Toxicity of The Aqueous aAnd Alcoholic Extracts of *Nicotiana Tabacum* (Tobacco Plant) on Histopathological and Haematological Parameters of Albino Rats

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Abstract: This research work exposed albino rats to dosage of aqueous and alcoholic extracts of *N. tabacum*. Rat samples showed continued loss of weight and general body weakness; especially in the limbs. The animals treated with alcoholic extract showed sluggishness after each round of dosage administration. The rats became less and less active as each day passes. Some of the rats were paralysed, which may be as a result of the intravascular injection. There was mortality and constant weight-loss recorded at the end of the experiment. *N. tabacum* exhibited some remarkable histopathological effects like haemorrhagic discharges and congestion in the liver and kidney of the treated rat samples. Severe vacuolar degeneration was also observed in the liver of exposed rats. Haematological effects of *N. tabacum* include; decrease in PCV, haemoglobin, RBC, WBC and platelet counts in the exposed rat specimen. This has led to reduction in amount of dissolved oxygen needed to be transported to body cells for normal metabolic activities and also made the body defenseless to foreign pathogenic organisms. However, the slight increase in lymphocytes as observed in the aqueous extract of *N. tabacum* treated rats could indicate presence of toxic agents in the plant. Therefore, further research work will be recommended to carry out a bioactivity and directed fractionation to detect which chemical compound(s) among the secondary metabolites is responsible for each of the pharmaco-toxicological manifestations associated with *N. tabacum*.
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1.Introduction

Toxicology is the study of the dynamic interaction of chemicals with living systems (Afshari and Hamadeh, 2004). The study is the science behind the numerous industries and regulatory agencies who are concerned about development and regulation of food additives and those concerned with use and remediation of hazardous chemicals. Over the years, with improved methodology and technology, toxicology slowly evolved from a science of high doses and insensitive end-points (e.g., death, changes in organ size or litter size) to a science of low (and environmentally-relevant) doses and of more sensitive end-points such as measurements of biochemical and functional changes in the immune system, endocrine system, and neurological system. Reduction in chemical exposure can, however, bring about less obvious effects which may be more difficult to detect. Therefore, results are more often expressed as ED50 of the effective dose (for whatever end-point selected for study) that leads to a 50% response of the test population. While these changes towards low doses and more sensitive end-points are encouraging, most current toxicology and regulatory policies that are based upon toxicological are still heavily dependent upon data derived from existing body of toxicological data which includes to a large extent high-dose studies. The result is that toxicologists and risk assessors, in order to relate this large database of high-dose data to relevant low-dose situations of today, must use 'high-dose to low-dose extrapolation', which is the practice of deriving effects thresholds for small concentrations of chemicals from studies that used much higher concentrations that often produce different types and severity of effects.

Tobacco (*Nicotiana tabacum L.*) is the dried leaf of a plant that grows in many parts of the world. Tobacco is also an agricultural product processed from the leaves of plants in the genus *Nicotiana*. It can be consumed, used as an organic pesticide and in the form nicotine tartrate (in medicine). It is commonly used as a recreational drug, and is a valuable cash crop for countries like Cuba, China and United States. Many plants contain nicotine, powerful neurotoxin to insects. However, tobacco contains a higher concentration of nicotine than most other plants. Unlike many other *Solanaceae*, they do not contain tropane alkaloids, which are often poisonous to humans and other animals. Despite containing enough nicotine and 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. Nonetheless, tobacco is unpalatable to many species, and therefore some tobacco plants (chiefly tree tobacco, *N. glauca*) have become established as invasive weeds in some species.

Tobacco contains the following phytochemicals; Nicotine, Anabasine (an alkaloid similar to nicotine but less active), Glucosides (tabacinine and tabacine), 2,3,6-Trimethyl-1,4-naphthoquinone, 2-Methylquinone, 2-Naphylamine, Propionic acid, Anatalline, Anthalin, Anethole, Acrolein, Anatabine, Cembrene, Choline, Nicotelline, Nicotianine, Pyrene (Cerami *et al*., 1997).

