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Phytochemical Screening of Tobacco (*Nicotiana tabacum*) and Its Effects on Some Haematological Parameters and Histopathology of Liver and Brain in Male Rats

Okere O. Shekins[1], Ejike Uju Dorathy[1*], Mubarak Liman Labaran[2] and Paul Joel[1]

[1]Department of Biochemistry, Bingham University, Karu, Nigeria.
[2]School of Apply Science, Nuhu Bamali Polytechnic, Zaria, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author OOS designed the study, wrote the protocol and supervised the work. Author PJ carried out all laboratories work and performed the statistical analysis. Author MLL managed the analyses of the study. Author EUD wrote the first draft of the manuscript, managed the literature searches and edited the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

This study investigated phytochemical constituents of *Nicotiana tabacum* (tobacco leaves), its effects on haematological parameters and its histopathology of brain and liver in albino rats. Eighteen (18) male albino rats were divided into three (3) groups with 6 rats in each group. Group A was administered 10 mg/kg body weight of aqueous extracts of tobacco, group B was administered 10 mg/kg body weight of methanol extracts of tobacco and group C was used as positive control. Two (2) rats from each group were sacrificed after every 7 days of administration for 21 days. There was a significant ($p<0.05$) decrease in rats' body weight, red blood cell (RBC), hematocrite (HCT or PCV), and haemoglobin (HGB) for aqueous extracts (7.45 ± 0.93, 14.90 ± 1.89, 44.70 ±5.60 RBC, HCT and HGB respectively). Methanolic extracts of tobacco significantly decreased the

**Corresponding author: E-mail: uju220@gmail.com;*

value of RBC, HCT, and HGB (6.63 ± 1.04, 13.27 ± 2.07 and 39.8 ± 6.27 respectively) when compared with the control group (7.72 ± 0.57, 15.43 ± 1.14 and 46.30 ± 3.43 respectivel) but no significant ($p>0.05$) increase in mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) for both extracts. The histopathological section of brain and liver were normal for the control group while neuronal, liver degeneration, acute necrosis and cirrhosis were observed in aqueous and methanolic extract groups. Tobacco has nutritional and medicinal potentials as seen from the phytochemical constituent results but long-time consumption of tobacco may lead to anaemia as a result of its high alkaloids or nicotinic contents which decreased the values of RBC, HCT, and HGB. The overall effect of methanolic extract of tobacco was greater than the aqueous extract suggesting that methanol is a better solvent for extraction of tobacco leaves than water.

Keywords: Phytoconstituent; tobacco (Nicotiana tabacum); haematology and histopathology.

1. INTRODUCTION

Tobacco is the dried and processed leaves of the plant *Nicotiana tabaccum* that is widely cultivated and commercially grown in many countries of the world. It is mostly consumed in the form of smoking, chewing, snuffing or dipping tobacco [1]. Its usage is an activity that is practiced by some 1.1 billion people, and up to one third of the adult population [1]. World Health Organization (WHO) describes tobacco use as the single most important preventable risk to human health in developed countries and an important cause of premature death worldwide [2]. One of the forms of smokeless tobacco is snuff. Snuff is a form of tobacco that is processed to grains and packaged either in cains or pouches. Its users take a pinch and place it between the lower lip or cheek and gum and suck on it [3]. Another route for the use of tobacco is by snuffing, i.e. nasal use which is common among Nigerians. People in many regions and Countries, including North America, Northern Europe, and India and other Asian countries, and part of Africa have a long history of using smokeless tobacco products. In Nigeria, tobacco snuff is mostly utilized for cultural and traditional purposes which are either inhaled through nose or being applied orally while some addicts also chew the dried leaves. Tobacco is used as pesticide and in the form of nicotine tartrate, it is used in medicines [4,5]. It is most commonly used as a drug, and is a valuable cash crop for countries such as Cuba, India, China, and the United States.

The frequency and speed of tobacco consumption and the effects which it has on the body is directly related to nicotine, the most prominent phytochemicals found in tobacco. All tobacco products, including smokeless tobacco, contain nicotine, which is addictive [6]. The amount of nicotine per gram of tobacco rang ed from 4.4 to 25 milligrams [7]. Other studies have shown that moist snuff had between 4.7 and 24.3 milligrams, dry snuff had between 10.5 and 24.8 milligrams per gram of tobacco. Nicotine stays in the blood longer for users of smokeless tobacco than for smokers [8]. The level of nicotine in the blood depends on the amount of nicotine in the smokeless tobacco products, the tobacco cut-size the products pH (a measure of its acidity or bacicity) and other factors [9].

