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Cardio-Haematological and Oxidative Assaults in Wistar Rats: The Roles of *Nicotiana tabacum* Leaf Extracts

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ABSTRACT

Background and Objective: Cow urine extract of *Nicotiana tabacum* leaf, "adimenu" is being used by a significant percentage of Nigerian youths to manage various conditions without enough scientific information to refute or support the usage. This research investigated the effect of single and repeated doses of oral administration of aqueous and cow urine extracts of *Nicotiana tabacum* leaf on the lipid profile and oxidative and haematological status of Wistar rats. **Materials and Methods:** Seven groups of five male rats each were used. The control group received 0.5 mL of distilled water, groups A and D received 8 mg kg^{-1} body weight of cow urine and aqueous extracts, respectively, orally, once daily for 28 days, groups B and E received 16 mg kg^{-1} body weight of cow urine and aqueous extracts, respectively, orally, thrice-a-day for 28 days while groups C and F received 32 mg kg^{-1} body weight of cow urine and aqueous extract, respectively, orally, thrice-a-day for 28 days. Biochemical parameters were analyzed in sera and blood samples. **Results:** Administration of single and repeated doses of the extracts revealed that single and repeated doses of the extracts significantly increased the activity of SOD and catalase as well as the level of LDL and atherogenic index while HDL, RBC, Hb, HCT, MCH and MPV significantly decreased. **Conclusion:** The administration of the extracts was found to induce oxidative stress, cause anaemia and impair heart functionality.

KEYWORDS

Haematology, cow urine extract, oxidative stress, *Nicotiana tabacum*, lipid profile, adimenu

INTRODUCTION

Nicotiana tabacum is an annual plant that is grown across many countries for its leaves, which can be refined into tobacco for commercial purposes[1]. The average height of the *N. tabacum* plant is between 1 and 2 m and it is widely distributed throughout the world's tropical regions[2]. Except for the seed, almost

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every part of the plant has a varied concentration of nicotine that increases with age depending on factors such as species, type of land and weather conditions[3]. Tobacco can be consumed in two forms: Smoking and smokeless tobacco. Snuff (dry and moist) and chewing tobacco are smokeless tobacco varieties, whereas cigarettes, cigars, bidis, kreteks, pipes, water pipes (also known as shisha) and paper-rolled cigarettes are smoking tobacco varieties[4,5].

Cigarette smoking, the far more popular way of using tobacco, exposes both active and passive smokers to a wide range of toxins. This can result in increased oxidative stress, decreased antioxidant availability, increased inflammation, altered lipid profile and nicotine addiction[6].

Nicotine, the primary component of tobacco, causes addiction to all tobacco products and alters brain chemistry, making abstinence impossible for heavy smokers and posing health risks[7]. Tobacco carcinogens cause a wide range of tumours, including lung, prostate, stomach, dental and bladder cancer, among others. Administration of the extract has been reported to cause liver damage[8].

Despite the numerous health risk posed by *Nicotiana tabacum* consumption, people continue to use cow urine as a solvent of extraction of *N. tabacum* leaf popularly known as "Adimenu or Grade 1" for acclaimed therapeutic uses. This prompted an investigation into the effect of the administration of the cow urine and aqueous extracts of *Nicotiana tabacum* leaf on the lipid profile and oxidative and haematological status of Wistar rats to engender the assessment of potentially harmful effects from repeated and long-term use of the *Nicotiana tabacum* leaf-based concoctions.

MATERIALS AND METHODS

Study area: The research was carried out between November, 2019 and December, 2020.

Plant material and authentication: A dried leaf of *N. tabacum* was purchased in November, 2019 from Ganmo market (8.4190°N, 4.6086°E) in Ifelodun LGA of Kwara State, Nigeria. The plant was identified and authenticated by Mr. Bolu Ajayi of the Herbarium of Plant Biology, University of Ilorin and the voucher number UIL/002/11011 was assigned. The plant name, *Nicotiana tabacum* L. was checked (data supplied on 2012-03-26) on http://theplantlist.org/tpl1.1/search?q=Nicotiana+tabacum+ and was found to be an accepted name.

