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The GC-MS fingerprints of *Nicotiana tabacum* L. extract and propensity for renal impairment and modulation of serum triglycerides in Wistar rats

[Las huellas digitales por GC-MS de extracto de *Nicotiana tabacum* L. y la propensión a la insuficiencia renal y la modulación de los triglicéridos en suero en ratas Wistar]

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Abstract

Context: *Nicotiana tabacum* is an herbaceous plant mostly known as tobacco. Locally, people do extract this plant with cow urine, they call it "Adimenu" and they claimed it is effective in managing various ailments, even with taking just a spoonful of the extract. Thus, profiling the toxicity and or otherwise of the cow-urine extracted tobacco plant cannot be overemphasized.

Aims: To characterize the cow urine extracts of *N. tabacum* and evaluated the sub-acute toxicity of smokeless exposure in male Wistar rats.

Methods: Forty male Wistar rats were randomly assigned into groups and exposed to different doses (50, 100 and 200 mg/kg body weight) of *N. tabacum* extract for 7 and 28 days, after which the animals were sacrificed for the biochemical assays.

Results: The GC-MS analysis revealed 21 compounds in the *N. tabacum* extract. As expected, nicotine was predominant among the identified compounds. The sub-acute exposure of rats to cow-urine extract of *N. tabacum* extract might have altered rat metabolic homeostasis, triggering adaptive mechanisms, while impairing renal functions. The *N. tabacum* extract also modulated ($p<0.05$) rat serum lipid profile when compared with control.

Conclusions: Findings in this study suggest that the cow-urine extract of *N. tabacum* exposure in male Wistar rats might have perturbed rat renal functions as well as modulated serum lipid profile. This research, therefore, recommends to local consumers to be cautious of the rate at which they consume their regular "Adimenu".

Keywords: biochemical toxicity; lipid profiling; medicinal biochemistry; safety assessment.

Resumen

Contexto: *Nicotiana tabacum* es una planta herbácea conocida principalmente como tabaco. A nivel local, las personas extraen esta planta con orina de vaca, la llaman "Adimenu" y afirman que es efectiva para controlar varias dolencias, incluso con solo tomar una cucharada del extracto. Por lo tanto, no se puede subestimar el perfil de la toxicidad de la planta de tabaco extraída de orina de vaca.

Objetivos: Caracterizar los extractos de orina de vaca de *N. tabacum* y evaluar la toxicidad subaguda de estos en ratas Wistar machos.

Métodos: Cuarenta ratas Wistar macho fueron asignadas al azar en grupos y expuestas a diferentes dosis (50, 100 y 200 mg/kg de peso corporal) de extracto de *N. tabacum* durante 7 y 28 días, después de lo cual los animales fueron sacrificados para los ensayos bioquímicos.

Resultados: El análisis GC-MS reveló 21 compuestos en el extracto de *N. tabacum*. Como se esperaba, la nicotina fue predominante entre los compuestos identificados. La exposición subaguda de las ratas al extracto podría haber alterado la homeostasis metabólica de la rata, desencadenando mecanismos de adaptación, al tiempo que afecta las funciones renales. El extracto de *N. tabacum* también moduló perfil lipídico de suero de rata en comparación con el control ($p<0.05$).

Conclusiones: Los resultados de este estudio sugieren que la exposición de ratas Wistar machos al extracto de *N. tabacum* en orina de vaca podría haber perturbado las funciones renales, así como modulado el perfil lipídico sérico. Por lo tanto, esta investigación recomienda a los consumidores locales precaución con la frecuencia a la que consumen de forma regular el "Adimenu".

Palabras Clave: bioquímica medicinal; evaluación de la seguridad; perfil lipídico; toxicidad bioquímica.

