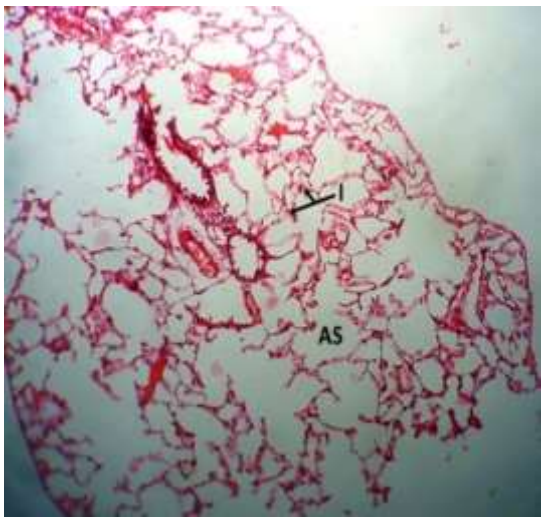


Plate 1: WEEK 1(CONTROL):
Photomicrograph of control animal lung(0.5g of sterile cotton wool burnt). showing normal alveolar spaces (AS) and interstitium (I) (H & E stain x 100).

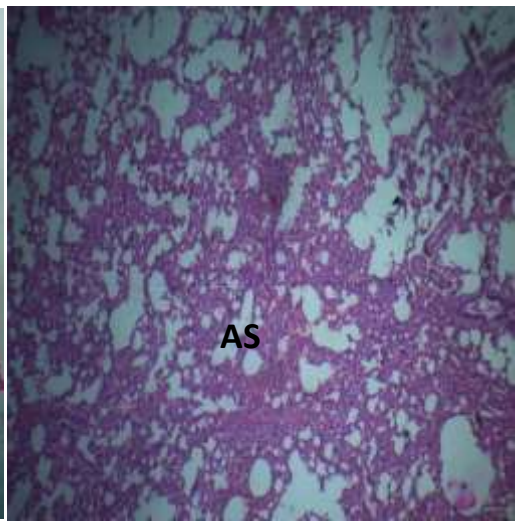


Plate 2: WEEK1 (TEST):
Photomicrograph of test animal lung at low dose(0.2g/kg + 0.5g sterile cotton wool) showing unremarkable change of the alveolar spaces. and Interstitium(I)(H & E stain x 100).

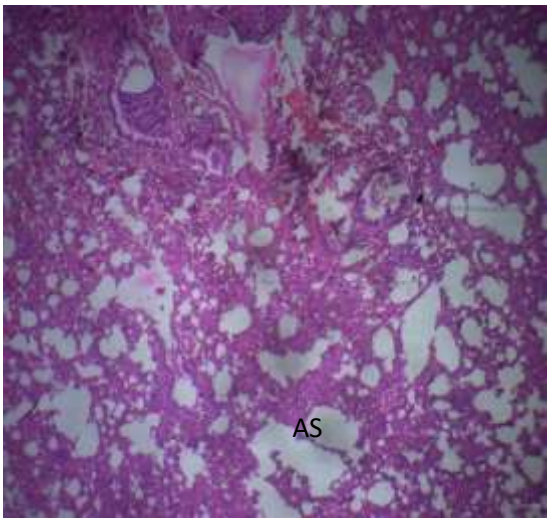


Plate 3: WEEK1(TEST): Photomicrograph of test animal lung at moderate dose(0.4g/kg of *N.tabaccum* + 0.5g sterile cotton wool) showing unremarkable change of the alveolar spaces and Interstitium. (H & E stain x 100).

Plate 4: WEEK1(TEST): Photomicrograph of test animal lung at high dose(0.8g/kg of *N.tabaccum* + 0.5g sterile cotton wool) showing mild lymphocytic infiltrate of the alveolar spaces.(AS) and Interstitium(I)(H & E stain x 100).

