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A comparative histological study on the effect of exposure to the smoke leaf extract of *tobacco nicotiana*, *cannabis sativa* and *datura stramonium* on the lungs of sprague-dawley rats

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ABSTRACT

Smoking may exert compromising effects on several organ systems of the body, but those in the lungs are the most deleterious. Therefore the aim of the present study was to investigate some of the effects of inhalation of smoke extract of *Tobacco nicotiana* (Tobacco), *Cannabis sativa* and *Datura stramonium* (Jimson Weed) on the respiratory system, especially on the lungs. Twenty male Sprague Dawley rats were divided into four groups A-D. The rats in the control group (A) were not subjected to any of the smoke extracts, while the rats in groups B, C, and D were exposed to smoke from a completely burnt 0.74g leaf extract of *Tobacco nicotiana, Cannabis sativa* and *Datura stramonium* each for 5 minutes three times daily (7am, 10am, and 1pm) respectively. The duration of exposure in all the groups was for five days. All the rats were sacrificed by decapitation and the lung tissues were obtained from each animal using thoracotomy, blotted dry and fixed in buffered neutral formalin for histopathological analysis using Hematoxylin and Eosin (H&E) stain. In the lung tissue of the rats in the control group (A), the histological profile of the lungs were preserved, whereas in groups B, C, and D the histological outline of the tissues obtained

revealed disruptive characteristics such as emphysema, evidence of bronchopneumonial features and fibrosis, occlusion of the bronchi, and dilation of the alveoli sac. In conclusion, the exposure of male Sprague Dawley rats to the smoke extract of *Tobacco nicotiana* (Tobacco), *Cannabis sativa* and *Datura stramonium* (Jimson Weed) has compromising effects on the histological integrity of the lungs of the rats and by extension may cause irreversible functional and morphological alterations in the lung tissue.

1. Introduction

Smoking has recently been recognized as a significant contributor to cardiovascular mortality (Well, 1994; Kawachi *et al,* 1997). Furthermore, in addition to the long-term effects of smoking, more recent research has also identified some acute sympathetic, hemostatic, and endothelial dysfunction often persistent with smoking (Raupach *et al,* 2006). The harmful vascular effects of smoking have pathophysiological pathways characterized by early endothelial dysfunction (Barnoya and Glantz, 2005). The effects of smoking on human health are serious and in many cases deadly. Smoking affects the function of the immune system and may increase the risk for respiratory and other infections. The respiratory system, which includes the nose, throat, windpipe (trachea) and lungs, brings air into the body during the process of inspiration. In the lungs, the oxygen from each breath is transferred to the bloodstream and sent to all the body's cells as life-sustaining fuel. Smoking represents the main stimulatory factor of the most common type of oral carcinoma, oral squamous cell carcinoma (Blot *et al,* 1988).

Tobacco is the dried leaves of a plant that grows in may parts of the world. Most tobacco are sold in the form of cigarettes, cigars and pipe tobacco. Tobacco smoke has long been recognized as a major cause of mortality and morbidity. It is responsible for approximately 434,000 deaths per annum in the United States. It is also a source of indoor air pollution due to the release of harmful chemicals, particles, and carcinogens. Tobacco smoke is made up of more than 4,000 different chemicals including carbon monoxide and formaldehyde. More than forty of these compounds are known to cause cancer in humans or animals, and many of them are strong irritants.

The burning of tobacco generates approximately 4000 compounds. The smoke can be separated into gas and particulate phases. The composition of the smoke delivered to the smoker depends on the composition of tobacco and how densely it is packed, the length of the column of tobacco, the characteristics of the filter and the paper and also the temperature at which the tobacco is burned. Some of the gaseous components of a burnt tobacco leaf include carbon monoxide, carbon dioxide, nitrogen oxides, ammonia, volatile nitrosamines, hydrogen cyanide, volatile sulfur containing compounds, volatile hydrocarbons, alcohols and aldehydes and ketones. Some of these compounds adversely affect ciliary movement in the lungs. The use of tobacco 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 and Cartwright, 1991). It also causes peripheral vascular disease and hypertension, all developed due to the exposure time and the level of dosage of tobacco. Furthermore, the earlier and the higher level of tar content in the tobacco filled cigarettes causes the greater risk of these diseases (Nichter and Cartwright, 1991).

