RESEARCH ARTICLE

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Aqueous leaf extracts of Tobacco Plant (*Nicotiana tabaccum*) Causes Hepatotoxicity in male Wistar albino rats

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ABSTRACT
Many medicinal plants use traditionally have been in use by man without the actual knowledge of their toxic potential. One of such plants used is tobacco plant leave (*Nicotiana tabacum*), which is chewed in order to relax the smoker. This work is aimed at determining the biochemical effects of aqueous leaves extracts of Tobacco plant (*Nicotiana tabacum*) in some liver indices in Wistar albino rats. Four groups of animals were studied. The Wistar rats were divided into four groups. Group 1 was treated with normal saline orally, group 2, 3 and 4 were treated with 0.5mL of the extracts, 200 mg/kg, 400 mg/kg and 600 mg/kg respectively. The animals were by a drop of proparicaine in each eye to minimise discomfort, blood sample was collected through Retro-orbital blood collection method 24 h after the last treatment, then alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) activities and bilirubin levels were assayed. The result obtained showed a significant ($P<0.05$) increase in the ALT, AST and ALP activity in the treated group when compared with the control. Also, bilirubin (total and direct) showed a significant ($P<0.05$) increase in concentration with the total bilirubin having the highest increase in the activity. The present results indicate that the aqueous leaves extracts of Tobacco plant (*Nicotiana tabacum*) might not be safe for human consumptions as they are associated with liver toxicity.

Keywords: *Nicotiana tabacum*, Hepatotoxicity, medicinal plants, liver indices

INTRODUCTION

In recent years, medicinal plants occupy an important position for being the paramount sources of the discovery of pharmacologically active compounds[1]. Many medicinal plants use traditionally have been in use by man without the actual knowledge of their toxic potential. In Nasarawa and other parts of Nigeria, one such plants used is tobacco plant leaves (*Nicotiana tabacum*), are used in two major forms which is the smoked and the smokeless, the tobacco snuff and chewing tobacco[2], used as an organic pesticide and in the form nicotine tartrate in medicine[3]. Tobacco snuff is the powdered form blended with potash as the main additive in Nigeria[4] and has been recommended as a substitution for nicotine in cigarette since it is devoid of hazardous elements such as tar and carbon monoxide[5]. Large number of people believes that using smokeless tobacco is safer than smoking it[3]. This however, is not true because smokeless tobacco can induce addiction to nicotine and leukoplakia[6,7].

Nicotiana tabacum (Family Solanaceae), is a small perennial herbaceous plant with long leaves, flowers and seeds that is the most commonly grown of all plants in the Nicotiana genus. It is widely grown in Northern Nigeria and Sudan. The leaves of the plants are commercially used and processed into tobacco products[8].

Tobacco has many constituents, but nicotine is singled out as having the highest concentration and most immediate pharmacological action[9]. Nicotine is extremely toxic, about as toxic cyanide, and only 60 mg are needed to kill humans but while smoking tobacco, it contain a small portion of nicotine which the body metabolize to a non-toxic substance[10]. Nicotine has major central nervous system (CNS) stimulant, though these effects are not as intense as what is observed with cocaine and amphetamines but nicotine enhance effect on alertness, learning, and memory[9]. Unlike many other plants Solanaceae family, do not contain tropane alkaloids, which are reported to be poisonous to humans and other animals[11]. However, other compounds such as germacrene, anabasine and other piperidine alkaloids (varying between species), which are powerful enough to deter most herbivores[12]. A number of such animals have developed the ability to feed on Nicotiana species without being harmed[11]. Nonetheless, tobacco is unpalatable to many species, and therefore some tobacco plants such as *Nicotiana glauca* have become established as invasive weeds in some species[11]. Tobacco either smoked or smokeless contains nicotine

and other metabolites such as tabacinine, tabacine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone), 2-Naphylamine, 4-methyl-nitrosamino)-4-(3-pyridyl)-butanal (NNA), 2,3,6-Trimethyl-1,4-naphthoquinone,2-Methylquinone, N-nitrosonornicotine, Cadmium (Cd), mercury (Hg), Propionic acid, Anatabine, Nicotelline, Choline, Pyrene, Cembrene and Choline which has been implicated with tobacco associated cancers and diseases[13,14,15,16,17,18,19]. These effects may account for part of nicotine reinforcing effects in humans. Nicotine affects cardiovascular system through its an autonomic effect[20].