Experimental animals: Thirty-five albino rats weighing 150.50±8.20 g were obtained from the Animal House of the Department of Chemical Sciences, Summit University Offa, Kwara State, Nigeria.

Assay kits and reagents: Total cholesterol and triglycerides High-Density Lipoprotein (HDL) kits were products of Randox Laboratories Ltd., Antrim, UK. Other chemicals and reagents used were of analytical grade.

Preparation of aqueous and cow urine extracts of *N. tabacum* leaf: The dried leaf of *N. tabacum* (400 and 200 g) was extracted in pre-boiled cow urine (4 L) and distilled water (1.5 L), respectively for 24 hrs at room temperature. The mixtures were separately filtered using Whatman no 1 filter paper and the filtrates were freeze-dried (Labconco Freeze Drier, Model 64132, Kansas City, Missouri, USA) to give the aqueous and cow urine extracts of 42.76% and 34% yields, respectively. The extracts were reconstituted in distilled water to give the desired doses of 8, 16 and 32 mg kg^{-1} body weight.

Ethical clearance: This study was carried out after ethical approval from the Summit University Ethical Review Committee with approval number SUERC/CONAS/2020/001.

Table 1: Animals grouping and extract administration

Groups	Dosage (mL)
Control	0.5 mL of distilled water
A	8 mg kg^{-1} body weight of cow urine extract of *N. tabacum* leaf once daily
B	16 mg kg^{-1} body weight of cow urine extract of *N. tabacum* leaf thrice daily
C	32 mg kg^{-1} body weight of cow urine extract of *N. tabacum* leaf thrice daily
D	8 mg kg^{-1} body weight of aqueous extract of *N. tabacum* leaf once daily
E	16 mg kg^{-1} body weight of aqueous extract of *N. tabacum* leaf thrice daily
F	32 mg kg^{-1} body weight of aqueous extract of *N. tabacum* leaf thrice daily

Animal grouping and extract administration: Healthy male Wistar rats weighing 150.50±8.20 g were kept in well-ventilated cages with free access to rat feed and clean water *ad libitum*. Thirty-five animals were divided into 7 groups (A, B, C, D, E, F and Control) of 5 rats each as outlined in Table 1.

Preparation of serum and determination of biochemical parameters: The procedure described by previously[9] was adopted for the preparation of serum. Briefly, the blood was collected by cutting the jugular vein and the blood was drained into sterile sample bottles (plain and anticoagulant). Blood samples in the plain sample bottles were left undisturbed at 25°C for 30 min to form clots after which the samples were centrifuged at 1282 g for 5 min. After centrifugation, the sera were collected by a means of Pasteur pipette into a clean sample bottle, appropriately labelled and stored in a freezer at 4°C not later than 72 hrs of preparation for the biochemical assays.

The methods employed[10] were used to assay for the activity of catalase and superoxide dismutase while glutathione transferase and reduced glutathione were assayed[11]. Cholesterol, triglyceride, high-density lipoprotein and low-density lipoproteins were determined using commercial assay kits (Randox Laboratory Limited, UK) ensuing the manufacturer's method while, very low-density lipoprotein (VLDL-c) was estimated[10]. Haematology was done using an automated haematological analyzer SYSMEX KX-21 (a product of SYSMEX Corporation, Harrier, Japan).

Data analysis: Data were expressed as the Mean±SEM of five determinations. Data were analyzed using a One-way Analysis of Variance followed by Tukey's *post-hoc* Test for multiple comparisons. Statistical significance was set at a 95% confidence interval ($p<0.05$) and graph Pad Statistical Package Version 6.0 was used for the statistical analyses.