Cannabis sativa is an annual plant in the *Cannabaceae* family. Humans have cultivated this herb throughout recorded history as a source of industrial fiber, seed oil, and food. Humans have long used the plant as a drug, as medicine, and as a spiritual tool. Each part of the plant is harvested differently, depending on the purpose of its use. When so used, preparations of *Cannabis sativa* are consumed by smoking, vaporizing and oral ingestion. The toxicological consequences of *Cannabis sativa* smoking on the lung, brain, immune system, and other organs is a subject of considerable importance (Adams and Martin, 1996; Ashton, 2001; Klein, 2005). Most of the toxic compounds found in tobacco smoke also are present in similar quantities in *Cannabis sativa* smoke, except that *Cannabis sativa* contains high concentrations of Δ^9-tetrahydrocannabinol (THC) and other cannabinoids in place of nicotine (Novotny *et al,* 1982). Furthermore, because of differences in the way that it is smoked, a single *Cannabis*

sativa cigarette may deposit 5–10 times as many smoke-related particulates into the lungs as does a regular tobacco cigarette (Wu *et al,* 1988).

Some of the chemical constituents of Cannabis sativa include about 100 compounds responsible for its characteristic aroma. These are mainly volatile terpenes and sesquiterpenes and they include; Δ^9-Tetrahydrocannabinol, α-Pinene, Myrcene, Trans-β-ocimene, α-Terpinolene, Trans-caryophyllene, α-Humulene and Caryophyllene-oxide (Novak *et al,* 2001). It is known that habitual *Cannabis sativa* smokers may consume only a few *Cannabis sativa* cigarettes per day, they often demonstrate symptoms of chronic bronchitis and signs of airway inflammation and mucosal epithelial injury much quite similar to those observed in tobacco smokers (Wu *et al,* 1988). Even with this evidence, there is a common perception that exposure to *Cannabis sativa* smoke poses no danger to humans and has little or no effects on the lung (Well, 1994). Datura stramonium is an erect annual plant measuring about 30 to 150 cm in height with erect purple stems. The leaves are large, measuring approximately 7 to 20 cm in length. Datura stramonium (Jimson Weed) is a common weed in the Nightshade Family (Richard *et al,* 1997). It contains tropane alkaloids that are sometimes used as a hallucinogen. The active ingredients are atropine, hyoscyamine and scopolamine which are classified as deliriants, or anticholinergics. Due to extremely high risk of overdose, many deaths and hospitalizations are reported from abused recreational use (Richard *et al,* 1997). Smoking is generally five times more prevalent among males than females (Thun *et al,* 2008), however the gender gap declines with younger age (Guindon *et al.,* 2003). In developed countries smoking rates for men have peaked and have begun to decline, however for women they continue to rise higher. As of 2002, about twenty percent of young teens smoke worldwide, with 80,000 to 100,000 children taking up the habit every day. The components of smoke are complex and vary considerably, in relation to conditions of incomplete combustion, the combustion materials, temperature, circumstances and timing (Richard *et al,* 2006) and since smoking has been recognized as a major contributor to mortality in man and previous studies has confirmed the effects of tobacco and/or *Cannabis sativa* exposure on the brain, nervous system and the general body system (Xie *et al,* 1998; Dagher *et al,* 2001; DiChiara *et al* 1992). With all of these in mind, the aim of this study was to investigate some of the effects of the smoke extract of *Tobacco nicotiana*, *Cannabis sativa*, and *Datura stramonium* on the lungs of rats as a marker of toxicity.

2. Materials and methods

2.1. Collection of plant and preparation of plant extracts

Fresh leaves of Tobacco nicotiana and Datura stramonium were from the premises of the Botanical garden of the University of Ilorin, Ilorin, Kwara State and were authenticated at the Department of Plant Science of the same University. The Cannabis sativa used was obtained from the Nigeria Drug Law Enforcement Agencies (NDLEA), Ilorin Command, Ilorin, Kwara State, Nigeria. The leaves of the plants were air-dried separately under standard laboratory conditions. The dried plant materials each were weighed using Gallenkomp (FA2104A, England) electronic weighing balance.

2.2. Animal care

Twenty male Sprague Dawley rats weighing 125 to 163 g were housed in a controlled environment and given free access to standard rat chow and distilled drinking water. This experimental investigation was done in accordance with the standard humane animal care as outlined in the "Guide for the care and use of Animals in research and teaching", as approved by the Institute of Laboratory Animal Resource, National Research Council, DHHS, Pub. No NIH 86 – 23 (NIH, 1985).