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INTRODUCTION

Tobacco is one of several herbal preparations commonly used for medicinal and social purposes. Tobacco is a name for any plant of the genus *Nicotiana* of the *Solanaceae* family (nightshade family) and products manufactured from its leaf include cigars, cigarettes, snuff and chewing tobacco (Ozoh et al., 2017). The smokeless tobacco is identified locally by various names, examples include Ntsu in South Africa, Toombak in Sudan, Shammah in South Arabia, plug chew in the United States, and Anwuru or Tabain Nigeria (Ozoh et al., 2017). *Nicotiana tabacum* L. is an annual plant that is usually grown in many countries for the commercial value of its leaf which can be processed into tobacco (Akomolafe et al., 2017). *N. tabacum* grows to heights between one and two meters and almost every part of the plant contains nicotine, but the concentration is relative to different factors such as species, type of land and weather conditions (Ben Saad et al., 2018). Other phytoconstituents found in *N. tabacum* include the polyphenols, pyridine alkaloids, and amino acids (Devlin et al., 2005). Nicotine accounts for 90-95% of the plant's pyridines (Nwangwa et al., 2015).

Tobacco has high nicotine content; therefore, it is readily used for several recreational purposes. For example, juice from tobacco leaves is used as intoxicating snuff or taken orally to induce vomiting and narcosis in Brazil. In addition, the fresh tobacco leaf is used as a poultice over boils and infected wounds, and as hair treatment to prevent baldness. In Cuba, tobacco leaves are used as antidysmenorrheal (Ben Saad et al., 2018). Also, separate studies have reported the extraction of a white–brown complex subfamily from tobacco leaves (Shekins et al., 2016); it is an odorless, tasteless white powder that can be added to cereal grains, vegetables, soft drinks and other foods as stabilizers (Shekins et al., 2016). In the present study, the research design was not to compare solvent extracts of the plant, but to evaluate the safety of its cow-urine extract as local consumption, among the people is on the increase daily. We characterized the cow-urine extract of *N. tabacum* and also evaluated the sub-acute toxicity of the extract in Wistar rats. Cow urine was used as the solvent in this study, to simulate the way it is used locally, no one locally prepares, nor uses the water extracted version of the plant, hence our reason for not comparing both in the first place, most importantly, to ensure that our research do not deviate from the actual investigation, which is the assessment of the safety of the concoction. The solvent was boiled to kill all germs that might be present in the urine prior to the extraction process.

MATERIAL AND METHODS

Reagents assay kits

All chemicals and reagents were of analytical grade and were used as supplied. Assay kits for alkaline phosphate (ALP), aspartate transaminase (AST), alanine transaminase (ALT), creatinine (CREA), urea, albumin (ALB), and bilirubin (BIL) were products of Randox Laboratories Limited (Crumlin, UK).

Preparation of plant extract

Fresh leaves of *N. tabacum* were purchased in February 2017 from Ganmo market (8.4190°N, 4.6086°E) in Ifelodun LGA of Kwara state, Nigeria. The plants were identified and authenticated with voucher number UIL/002/11011 by Mr. Bolu Ajayi of the Herbarium unit of the Department of Plant Biology, University of Ilorin, Kwara State, Nigeria. The plants were cut into smaller pieces, air-dried and pulverized. The powder was then weighed, and 200 g of the powdered plant was thereafter mixed with freshly collected and treated 1 L of cow urine to simulate local and traditional extraction methods. Prior to this, the solvent used, the cow urine, was subjected to a purification treatment by boiling it first, to kill all germs that might be present in the urine before it was used in the extraction process. The mixture was shaken for 72 h at room temperature. Each sample solution was filtered with Whatman No. 1 filter paper and concentrated at 55°C under reduced pressure, using a rotary evaporator connected to cold water circulator and pressure pump (BuchiRotavapor –

R110, Switzerland). The plant extracts were stored in a refrigerator (4°C) until required for experimental use.

Experimental animals

A total number of forty (40) apparently healthy adult male Wistar rats were sourced from the Animal House of the Biochemistry Department, University of Ilorin, Ilorin, Kwara state, Nigeria. They were acclimated in a 12-hour light and 12-hour dark cycles at room temperature for a week in the animal house of the Biochemistry Department of the University of Ilorin, Ilorin, Kwara state, Nigeria. They were housed in well-ventilated plastic cages with saw dust as bedding and proper aeration made available through the presence of large windows in the animal house. They were maintained on standard rat pellets and allowed free access to water *ad libitum*. Handling of animals was consistent with relevant guidelines on the care and use of laboratory animals (National Research Council, 2011) under the guidance of the Animal Ethics Committee of the University of Ilorin. Approval number 20320 was dated 15 March 2017.