RESULTS

Antioxidant enzyme activity: The activity of Superoxide Dismutase (SOD) significantly ($p<0.05$) increased from 2.28 to 3.11 (U mg^{-1} protein) while, catalase activity significantly ($p<0.05$) increased from 0.36 to 0.43 (U mg^{-1} protein) following oral administration of single and repeated daily doses of aqueous and cow urine extracts of *N. tabacum* leaf. Glutathione Peroxidase (GPX), Glutathione-S-Transferase (GST) and Glutathione Reductase (GSH) activities were not significantly ($p>0.05$) different from the control in Table 2.

Lipid profile: Oral administration of aqueous and cow urine extracts of *N. tabacum* leaf produced no significant ($p>0.05$) difference in cholesterol, triglyceride and VLDL of the animals administered all doses of aqueous and cow urine extract of *N. tabacum* when compared with the control. However, there was a significant ($p<0.05$) decrease from 16.32±2.22 to as low as 8.08±0.90 in HDL levels of wistar rats administered doses of aqueous and cow urine extracts of *N. tabacum* leaf except for the group administered 16 mg kg^{-1} body weight of the aqueous extract of *N. tabacum* leaf three times daily that was not significantly different from the control group administered distilled water (16.32±2.22). Also, there was a significant ($p<0.05$) increase in LDL level from 11.25±2.94 to 22.12±1.04 and the atherogenic index of all groups administered doses of the extracts up to 2.71±0.53 when compared with the control in Table 3.

Table 2: Antioxidant enzyme activity of rats administered aqueous and cow urine extracts of *N. tabacum* leaf

Groups	Superoxide dismutase (U mg^{-1} protein)	Catalase (U mg^{-1} protein)	Glutathione peroxidase (U mg^{-1} protein)	Glutathione transferase (U mg^{-1} protein)	Glutathione reductase (U mg^{-1} protein)
Control	2.28±0.17^{a}	0.36±0.01^{a}	4.71±0.73^{a}	0.09±0.02^{a}	21.00±3.24^{a}
A	3.00±0.13^{b}	0.42±0.02^{b}	3.36±0.18^{a}	0.09±0.00^{a}	24.06±4.44^{a}
B	3.05±0.85^{b}	0.40±0.01^{b}	2.99±0.71^{a}	0.11±0.02^{a}	24.70±3.84^{a}
C	2.94±0.08^{b}	0.42±0.02^{b}	3.21±0.90^{a}	0.08±0.01^{a}	23.07±5.07^{a}
D	2.79±0.16^{b}	0.39±0.01^{b}	3.41±0.65^{a}	0.11±0.02^{a}	27.03±7.11^{a}
E	3.11±0.26^{b}	0. 41±0.01^{b}	3.86±0.82^{a}	0.09±0.02^{a}	27.24±5.41^{a}
F	2.90±0.46^{b}	0.43±0.02^{b}	4.36±0.81^{a}	0.08±0.01^{a}	24.63±3.34^{a}

Values are Means±SEM of five replicates and values with different superscripts are significantly ($p<0.05$) different from one another

Table 3: Lipid profile of rats administered aqueous and cow urine extracts of *N. tabacum* leaf

