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DRUG DISCOVERY

Hepatotoxicity, Hyperglycemic and hyperlipidemic effect of Aqeous Leaf Extract of *Nicotianatabaccum* in male Wistar albino rats

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The leaves of *Nicotianatobacum* is used as a medicinal plant in Northern Nigeria. The present study aimed at evaluating the biochemical effects of aqueous leaves extracts of *Nicotianatabacum* in liver indices, blood glucose and some lipid profile in male Wistar albino rats. The animals were divided into four groups: Group 1 was treated with normal saline, group 2, 3 and 4 with 0.5mL of the extracts, at 150 mg/kg, 300 mg/kg and 450 mg/kg respectively. The treatment was given daily for fourteen days. Blood samples were collected and assayed. The results showed significantly (P<0.05) elevated blood glucose level accompanied by significant increase in alanine amino transferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activities in the treated groups (1,2, and 3) when compared with group 1. Bilirubin (total and direct) showed a significant (P<0.05) increase in concentration in groups 2, 3, and 4 with the total bilirubin having the highest increase in the activity compared to group 1. Total cholesterol, triglyceride and low density lipoproteins were significantly elevated in groups 2, 3, and 4; while high density lipoprotein was significantly (p<0.05) reduced compared to group 1. The leaves extract at different concentrations (doses) showed that group 4 (600mg/kg) had a significant (p<0.05) increase in blood biochemical samples compared to lower concentrations in group 2 and 3. Also group 3 showed a significant (p<0.05) increase compared to group 2. Therefore, the present studyrevealed that the aqueous leaves extracts of *Nicotianatabacum* might not be safe for human consumptions as they are associated with liver toxicity, hyperglycemia and hyperlipidemia.

INTRODUCTION

In recent years, medicinal plants occupy an important position for being the paramount sources of the discovery of pharmacologically active compounds (Mustapha, 2014). 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 northern Nigeria, one such plants used is tobacco plant leaves (*Nicotianatabacum*), are used in two major forms which is the smoked and the smokeless, the tobacco snuff and chewing tobacco (Aduema et al., 2012), used as an organic pesticide and in the form nicotine tartrate in medicine (Ugbor et al., 2013). Tobacco snuff is the powdered form blended with potash as the main additive in Nigeria (Ureme et al., 2007) and has been recommended as a substitution for nicotine in cigarette since it is devoid of hazardous elements such as tar and carbon monoxide (Russel et al, 1980). Large number of people believes that using smokeless tobacco is safer than smoking it (Ugbor et al., 2013). This however, is not true because smokeless tobacco can induce addiction to nicotine and leukoplakia (Armitage et al., 1978; Armitage and Turner, 1970).

Nicotianatabacum (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 (Ren and Timko, 2001).

Tobacco has many constituents, but nicotine is singled out as having the highest concentration and most immediate pharmacological action (Heishman et al., 1994). 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 (Dasofunjo et al., 2014). 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 (Heishman et al., 1994). Unlike many other plants Solanaceae family, do not contain tropane alkaloids, which are reported to be poisonous to humans and other animals (Fafioye and John-Dewole, 2013). However, other compounds such as germacrene, anabasine and other piperidine alkaloids (varying between species), which are powerful enough to deter most herbivores (Benowitz, 1990). A number of such animals have developed the ability to feed on Nicotiana species without being harmed (Fafioye and John-Dewole, 2013). Nonetheless, tobacco is unpalatable to many species, and therefore some tobacco plants such as *Nicotianaglauca* have become established as invasive weeds in some species (Fafioye and John-Dewole, 2013). Tobacco either smoked or

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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 (Adedayo et al., 2011; Stepanov et al., 2010; Addo et al., 2008; Hecht et al., 2007; Cerami et al., 1997; Chiba and Masironi, 1992; Hecht and Hoffmann, 1988; Hoffmann and Hecht, 1985; Hecht et al., 1978). These effects may account for part of nicotine reinforcing effects in humans. Nicotine affects cardiovascular system through its an autonomic effect (Checkoway et al., 2002).