Animal grouping

The dosages of 50, 100 and 200 mg/kg rat body weights of *N. tabacum* extract were re-suspended in distilled water prior to their administration to the rats. The extract was orally administered to the rats by using an oropharyngeal tube. Following the International guidelines for this type of toxicological study (OECD, 2008), in Phase 1, the rats were subjected to sub-acute treatment, where the first four groups of 20 rats, 5 rats per group, were treated for seven (7) days and observed for any sign of toxicity and/or mortality. Doses as low as 50, 100 and 200 mg/kg rat body weights, were used because of the acclaimed very strong effect of the concoction on the body of its regular local consumers, even at very low concentration of the local concoction, "Adimenu". On the 7th day, the 5 rats from each of the groups were sacrificed for biochemical assay.

Group A: 200 mg/kg bw of cow urine extract of *N. tabacum* (Five rats per group).

Group B: 100 mg/kg bw of cow urine extract of *N. tabacum* (Five rats per group).

Group C: 50 mg/kg bw of cow urine extract of *N. tabacum* (Five rats per group).

Group D: Negative drug Control (no extract administered but given distilled water) (Five rats per group).

In the Phase 2, the second four groups of 20 rats, 5 rats per group, were treated with 50, 100 and 200 mg/kg rat body weights and observed for twenty-eight (28) days. This study was done to give us information on the effect of longer exposure to the concussion at the same low dosage. The five rats from each of the groups were also sacrificed on the 28th day. Additional details on the animal groupings and treatments are as stated below:

Group A: 200 mg/kg bw of cow urine extract of *N. tabacum* (Five rats per group).

Group B: 100 mg/kg bw of cow urine extract of *N. tabacum* (Five rats per group).

Group C: 50 mg/kg bw of cow urine extract of *N. tabacum* (Five rats per group).

Group D: Negative drug control (no extract administered but given distilled water) (Five rats per group).

Necroscopy

After seven and/or twenty-eight days of the administration of *N. tabacum* extracts, the animals were fasted overnight. Thereafter, rats were sacrificed after mild anaesthetization with diethyl ether. The rat jugular vein was incised, and blood samples were collected into sterile sample tubes. The rats were then dissected and organs of interest (kidneys, brain, heart and liver) were collected, weighed and preserved in 0.25M sucrose solution (Adeyemi and Orekoya, 2014).

Preparation of serum

After 30 min at room temperature, blood samples were centrifuged at 1000 g for 10 min (Uniscope Centrifuge Model SM800B, England, UK). The supernatant was collected using Pasteur's pi-

pette and stored frozen at -4°C until required for the biochemical assay (Adeyemi and Elebiyo, 2014).

Preparation of tissue homogenate

Rat tissues in 0.25 M sucrose solution were homogenized using a mortar and pestle on ice. The homogenates were collected and centrifuged at 4000*g* for 15 min. The supernatant was diluted with 0.9% saline (1:5 v/v) and kept frozen until required for the biochemical assay (Adeyemi et al., 2012).

Biochemical assays

The levels of rat serum and tissue total protein (TP), albumin, AST (EC: 2.6.1.1), ALT (EC: 2.6.1.2), ALP (EC: 3.1.3.1), bilirubin, urea and creatinine were determined using Randox assay kits (Crumlin, UK).

GC-MS analysis

The GC-MS analysis was carried out using Agilent 7890 A. Initially, an aliquot of the *N. tabacum* extract concentrate was reconstituted in methanol and thereafter, the extract was injected into the GC-MS and vaporized at 250°C. This is followed by compound separation in HP5MS column fused with phenylmethylsiloxane. The carrier gas was helium operated at a flow rate of 1 mL per minute. The separated compounds were detected using GC-FID detector and each signal was fragmented on a mass spectrometry for compound identification (Sulaiman 2017).