Treatment groups	Cholesterol (mmol L^{-1})	Triglyceride (mmol L^{-1})	HDL (mmol L^{-1})	LDL-C (mmol L^{-1})	VLDL-C (mmol L^{-1})	Atherogenic index (LDL-C/HDL-C)
Control	29.98±1.68^{a}	12.00±2.26^{a}	16.32±2.22^{a}	11.25±2.94^{a}	2.40±0.45^{a}	0.71±0.25^{a}
A	29.99±1.58^{a}	12.28±1.31^{a}	9.94±1.50^{b}	17.59±1.60^{b}	2.46±0.26^{a}	1.82±0.43^{b}
B	31.34±2.15^{a}	12.61±1.14^{a}	11.78±0.92^{b}	17.04±1.74^{b}	2.52±0.23^{a}	1.45±0.16^{b}
C	33.90±1.01^{a}	14.29±1.12^{a}	08.92±0.64^{b}	22.12±1.04^{b}	2.86±0.22^{a}	2.49±0.27^{c}
D	31.07±2.80^{a}	11.12±0.71^{a}	10.97±1.70^{b}	17.88±2.27^{b}	2.22±0.14^{a}	1.66±0.32^{b}
E	35.82±1.46^{a}	11.65±1.66^{a}	14.52±1.13^{a}	18.97±2.49^{b}	2.33±0.33^{a}	1.32±0.27^{b}
F	31.86±2.23^{a}	11.16±1.08^{a}	08.08±0.90^{b}	21.55±2.66^{b}	2.23±0.22^{a}	2.71±0.53^{c}

Values are Means±SEM of five replicates, values with different superscripts in a column are significantly ($p<0.05$) different from one another, HDL: High-density lipoprotein, LDL-C: Low-density lipoprotein-cholesterol and VLDL-C: Very low-density lipoprotein-cholesterol

Table 4: Hematological parameters of rats administered aqueous and cow urine extracts of *N. tabacum* leaf

Groups/ parameters	Control	A	B	C	D	E	F
RBC (×10^{6})	6.27±0.15^{a}	3.72±0.13^{c}	5.53±0.28^{b}	5.10±0.51^{b}	5.50±0.14^{b}	6.40±0.22^{a}	6.25±0.38^{a}
Hb (g dL^{-1})	12.6±0.35^{a}	9.98±0.38^{b}	8.67±0.55^{b}	10.07±0.67^{b}	10.58±0.85^{b}	9.37±0.42^{b}	9.37±0.21^{b}
WBC (10^{3} μL^{-1})	19.70±5.32^{a}	15.73±2.34^{a}	13.03±2.31^{a}	22.13±7.25^{a}	19.04±5.14^{a}	15.37±4.79^{a}	13.47±3.44^{a}
HCT (%)	46.92±2.19^{a}	39.1±4.01^{b}	33.43±2.42^{b}	38.93±0.15^{b}	40.16±3.08^{b}	34.33±0.70^{b}	35.30±2.07^{b}
MCV (fL)	64.04±3.21^{a}	57.68±4.58^{a}	55.97±1.17^{a}	58.2±3.50^{a}	57.2±3.67^{a}	52.53±0.06^{a}	53.47±1.03^{a}
MCH (pg)	17.2±0.47^{a}	14.73±0.38^{b}	14.53±0.83^{b}	15.00±0.92^{b}	15.02±0.66^{b}	14.3±0.46^{b}	14.2±0.36^{b}
MCHC (g dL^{-1})	26.92±1.88^{a}	25. 68±2.52^{a}	25.97±1.88^{a}	25.83±1.63^{a}	26.40±2.02^{a}	27.27±0.86^{a}	26.57±0.97^{a}
PLT (10^{3} μL^{-1})	761.40±132.8^{a}	1065.00±154.22^{a}	1216.00±327.38^{a}	885.33±33.65^{a}	995.6±227.77^{a}	1032.76±108.36^{a}	1105. 33±351.76^{a}
RDW-SD (fL)	44.34±5.77^{a}	41.35±9.76^{a}	37.17±1.98^{a}	39.80±3.42^{a}	35.70±3.81^{a}	34.50±2.05^{a}	34.33±0.75^{a}
RDW-CV (%)	761.40±132.8^{a}	1065.00±154.22^{a}	1216.00±327.38^{a}	885.33±33.65^{a}	995.60±227.77^{a}	1032.67±108.36^{a}	1105.35±351.76^{a}
PWD (fL)	12.86±1.82^{a}	10.80±1.09^{a}	9.47±0.71^{a,b}	11.50±0.30^{a}	11.22±1.39^{a}	9.73±0.38^{a,b}	9.83±0.68^{a,b}
MPV (fL)	9.00±0.70^{a}	7.93±0.57^{b}	7.57±0.31^{b}	8.21±0.17^{b}	8.06±0.32^{b}	7.37±0.25^{b}	7.40±0.10^{b}
P-LCR (%)	20.54±4.96^{a}	13.28±3.77^{a}	10.47±2.65^{a,b}	15.77±0.70^{a}	14.08±3.04^{a}	9.90±0.89^{a,b}	10.20±0.62^{a,b}
PCT (%)	0.69±0.13^{a}	0.85±0.17^{a}	0.92±0.23^{a}	0.73±0.03^{a}	0.80±0.22^{a}	0.76±0.17^{a}	0.82±0.25^{a}