Nicotine binds stereo-specifically to acetylcholine receptors at autonomic ganglia, the adrenal medulla, neuromuscular junction and the brain. As a consequence of the stimulation of nicotinic receptors possibly located on presynaptic sites, short-term exposure to nicotine results in the activation of several central nervous system neuro-human pathways, leading to the release of acetylcholine, norepinephrine, dopamine, serotonin, vasopressin, growth hormone and ACTH.

Nicotianas are highly toxic plants due to their nicotine alkaloid content. The effects of nicotine alkaloid are as a result of the summation of actions at ganglionic sites, motor end-plates and smooth muscle. The central nervous system is affected, initially by stimulation, resulting in tremors and convulsions, progressing to depression. Death occurs from respiratory failure. Vomiting is a result of stimulation of the emetic chemoreceptor trigger zone.

The cardiovascular responses are generally due to stimulation of sympathetic ganglia and adrenal medulla combined with discharge of catecholamines. The target organs are nervous system and heart.

However, Tobacco has been used as an antispasmodic, a diuretic, an emetic, an expectorant, a sedative, and a sialagogue, and in homeopathy. Tobacco has a long history of use by medical herbalists as a relaxant, though since it is a highly addictive drug it is seldom employed internally or externally at present. The leaves act as antispasmodics, discutients, diuretics, emetics, expectorants, irritants, sedatives and sialagogues. They are used externally in the treatment of rheumatic swelling, skin diseases and scorpion stings. The plant should be used with great caution, when taken internally it is additive. The active ingredients can also be absorbed through the skin. Wet tobacco leaves can be applied to stings in order to relieve the pain. They are also a certain cure for painful piles. A homeopathic remedy is made from the dried leaves. It is used in the treatment of nausea and travel sickness. Some other activities reported for *N. tabacum* are: Anagelsic activity, anaesthetic activity, angiogenesis inhibition, antibacterial activity, anti-cunvulsant activity, anti-estrogenic effect, antifungal activity, aromatase inhition, arrhythmogenic effect, anti-stress effect, antiviral activity, antiglauconic activity, antioxidant activity, carcinogenic activity, broncho-constrictor activity and bupivacaine kinetics.

The aim of this research is therefore to investigate the effects of *N. tabacum* on the tissues of white rats, so as to get baseline information as to predict the effects of tobacco consumption on human tissues.

Materials and Methods

11 Albino rats (*Rattus novegicus*) with average age of 4 months and average weight of 175.61g were gotten from the same source in the Department of Zoology, Olabisi Onabanjo University, Ago-Iwoye Ogun State, Nigeria. 7 rats were selected for the research out of which 6 were used to test for the toxicity of each extract, i.e. 3 rats for alcoholic extract and the other 3 for aqueous extract analysis. The last rat was used as a control test. The fresh leaves of *N. tabacum* used in this research work was gotten from a farmland in Ago-Iwoye, Ogun State and was identified by Mr. Akasoro of the Department of Plant and Applied Zoology, Olabisi Onabanjo University, Ago-Iwoye Ogun State.

Preparation of Animal Samples

The rats were weighed and allowed to acclimatize for 7 days. They were allowed pure distilled water, uncontaminated grower mash and a conducive environment. No mortality was however recorded during the process of acclimatization. After the expiration of 7 days, the rats were divided into three groups and were selected in relevance to their similarity in body weight. Group 1 contained the control specimen. Group 2 contained the rats administered with aqueous extract while Group 3 consisted of rats administered with alcoholic extracts.

Extraction of the Botanicals

Aqueous Extract: The aqueous extract was prepared in line with USDHHS (2008) specifications for tobacco extraction methods and formulations. The fresh plant was crushed in mortar, 90g of the finely ground fresh plant was weighed and put in a clean jar. 1 Lt of distilled water was brought to 85^{o}C and poured over the weighed and crushed plant and then allowed to steep and cool for about 2 h. The mixture was then filtered to remove the shaft.