Statistical analysis

The data were statistically analyzed using the one-way analysis of variance (ANOVA) on SPSS 20.0 version statistical package program (SPSS, Chicago, IL). The results were presented as mean of five replicates ± standard error of mean (SEM) n = 5. The Duncan Multiple Range test was used for post-hoc analysis and mean values at $p<0.05$ taken as significant.

RESULTS

Characterization of a cow urine extract of *N. tabacum* using Gas Chromatography-Mass Spectrometry

The chemical composition of a cow urine extract of *N. tabacum* was predominated by α-nicotine, methyl palmitate, and methyl elaidate, in decreasing order (Table 1, Supplementary Data), while, thymol and butanoic acid had the least concentration in the extract.

The average weight of animals

During the sub-acute treatment, no sign of toxicity and/or mortality of rats was recorded.

The oral administration of *N. tabacum* at various doses caused reduction in average rat weights for both the 7- and 28- day treatments (Fig. 1A). Similarly, the oral extract administration to rats inconsistently affected the rat organ weights as the organ-body weight ratio for both the 7- and 28-day treatments when compared to control (Fig. 1B-C).

Biochemical evaluations

The rat liver and kidney function indices were determined to evaluate the effect of the *N. tabacum* extract administration. For the 7-day treatment, administration of extract (50 mg/kg bw) elevated ($p<0.05$) the rat serum ALT activity compared to control (Fig. 2A), while for the 28-day treatment, the extract caused inconsistent alterations in rat serum ALT activity. Additionally, *N. tabacum* extract caused a reduction in rat serum AST activity (Fig. 2B). In the same manner, extract administration altered ($p<0.05$) rat serum and tissue protein levels compared to control (Fig. 3A). Meanwhile, extract at the highest dose (200 mg/kg bw) reduced rat serum albumin level while elevating the same at a lower dose (100 mg/kg bw) (Fig. 2C). Furthermore, the extract administration caused an elevation ($p<0.05$) of total bilirubin level for the 7-day treatment, but at the 28-day treatment, total bilirubin level was reduced ($p<0.05$) compared to

control (Fig. 2D). The administration of the extract dose-dependently elevated ($p<0.05$) rat serum triglyceride level compared to control (Fig. 2E). Likewise, the extract administration led to elevated ($p<0.05$) rat serum urea concentration for the 7-day treatment (Fig. 3B). In contrast, rat serum urea level was decreased following the 28-day administration of the extract. Meantime, at 7- and 28- day treatments, rat serum creatinine level was increased ($p<0.05$) compared to control (Fig. 2F).

DISCUSSION

The role of traditional medicines in the solution of health problems is globally invaluable; medicinal plants have been used as therapeutic agents, both in modern and traditional medicine (Atolani et al., 2011; 2014; Fabiyi et al., 2012; Owa et al., 2016a;b). In parallel, no sufficient evidence exists on the safety/toxicity profiling of these traditionally applied medicinal plants.

Table 1. Chemical composition of cow urine extract of *Nicotiana tabacum* as determined by using Gas Chromatography-Mass Spectrometry (GC-MS).

S/N	RT	Chemical compound	Concentration (%)
1	4.86	p-Cresol	1.93
2	5.29	Octanoic acid	0.76
3	7.53	Thymol	0.32
4	7.84	Nicotine	2.93
5	8.00	Butanoic acid	0.33
6	8.34	α –Nicotine	32.46
7	9.92	1'S2'S-Nicotine-n-oxide	0.64
8	0.45	Dodecanoic acid	2.72
9	12.24	Methyl tetradecanoic	2.29
10	13.71	Methyl palmitate	14.74
11	14.48	1-Naphalenemethanol	0.52
12	14.82	9-12 Octadecadienoic acid	8.02
13	14.86	Methyl elaidate	13.45
14	15.89	7-Octadecadienoic acid	1.24
15	15.37	Methyl 9 cis, 11 transoctadecadienoate	3.24
16	15.018	Methyl stearate	2.42
17	15.018	Valeric acid	1.12
18	15.79	Copaiferic acid	4.57
19	16.19	Methyl 18 methylnonadecanoate	1.65
20	18.13	Octacosine	2.42
21	19.06	2-Methyltetracosine	1.09