2.3. Experimental design

The twenty male Sprague Dawley rats were randomly assigned into four experimental groups; A, B, C, and D. Three closed glass chambers of approximately 0.1 m^3 volume (38 cm X 88 cm X 30cm) (Onarlioglu *et al,* 1999) was used for exposing the animals to the smoke extract of the plant samples. In the upper surface of the chambers an opening of 1 cm was made. The chambers were designated as A, B and C respectively. Animals in groups A, B, and C (treatment groups) were each exposed to smoke from a completely burnt 0.74g leaf of Tobacco nicotiana, Cannabis sativa and Datura stramonium for 5minutes three times daily (7am, 10am, and 1pm) in each of the

designated closed glass chambers respectively. The animals in group D (control group) were not exposed to smoke from any of the plant samples. The duration of investigation was for five days.

2.4. Animal sacrifice and histological parameters

Three hours after the termination of investigation, the animals were sacrificed by decapitation, the lung tissue samples were taken by thoracotomy. The samples were fixed for 48 hours in buffered neutral formalin. Following the fixation procedure, the tissues were dehydrated through increasing concentrations of ethanol. They were then embedded in paraffin and 5µm thick sections were cut from the paraffin blocks on a Leitz Rotary microtome (Leitz 1512 Microtome). These sections were stained with Hematoxylin-eosin for routine histological procedures and the histological examination was done with the aid of the Olympus binocular light research microscope (XSZ-107BN, No. 071771). The permanent photomicrographs of each slide were recorded with a Kodak Digital Camera (Kodak Easyshare C183) for subsequent histological analysis.

3. Results

Using the Olympus binocular light microscope (XSZ-107BN, Nọ. 071771) for the histological observation of the processed lung tissue samples obtained from the rats in the control group (group A), it was observed that the bronchial structures were surrounded by a smooth muscle layer. Surrounding these structures are the saccus alveolaris, alveoli with regular walls, interalveolar septa seen in the lung parenchyma. The histological outline of the lungs of the animals in the control group were preserved (Fig. 1).

Clear histological observation of the lungs of the rats exposed to *Tobacco nicotiana* smoke (group B) demonstrated cell death as evidenced by the presence of progressive sloughing of the bronchiolar epithelium, cytoplasmic blebbing, cytoplasmic vacuolation, and pronounced vascular congestion. The histoarchitectural organization of the alveoli seemed to be disturbed and the interalveolar septa were distorted. These findings may indicate that the lung tissue had undergone fibrosis (Fig. 2).

The light microscopic examination of the processed lung tissues of the rats exposed to *Cannabis sativa* smoke extract (group C) revealed sloughing of the bronchiolar epithelium, cytoplasmic vacuolization of the bronchiolar epithelium, occlusion of the vascular structures, and dilation of the alveoli and the alveolar sacs (Figure 3). The histostructural make-up of the lungs of the rats was distorted and this may lead to fibrosis of the lung tissues.

The microscopic examination of the processed lung tissues of the rats exposed to the smoke extract of *Datura stramonium* (group D) revealed histological deviations such as dilation of alveoli and alveolar sacs, sloughing of the bronchiolar epithelium occlusion of vascular structures, patchy hemorrhages of lung tissues and cystic segments. Also, the histological profile of the lung tissues was distorted (Fig. 4).

4. Discussion

In this investigation, we examined the histopathological effects of the inhalation of the smoke extracts of *Tobacco nicotiana, Cannabis sativa*, and *Datura stramonium* on the lungs of male Sprague Dawley rats. Evidence from this investigation through the use of the Olympus binocular light microscope (XSZ-107BN, Nọ. 071771), showed that the smoke extracts of *Tobacco nicotiana*, *Cannabis sativa*, and *Datura stramonium* has adverse and severe effects on the histology of the lungs of male Sprague Dawley rats when compared with the control rats.

Clear microscopic observation of the processed lung tissue samples form the treated groups (i.e. groups A, B and C) revealed several histological derangements The sections of the lungs obtained from the treatment groups (A, B and C) has disrupted histological organization compared with the control group. This confirmed that smoking *Tobacco nicotiana*, *Cannabis sativa*, and *Datura stramonium* has toxic and disruptive interference on cellular integrity of the lungs in the experimental rats. The lungs of the rats in the control group (D) showed better histological features (Fig. 1). There were no degenerative changes, cellular hypertrophy in the sections obtained from the rats in the control group.