The stimulation of the heart and its resultant increased demand for oxygen underlie the association of nicotine and disease resulted. Moreover, when there is a failure to deliver an adequate supply of oxygen to the heart, it may result in chest pain (angina) or a heart attack[21] due to stimulation of sympathetic ganglia and adrenal medulla combined with discharge of catecholamines[11]. One of the famous acute effects of nicotine is its relationship to lower body weight[22]. In this regard, nicotine decrease appetite for sweet foods and increase the amount of energy the body uses both while resting and exercising[23]. These features of nicotine helps to explain the research which show that smokers tend to weigh less compare to non-smokers. On the other hand, quitting from smoking is associated with weight gain[24]. Tobacco has a long history of use by traditional medical practitioners as a relaxant, but because of its highly addictive property, it is seldom employed internally to treat depression and heart diseases or externally to treat rheumatic swelling, skin diseases and scorpion stings[11].

However, every drug is associated with hepatotoxicity almost certainly due to its ability to generate free radicals and to cause disturbance in hepatocyte biochemistry[25]. However, the increasing demand and consumption of Nicotine, either smoked or chewed has highlighted researchers to assess its toxicological effect in the liver since the liver is responsible for the detoxification of foreign substances.

Materials and Methods

Plant Material

Fresh tobacco plant leaves were collected from Nasarawa Eggon of Nasarawa State which was identified at the herbarium of the Plant Science and Biotechnology Unit, Department of Biological Sciences of Nasarawa State University, Keffi.

Preparation of Extract

Fresh plant leaves were allowed to dry at room temperature which was letter grounded to powder form using mortar and pestle. 100 g of the powdered extract were soaked into 1000ml of distilled water for 24 h[26]. After 24 h, the soaked extract was filtered with sieve and then Whatman filter paper (No 1) was used to re-filter it and pure extract was obtained. The residue was discarded. The filtered extract was subjected to water bath for evaporation at 98^{o}C. Solid extract was obtained which was used for analysis.

Animal Studies/Treatments

Male albino rats weighing (170-175g) were purchased from the animal house of the National Vetenary Research Institute, Vom, Jos, Plateau State. The animals were acclimatised to environmental condition for a week prior to the experiment and had access to food (rat chow) and distilled water.

The animals were grouped into four groups of five rats each. The first group were orally administered normal saline and were sacrifice 24 h after the last administration. This group represent the control animals. The second groups were given 0.5 mL of aqueous leaves extract dose of 200 mg/kg body weight was orally administered and the animals were sacrifice 24 h after the last administration. The third groups were administered aqueous leaves extract dose of 400 mg/kg body weight was orally administered and the animals were sacrifice 24 h after the last administration. The fourth group were orally administered with an aqueous leaves extract dose of 600 mg/kg body weight was orally administered and the animals were sacrifice 24 h after the last administration. However, the treatment periods lasted for fourteen days.

Collection of Blood Sample

The blood was collected according to manual used by Institutional Animal Care and Use Committee (IACUC). Retro-orbital blood collection method was employed. Briefly, the animals were fully anesthetised by a drop of proparicaine in each eye to minimise discomfort. The animal was held by the back of the neck and the loose skin of the head was tightened with thumb and middle finger to keep the animal stable. The tip of non-heparinised capillary tube was place at the medial can thus of the eye under the nictitating membrane. With gentle thrust and rotation motion past the eyeball the tube will enter the slightly resistant sinus membrane and the eye ball remained uninjured. As soon as the sinus was punctured, the blood enters the tubing was capillary action. The blood was allowed to clot and then centrifuged at 3500 rpm for 5 min using table centrifuge. The serum was then separated from the blood and further used for analysis.

Biochemical Parameters

The serum separated was used to assay for liver enzymes; alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were assayed by the method described by Reitman and Frankel[27], alkaline phosphatase (ALP) was also analysed by method of Rec[28]. Serum Bilirubin was determined using colorimetric method as previously described by Jendrassik and Grof[29].

Statistical Analysis

The result is expressed as Mean ± Standard Deviation for five animals in each group. The biochemical parameters were analysed statistically using one-way analysis of variance ANOVA using Smith's Statistical Package, followed by Student t-test for comparism. Statistical significant difference was considered at 95% probability level.

