

Effect of Aqueous Leaf Extract of Tobacco on Plasma Glucose Level and Biochemical Indices of Male Albino Wister Rats

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Original Research Article

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Article History

Received: 19.12.2017

Accepted: 02.01.2018

Published: 30.01.2018

DOI:

10.21276/sjpm.2018.3.1.3



Abstract: Several studies have highlighted the health hazards of cigarette smoking and other forms of tobacco consumption to include addiction, cancers, cardiovascular diseases, respiratory disorders, and electrolyte disturbances. However, none has reported the effect of aqueous leaf extract of tobacco on blood glucose and plasma concentrations of many electrolytes. This study was designed to find answers to these questions. Ten male albino Wister rats were randomly divided into test and control groups of 5 rats each. Each test rat was given 100 mg of aqueous tobacco leaf extract dissolved in 1.5 mls distilled water once daily for 21 days through an orogastric tube. Each control rat received 1.5 mls distilled water once daily for the same duration. After 21 days, blood samples were collected from each rat for the estimation of plasma concentrations of glucose, sodium, potassium, chloride, bicarbonate, magnesium, and calcium. Blood samples were collected and plasma concentrations of glucose, sodium, potassium, chloride, and calcium determined. There were no significant differences in plasma glucose, sodium, chloride, bicarbonate, magnesium, and calcium in the test and control groups of rats. However, there was a significant decrease in plasma potassium in the test group compared to the control group of rats as shown by the mean values of 5.7 ± 0.25 mmol/L and 6.60 ± 0.25 mmol/L respectively and P value of 0.0005. Orogastric administration of aqueous tobacco leaf extract produced statistically significant reduction in plasma potassium, but not in plasma glucose, sodium, chloride, bicarbonate, magnesium, or calcium in male albino Wister rats.

Keywords: Biochemical indices, electrolytes, glucose, rats, tobacco extract.

INTRODUCTION

Tobacco is the dried and processed leaves of the plant *Nicotiana tabacum*. It is mostly consumed in the form of smoking, chewing, or snuffing. About 1.1 billion people are involved in one form of tobacco related activity or another [1].

Nicotiana tabacum, the botanical name of tobacco was derived from the surname of Jean Nicot, French ambassador to Portugal who introduced tobacco snuff to France from Portugal [2]. One of the forms of smokeless tobacco is snuff which is a form of tobacco that is processed to fine powder and packaged either in cans or pouch. It can be inhaled (sniffed) or licked. The sniffing route is very common among Nigerians.

People in many regions of the world have a long history of using smokeless tobacco products. The uses of tobacco in those cultures were for several reasons such as treatment, rituals, and also for prevention of hunger and fatigue on long distance trek. In Nigeria, tobacco is also used for cultural and traditional purposes.

In 2012, the United States Food and Drug Administration listed 93 harmful and potentially harmful constituents of tobacco [3]. Most of them are original tobacco constituents while the rest are derived during the period of cultivation, harvesting and processing. The major constituents include aromatic hydrocarbons, aldehydes, ketones, alcohols, phenols, amines, amides, alkaloids, metals, and radio elements. Tobacco specific nitrosamines (TSNA) are formed from alkaloids during the processing of tobacco leaves.

The risks associated with tobacco use include cancers (particularly oral, oesophageal, and pancreatic cancers), heart diseases, gum diseases, and oral lesions other than cancer [4]. The World Health Organization estimated that the use of tobacco caused 5.4 million deaths in 2004 and 100 million deaths over the course of the 20th century [5]. All tobacco products including smokeless tobacco contain nicotine which is addictive [4]. Nicotine which constitutes 0.3-5% of the tobacco plant by dry weight was first isolated in 1828 by German chemists Posselt and Reimann [6]. There is sufficient evidence for a causal association between

smokeless tobacco use and oral cancer, but studies from various countries show contradicting results. These differences in result could be due to differences in tobacco species or in tobacco specific nitrosamine (TSNA) content. The presence of TSNA in tobacco is seen as the most potent factor for carcinogenicity [7].

Nicotine, one of the major constituents of tobacco has profound effect on cardiovascular physiology. It increases heart rate and systemic blood pressure. It also induces atherosclerosis by increasing oxidative stress and nitric oxide inactivation in vascular endothelium [8]. Nicotine increases lipolysis thereby increasing free fatty acid concentration. This results in increased very low density lipoproteins (VLDL) and reduced high density lipoproteins (HDL) concentrations.

Consumption of smokeless tobacco is toxic to the kidney. Animal studies have shown that extracts of tobacco impairs enzymatic antioxidant system which induces oxidative stress and lipid peroxidation in liver, lung and kidney [9]. The effect of heavy metals in tobacco such as cadmium, mercury, and lead might be another possible mechanism for tobacco-induced renal damage [10].

