



## Pathological Changes among *Norvegicus Rattus* Exposed on Novel Smoked *Bambusa Vulgaris* (Bamboo) Leaf: Cigarette Substitute during COVID-19 Lockdown in Nigeria

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### Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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### ABSTRACT

**Background:** This research titled; Pathology changes among *Norvegicus Rattus* exposed on novel smoked *Bambusa vulgaris*: Cigarette substitute during COVID 19 lockdown in Nigeria is stimulated following increase use of the aforementioned substances as a substitute to drugs during covid -19 lock down in Nigeria where a number of psychoactive substances were limited and restricted for months, both known addicts to cannabis and cigarette smoking and frustration induced initial smokers engaged massively on the use of bamboo leaf and that had continued after years of the lockdown.

**Methods:** The experimental subjects were grouped into acute and chronic for both smoked and the control. *Norvegicus rattus* subjects were exposed to the 0.01g/g *bamboo vulgaris* dry leaf smoke twice daily for 21 and 42 days (acute and chronic) exposure respectively. The Animals were sacrificed and the organs harvested following ethical procedures for animal killing. The brain and lungs were divided and parts subjected into histopathological examination using formalin fixed

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paraffin processed methods stained with both routine and special stains, and the other parts were homogenized to investigate the oxidative stress biomarkers.

**Results:** Histopathology results first revealed a severe cellular injury in both lungs and brain compare to the normal control; significance ( $p < 0.5$ ) elevation found in MDA and reduced SOD, Gpx, GSH and catalase correlate the histology results with oxidative stress biomarkers.

**Conclusion:** Smoking *Bambusa vulgaris* dry leaf is been found in this study to be more harmful to the respiratory system and brain in more rapid manner compared to conventional abused substances and should be discourage using state agency and enlightenment campaign

**Keywords:** Pathological changes; *Norvegicus rattus*; smoking; *Bambusa vulgaris* (bamboo) leaf; cigarette; COVID-19; Nigeria.

## 1. INTRODUCTION

Smoking is a major risk factor for cardiovascular morbidity and mortality, and is considered to be the leading preventable cause of death in the world [1]. Internationally, 25% of middle-aged cardiovascular deaths are attributable to smoking [2]. The European Society of Cardiology reported recently that smoking causes 28% of cardiovascular deaths in men aged 35 to 69 years and 13% in women of the same age [3]. In the European Region of the World Health Organization (WHO), smoking is the second most important risk factor in the burden of disability-adjusted life years and is the primary risk factor for premature mortality, associated with about 1.6 million deaths each year [4]. Smoking is an intractable and preventable public health problem. Marijuana is a combination of shredded leaves, stems and flower buds of the *Cannabis sativa* plant. Marijuana can be smoked, eaten, vaporized, brewed and even taken topically, but most people smoke it. A growing body of evidence suggests that Marijuana (*Cannabis sativa*) use may negatively impact several aspects of people's lives, including mental and physical health, cognitive functioning, ability to drive a motor vehicle, and pre- and post-natal development among offspring. Regular cannabis smoking can lead to respiratory bronchitis in a high proportion of people who use cannabis.

The observed reduction in heart weight and presence of dead cells following smoking may be due to the release of epinephrine which causes vasoconstriction and hence reduced blood supply to the heart tissues. Thrombus seen on the histological section through the heart is consistent with the observed coronary thrombosis in smokers [5].

Despite the significant health impacts arising from smoking, little attention is paid to combat

the neurological and other organ changes associated with it and it becomes the growing popularity of medical and recreational for users. This ugly trend of high desire and subsequent addiction of smoking Bamboo leaf (*Bambusa vulgaris*) is gaining ground among Nigerians, phytochemical analysis of the bambusa vulgaris o leaf vugaris has shown to contain the following; alkaloids, tannins, phenolics, glycosides, saponins, flavonoids, and anthraquinones [6]. Oral dose administration of *bambusa vulgaris* has been associated with dramatic increase in abortion frequency and decrease survival rate among pregnant rabbits at varied doses of between 250-500 mg/kg, this is as a result of its effect on increased resorption index and implantation loss, decreased serum progesterone, follicle-stimulating and luteinizing hormones, decrease alkaline phopatase activity and glucose concentration in the uterus [6]. Meanwhile, the leaf has also been used over the years to relief labor pains and postpartum cleansers for livestock. Recently, it has become one of the commonly abuse smoke substance among Nigerians and it demand research to unveil the implication of this already addictive substance among Nigerians. This informed the country regulating body to enlist such drug among controlled drug and recently other form of enzolyn cough syrup expectorant is also similarly abused among other cough syrups. Another form of opioid that is grossly abused by Nigerians is Tramadol HCL, it is use to relief moderate to moderately severe pain, and usually it's taken orally based on clinician prescription. It has been observed to be heavily consumed among farmers, daily laborers at construction sites and students for various reasons, though a control drug but it's still been sold secretly to the consumers without considering the potential effects of its administration. Adverse reactions of therapeutic use of tramadol include nausea, dizziness, drowsiness, fatigue, headache, increased sweating, vomiting, dry mouth,