Result

The results are presented in Table 1. There were significant ($p<0.05$) increase in the level of ALT, AST, ALP, and serum total and direct bilirubin.

Treatment ($Mgkg^{-1}$)	ALT (µ/L)	AST (µ/L)	ALP (µ/L)	TB (µmol/L)	DB (µmol/L)
Control	10.2± 5.54	12.4 ± 4.53	128.0 ± 9.54	6.2 ± 1.92	19.8 ± 2.78
200 (AE)	26.0± 4.53*	26.4 ± 4.04	295.8 ± 13.07*	12.0 ± 2.55	26.0 ± 2.35*
400 (AE)	45.4± 6.07*	46.6 ± 4.98*	192.0 ± 5.95	13.2 ± 2.39*	40.0 ± 3.39*
600 (AE)	63.0± 6.88*	71.8 ± 8.04*	311.4 ± 16.12*	42.0 ± 3.61*	49.0 ± 3.08*

Table 1: Some selected serum liver indices in rat administered with aqueous leaves extract of tobacco plant (*Nicotiana tabacum*).

Values are mean ± Standard Deviation, *Significant different from the control ($P<0.05$), ($n=5$).
ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline aminotransferase; TB: Total Bilirubin; TD: Direct Bilirubin.

Discussion

The measurement of activities of enzymes in tissues and body fluids plays a significant role in the investigation and diagnosis of diseases[30]. The liver plays a key role in many metabolic processes. This fact demonstrates the biochemical importance of the organ, severe hepatic injury as a result of the metabolism of some toxic phytochemicals found in plants and failure of metabolic produces to be found in plants and the metabolic products to be eliminated by the liver[31] may be associated with distortion of these function thereby leading to increase in the serum concentration of Aspartate aminotransferase (ASP), Alanine aminotransferase (ALT) and alkaline phosphatase activities (ALP) as found in the present study.

ALP is widely distributed within different tissues possessing one or more of the isoenzymes in bone, liver and intestine[32]. ALP is useful in diagnosis hepatobiliary lesions and Oesteoblastic bone diseases[33]. Elevated activities of liver ALP immediately after the administration of the extract implied activation of the enzyme molecule. However, since there was significant change in the levels of serum ALP, the elevation in the liver ALP activity could be therefore attributed to enzyme activation, ALT, is a more specific enzyme of liver damage than AST. This is probably as a result of induction of enzyme synthesis due to defence reaction of the organ to the exogenous substances or loss of other proteins[34]. This trend may have consequential effect on amino acid metabolism.

The result in Table 1 showed that there is significant increase in alkaline phosphatase in all treated groups compared to the control. Elevated levels indicate that there was an impaired bile formation (Cholestasis), intrahepatic cholestasis or infiltrative disease of the liver.

The ALT and AST are useful indicators for identifying inflammation and necrosis of the liver[35]. ALT has the highest concentration in the liver while has a lesser activity concentration in the kidney and skeletal muscle[35]. The activity of AST is located in the microsomal and mitochondrial portions of the liver cells as well as in the skeletal and cardiac muscles, pancreas and kidney[32]. ALT measurements are more liver specific than the AST and its activity is usually greater at early or acute hepatocellular diseases[32].

A marked elevation of ALT, however in the presence of mild to moderate elevation of AST indicate either hepatic disease or hepatic disease combined with other conditions[35].

The result of this study showed significant increase in the activity of both ALT and AST as compared to the control in all the groups treated (see Table 1). Thus, this finding did not provide evidence of clinical safety of the plant at the higher concentrations as indicated by the results. The significant increase of serum total and direct bilirubin, indicate defective liver excretory function[36] and impaired synthetic function of the liver[37]. The result of these findings is in accordance with the one reported elsewhere[38].

From the result obtained in this study, oral administration of aqueous extract of Tobacco plant (*Nicotiana tobaccum*), it may be apparent to suggest that the plant leaves extract may not be safe for humans especially at higher concentrations.

Furthermore this study has found that effect of nicotine in the serum is dose dependent. However these factors need further justification by further studies. For the reason that the method administration used by this study is oral, other ways of administration might be more toxic to the liver and other cells. Therefore to determine the toxicological effect of this study at moderate level, it oral administration method was chosen. Finally it was to compare its effect with other part of the world, thus the results were found to be comparable with that in the rest of the world as explained in the discussion section.

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