Justification for the study

In recent times, preparations of tobacco are widely consumed in many countries of the world including Nigeria. There are studies highlighting the effects of cigarette smoking and other modes of tobacco consumption [11, 12]. However, little is known about the effect of aqueous tobacco extracts on blood sugar and serum electrolytes. This necessitated the present study.

Objectives of the study

The general objective of the study was to find out the effect of orogastric administration of aqueous leaf extract of *Nicotiana tobacum* on plasma levels of glucose and some electrolytes of male albino rats. Specific objectives included:

- To determine the effect of aqueous leaf extract of *Nicotiana tobacum* on plasma glucose level in male albino rats.
- To determine the effect of aqueous leaf extract of *Nicotiana tobacum* on plasma concentrations of potassium, sodium, magnesium, chloride, and bicarbonate in male albino rats.

Hypotheses

The Null hypothesis stated that aqueous leaf extract of *Nicotiana tobacum* does not affect the plasma concentrations of glucose, potassium, sodium, magnesium, chloride, and bicarbonate in male albino rats. The alternative hypothesis stated that aqueous leaf extract of *Nicotiana tobacum* affects the plasma concentrations of glucose, potassium, sodium, magnesium, chloride, and bicarbonate in male albino

rats. The null hypothesis would be tested at a significant level (p value) of 0.05. It would be rejected if the p value is < 0.05 and accepted if p value > 0.05. At p value < 0.05, the null hypothesis would be rejected implying that aqueous leaf extract of *Nicotiana tobacum* affects plasma concentrations of glucose, potassium, sodium, magnesium, chloride, and bicarbonate in male albino rats.

MATERIALS AND METHODS

This study was conducted at the Physiology laboratory, Department of Physiology, College of Medicine, Chukwuemeka Odumegwu Ojukwu University.

Calculation of sample size

Sample size of 10 rats at 95% power to detect a difference between means of 5.0 at a significant level (alpha) of 0.05 (two tailed) was chosen using the special formula for the calculation of sample size for laboratory animal experiments [13]:

$$N = 1 + 2C[s/d]^2$$

Where, C = a constant (7.8) at 0.05 level of significance; s = 2.75 (standard deviation from a similar previous study); d = difference between means desired in present study.

Animal source

Ten (10) male albinos wister rats, 6-8 weeks old, weighing 150-300 grams were obtained from the animal house, Department of Pharmacology and Therapeutics, Anambra State University, Awka Campus, Nigeria. The animals were certified healthy by a veterinarian. Each group of 5 rats was housed in a metal cage measuring 60cm x 45cm x 30cm and was allowed free access to animal feeds (Growers, Top Feeds, and Nigeria) and clean drinking water. Left over feeds and water were discarded and the cages cleaned with chlorhexidine antiseptic solution every 12 hours. Artificial light was provided by fluorescent lamp (Philips, Holland; 18 watts) and light-dark cycle of 12-12 hours maintained. The animals were maintained in this arrangement for two weeks for acclimatization.

Preparation of tobacco extracts

Dry leaves of *Nicotiana tobacum* were purchased from a local market, Afor Egbu, in Egbu town, Imo State and identified by a lecturer in the Department of Biological Sciences, Chukwuemeka Odumegwu Ojukwu University.

The tobacco leaves were blended into powder using a mortar and iron pestle. Then 100 grams of the blended tobacco leaves were dissolved in 1 litre of distilled water and shaken vigorously at intervals of ten minutes for twelve hours daily for two days. Thereafter, the mixture was filtered using Whatman filter paper 125 mm and a funnel. The filtrate was evaporated to dryness

yielding 30 grams of the extract which was stored at 4°C until use.

Experimental procedure

The rats were randomly divided into two groups (control and test) of 5 rats per group. Thereafter, 100 mg of extract dissolved in 1.5 mls distilled water was administered daily to each rat in the test group between 8 am-10am for 21days through an orogastric tube. The control group of rats received 1.5 mls of distilled water through the same route and at the same time for the same duration. A treatment chart was maintained.

After 21 days, blood samples were collected from each rat for the estimation of plasma concentrations of glucose, sodium, potassium, chloride, bicarbonate, magnesium, and calcium.

Collection of blood samples

The rats were anaesthetized, one at a time, using intramuscular ketamine (Nirma, India) and diazepam (Norris Medicals, India) 50mg/kg and 5mg/kg respectively [14]. Thereafter, blood samples were collected using the method described by Hoff [15]. Briefly, the skin over the jugular vein was cleaned with methylated spirit-soaked cotton wool and 1.0-1.5 mls of whole blood withdrawn through the jugular vein using a 25G hypodermic needle fitted unto a 2ml syringe. The blood samples were stored in EDTA bottles (for potassium, sodium, magnesium, chloride, and bicarbonate) and fluoride-oxalate bottle (for glucose) and allowed to stand for 30 minutes to separate into plasma and cellular components. The plasma was separated into separate tubes, stored at 2-8° C and chemical estimation of glucose and electrolytes done within 24 hours.