constipation diarrhea, and cardiovascular dysregulation (palpitations, tachycardia, and postural hypotension - particularly after rapid intravenous administration. COVID-19 lockdown in Nigeria practically restrict the supply and availability of the above abuse pharmaceutical products with a cobra effect of discovery the backhouse tree in some Nigeria homes and forest- *bambusa vulgaris* leaf; whom users confirmed that it is far much active than most smoking substances commonly sold in markets and other substances already declared illegal and enlisted among illicit drugs and substance addictive by Nigeria Drug Law enforcement Agency).

## 2. MATERIALS AND MATHODS

### 2.1 Study Area

This research was carried out in Animal House of Ebonyi State University Abakaliki, Histopathology Department Federal Teaching Hospital Abakaliki, 161 Nigerian Air Hospital Laboratory Makurdi Benue State and Divine Medical Laboratory Enugu

### 2.2 Sample Size Estimation

Resource Equation method (E) was used for the sample size estimation; the formular:

E = (Total number of animals in a group multiply by number of groups) - Number of groups as recommended by Jaykara and Kantharia (2013) on how to calculate sample size in animal studies

$$= (7 \times 3) - 6, = 21 - 6 = 14$$

### Sample size (E):

**Corrected sample size** = sample size E ÷ (1-% attrition)

In this study 10% attrition is expected hence,

$$\begin{aligned} \text{Corrected sample size} &= 14 / 1 - 10 / 100 \\ &= 14 / 0.9 \\ &= 16 \end{aligned}$$

The above sample size also conformed to the sample size used by Hebert et al; (2009) on similar study.

**Inclusion criteria:** Eligibility of the subjects includes; healthy *Rattus norvegicus*, body weight between 150g to 200 g and must be male *Rattus norvegicus*.

**Exclusive criteria:** Other species of rats except *Rattus norvegicus*, non-healthy *Rattus norvegicus*, body weight below 150g or above 200 g are excluded in this study.

### 2.3 Experimental Animal

Twenty-one (21) *Rattus norvegicus* with average weight of 140 g was procured from the central animal house of the College of Health Sciences of Ebonyi State University, Abakaliki. They were housed in Histopathology laboratory of the University, allowed to acclimatize to the standard laboratory conditions of good ventilation and lighting, moderate temperature and adequate humidity were all adequately cared for before and during the experiment.

### 2.4 Sacrifice and Collection of Samples

The animals was anaesthetized using chloroform vapour in an enclosed transparent plastic jar, blood samples collected through cardiac puncture in to plain containers and thereafter dissected to remove the brain and lungs fixed in 10% Buffered formal saline for histopathological evaluation, parts homogenized and tested for oxidative stress biomarkers and the blood in plain container allow to clot, retracted and serum separated for biochemical analysis and EDTA blood sample send to the laboratory for laboratory analysis.

**Table 1. Experimental design**

Experimental groups	Treatment given	Duration of treatment (Days)
T1 (7 rats)	Distilled water and diet	42
T2 (7 rats)	0.01g of Dried <i>bambusa vulgaris</i> leaf smoked per gram weight of the rat and diet, Twice daily	21
T3 (7 rats)	0.01g of Dried <i>bambusa vulgaris</i> leaf smoked per gram weight of the rat and diet, Twice daily	42