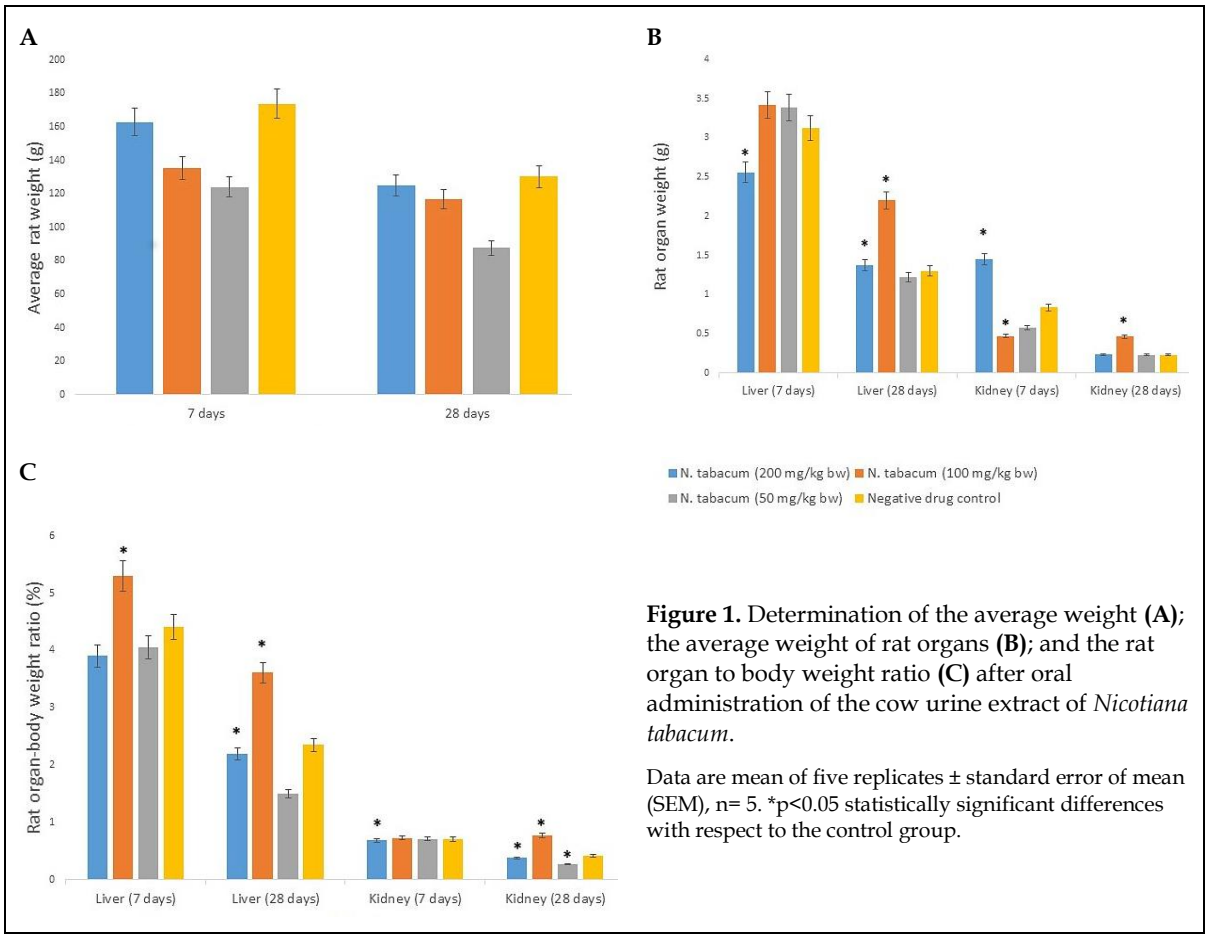

Figure 1. Determination of the average weight **(A)**; the average weight of rat organs **(B)**; and the rat organ to body weight ratio **(C)** after oral administration of the cow urine extract of *Nicotiana tabacum*.

Data are mean of five replicates ± standard error of mean (SEM), n= 5. *p<0.05 statistically significant differences with respect to the control group.