Alcoholic Extract: The alcoholic extract was prepared using fresh leaves and stem of the plant. The plant parts used were crushed with pestle and mortar. 25g the crushed plants was mixed with 1 Lt of 100% alcohol according to IARC (2009). The mixture was

sieved with a fine cloth and the residue was collected after filtration.

Method of Administration

Aqueous Extract: The dosage administered to the rats was 5ml/kg body-weight of each rat (Adeniyi *et al*., 2010). The rats were water-starved for 4 h by removing all water cans from their cells from 8.00 am to 12.00 noon, before the aqueous extract was administered on them. The extract was suctioned into a needle hypodermic syringe with relevant dosage (according to the rats' body-weight) was pumped gently into the rat's mouth according to Adeniyi *et al.*, 2010. The extract was administered on the rats for 21 days continuously; each rat is being sacrificed at 7 days' interval.

Alcoholic Extract: The alcoholic extract was injected into the body of the rats via the region of intravascular injection, *gluteous meteaus*. Each rat was given a dosage of 2ml/kg of body-weight. The rats were injected 12.00 pm daily and were not starved of clean water and animal feed. The extract was administered on the rats for 21 days continuously; each rat is being sacrificed at 7 days' interval.

Sample Collections

Group 2 Specimen (Aqueous Extract): After administration of the aqueous extract, the rats were reweighed and each of the 3 rats was sacrificed at of 7 days interval until the 21st day. The blood was collected with hypodermic syringe and stored in EDTA bottle. The heart, pancreas, liver, kidneys and brain were removed and stored separately in universal bottles containing bovine solution as the preservative. The samples were kept cooler and taken to the laboratory for analysis.

Group 3 Specimen (Alcoholic Extract): After administration of the alcoholic extract, the rats were reweighed and each rat was sacrificed at interval of 7 days until the 21st day. The blood was collected with a hypodermic syringe and stored in EDTA bottle. The heart, pancreas, liver, brain and kidneys were removed and stored separately in universal bottles containing bouin solution as the preservative. The samples were kept cooler and taken to the laboratory for analysis.

Analysis of Samples

All the collected samples were taken to the Department of Physiology, University of Ibadan, Nigeria for the histopathology and haematology analysis. Haematology parameters investigated are: Packed Cell Volume (PCV), Red blood Cell (RBC), White Blood Cell (WBC), Platelet (Plt), Lymphocyte (Lym), Eosinophil (EOS), Neutrophil (N), Monocytes (M) and Haemoglobin count (Hb).

Results and Discussion

After the dosage administration of the extracts, the behavioural, physiological, histopathological and haemotological observations of the rats were recorded.

Behavioural and Physiological Changes: After each round of dosage administration, the rats were observed for 1 h to check if the dosage is the lethal dosage (LD50) or if the concentration is the lethal concentration (LC50).

Table 1: Weight Changes as Observed in the Groups

Group	Duration of Exposure (days)	Mortality rate	Initial body weight (g)	Final body weight (g)	Body weight difference	% weight difference
Control	0	0	157.43	157.43	0	0
Control	7	0	157.43	163.01	5.58	3.54
Control	14	0	163.01	179.80	16.79	10.30
Control	21	0	179.80	221.32	41.52	23.09
Aqueous Extract	0	0	174.87	174.87	0	0
Aqueous Extract	7	1	174.87	179.26	4.39	2.51
Aqueous Extract	14	0	179.26	182.94	3.68	2.05
Aqueous Extract	21	0	182.94	183.66	0.72	0.39
Alcoholic Extract	0	0	194.53	194.53	0	0
Alcoholic Extract	7	1	194.53	198.78	4.25	2.18
Alcoholic Extract	14	0	198.78	183.44	-15.34	-7.72
Alcoholic Extract	21	0	183.44	162.24	-21.2	-11.56