Aside tobacco uses, it also have some negative effects such as excess saliva production, bad breath, patches and lumps in mouth or neck, etc. Therefore, this work was designed to determine the nutritional as well as its negative effects in rat's blood and organs.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Methanol purchased from Sigma chemical Co, Ltd (USA) and are of analytical grades.

2.2 Plant Collection and Authentication

The leaves of tobacco (*Nicotiana. tabacum*), were obtained from Kukui village, Kagarko local Government, Kaduna state, Nigeria, and was identified in the herbarium unit of Biological Science Department, Bingham University, Karu, Nigeria.

2.3 Sample Preparation and Extraction

The tobacco leaves were air-dried at room temperature for two weeks in the Biochemistry laboratory and pulverized into powder form using laboratory mortar and pestle. Pulverized material

(100 g) was placed in the thimble of soxhlex extractor and extracted using methanol (1000 mL) 5 hours each. The methanol extracts were dried *in vacuo* at 64.7℃ using a rotary evaporator (Buchi Labortechnik AG, Switzerland). Aqueous extract of the pulverized tobacco sample was gotten by soaking 100 grams in 500ml of distilled water with occasional swirling and then sieved using a white handkerchief after 24 hrs of soaking. The filtrate was centrifuged and decanted, filtered using a watman1 filter paper. The chaff was soaked again in another 500 ml of distilled water and processed like the first one. The filtrate obtained was evaporated to dryness using water bath at 100℃ [10].

2.4 Experimental Animals and Management

Male albino rats (7-8 weeks old and weighing about 120-150 kg) were purchased from the animal house of National Veterinary Institute Vom Jos Plateau State, Nigeria. They were acclimatized for two weeks prior to commencement of experiment. They were kept at room temperature and were maintained *ad libitum* on tap water and growers mash (Vita feeds, Jos, Plateau State Nigeria). They were weighed prior to commencement and termination of the experiment.

2.5 Phytochemical Analysis

Test for phenols, carbohydrate and free reducing sugar was according to Sofowara [11]. Terpenoids, saponins and steroids was as described by Edeoga [12]. Flavonoids and alkaloids test were carried out as described by Harborne [13]. Glycosides was done according to Parekh [14]. Tannin was as described by Kumar [15], while starch was as described by Ganesan [16].

2.6 Administration Method of Plant Extracts

Rats were divided into 3 groups of six rats each. Group A was the control group, Group B was administered aquous extract, while Group C was administered methanol extract. The extracts were administered at a dose of 10 mg in 0.5 mL/kg body weight orally and intraperitonially for aqueous and methanol extracts respectively. The extracts administration lasted for 21 days. 2 rats from each group were weighed and sacrificed at interval of 7 days until the 21st day.

2.7 Collection and Storage of Blood and Tissues

The blood was collected through intraocular method of blood collection and stored inside tri-potasium ethlenediamine tetra-acetic acid (K_3EDTA) bottles, while the hearts, livers and kidneys were stored in formal saline and the brains were stored in formal calcium for histopathological assay.

2.8 Full Blood Count (FBC)

Blood samples were collected from all the experimental rats into (k_3EDTA) anticoagulant bottle, mixed by gentle inversion for complete blood count (CBC) analysis using haematology auto analyser sysmex. The automated analysis was done following the manufacturer's operational guidelines using automated sysmex XE-2100 haematology analyzer.

2.9 Histopatological Examination

This was as described by Lamb [17]. The organ pieces (3 - 5 micrometers thick) was fixed in 10% formalin for 24 h and washed in running water for 24 h. Samples were dehydrated in an autotechnicon and then cleared in benzene. To remove absolute embedding was done by passing the cleared samples through three cups containing molten paraffin at 50℃ and then in a cubical block of paraffin made by the “L” moulds, followed by microtome. The specimens were then stained with Haematoxylin and Eosin. The H&E stained specimens were examined by a pathologist to histopathologically classify the organ as described by [17].

3. RESULTS

Phytochemical screening revealed the presence of alkaloids, flavonoids, glycosides, reducing sugars, starch and steroids, phenols and tannins in the methanol extract. There were no phenol and starch in the aqueous extract, while there was only trace of reducing sugar (Table 1).