The *N. tabacum* is an annual herbaceous plant reported to have a variety compounds with several biological activities (Alwar et al., 2013). In this study we detected 21 different phytoconstituents in *N. tabacum*, with α- nicotine been predominant. This finding supports earlier report (Alwar et al., 2013). Among the compounds found in *N. tabacum* is nicotine, which is predominant and has been employed in various form for human use. Reports from WHO, stated that an approximate of 1.3 billion people use nicotine in the form of tobacco products, with a likelihood for its consumption to increase due to its addictive nature (Mosbah et al., 2015). We also observed that the extract of *N. tabacum* caused a weight loss unexposed rats compared to control. This effect may be attributed to the modulating effect of nicotine on leptin secretion, thus affecting fat storage as reported elsewhere (Mahmoud and Amer, 2014).

In our assessment of liver function, rat serum AST and ALT levels were inconsistently altered, with pronounced reduction in the enzyme activities when compared with control. This is contrary to previous studies which reported elevated activities of these enzymes, indicative of liver damage (Mahmoud and Amer, 2014; Nwangwa et al., 2015; Shekins et al., 2016; Ben Saad et al., 2018). We suspect that the sub-acute administration of the extract of *N. tabacum* might have imposed a stress on the rat liver leading to metabolic adaptations. This is likely so, if we consider that the extract administration raised the rat serum albumin level in a manner that suggests cellular adaptation. However, as the dose increased, the rat serum albumin level dropped drastically, suggestive of early signs of impaired liver synthetic function. Additionally, that the administration of the extract inconsistently altered rat serum protein and bilirubin levels, may