The components of smoke are complex and vary considerably, in relation to conditions of incomplete combustion, the combustion materials, temperature, circumstances and timing. The inhalation of the several composition of smoke may cause degeneration of airway mucosal proteins, loss of cilia and the respiratory epithelium, and activation of alveolar macrophages, leading to inflammatory reactions that result in damage to the alveolar capillary membrane, an increase in its permeability, and pulmonary oedema (Richard *et al,* 2006).

Tobacco use leads most commonly to diseases affecting the heart and lungs. It also causes peripheral vascular disease and hypertension, all developed due to the exposure time and the level of dosage of tobacco. The higher level of tar content in the tobacco filled cigarettes causes the greater risk of these diseases. Cigarettes sold in developing nations tend to have higher tar content, and are less likely to be filtered, potentially increasing vulnerability to tobacco-related disease in these regions (Nichter and Cartwright, 1991).

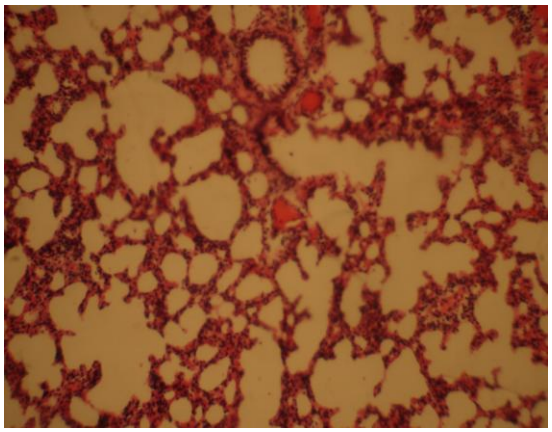

Fig. 1. Photomicrograph of the lungs of the rats in the control group (H&E x 480).

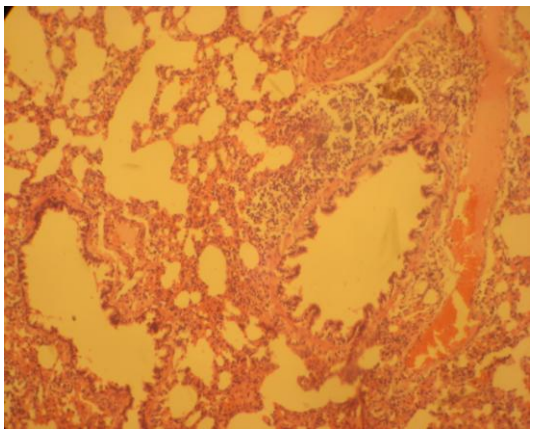

Fig. 2. Photomicrograph of the lungs of the rats in the treatment group B exposed to Tobacco nicotiana smoke showing enlarged alveoli and alveoli sacs, sloughing of the bronchiolar epithelium, cytoplasmic blebbing and vascular congestion (H&E x 480).

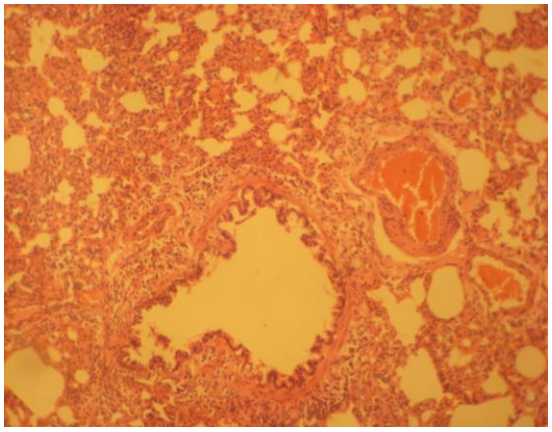

Fig. 3. Photomicrograph of the lungs of the rats in the treatment group showing pronounced occlusion of the vascular structures, dilation of the alveoli and alveolar sacs, cytoplasmic vacuolization of the bronchiolar epithelium, and sloughing of the bronchiolar epithelium (H&E x 480).

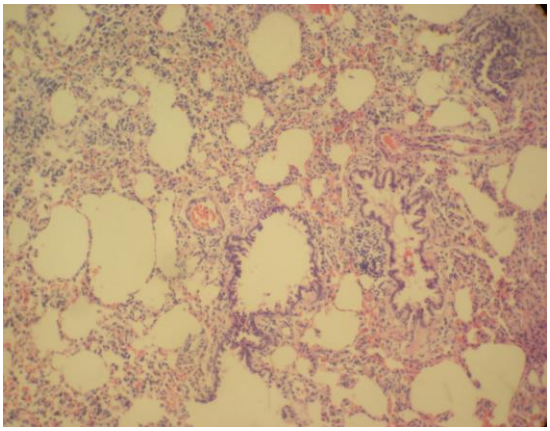

Fig. 4. Photomicrograph of the lungs of the rats in the treatment group showing dilation of the alveoli and alveoli sacs, occlusion of vascular structures, presence of cystic segments, and sloughing of the bronchiolar epithelium (H&E x 480).