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 (Carlisle et al., 2004) due to stimulation of sympathetic ganglia and adrenal medulla combined with discharge of catecholamines (Fafioye and John-Dewole, 2013). One of the famous acute effects of nicotine is its relationship to lower body weight (Julien, 1996). In this regard, nicotine decrease appetite for sweet foods and increase the amount of energy the body uses both while resting and exercising (Vik et al., 1996). 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 (Meyer et al., 1997). 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 (Fafioye and John-Dewole, 2013).

However, every drug is associated with hepatotoxicity almost certainly due to its ability to generate free radicals and to cause disturbance in hepatocyte biochemistry (Fernandez-checa and Kaplowitz, 2005). 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. Diabetes mellitus refers to a group of metabolic diseases characterized by high blood glucose (hyperglycaemia) concentration result from defects in insulin secretion, or action. It is a morbid condition with high prevalence worldwide (Etuk, 2010). Hyperlipidemia has been shown to be associated with coronary heart diseases and oftentimes are associated with diabetes mellitus (Lotfy *et al*., 2013).

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 grinded to powder form using mortar and pestle. 100 g of the powdered extract were soaked into 1000ml of distilled water for 24 h (Mustapha et al., 2014). 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 98ºC. Solid extract was obtained which was used for analysis.

Experimental Design

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 represents the control animals. The second groups were given 0.5mL of aqueous leaves extract dose of 150 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 300 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 450 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.

The blood was collected as previously described by the 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 Analysis

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, (1957), alkaline phosphatase (ALP) was also analysed by method of Rec (1972). Serum Bilirubin was determined using colorimetric method as previously described by Jendrassik and Grof, (1938). The serum glucose and total cholesterol (TC) were determined by the method of Trinda, (1969); Searcy and Berquist, (1960) respectively. High density lipoprotein cholesterol (HDLc) and triglyceride (TG) were determined by the method of Friedwald *et al*. (1972); Assman *et al*. (1984) respectively. Low density lipoprotein cholesterol (LDLc) was calculated using formula (Albers *et. al*., 1978)

LDL = TC – TG/5 – HDL

Statistical Analysis

Statistical analysis of the result analysed in one-way analysis of variance ANOVA using Smith's Statistical Package, followed by Student t-test for comparison. Statistical significant difference was set at $p < 0.05$.

RESULTS AND DISCUSSION

The measurement of activities of enzymes in tissues and body fluids plays a significant role in the investigation and diagnosis of diseases

Table 1 Effect of aqeous leaves extract of *Nicotianatabacum* on Some selected serum liver indices in male Wister rats

Treatment (Mgkg^{-1})	ALT (µ/L)	AST (µ/L)	ALP (µ/L)	TB (µmol/L)	DB (µmol/L)
Control	12.4±4.32	11.6 ± 5.37	137.3 ± 9.54	5.7 ± 2.64	17.9 ± 5.16
150 (AE)	25.5±3.71*	28.6 ± 7.20	*289.9 ± 13.07*	10.5 ± 4.43*	28.3 ± 7.00*
300 (AE)	49.8±2.96*[a]	49.00 ± 4.81*	[a]342.0 ± 6.75*[a]	11.6 ± 3.50	39.6 ± 7.45*[a]
450 (AE)	66.4±5.34*[b]	70.3 ± 6.62*	[b]394.6 ± 1.43*	[b]26.7 ± 6.28*	[b]51.1 ± 4.60*[b]

Data expressed as mean ± SD, *Significant different from the control (P<0.05), (n=5); [a] significantly different from group 1 150(AE); [b] significantly different from group 2 300 (AE)

Table 2 Effect of aqeous leaves extract of *Nicotianatabacum* on serum glucose of male Wister rats

Treatment	Serum glucose (mg/Dl) at 7 days	Serum glucose (mg/Dl) at 14 days
Control	72.62±22.61	75.03±43.51
150 (AE)	98.93±10.35*	121.85±50.06*[c]
300 (AE)	121.06±18.27*[a]	163.29±17.02*[ac]
450 (AE)	140.22±19.03*[b]	201-68±64.90*[bc]