## 2.5 Laboratory Analysis

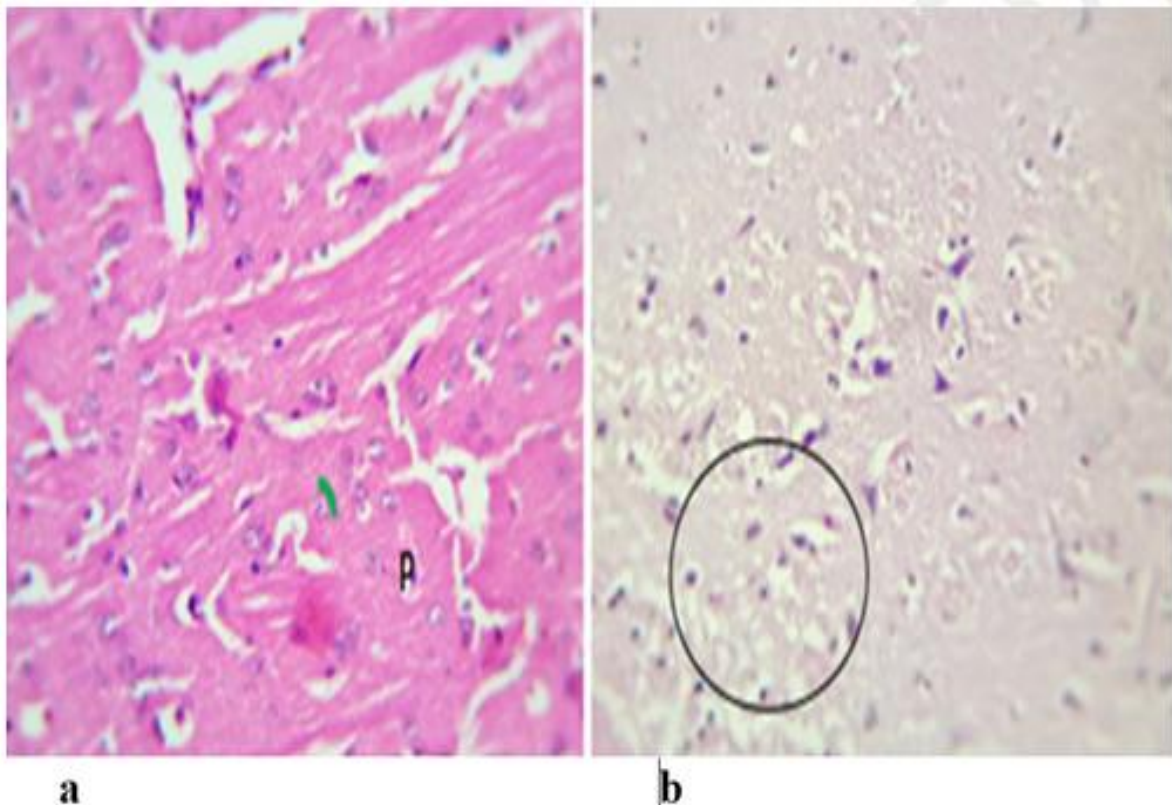
Histology specimens were grossed into triplicates, processing using conventional paraffin wax methods, using automatic Tissue Processor and embed using standard embedding centre, sectioned and stained using both routine (H&E) and special stains; PAS, Haematoxylin Van Gieson Stain, Phosphotungstic acid Haematoxylin, silver stains techniques and photomicrograph at  $\times 40$  Magnification and  $\times 10$  Magnification and oxidative stress biomarkers were done using standard method.

## 3. RESULTS

Plate 1a Mag. X40 is a brain section of *Norvegicus rattus* stained with haematoxylin and Eosin on acute smoked *Bambusa vulgaris* leaf administration; the brain cells are moderately pleomorphic, few astrocytes are degenerated (green arc), and apoptotic cells with pyknotic nuclei (p) are also seen with a focus of cellular lesions and the infiltrating polymorphs. Plate 1b

Mag. X40 is a brain section with same smoked substance with plate 1a but on chronic administration, section stained with periodic acid Schiff. The section shown moderate degeneration of astrocyte with background vacuoles (oval) presenting infarction and degenerated axon skeleton is also evidenced as observed in neuro behavioral experiment.

Plate 2a Mag. X40 is a negative control brain section of *Norvegicus Rattus* with hematoxylin and eosin technique; the section revealed well differentiated neurons with somatic body and dendrites (black arrow), astrocytes (blue triangle) were all well demonstrated with few microglial cells at the background. Plate 2b is a brain tissue section of *Norvegicus Rattus* exposed to smoke *Bambusa vulgaris*, section stained with H&E revealed characteristics inflammatory background, aggregation of oligodendrocyte and microgliosis (green arrow). This revealed relative brain injury as demonstrated by accumulation of brain macrophages, the microglial at the site of injury



Plates 1a and b. Section of *Norvegicus Rattus* brain on acute and chronic administration of smoked *Bambusa vulgaris*