Table 2: Histopathological Changes Observed in the Heart, Kidney, Brain, Spleen and Liver

	Organ	Lession	Haemorrhages	Hepatocytes	Necrosis	Congestion	Vacuolar Degeneration
Control	Heart	-	-	-	-	-	-
	Kidney	-	-	-	-	-	-
	Brain	-	-	-	-	-	-
	Liver	-	-	-	-	-	-
	Spleen	-	-	-	-	-	-
Aq. Extract	Heart	-					
	Kidney		++			++	
	Brain	-					
	Liver		++			++	+++
	Spleen	-					
Alc. Extract	Heart	-					
	Brain	-					
	Kidney	-				+++	
	Liver					+	
	Spleen	-					

Key: - = completely absent, + = present, ++ = mild, +++ = severe

Table 3: Haematological Data for the Groups of the Rats for the Duration of Study

Week	Group	PCV%	Hb(g/dl)	RBC (10^{-2}/C)	WBC (x 10^9/L)	PLT (x 10^9/L)	LYM	Neu	Eos	M
1	Aqueous	30	9.6	4.32	9600	54000	89	18	1	11
	Alcohol	16	4.8	2.41	4900	5000	60	37	1	2
	Control	46	14.6	7.31	10500	108000	75	21	1	2
2	Aqueous	10	2.8	1.04	2400	60000	53	42	3	2
	Alcohol	36	11.7	5.91	12900	1945000	21	67	1	2
	Control	46	14.6	7.31	10500	108000	75	21	1	2
3	Aqueous	-	-	-	-	-	-	-	-	-
	Alcohol	-	-	-	-	-	-	-	-	-
	Control	-	-	-	-	-	-	-	-	-

Table 4: ANOVA of Haematological Data

		Sum of Sq.	Df	Mean Square	F	Sig. Dif.
PCV	Between Groups	9.54	2	4.77	0.1022	0.063
	Within Groups	140	3	46.66		
	Total	149.54	5			
Haemoglobin	Between Groups	15.65	2	7.825	0.7931	0.346
	Within Groups	29.6	3	9.866		
	Total	45.25	5			
RBC counts	Between Groups	2.54	2	1.27	0.3202	0.1759
	Within Groups	11.9	3	3.966		
	Total	14.4	5			
Platelet Counts	Between Groups	1.28±0.02	2	6.40±0.01	1.509	0.501
	Within Groups	1.27±0.02	3	4.24±0.01		
	Total	2.55±0.02	5			
WBC Counts	Between Groups	1.25±0.02	2	6.26±0.01	1.543	0.9039
	Within Groups	1.21±0.02	3	4.04±0.01		
	Total	1.38±0.02	5			
Lymph	Between Groups	189.7	2	948.55	18.9	0.9267
	Within Groups	150	3	50		
	Total	2047.1	5			
Neutrophil	Between Groups	150.5	2	75.25	28.28	0.949
	Within Groups	8	3	2.66		
	Total	158.5	5			

Group 1 animals were active and healthy as they gain weight (Table 1) constantly throughout the period of the experiment.

Group 2 animals showed no behavioural changes but moved on with their normal activities. They were also physiological sound. There was a single mortality (Table 1) recorded at a particular time in the group and constant weight-loss during the experiment.

Group 3 animals showed sluggishness after each round dosage administration. This could be due to the effect of alcohol in the extract. As each day passes, the rats became less and less active. Some of the rats were paralysed, which may be as a result of the intravascular injection. There was mortality (Table 1) and constant weight-loss recorded at the end of the experiment.