The hematology result shows significant ($P<0.05$) low level of RBC, HGB and HCT in the group treated with the methanol extract when compared with the control and the aqueous extract treated. There were no significant difference ($P>0.05$) in the levels of MCV, MCH and MCHC in all the groups (Table 2).

Table 1. Phytochemical result for aqueous and methanolic extracts of tobacco

Class of compounds	Methanolic extracts	Aqueous extracts
Alkaloids	+++	+
Steroids	+++	+
Phenols	++	-
Glycosides	+++	+
Flavonoids	+++	+
Tannins	++	+
Reducing sugars	++	±
Starch	+	-

Key: Positive (+), Strong positive (++), very strong positive (+++), Trace (±), Negative (-)

Table 2. Haematology results for experimental groups

Parameters	Control GRP (Mean ±SD)	Aqueous (Mean ± SD)	Methanol (Mean ± SD)
RBC (10^6/µL)	7.72 ± 0.57	7.45 ± 0.93	6.63 ± 1.04*
HGB (g/dl)	15.43 ± 1.14	14.90 ± 1.89	13.27 ± 2.07*
HCT (%)	46.30 ± 3.43	44.70 ±5.60	39.8 ± 6.27*
MCV (fl)	59.98 ± 0.02	60.03 ± 0.03	60.01 ± 0.03
MCH (pg)	19.20 ± 0.01	20.01 ± 0.01	20.00 ± 0.01
MCHC (g/dl)	33.33 ± 0.00	33.34 ± 0.01	33.33 ± 0.01

*Mean ± SD of five determinations; * represents P<0.05 significantly different*

Photomicrogragh of histopathological section of liver in the control group indicating normal liver cells: PV=portal vein, S=sinusoid, C=cords and CV=central vein (Fig. 1). Photomicrograph of histopathological section of liver in the group treated with 10 mg/kg rat body weight of aqueous extracts of *Nicotiana tabacum* after 7 days indicated: TN=tissue necrosis, KC=kupffer (Fig. 2). Fig. 3 shows the photomicrograph of histopathological section of liver in the group treated with 10 mg/kg rat body weight of aqueous extracts of *Nicotiana tabacum* after 14days indicated: TD=tissue degeneration, AH=acute hepatic necrosis, KC=kupffer. Fig. 4 shows the photomicrograph of histopathological section of liver in the group treated with 10 mg/kg rat body weight of aqueous extracts of *Nicotiana tabacum* after 21 days indicated: AHN=acute hepatic necrosis, TD=tissue degeneration, CVI=central vein inflammation. Photomicrograph of histopathological section of liver in the group treated with 10 mg/kg rat body weight of methanolic extracts of *Nicotiana tabacum* after 7 days indicated: HD=hepatocellular degeneration and AHN= (Fig. 5). Photomicrograph of histopathological section of liver in the group treated with 10 mg/kg rat body weight of methanolic extracts of *Nicotiana tabacum* after 14days indicated: HD=hepatocellular degeneration and AHN= acute hepatocellular necrosis and CVI= central vein inflammation Figs. 6 and 7 shows the photomicrograph of histopathological section of liver in the group treated with 10 mg/kg rat body weight of methanolic extracts of *Nicotiana tabacum* after 21 days indicated: HD=hepatocellular degeneration and AHN= acute hepatocellular.

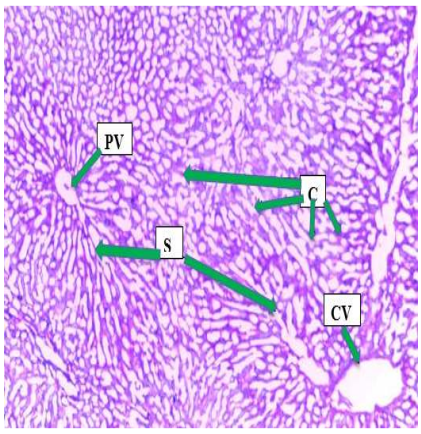

Fig. 1. Section of liver in the control group

Fig. 8 shows the photomicrogragh of histopathological section of brain in the control group indicating normal neuronal cells: PY= pyramidal cell, P = p erivascular space, V=vacuole and NC = neuronal cells. Photomicrograph of histopathological section of brain in the group treated with 10 mg/kg rat body weight of aqueous extracts of *Nicotiana tabacum* after 7 days indicated: ND=neuronal (Fig. 9). Photomicrograph of histopathological section of brain in the group treated with 10 mg/kg rat body