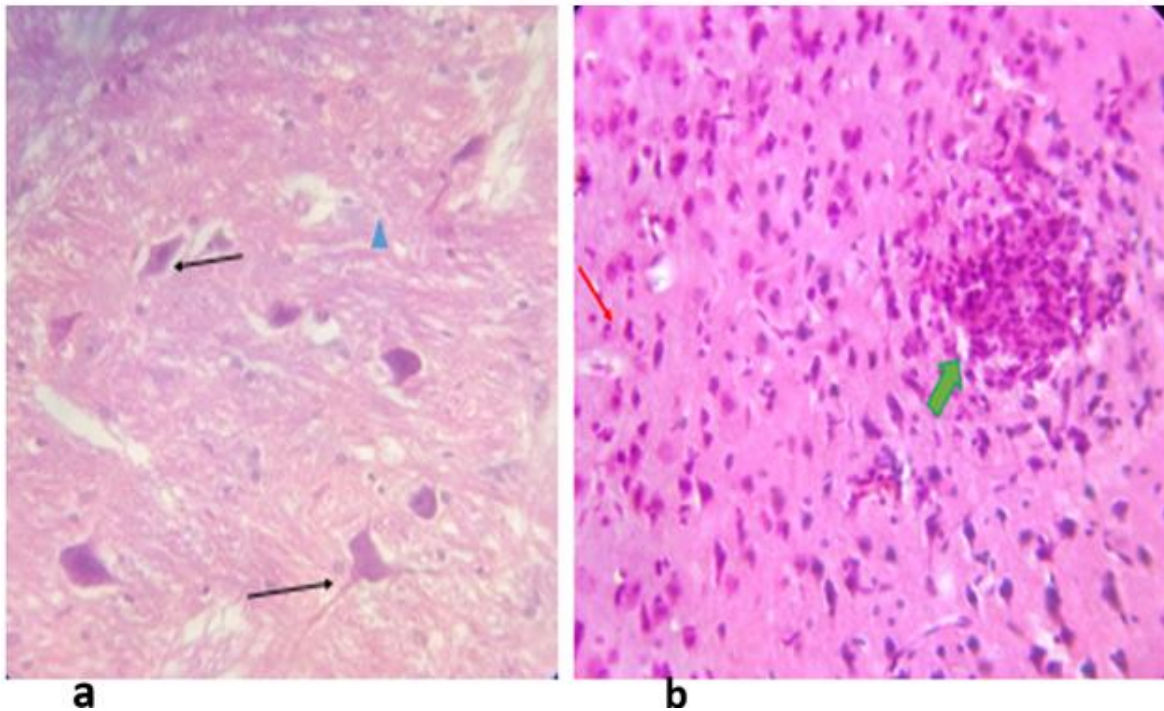


Plate 2a and b. Negative control brain section of *Norvegicus Rattus* with hematoxylin and eosin technique and *Norvegicus Rattus* exposed to smoke *Bambusa vulgaris*

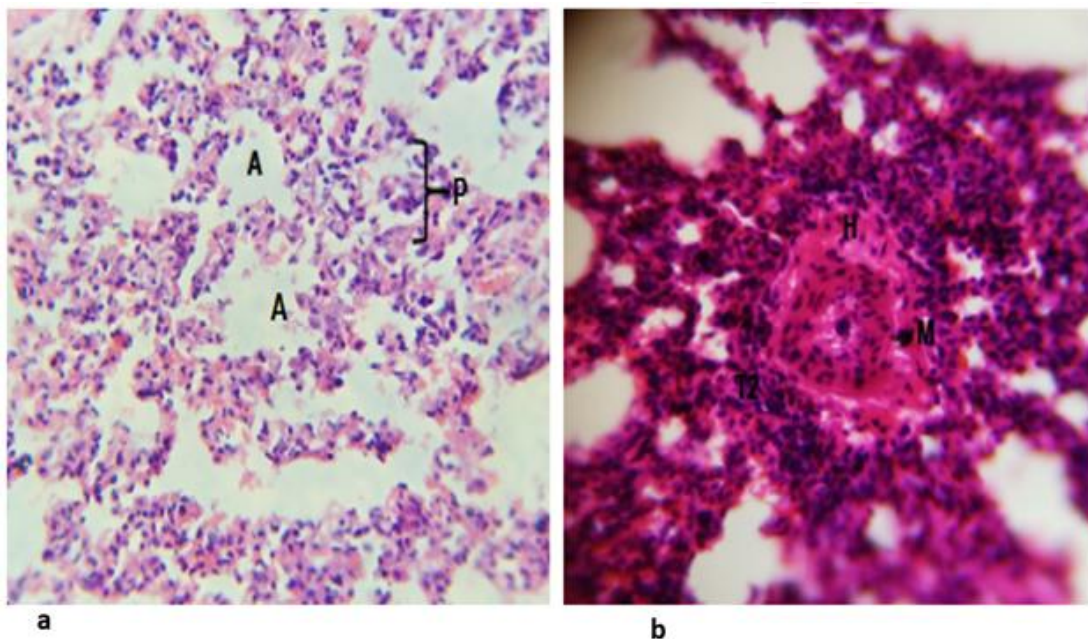


Plate 3a and b is a lung section of *Norvegicus Rattus* on acute administration of smoked *Bambusa vulgaris* and its control