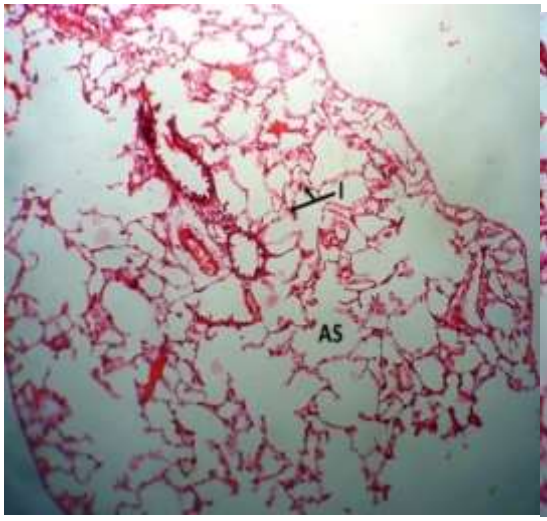


Plate 5. WEEK2 (CONTROL): Photomicrograph of control animal lung(0.5g of sterile cotton wool burnt.) showing normal alveolar spaces (AS) and interstitium (I) (H & E 100)

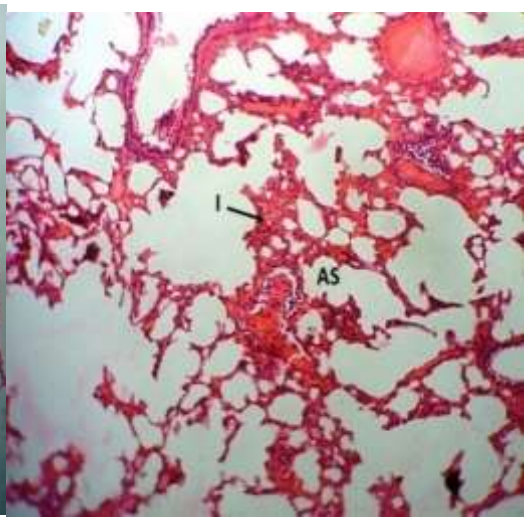
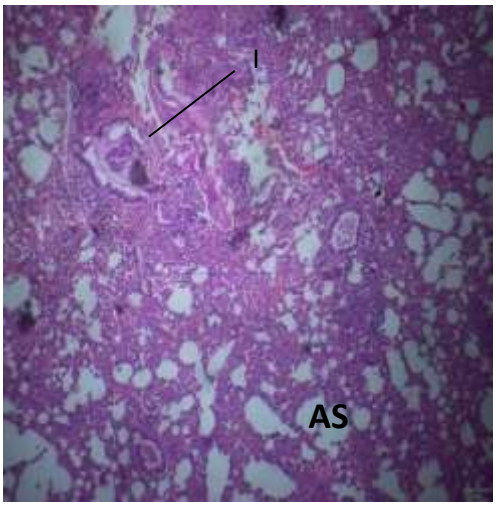


Plate 6: WEEK2(TEST): Photomicrograph of test animal lung at low dose(0.2g/kg of *N.tabaccum* + 0.5g sterile cotton wool) showing mild lymphocytic infiltrate of the alveolar spaces.(AS) and Interstitium(I)(H & E stain x 100).



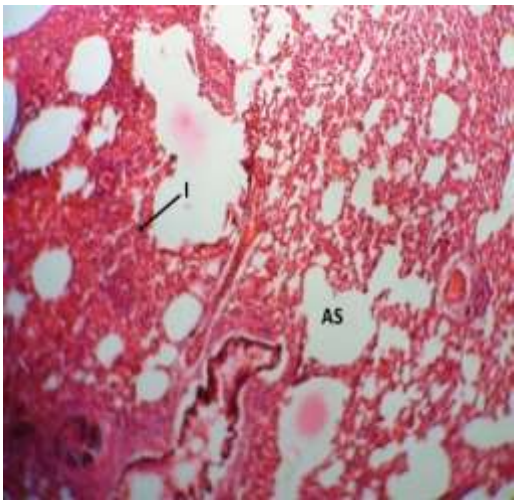
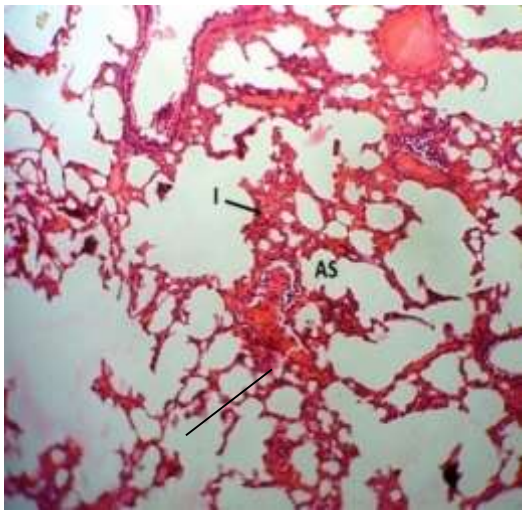


Plate 7: WEEK2(TEST): Photomicrograph of test animal lung at moderate dose(0.4g/kg of *N.tabaccum* + 0.5g sterile cotton wool) showing mild to moderate lymphocytic infiltrate of the alveolar spaces and Interstitium (H & E stain x 100).