Values are Means±SEM of five replicates, values with different superscripts in a row are significantly ($p<0.05$) different from one another, RBC: Red blood cell count, HB: Haemoglobin, WBC: White blood cell, HCT: Hematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular haemoglobin, MCHC: Mean corpuscular haemoglobin concentration, PLT: Platelet, RDW-SD: Red blood cell distribution width standard deviation, RDW-CV: Red blood cell distribution width coefficient of variation, PDW: Platelet distribution width, MPV: Platelet volume, P-LCR: Platelet-large cell rate and PCT: Platelet crit

Hematological parameters: Administration of single and repeated daily doses of *N. tabacum* leaf extracts produced a significant ($p<0.05$) decrease in Mean Platelet Volume (MPV (fL)) from 9.00±0.70-7.37±0.25, Mean Corpuscular Hemoglobin (MCH (pg)) from 17.2±0.47-14.2±0.36, Hematocrit (HCT (%)) from 46.92±2.19-33.43±2.42, Hemoglobin (HB (g dL^{-1})) from 12.6±0.35-8.67±0.55 and Red Blood Cell count (RBC (×10^{6})) from 6.27±0.15 to as low as 3.72±0.13 except the RBC of the repeated doses of 16 and 32 mg kg^{-1} body weight of aqueous extract of *N. tabacum* leaf that were not significantly different from the control in Table 4. However, the White Blood Cell (WBC (10^{3} μL^{-1})), Mean Corpuscular Volume (MCV (fL)), Platelet (PLT (10^{3} μL^{-1})), Platelet Distribution Width (PDW (fL)), Platelet-Large Cell Rate (P-LCR (%)), Platelet Crit (PCT (%)), Red Blood Cell Distribution Width Standard Deviation (RDW-SD (fL)), Red Blood Cell Distribution Width Coefficient of Variation (RDW-CV %)) were not significantly different from the control.

DISCUSSION

The surge in the use of herbal medicines for healthcare[12] is attributed partly to its safety, acceptability and affordability by the poor masses. Patients in some cases became addicted to some herbal medications/concoctions, the most notable in Southwest Nigeria is the extracts of *Nicotiana tabacum* leaf popularly known as "adimenu".

Endogenous antioxidants such as Superoxide Dismutase (SOD), catalase and glutathione peroxidase are known to attenuate the generation of Reactive Oxygen Species (ROS) by removing potential oxidants or by transforming ROS/RNS into relatively stable compounds. The cell protects itself from oxidative damage by recruiting GSH and scavenging enzymes such as SOD and GST as a first-line cellular defence in response to oxidative challenges to protect cellular integrity[13]. The significant increase in the antioxidant activities of SOD and catalase as evident in this study might be due to the presence of saponins, polyphenols and flavonoids that have been earlier reported to have prolific antioxidant capacity[14]. The increased activity of SOD and catalase suggests that there were a lot of superoxide radicals produced during the metabolism of nicotine.

The lipid profile assay presented a unique trend of results in the concentration of total cholesterol, triglyceride, very low-density lipoprotein and atherogenic index. The alterations in lipid profiles have also been linked to tobacco use. The significant increase and decrease in the level of LDL and HDL concentration can lead to heart disease, particularly atherosclerosis which is supported by a significant increase in the level of the calculated Atherogenic Index (AI)[15].