Data expressed as mean ± SD; n – 5; *Significant different from the control (P<0.05), (n=5); [a] significantly different from group 1 150(AE); [b] significantly different from group 2 300 (AE); [c] different concentrations significantly different from serum glucose level at 7days

Table 3 Effect of aqeous leaves extract of *Nicotianatabacum* on serum lipid profiles (mmol/L) of male Wister rats

Treatment (Mgkg^{-1})	Cholesterol	Triglyceride	HDLcLDLc	
Control	2.98±0.05	2.36±0.02	1.98±0.02	0.69±0.01
150 (AE)	4.64±0.02*	3.71±0.05*	0.92±0.05*	1.16±0.03*
300 (AE)	5.86±0.07*[a]	4.39±0.06*[a]	1.08±0.07*	1.44±0.05*[a]
450 (AE)	6.00±0.03*[b]	4.98±0.09*[b]	0.71±0.04*[b]	1.68±0.08*[b]

Data expressed as mean ± SD, *Significant different from the control (P<0.05), (n=5); [a] significantly different from group 1 150(AE); [b] significantly different from group 2 300 (AE)

(Molowo, 2000). 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 (Giedan et al., 2004) 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 (Whitby et al., 1989). ALP is useful in diagnosis hepatobiliary lesions and Oesteoblastic bone diseases (Wolf, 1978). 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 (Akanji, 1989). 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 (Tikian et al., 1979). ALT has the highest concentration in the liver while AST has a lesser activity concentration in the kidney and skeletal muscle (Tikian et al., 1979). 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 (Whitby et al., 1989). ALT measurements are more liver specific than the AST and its activity is usually greater at early or acute hepatocellular diseases (Whitby et al., 1989). 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 (Tikian et al., 1979). 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 (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 (Chessbrough, 1991) and impaired synthetic function of the liver (Harold et al., 1980). The result of these findings in liver enzymes is in accordance with the one reported elsewhere (Mitra et al., 1980) on seed aqeous extract of *Nicotianatobaccum*.

Diabetes mellitus is a metabolic disease characterized by high blood glucose (hyperlipidemia) level. In the present study, administration of aqueous extract of *Nicotianatobaccum* in different concentrations of 0.5mL in 150mg/kg, 300mg/kg and 450mg/kg body weight (Table 2) to the rats caused significant ($p<0.05$) elevation of serum blood glucose level as compared to control. This can be as result of defects in the insulin secreation. *Nicotianatobaccum*is a Beta-cytotoxic agents and it caused beta-cytotoxic action that is associated with the sudden release of insulin leading to severe transient hypoglycaemia followed by chronic hyperglycaemia resulting from the complete destruction of the pancreatic β-cells that secretinsulin. The finding in this study is in agreement with other studies that an agent of beta-toxicity impairs the release of insulin (Nweze et al., 2017).

The serum lipids level is usually raised in diabetes and present a risk factor for the development of cardiovascular diseases most especially coronary heart diseases (Luka *et al*., 2013). Administration of aqueous extract of *Nicotianatobaccum* in different concentrations of 0.5mL in 150mg/kg, 300mg/kg and 450mg/kg body weight (Table 3) showed significant ($p<0.05$) increase in cholesterol (CHOL), triglyceride (TG) and low density lipoprotein (LDLc), with significant ($p<0.05$) reduction in high density lipoprotein (HDLc). Diabetic states affect insulin and thus, may lead to inadequate utilization and activations of hormone-sensitive lipases, glucagon and cathecolamines which in turns increase the breakdown of lipids and mobilization of free fatty acids from the peripheral depots (Yakubu et al, 2003). Activation of 3-hydroxy-3-methylglutaryl coenzyme responsible for the biosynthesis of cholesterol due to insulin deficiency might have been responsible for the observed hyperlipidermia. The hyperlipidemic effect of was compared to other studies that bark of the stem (Mustapha *et al*., 2014)

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.It may be apparent to suggest that the plant leaves extract may not be safe for humans especially at higher concentrations.

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Authors Declaration
The Authors hereby declare that this study is original research work and that we shall be liable for any claims relating to the content of this article.

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