Determination of plasma glucose and electrolytes

The determination of plasma glucose and the electrolytes were done as described by Sood for glucose [16]; Berry *et al.* for sodium, potassium, chloride, and calcium [17]; Chromy *et al.* for magnesium [18]; and Van Slyke *et al.* for bicarbonate [19].

STATISTICAL ANALYSIS

The mean values ± S.E.M. of plasma glucose, potassium, sodium, magnesium, chloride, and bicarbonate for the test and control groups of rat were calculated. Then the data were tested for normality using D’Augustino and Pearson omnibus normality test. Thereafter, values of plasma glucose and electrolytes (sodium, potassium, chloride, bicarbonate, magnesium, and calcium) obtained for the test and control groups of rats were tested for statistically significant differences using t-test. There was no transformation of data. All the statistical tests were performed using Graph Pad Prism 6.0 and the results taken as statistically significant if the p value < 0.05.

RESULTS

Yield of extract

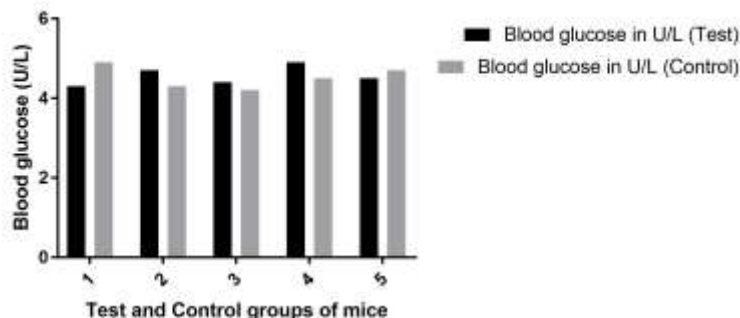
100 grams of ground tobacco leaves was used to produce 30 grams of aqueous extract thereby giving a yield of 30%. None of the animals died i the course of the experiment.

Plasma glucose and electrolytes

There were no significant alterations in plasma glucose, sodium, chloride, bicarbonate, magnesium, and calcium in the test and control groups of rats as shown in Table I and Figures 1, 2. However, there was a significant decrease in plasma potassium in the test group compared to the control group of rats as shown by the mean values of 5.7 ± 0.25 mmol/L and 6.60 ± 0.25 mmol/L respectively and P value of 0.0005 (Table 1, Figure 2).

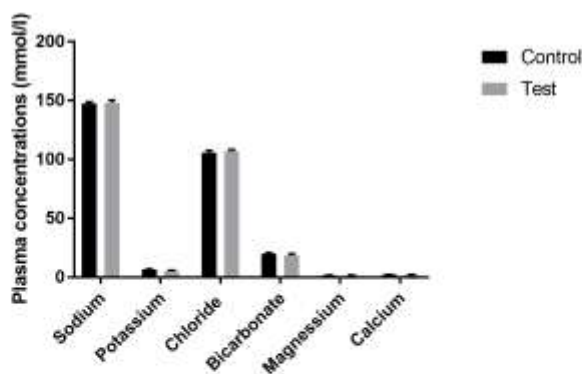
Table-1: Mean (± S.D.) values of plasma glucose and electrolytes of control (C₁-C₅) and test (T₁-T₅) groups of rats after oral administration of tobacco extract for 21 days

	C ₁	C ₂	C ₃	C ₄	C ₅	Mean± SD	T ₁	T ₂	T ₃	T ₄	T ₅	Mean± SD
GLU(U/L)	4.9	4.3	4.2	4.5	4.7	4.82 ± 0.29	4.3	4.7	4.4	4.9	4.5	4.56 ± 0.24
Na(mmol/L)	147	148	147	149	145	147.2±1.48	147	145	148	149	151	148.0±2.24
K (mmol/L)	6.6	6.6	6.7	6.9	6.2	6.60±0.25	5.7	5.9	6.0	5.5	5.4	5.7±0.25
CL (mmol/L)	105	107	105	108	104	105.8±1.64	106	106	107	107	109	107.7±1.22
HCO ₃ (mmol/L)	20	20	19	21	20	20.0±0.71	18	17	20	18	20	18.6±1.34
Mg (mmol/L)	1.7	1.6	1.9	1.6	1.5	1.66±0.15	1.7	1.4	1.6	1.5	1.6	1.56±0.11
Ca (mmol/L)	2.33	2.16	2.16	2.31	2.24	2.24±0.08	2.16	2.28	2.03	2.15	2.24	2.17±0.10