Plate 3a is a negative control lung tissue section of *Norvegicus rattus* stained with haematoxylin and eosin staining technique; pneumocytic cells (P) and air spaces (A) are well demonstrated. All the cellular details appeared normal; Plate 3b is

a lung section of *Norvegicus Rattus* on acute smoke administration of *Bambusa vulgaris*; the section revealed hyaline of the air space (H) with few smokers' macrophages (M) and abundance type II pneumocyte.

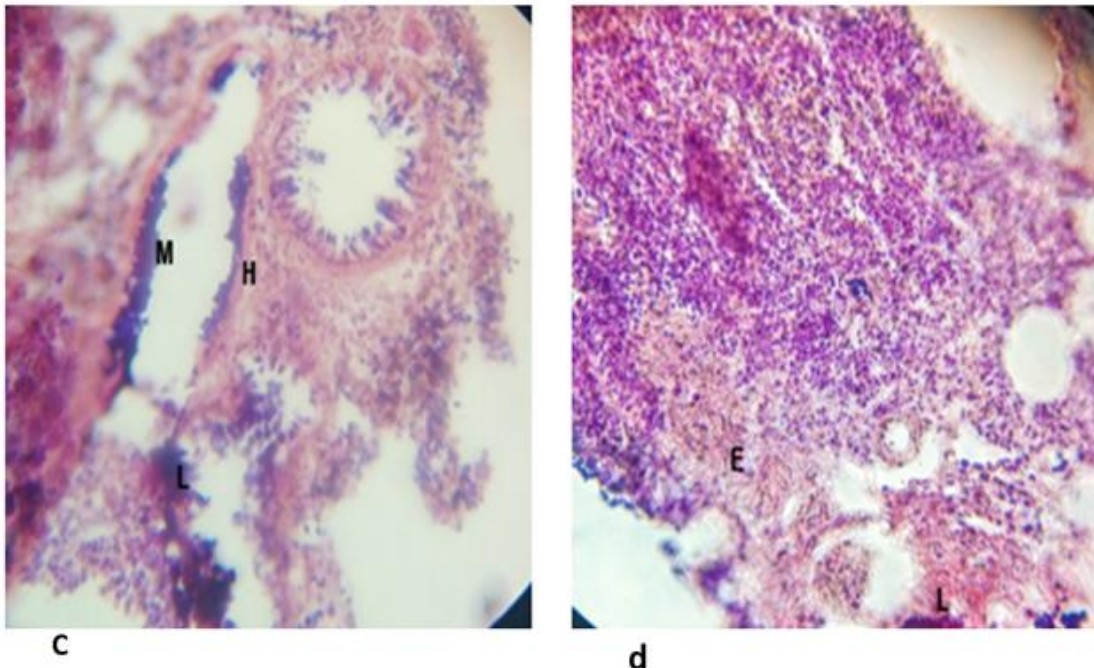


Plate 3c and d is a lung section of *Norvegicus Rattus* on chronic administration of smoked *Bambusa vulgaris*

Plate 3c Mag. X10 is a lung section of *Norvegicus Rattus* on chronic administration of smoked *Bambusa vulgaris* leaf stained with phosphotungstic acid haematoxylin. The section shown hyalinization of the alveoli septa (H), occlusion of alveoli space with smokers' macrophages (M) and progressive lesion of the septa (L). Plate 3d Mag. X10 is lung section from *Norvegicus rattus* on chronic administration of smoked *Bambusa Vulgaris* leaf; shown includes

edematous (E) and inflammatory lesion (L) infiltrated with polymorphs within the air space

#### 4.1 Effects of Smoked *Bambusa Vulgaris* (bamboo) Leaf on the Body Weight in Rat

Administration of smoked *bambusa vulgaris* leaf in rats significantly ( $p < 0.05$ ) reduced the body weight of the experimental rats (Fig. 1).