Plate 8: WEEK2(TEST): Photomicrograph of test animal lung at high dose(0.8g/kg of *N.tabaccum* + 0.5g sterile cotton wool) showing moderate to severe lymphocytic infiltrate of the alveolar spaces.(AS) and Interstitium(I)(H & E stain x 100).



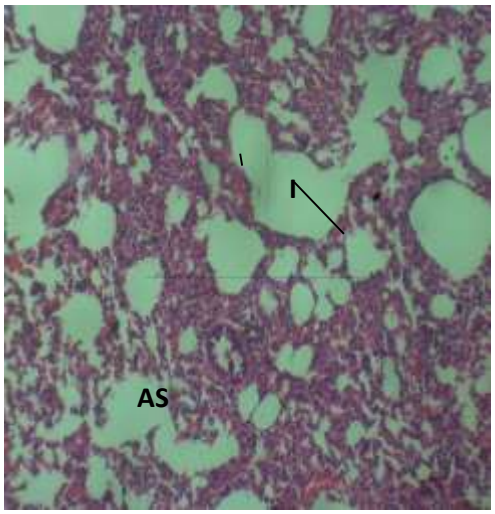


Plate 9: WEEK4(CONTROL): Photomicrograph of test animal lung(burnt 0.5g of sterile cotton wool) showing mild lymphocytic infiltration of the interstitium (I) [interstitial pneumonitis] (H & E stain x 100).

Plate 10:WEEK4(TEST): Photomicrograph of test animal lung at low dose(0.2g/kg + 0.5g of sterile cotton wool) showing mild to moderate lymphocytic infiltration of the interstitium [interstitial pneumonitis] (H & E stain x 100).

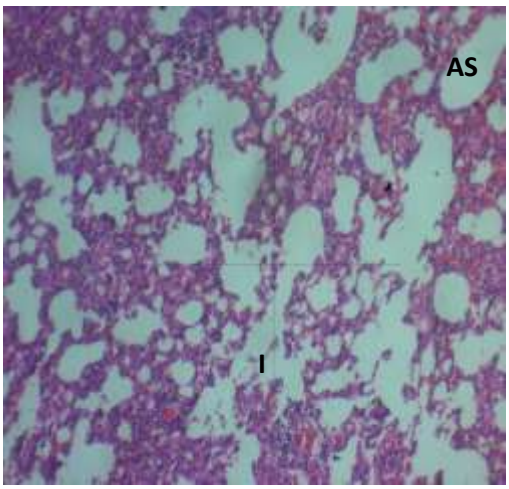


Plate 11:WEEK4(TEST):
Photomicrograph of test animal lung at moderate dose(0.4g/kg + 0.5g of sterile cotton wool) showing moderate to severe lymphocytic infiltration of the interstitium [interstitial pneumonitis] (H & E stain x 100).

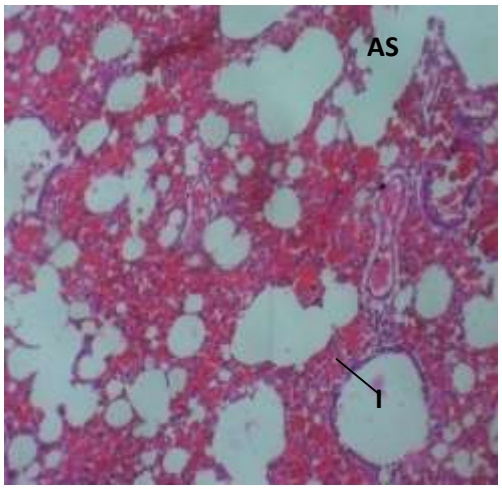


Plate 12:WEEK4(TEST):
Photomicrograph of test animal lung at high dose(0.8g/kg + 0.5g of sterile cotton wool) showing moderate to severe lymphocytic infiltration of the interstitium (I) [interstitial pneumonitis] (H & E stain x 100).