Unpaired t test	
P value	0.8171
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=0.239 df=8
How big is the difference?	
Mean ± SEM of column A	4.56 ± 0.1077, n=5
Mean ± SEM of column B	4.52 ± 0.1281, n=5
Difference between means	-0.04 ± 0.1673
95% confidence interval	-0.4259 to 0.3459
R squared (eta squared)	0.007092
F test to compare variances	
F, DFn, Dfd	1.414, 4, 4
P value	0.7454
P value summary	ns
Significantly different (P < 0.05)?	No

Fig-1: Plasma concentration of glucose (U/L) in test and control Wister rats after oral administration of 100 mg aqueous tobacco leaf extract to the test rats for 21 days



Discovery?	P value	Mean1	Mean2	Difference	SE of difference	t ratio	df	q value
No	0.523742435547025	147.2	148	-0.8	1.2	0.6667	8	0.440816549918746
Yes	0.000521347579224	6.6	5.7	0.9	0.1612	5.582	8	0.002632805275079
No	0.226782039495303	105.8	107	-1.2	0.9165	1.309	8	0.275180271113024
No	0.072884353005723	20	18.6	1.4	0.6782	2.064	8	0.184032991339452
No	0.272455713973291	1.66	1.56	0.1	0.08485	1.179	8	0.275180271113024
No	0.259822711126502	2.24	2.172	0.068	0.05607	1.213	8	0.275180271113024

Fig-2: Plasma concentrations of glucose (U/L), chloride, bicarbonate, magnesium, and calcium (mmol/L) in test and control Wister rats after oral administration of 100 mg aqueous tobacco leaf extract to the test rats for 21 days

DISCUSSION

The results from the study showed that orally administered aqueous extract of *Nicotiana tabacum*

leaves to adult male albino rats did not significantly affect plasma levels of sodium, chloride, bicarbonate, magnesium, calcium, and glucose.

The results obtained for plasma glucose conflicts with those from previous similar studies: some for, others against. For instance, Kumar *et al.* demonstrated an increase plasma glucose levels in consumers of chewing tobacco compared to non-consumers [12]. This contradicts Obeten *et al.* who found that oral administration of smokeless tobacco to Wister rats significantly reduced plasma glucose levels compared to controls [20]. This later finding and that of the present study is very questionable since it is known that nicotine from cigarette produces hyperglycaemia by increasing the production of catecholamines which impair insulin action [21]. It also produces reduction in plasma adinopectin levels [22]. The differences in the results obtained could be as a result of differences in dosage. Other factors could also be responsible for the differences in the results. For instance, the age of plant at harvest, soil characteristics, and other environmental variables affect the active constituents of medicinal plants [23]. Equally, probable genetic differences between the groups of rat and those used in earlier studies could be contributory.

However there was a statistically significant decrease in the plasma potassium levels in the test group of rats compared to the controls. The observed hypokalaemia in this study could be due to the stimulatory effect of nicotine on the release of catecholamines which in turn increased the cellular uptake of potassium [24]. Another possible cause of hypokalaemia is starvation. Though this can occur in experimental (restricted) animals, it did not apply here since both the test and control groups of rats were kept under the same experimental conditions.

The hypokalaemia observed in this study corroborated with findings from other studies as there is often a correlation between tobacco use and hypokalaemia [25]. In the light of this, it is interesting to note that some studies observed no significant differences in the plasma levels of potassium in smokers and non-smokers [26], whereas orally administered tobacco often always produces hypoglycaemia. Indeed, it has been documented that orally administered tobacco produces greater hypokalaemia (because of greater kaliuretic effect) compared to those administered intravenously or by inhalation [27]. This phenomenon which is also established in the case of orally versus intravenously administered glucose and insulin release suggests that orally administered tobacco can stimulate insulin release which drives plasma potassium into the cells resulting in the observed hypokalaemia.

It is also known that potassium in the glomerular filtrate is reabsorbed in the proximal convoluted tubule but reabsorbed in the collecting duct, principally under the influence of aldosterone thereby resulting in hypokalaemia. It is therefore possible that this tobacco extract produced hypokalaemia in the test

group of rats by stimulating the rennin-angiotensin system [28]. This also explains the hypertensive and other cardiovascular risks associated with tobacco use.

CONCLUSION

We conclude that orogastric administration of tobacco leaf extract produced statistically significant reduction in plasma potassium, but not in plasma glucose, sodium, chloride, bicarbonate, magnesium, or calcium in albino Wister rats. When extrapolated to humans, this finding highlights the possible risk of hypokalaemia in users of chewing tobacco.

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