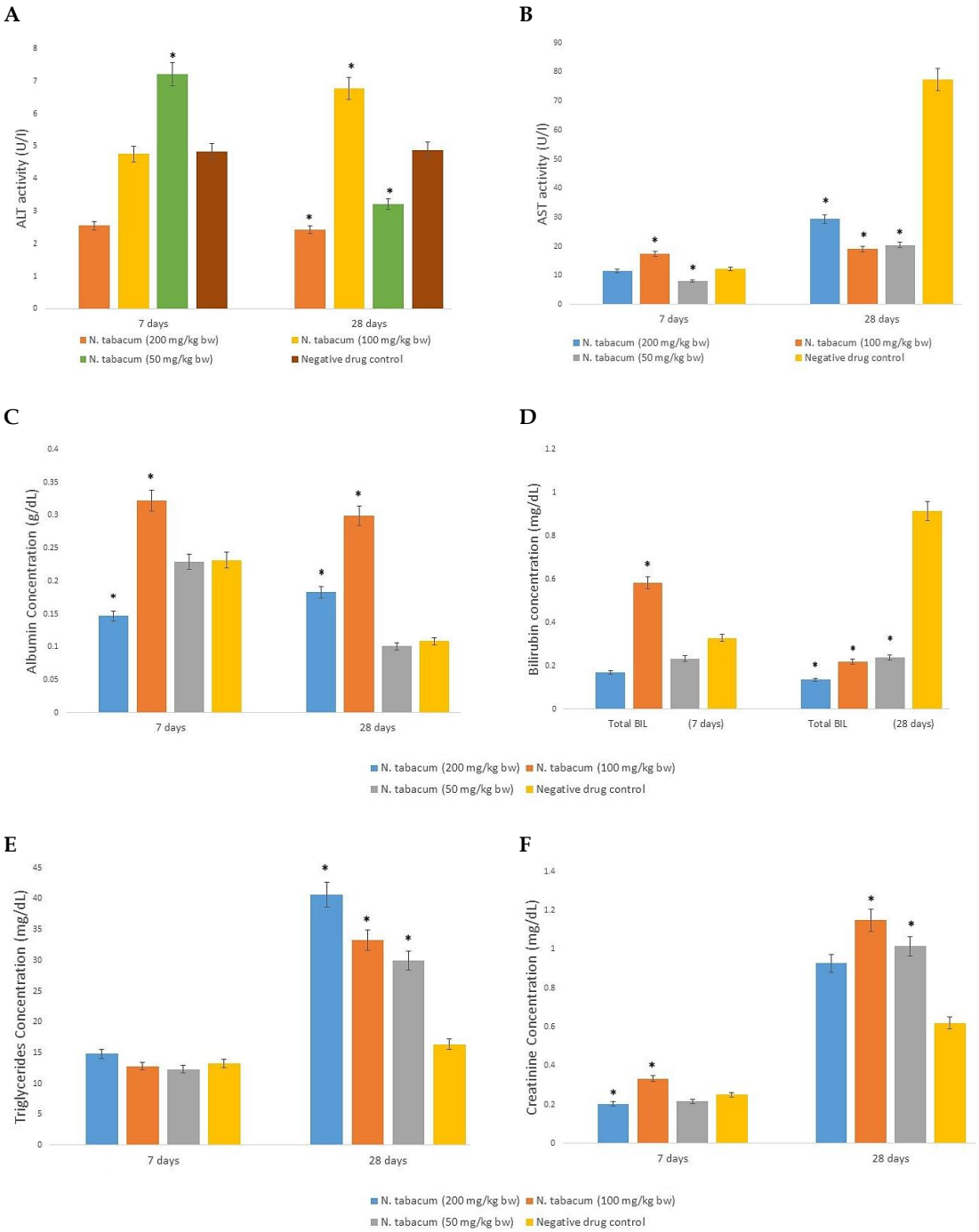

Figure 2. The alanine transaminase (ALT) activity **(A)**; the aspartate transaminase (AST) activity **(B)**; the albumin level **(C)**; the total bilirubin level **(D)**; the triglyceride level **(E)**; the creatinine level **(F)** in rat serum after oral administration of the cow urine extract of *Nicotiana tabacum*.

Data are mean of five replicates ± standard error of mean (SEM), n= 5. *p<0.05 statistically significant differences with respect to the control group.