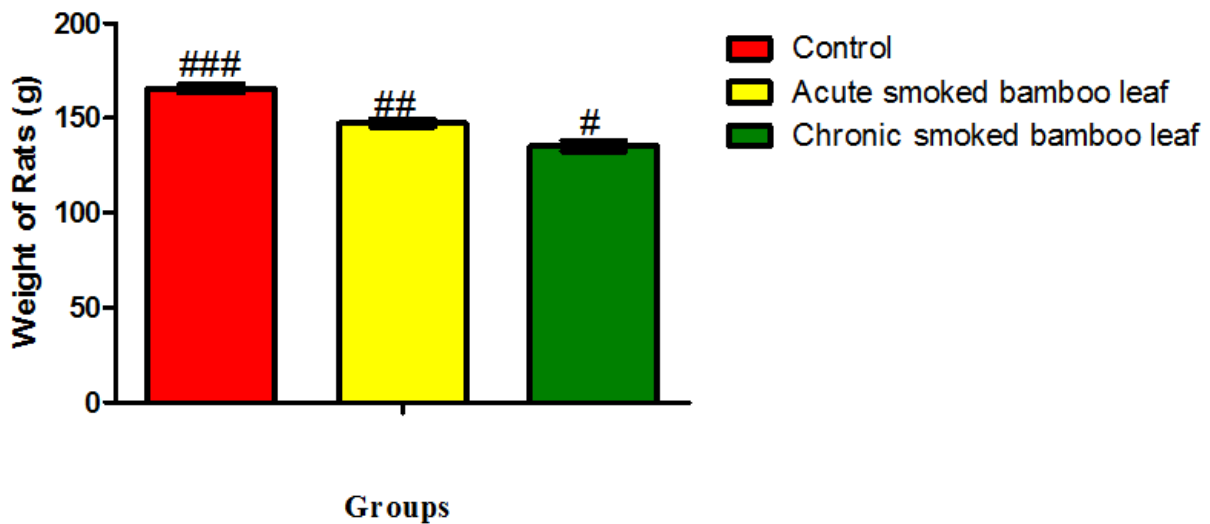


Fig. 1. Effects of smoked *Bambusa vulgaris* leaf on the body weight of rats. Mean values with different sign are significantly different at  $p < 0.05$

#### 4.2 Effects of Smoked *Bambusa vulgaris* Leaf on Oxidative Stress Markers in Rat Lung

Exposure of smoked *bambusa vulgaris* leaf in rats significantly ( $p < 0.05$ ) elevated the level of MDA and significantly ( $p < 0.05$ ) reduced the activities of catalase, SOD, GPx and level of GSH in rat lung in time dependent manner as shown in (Fig. 2).

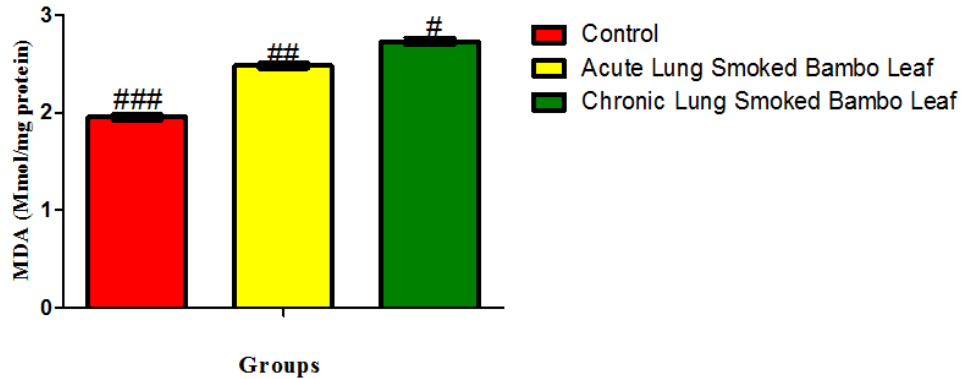


Fig. 2a. Effects of smoked *Bambusa vulgaris* leaf on lung MDA Level in Rats. Mean values with different sign are significantly different at  $p < 0.05$

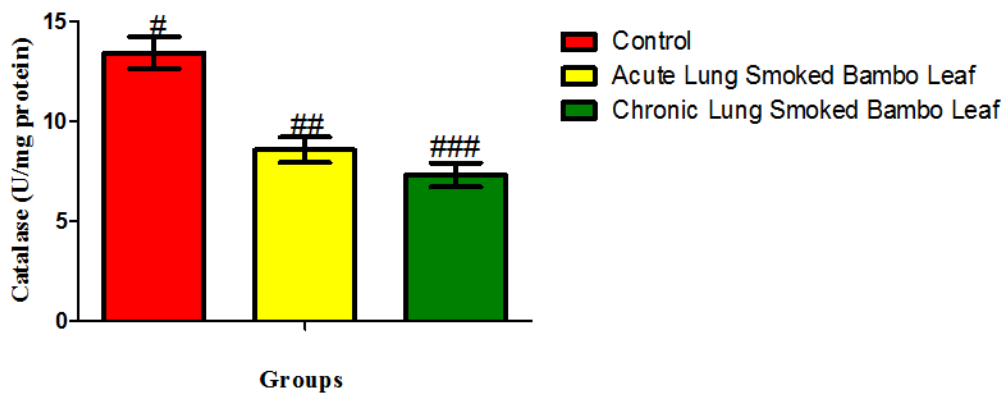


Fig. 2b. Effects of smoked *Bambusa vulgaris* leaf on lung catalase activity in rats. Mean values with different sign are significantly different at  $p < 0.05$