weight of aqueous extracts of *Nicotiana tabacum* after 14 days indicated: ND=neuronal degeneration (Fig. 10). Fig. 11 shows the photomicrograph of histopathological section of brain in the group treated with 10 mg/kg rat body weight of aqueous extracts of *Nicotiana tabacum* after 21 days indicated: H = hemorrhage, ND = neuronal. Fig. 12 shows the photomicrograph of histopathological section of brain in the group treated with 10 mg/kg rat body weight of methanolic extracts of *Nicotiana tabacum* after 7 days indicated: ND=neuronal degeneration. Photomicrograph of histopathological section of brain in the group treated with 10 mg/kg rat body weight of methanolic extracts of *Nicotiana tabacum* after 14 days indicated: ND=neuronal degeneration and N=necrosis (Fig. 13) and the photomicrograph of histopathological section of brain in the group treated with 10 mg/kg rat body weight of methanolic extracts of *Nicotiana tabacum* after 21 days indicated: ND=neuronal degeneration and N=necrosis (Fig. 14).

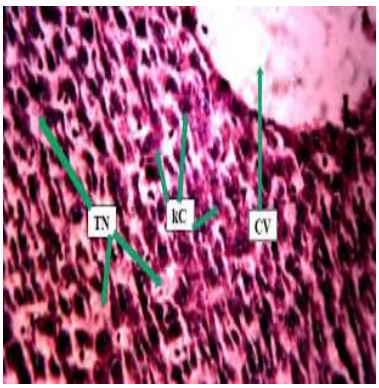

Fig. 2. Liver section in the group treated with 10 mg/kg of aqueous extracts of *Nicotiana tabacum* after 7 days

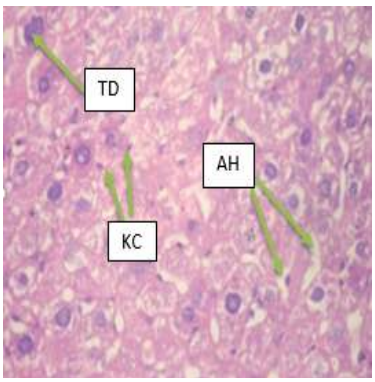

Fig. 3. Section of liver in the group treated with 10 mg/kg rat body weight of aqueous extracts of *Nicotiana tabacum* after 14 days

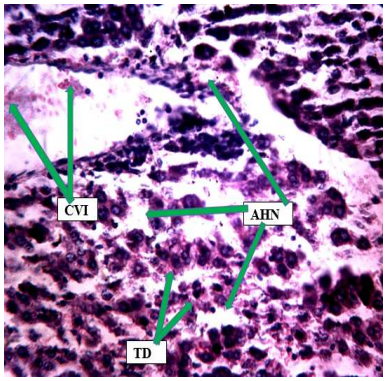

Fig. 4. Liver section in the group treated with 10 mg/kg rat body weight of aqueous extracts of *Nicotiana tabacum* after 21 days

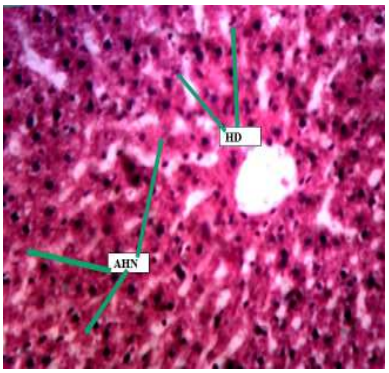

Fig. 5. Liver section in the group treated with 10 mg/kg rat body weight of methanolic extracts of *Nicotiana tabacum* after 7 days

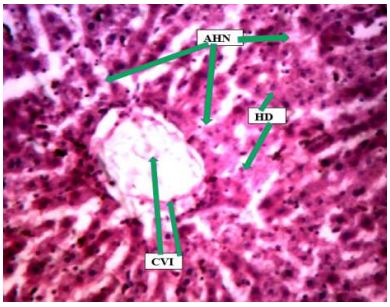

Fig. 6. Liver section in the group treated with 10 mg/kg rat body weight of methanolic extracts of *Nicotiana tabacum* after 14 days