Smoking affects the function of the immune system and may increase the risk for respiratory and other infections (Moir *et al,* 2008). There are several likely ways that smoking does its damage. One is oxidative stress that mutates DNA, promotes atherosclerosis, and leads to chronic lung injury. Oxidative stress is proposed to be the general mechanism behind the aging process, contributing to the development of cancer, cardiovascular disease, and chronic obstructive pulmonary disease. The body produces antioxidants to help repair damaged cells.

Smokers have lower levels of antioxidants in their blood than do nonsmokers. Smoking is associated with higher levels of chronic inflammation, another damaging process that may result in oxidative stress (Moir *et al,* 2008).

According to Ohlsson *et. al.,* (1980), it was reported that if *Cannabis sativa* is consumed in food or drink, the short-term effects begin more slowly, usually in about 30 minutes to 1 hour, and may last longer, for as long as 4 hours. Smoking *Cannabis sativa* deposits several times more THC into the blood than does eating or drinking the drug. Within a few minutes after inhaling *Cannabis sativa* smoke, an individual's heart begins beating more rapidly, the bronchial passages relax and become enlarged. The heart rate, normally 70 to 80 beats per minute, may abnormally increase by 20 to 50 beats per minute or, in some cases, even double (Ohlsson *et al.,* 1980). This effect can be greater if other drugs are taken with *Cannabis sativa* (Gilman *et al,* 1998).

Cannabis sativa smoking has the potential to promote cancer of the lungs and other parts of the respiratory tract because it contains irritants and carcinogens (Volkow, 2005). *Cannabis sativa* smoke contains 50 to 70 percent more carcinogenic hydrocarbons than tobacco smoke (Sridhar *et al.,* 1994). It also produces high levels (a very high level that may accelerate the changes that ultimately produce malignant cells) of an enzyme that converts certain hydrocarbons into their carcinogenic form (Hoffman *et al.,* 1975).

Effects of exposure of the treated rats to the smoke extract of *Cannabis sativa* on the lungs indicate similar pattern of toxicity. The massive dilation of the alveoli and occlusion of the vascular structures could have been a result of direct toxicity or could have resulted from transportation of toxic substances from other organs like the liver and kidneys to the lungs. The sections of the processed lung tissues obtained from the animals in the treated groups B, C and D showed derangement in their histoarchitectural profile (Figures 2-4), whereas the histostructural outline of the processed lung tissues of the animals in the control group showed a well preserved histological outline.

The bronchiolar sloughing was also another indication of cell death showing the extent of damage to the lung tissues. Sloughed cells may form respiratory casts which may ultimately produce airway obstruction and respiratory maladies. The cell death may recorded in this investigation may be as a result of several factors such as tissue hypoxia (Cohen, 1981; Cahalane and Demling, 1984) or damage by deleterious and harmful chemicals that have adhered to the smoke particles, reactive oxygen species (Lentz and Peterson, 1997) and other inflammatory mediators. With these histological abnormalities, the anatomical, physiological and biochemical activities of the lungs could be compromised thereby leading to medical deviations and clinical maladies affecting the respiratory system.

In the present study, rat lungs exposed to smoke extracts of *Tobacco nicotiana*, *Cannabis sativa*, and *Datura stramonium* displayed irregularly organized histological outlines. It was observed that the exposure of male Sprague Dawley rats to the smoke extract of *Tobacco nicotiana, Cannabis sativa,* and *Datura stramonium* have the potentials of causing structural damage to the lungs.

4. Conclusion

The effects of smoking on human health are serious and in many cases, deadly. Smoking is associated with higher levels of chronic inflammation. Data obtained from this study showed that exposure to the smoke extract of *Tobacco nicotiana, Cannabis sativa*, and *Datura stramonium* on the lungs have deleterious effects on the cytoarchitecture of this organ in male Sprague Dawley rats. Considering some of the effects of the smoke extract of *Tobacco nicotiana*, *Cannabis sativa,* and *Datura stramonium* on the histological integrity of the lungs in male Sprague Dawley rats, drug abusers should be properly educated on the use of the plant considering the negative impact it conferred on the lung of the treated rats in this investigation.

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