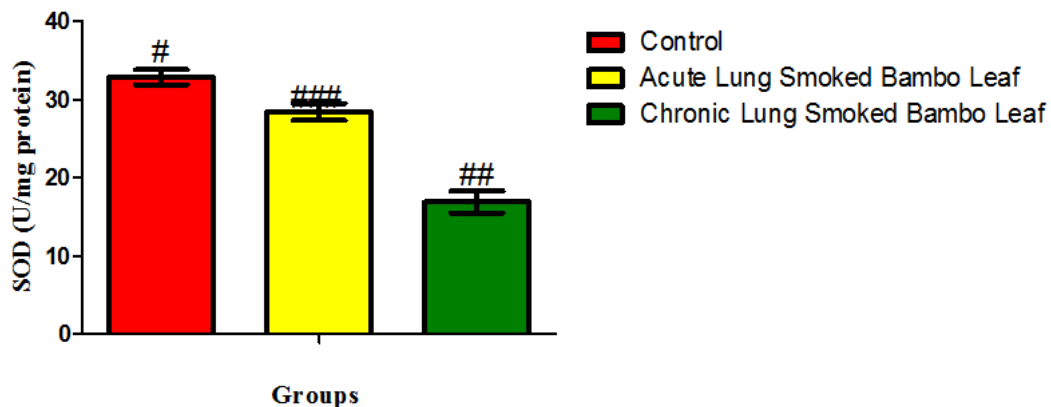


Fig. 2c. Effects of smoked *Bambusa vulgaris* leaf on lung SOD Activity in Rats. Mean values with different sign are significantly different at  $P < 0.05$

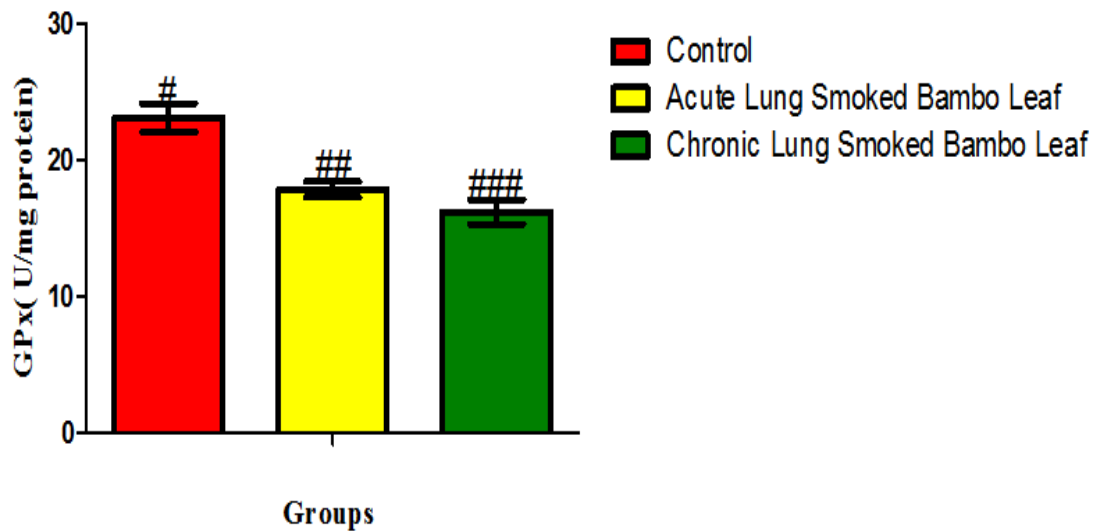


Fig. 2d. Effects of smoked *Bambusa vulgaris* o leaf on lung GPx Activity Level in Rats. Mean values with different sign are significantly different at P<0.05