Assessment of haematological parameters can be used to determine the extent of the harmful effect of the foreign compound on the blood and also explain blood relating functions of biochemical compounds[16]. Erythrocytes are particularly susceptible to oxidative damage as a result of high polyunsaturated fatty acid content in cell membranes and high concentrations of oxygen and haemoglobin, which promotes oxidative processes[17]. This was evidenced by the decrease in the number of Red Blood Cells (RBCs), haemoglobin, hematocrit, MCH and MPV level in rats administered extracts of *N. tabacum* leaf in this study. The observed decrease in these 16 parameters could be because nicotine interferes with vitamin C levels, which is crucial for functional iron absorption and inhibits iron uptake[18]. Also, the nicotine reduction of red blood cells may be due to peroxidative membrane damage via ROS production[17] and/or by ROS reacting with haemoglobin, altering its structure and releasing free iron ions that, in turn, increase the generation of ROS[19]. The decreased RBC, MPV and Hb levels agree with the previous study on rats administered aqueous extract of *N. tabacum* leaf[20].

CONCLUSION

The cow urine and aqueous extracts of *Nicotiana tabacum* leaf alter the lipid profile, antioxidant and haematological status. This can result in oxidative stress, anaemia and heart disease. Based on this result, users of this concoction should do so with care and not take the extract daily.

SIGNIFICANCE STATEMENT

The study discovered that aqueous and cow urine extracts of *N. tabacum* leaf are capable of inducing toxicological assaults on the oxidative status and cardiovascular system of Wistar rats. The results of this research will serve to inform researchers of the toxicological effects of *N. tabacum* extracts and thus prompt research into the molecular mechanism of its toxicity.

REFERENCES

1. Akomolafe, O.R., C.E. Imafidon, O.S. Olukiran, A.A. Oladele and B.O. Akanji, 2017. Sub-acute administration of lower doses of nicotine caused sex-dependent improvement of renal function in Wistar rats. Toxicol. Rep., 4: 535-542.