4. DISCUSSION

The phytochemical screening of *Nicotiana tabacum* shows the presence of high concentration of alkaloid, phenols, glycoside, flavonoid, tannins, reducing sugar and starch for methanolic extract compared to the aqueous

extract that gave little result. Hematology results indicated that the aqueous and methanolic extracts of *Nicotiana tabacum* decreased RBC, HGB and HCT but increased MCV, MCH and MCHC when compared with the control group. The effects of methanolic extracts on RBC, HGB and HCT has greater decrease when compared to the aqueous extracts but no significant difference on MCV, MCH and MCHC as seen in Table 2. These results tallied with the work of Fafioye [18]. The results also showed decrease in the value of RBC, HGB and HCT for aqueous extracts at the duration of treatment from week1 to week 2 then to week 3 but increase the value of RBC, HGB and HCT for methonolic extracts during the weeks of treatment. Despite the increment from week1 to week2 and then to week3, the increase is without significance as compared with aqueous group. The overall effects on the duration of weekly treatment showed increase in RBC, HGB and HCT but no significant different on MCV, MCH and MCHC, but when compared with the control it showed a significant decrease. These results also tallied with the work of [18].

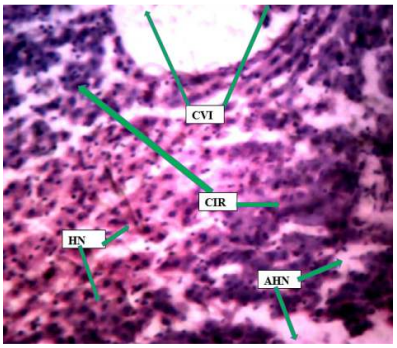

Fig. 7. Liver section in the group treated with 10 mg/kg rat body weight of methanolic extracts of *Nicotiana tabacum* after 21 days

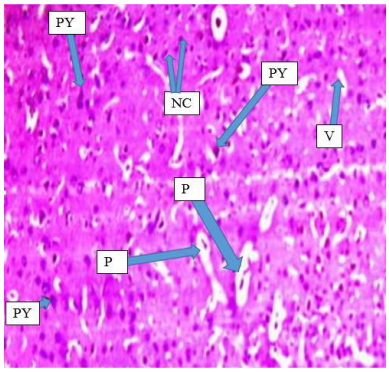

Fig. 8. Section of brain in the control group

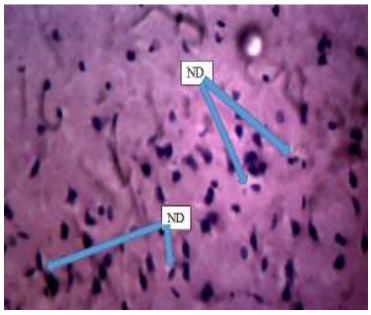

Fig. 9. Brain section in the group treated with 10 mg/kg rat body weight of aqueous extracts of *Nicotiana tabacum* after 7 days

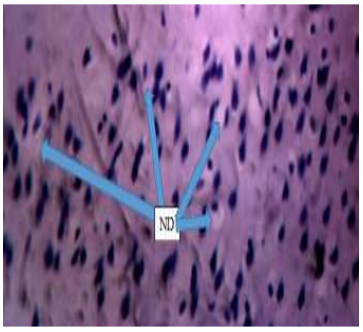

Fig. 10. Brain section in the group treated with 10 mg/kg rat body weight of aqueous extracts of *Nicotiana tabacum* after 14 days

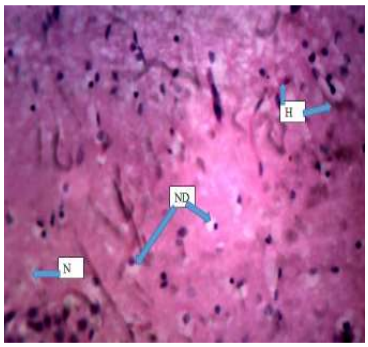

Fig. 11. Brain section in the group treated with 10 mg/kg rat body weight of aqueous extracts of *Nicotiana tabacum* after 21 days