Histopathological analysis (Table 2) showed that the organs of control animals were not adversely affected over the period of dosage administration. However, the livers and kidneys were affected with haemorrhages and congestion from the dosage administration of aqueous extract of *N. tabacum*. The liver also suffered a considerable degree of vacuolar degeneration (Table 2) from exposure to aqueous extract of *N. tabacum*. The kidney was badly congested with alcoholic extract of *N. tabacum*. This is similar to reports of (El Gamal, 2006) where the hearts, livers and kidneys of the sample rats showed severe congestion over exposure to alcoholic extract of *N. tabacum*. Catarrhal enteritis and fatty discharge are always the physical manifestations accompany acute liver and kidney congestions over a longer period of continued exposure (Khalid, 2002).

The PCV measured at the end of week 2 indicated a significant difference of LSD (0.063) which is > 0.05, indicating that there is no significant difference in the results observed during the weeks of exposure. There was an increase in PCV from week 1 to week 2 but lower than that of the control at the end of week 2. The haemoglobin measured at the end of week 2 indicated a significant difference of LSD (0.346). Since this value is greater than 0.05, it also indicate that there is no significant difference during the exposure period. There was an increase in haemoglobin from week 1 to week 2 but lower than that of the control at the end of week 2.

The haemalogical data (Table 3) showed decrease in PCV from Control through aqueous extract to alcoholic extract. The haemoglobin counts also reduce in the like manner. Similarly, the RBC, WBC and platelets reduced considerably in exposure of the sample animals to alcoholic extract of *N. tabacum*. However, there was increase in lymphocytes counts with aqueous extract administration. This could be connected with the aqueous nature of the active ingredients in the extract. Similar reports (Kausal *et al*., 2008*)* showed congestion of the sinusoids and accumulation of lymphocytes in the liver of rats treated extracts of *N. tabacum*. The effects of the exposure also include degeneration and necrosis of the renal glomeruli and cortical tubular cells. These tubules contain desquamated cells or acidophilic homogenous material. Ranivar and Chatterjee (2009) reported presence of lymphocytic aggregates in the cortex coupled with dilation of renal convoluted tubules and scattered haemorrhagic foci in the renal interstitial tissue of the rats exposed to extracts of *N. tabacum*. (Kausal *et al*., 2008) suggested that the hepatic microsomal enzymes as well as the mitochondrial membranes are vulnerable to the peroxidative attack of *N. tabacum* and may be instrumental in leading to the hepatotoxicity symptoms observed in *N. tabacum* treated animals. The intermittent diarrhea may attribute to gastroenteritis or to the parasympathomimthic cholinergic effect of the plant constituents (El Gamal, 2006). Hyperaesthesia, depression and weakness of the limbs may be attributed to hepatornal insufficiency or significant reduction of the cardiac muscles which may lead lately to congestive heart failure and/or the involvement of the nervous system.

Conclusion

Exposure of rat samples to *N. tabacum* showed continued loss of weight and general body weakness; especially in the limbs. This could have affected the muscles, and as a result, have some connections with and interruptions of the CNS. *N. tabacum* exhibited some remarkable histopathological effects by creating haemorrhagic discharges and congestion in the liver and kidney of the treated rat samples. Severe vacuolar degeneration was also observed in the liver of exposed rats. Liver vacuolar degeneration could be so dangerous leading to cirrhosis (liver damage), widespread formation of nodules and fibrosis in the liver, poor metabolic activities of vital organs in the body and eventually (inevitably) death. Haematological effects of *N. tabacum* include; decrease in PCV, haemoglobin, RBC, WBC and platelet counts in the exposed rat specimen. This has led to reduction in amount of dissolved oxygen needed to be transported to body cells for normal metabolic activities and also made the body defenseless to foreign pathogenic organisms. However, the slight increase in lymphocytes as observed in the aqueous extract of *N. tabacum* treated rats could indicate presence of toxic agents in the plant. Therefore, further research work will be recommended to carry out a bioactivity and directed fractionation to detect which chemical compound(s) among the secondary metabolites is responsible for each of the pharmaco-

toxicological manifestations associated with *N. tabacum*.

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