This study investigated the effects of second degree exposure of smoked Nicotianum tabaccum leaf on the alveoli cells using adult wistar rats. These exposures had significant weight differences of the animals used for this studywith $P \leq 0.05$**.** Tables 2a, b and c showed there was an increase in weight across all control groups in animals used after weeks 1, 2 and 4 while animals in the experimental groups exhibited gradual weight reduction. Table 1b showed significant weight loss across animals in animals in subgroup 2A ($P = 0.002$) and subgroup 2B ($P = 0.005$) compared to the control group while table 1C showed significant weight loss in animals in subgroup 3C ($P = 0.004$).There was also, a progressive and significant ($P < 0.05$), increase in body weight of the rats in the control groups in respect to duration (4 weeks). Loss of body weight has been attributed to nicotine-mediated (nicotine cholinergic receptors in the brain) and thus, lead to anorexia (Adeniyi P.A; 2010). This was observed in the experimental animals which showed marked reduction in appetite and this will resultantly lead to weight loss due to reduced metabolism. This will also inhibit neuroendocrine cells in the GIT that play significant roles in neural and hormonal roles in

appetite and digestion leading to anorexia (Benowitz N.L; 2010) and possible increased lipoprotein lipase activity in adipose tissue that can provide a counter-regulatory role in weight gain.

Photomicrograph showed lymphocyte infiltration on the alveolar septa especially on animals exposed to the smoked *nicotianna tabacum*for two weeks (stage 2) and four weeks (stage 3).The results showed that the doses of 0.4 and 0.8g/kg bw of the nicotianum tabaccum smoke significantly increased the percentage of lymphocytic infiltrations in the alveolar septa ($P<0.05$) from 1.0±30.5 at the end of week 1 (1^{st} stage) to 0.8±65.6 at the end of week 4 (3^{rd} stage), while the lymphocytic infiltrationssignificantly increased ($P<0.05$) from 107.5± 2.00 at week 1 to 347.5±43.1 *(ml-1)* at the week 4.Moreso, plates 10,11 and 12 showed mild levels of intestinal Pneumonitis and degeneration of alveolar epithelium in the alveolar tissue of animals exposed to).8g/kg of nicotianum tabaccum in week 4(stage 3).

AlAwaida in his work on Chinese tea consumption reduces oxidative stress and inflammed tissue dmage, he assessed tobacco smoke toxicity and revealed changes in the trachea epithelium, heart ventricles and lung alveoli. A thin section of the trachea epithelium showed a high degree of cytoplasmic vacuolization. The alveolar epithelium showed damaged multilamellar bodies of type 2 pneumocytes, cytoplasmic vacuolization and degeneration of alveolar epithelium (Al waida, 2014).

IMPLICATION TO RESEARCH AND PRACTICE

This work has highlighted the health hazards that people who are passive smokers or second degree smokers may be exposed to. In normal physiological reaction, macrophages in the alveolar septum usually migrate to alveoli areas loaded with tobacco for normal inflammatory reactions. Thus, macrophage –defence system reaction. This work analysed the biochemical and histological effect of cigarette smoke on the alveoli and it showed that there was significant increase in the number of macrophages, mono-aldehyde levels of pulmonary tissue and gross distortion of the alveolar membrane. This is also in line with the result of the research work carried out by Devici et al in 2004.

CONCLUSION

This study accessed the histomorphological alterations of the alveoli cells of adult wistar rats following second degree exposure to smoked leaf of *Nicotiana tabaccum. Nicotianna tabacum* second degree exposure caused lymphocytic infiltrations in the alveolar septa and interstitial pneumonitis which led to alveolar degeneration. These changes could correlate with the ballooning and destruction of the aveolar wall (emphysematous changes) as seen in chronic obstructive pulmonary disease caused by chronic tobacco smoking in man.

FUTURE RESEARCH

Further studies is recommended to assess the impact of *Nicotiana tabaccun* smoke exposure to surfactant cells of the alveoli and also assess any possible physiological effect on lung volumes.

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