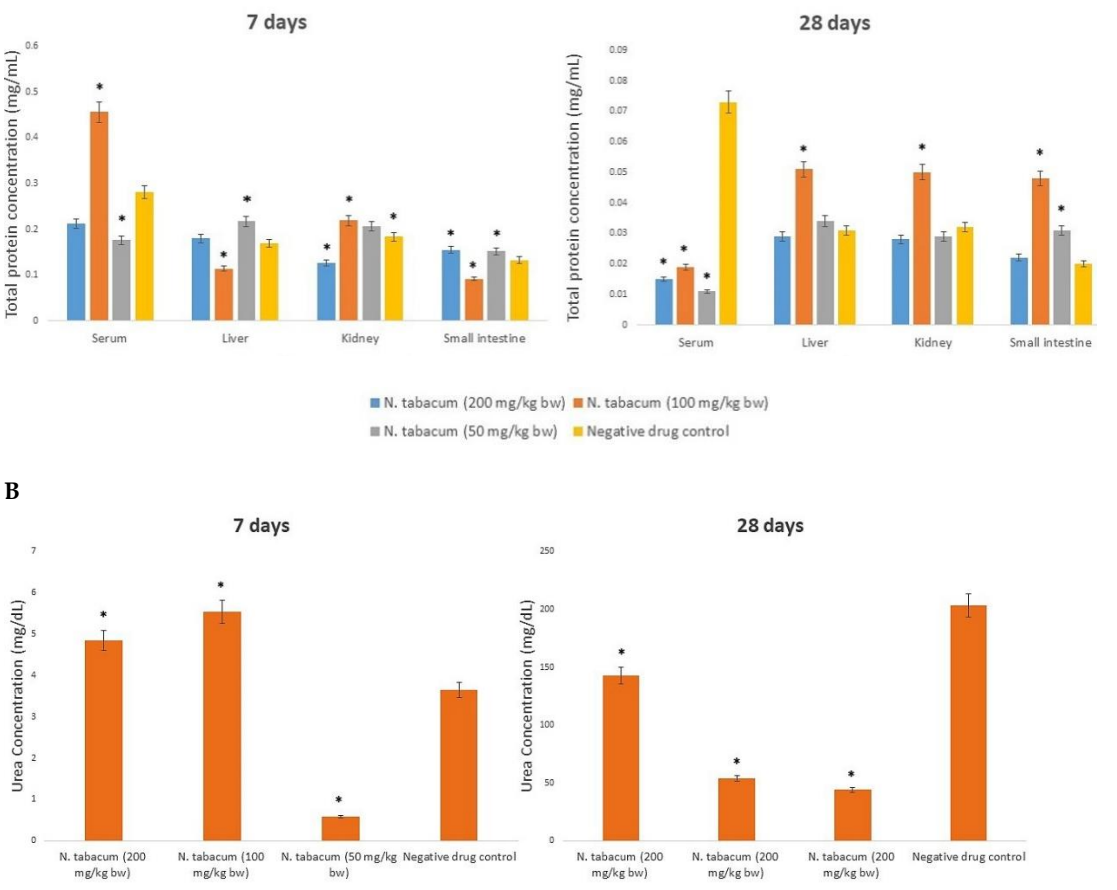

Figure 3. The total protein levels in rat serum and tissues **(A)**; and the urea level in rat serum **(B)**, after oral administration of the cow urine extract of *Nicotiana tabacum*.

Data are mean of five replicates ± standard error of mean (SEM), n= 5. *p<0.05 statistically significant differences with respect to the control group.

further reinforce the plausibility of the adaptive mechanisms in rats due to likely stress imposed by exposure. Notwithstanding that the extract of *N. tabacum* might have triggered an adaptive mechanism in rats. It is also within the realm of possibility that the reduced activities of rat serum ALT and AST might be attributable to inhibitory action by the extract or the extract metabolites. This line of thought would be consistent with earlier findings that showed that plant extracts or its metabolites are capable of inhibiting enzyme activities in rats (Adeyemi and Akanji, 2011; Adeyemi et al., 2012). Furthermore, the extract administration led to elevated levels of rat serum urea and creatinine suggesting likely renal impairment. Moreover, considering that nicotine, which is the predominant ingredient found in the *N. tabacum* extract has been linked with impaired renal reabsorption and clearance in rats (Rezonzew et al., 2012; Akomolafe et al., 2017). In addition, the administration of *N. tabacum* extract caused a dose-dependent elevation of rat serum triglycerides. This finding may not only indicate potential to modulate the rat lipid profile but suggest a predisposition to cardiac disturbances. Taken together, data do not only suggest adaptive mechanisms by the rats, but the ensuing renal perturbations due to the extract administration, even at low doses as used in this study.

CONCLUSIONS

This study revealed the biochemical effects of *N. tabacum* extract in Wistar rats following a sub-acute oral exposure. Our findings support adaptive mechanisms in rats as a result of oral exposure to *N. tabacum* extract. Conversely, the extract administration to rats might have led to the ensuing renal impairment. Additionally, the sub-acute administration of *N. tabacum* extract raised the rat serum triglycerides in a manner that may predispose to cardiac agitation. This research therefore recommends to local consumers of the concoction to be cautious of the rate at which they consume their regular "Adimenu", the cow-urine extracted *N. tabacum*.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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SUPPLEMENTARY DATA

Supplementary data associated with this article can be found at http://jppres.com/jppres/pdf/vol8/jppres19.594_8.3.191.suppl.pdf

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AUTHOR CONTRIBUTION:

Contribution	Sulaiman FA	Nafiu MO	Muritala HF	Adeyemi SB	Yusuf BO	Omar SA	Dosumu KA	Adeoti ZJ	Adegbesan OA	Busari BO	Otohinoyi DA	Romiti D	Batiha GE	Adeyemi OS
Concepts or ideas	x				x									
Design	x	x	x		x									
Definition of intellectual content	x	x	x											
Literature search											x	x	x	
Experimental studies				x	x		x	x	x	x				
Data acquisition					x	x	x	x	x	x				
Data analysis	x				x	x								
Statistical analysis				x	x	x								
Manuscript preparation											x	x	x	x
Manuscript editing														x
Manuscript review	x	x	x	x	x	x	x	x	x	x	x	x	x	x

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