2. Liu, X., Z. Wei, Y. Ma, J. Liu and F. Liu, 2021. Effects of biochar amendment and reduced irrigation on growth, physiology, water-use efficiency and nutrients uptake of tobacco (*Nicotiana tabacum* L.) on two different soil types. Sci. Total Environ., Vol. 770. 10.1016/j.scitotenv.2020.144769.
3. Djordjevic, M.V. and K.A. Doran, 2009. Nicotine Content and Delivery Across Tobacco Products. In: Nicotine Psychopharmacology: Handbook of Experimental Pharmacology 192, Henningfield, J.E., E.D. London, S. Pogun (Eds.), Springer, Berlin, Heidelberg, Germany, ISBN: 978-3-540-69248-5, pp: 61-82.
4. Ozoh, O.B., M.O. Akanbi, C.E. Amadi, W.M. Vollmer and N.G. Bruce, 2017. The prevalence of and factors associated with tobacco smoking behavior among long-distance drivers in Lagos, Nigeria. Afr. Health Sci., 17: 1110-1119.
5. Prignot, J.J., A.J. Sasco, E. Poulet, P.C. Gupta and T.Y. Aditama, 2008. Alternative forms of tobacco use. Int. J. Tuberculosis Lung Dis., 12: 718-727.
6. Carnevale, R., V. Cammisotto, F. Pagano and C. Nocella, 2018. Effects of Smoking on Oxidative Stress and Vascular Function. In: Smoking Prevention and Cessation, Rajer, M. (Ed.), IntechOpen, London, UK, ISBN: 978-1-83881-574-5, pp: 25-48.
7. Benowitz, N.L., 2009. Pharmacology of nicotine: Addiction, smoking-induced disease and therapeutics. Annu. Rev. Pharmacol. Toxicol., 49: 57-71.
8. Andong, F.A., E.A. Orji, N.E. Ezenwaji, A.O. Nkemakolam and T.D. Melefa *et al*., 2021. Sub-acute oral toxicity study of aqueous extract of tobacco leaves (*Nicotiana tabacum* L.) on lipid profile, the tissue, and serum of the liver and kidney of male Wistar rats. Biomarkers, 26: 127-137.
9. Yakubu, M.T., M.A. Akanji and A.T. Oladiji, 2005. Aphrodisiac potentials of the aqueous extract of *Fadogia agrestis* (Schweinf. Ex Hiern) stem in male albino rats. Asian J. Androl., 7: 399-404.
10. Yusuf, B.O., M.T. Yakubu and M.A. Akanji, 2021. Chromatographic fractions from *Chrysophyllum albidum* stem bark boost antioxidant enzyme activity and ameliorate some markers of diabetes complications. J. Traditional Complementary Med., 11: 336-342.
11. Ejeh, S.A., S.E. Abalaka, I.L. Usende, Y.A. Alimi and F.O. Oyelowo, 2019. Acute toxicity, oxidative stress response and clinicopathological changes in Wistar rats exposed to aqueous extract of *Uvaria chamae* leaves. Sci. Afr., Vol. 3. 10.1016/j.sciaf.2019.e00068.
12. Mensah, M.L.K., G. Komlaga, A.D. Forkuo, C. Firempong, A.K. Anning and R.A. Dickson, 2019. Toxicity and Safety Implications of Herbal Medicines Used in Africa. In: Herbal Medicine, Builders, P. (Ed.), IntechOpen, London, Uk, ISBN: 978-1-83881-386-4, pp: 63-86.
13. Nwozo, S.O., A.A. Ajagbe and B.E. Oyinloye, 2012. Hepatoprotective effect of *Piper guineense* aqueous extract against ethanol-induced toxicity in male rats. J. Exp. Integr. Med., 2: 71-76.
14. Jannet, S.B., N. Hymery, S. Bourgou, A. Jdey, M. Lachaal, C. Magné and R. Ksouri, 2017. Antioxidant and selective anticancer activities of two *Euphorbia* species in human acute myeloid leukemia. Biomed. Pharmacother., 90: 375-385.
15. Santos, H.O. and C.J. Lavie, 2021. Weight loss and its influence on high-density lipoprotein cholesterol (HDL-C) concentrations: A noble clinical hesitation. Clin. Nutr. ESPEN, 42: 90-92.
16. Mehany, H.M., N.M. El-Shafai, A.M. Attia, M.M. Ibrahim and I.M. El-Mehasseb, 2022. Potential of chitosan nanoparticle/fluoride nanocomposite for reducing the toxicity of fluoride an *in-vivo* study on the rat heart functions: Hematopoietic and immune systems. Int. J. Biol. Macromol., 216: 251-262.
17. Tedesco, I., M. Russo, P. Russo, G. Iacomino and G.L. Russo *et al*., 2000. Antioxidant effect of red wine polyphenols on red blood cells. J. Nutr. Biochem., 11: 114-119.
18. Salunke, R., B. Sharma, K. Saraf and P. Roy, 2011. Smokeless tobacco extract impairs iron homeostasis in rats and human hepatoma, HepG2 cells. Toxicol. Environ. Chem., 93: 1028-1044.
19. Cimen, M.Y.B., 2008. Free radical metabolism in human erythrocytes. Clin. Chim. Acta, 390: 1-11.
20. Andong, F.A., E.S. Okwuonu, T.D. Melefa, C.O. Okoye and A.O. Nkemakolam *et al*., 2021. The consequence of aqueous extract of tobacco leaves (*Nicotiana tabacum* L.) on feed intake, body mass, and hematological indices of male wistar rats fed under equal environmental conditions. J. Am. Coll. Nutr., 40: 429-442.