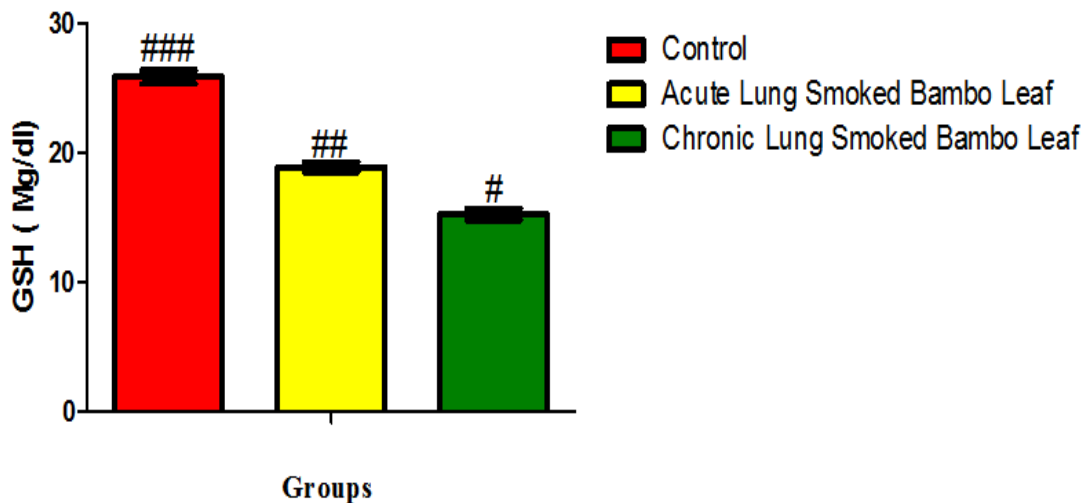


Fig. 2e. Effects of smoked *bambusa vulgaris* leaf on Lung GSH Level in Rats. Mean values with different sign are significantly different at P<0.05

## 5. DISCUSSION

Plate 1a Mag. X40 is a brain section of *Norvegicus rattus* stained with haematoxylin and Eosin on acute smoked dry *Bambusa vulgaris* leaf administration; the brain cells were moderately pleomorphic, few astrocytes were degenerated (green arc), and apoptotic cells with pyknotic nuclei (p) were also seen with a focus of cellular lesions and the infiltrating polymorphs. Plate 1b Mag. X40 is a brain section with same smoked substance with plate 1a but on chronic administration, section stained with periodic acid Schiff. The section shown moderate

degeneration of astrocyte with background vacuoles (oval) presenting infarction and degenerated axon skeleton is also evidenced as observed in neuro behavioral experiment; these confirmed the position of Danian et al. [7] on a similar report concerning the effect of psychotic substances and other drug abuses. Plate 2b shown lung section of *Norvegicus Rattus* on acute smoke administration of *Bambusa vulgaris*; the section revealed hylination of the air space (H) with few smokers' macrophages (M) and abundance type II pneumocyte. Plate 2c Mag. X10 is a lung section of *Norvegicus Rattus* on chronic administration of smoked *Bambusa*



*vulgaris* leaf stained with phosphotungstic acid haematoxylin. The section shown hyalinization of the alveoli septa (H), occlusion of alveoli space with smokers' macrophages (M) and progressive lesion of the septa (L). while Plate 2d Mag. X10 is lung section from *Norvegicus rattus* on chronic administration of smoked dry *Bambusa Vulgaris* leaf; among the pathological changes observed includes edematous (E) and inflammatory lesion (L) infiltrated with polymorphs within the air space; this report on smoking conformed with other researchers' reports. Emeka et al. [8] and Dubili [9] reports on effects of smoke substances on the lungs affirmed the consistence of this research work. Plate 3a Mag. X40 is a negative control brain section of *Norvegicus Rattus* with hematoxylin and eosin technique; the section revealed well differentiated neurons with somatic body and dendrites (black arrow), astrocytes (blue triangle) were all well demonstrated with few microglial cells at the background. Exposure of smoked *bambusa vulgaris* leaf in rats significantly ( $p<0.05$ ), elevated level of MDA and significantly ( $p<0.05$ ) reduced the activities of catalase, SOD, GPx and level of GSH in *rattus Norvegicus* lung and brain in time dependent manner as shown in, has proven the smoke induced production of free radicals which is the major cause of over production of MDA; a stress biomarker for even cancer and the administration of all the substances both in acute and chronic reduces the production of antioxidant stress biomarkers in time dependent manner [10].

## 6. CONCLUSION

The smoking of the novel dry *Bambusa vulgaris* leaf was noticed more during the covid-19 lockdown in Nigeria, where people were not allowed to move from one place to the other, especially those that are already addicted to illicit drugs indulges more on the use of *Bambusa vulgaris* leaf smoking and this study has demonstrated its dangerous effect on both brain and lungs. It also expresses elevation in production of free radicals which in no doubt is a precursor to all kind of cancers

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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