The histopathology results showed progressive degeneration of liver cells and necrosis for both aqueous and methanolic extracts of *Nicotiana tabacum* from week 1 to week 3. But the effects of methanolic extracts of *Nicotiana tabacum* on the liver cells are greater than that of aqueous extracts of *Nicotiana tabacum.* This may be as a result of high alkaloid (nicotinic content) of tobacco leaves. The results also showed

progressive degeneration of neuronal cells and necrosis. The effects of methanolic extracts of *Nicotiana tabacum* on neuronal cells is greater than the aqueous extracts.The section of the liver and brain obtained from the treated group has disrupted histological organization compared with the control group. Some of the deleterious effects seen in the section of the liver and brain obtained from the treated group following the use of aqueous and methanolic extracts include: degeneration and necrosis of the hepatocytes and the neuronal cells as well as the degeneration of the cells lining the bile ducts. With these histological abnormalities, the functional integrity of the liver and brain could be compromised. It is known that the hepatocytes play a vital role in the proper functioning of the liver as the hepatocytes are the main functional cells of the liver. The hepatocytes frequently contain glycogen and the hepatocytes maintain a steady level of blood glucose. This is one of the main sources of energy for the body [19]. A compromise in the integrity of the hepatocytes could lead to improper functioning of the liver. This study corresponds with the findings of Adekomi [19] and Adeniyi [20]. Also, Durazzo [21] states that chronic smoking is related to global brain atrophy and to structural and biochemical abnormalities in anterior frontal regions, subcortical nuclei and commissural white matter. Chronic smoking may also be associated with an increased risk for various forms of neurodegenerative diseases. Recent research indicates chronic cigarette smoking is associated with increased risk for numerous biomedical conditions that may directly or indirectly compromise brain neurobiologyand neurocognition [22-25].

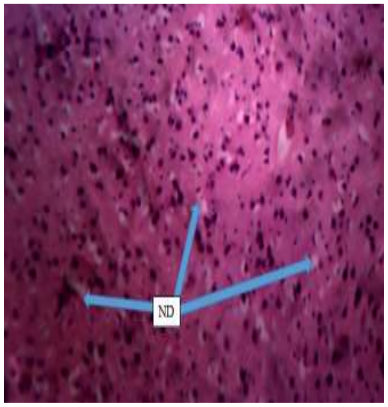

Fig. 12. Brain section in the group treated with 10 mg/kg rat body weight of methanolic extracts of *Nicotiana tabacum* after 7 days

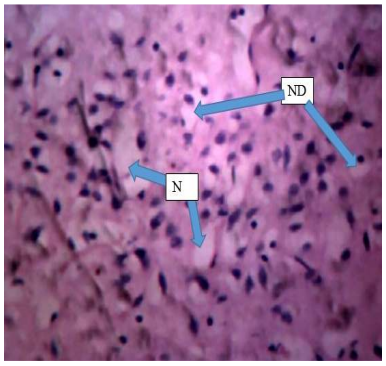

Fig. 13. Brain section in the group treated with 10 mg/kg rat body weight of methanolic extracts of *Nicotiana tabacum* after 14 days

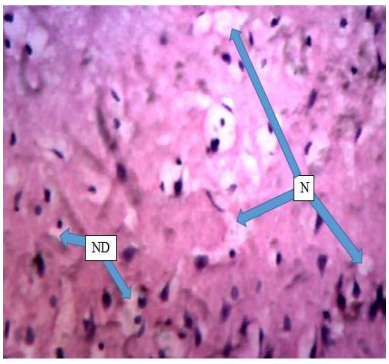

Fig. 14. Brain section in the group treated with 10 mg/kg rat body weight of methanolic extracts of *Nicotiana tabacum* after 21 days

5. CONCLUSION

The results obtained from phytochemical analysis indicated that the leaves of *Nicotiana tabacum* (tobacco) has nutritional and medicinal potentials. Despite its nutritional potentials, it is found to have some negative effects on hematological parameters by decreasing RBC, HGB, and HCT. Decrease in RBC, HGB, and HCT below normal range may result to anemia which is unhealthy to individuals. Tobacco also showed tissue and neuronal degeneration, inflammation, cirrhosis and necrosis. The effects of methanolic extracts is greater than aqueous extracts. From this research, it is shown that tobacco can do more harm than good.

6. RECOMMENDATION

Government should create thorough awareness to the general public through Newspapers and medias, also send educators to the rural areas to

enlighten them about the effects of tobacco on human health and therefore advice them to stay away from it.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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