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Evaluation of the Anti-diabetic, Haematological and Hypolipidemic Effects of Methanol Extract of *Annona muricata* (Annonaceae) Seeds in Alloxan-Induced Diabetic Albino Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The study evaluated the antidiabetic, haematological, and hypolipidemic effects of Annona muricata seed methanol extract (AMSME) in alloxan-induced diabetic rats. Twenty-four (24) Wistar rats were grouped into five groups: group 1 served as the normal control, group 2 served as the positive control treated with glibenclamide (5 mg/kg body weight), group 3 had diabetic rats not given any intervention, and group 4-5 served as the treatment group and contained diabetic rats treated with AMSME (50 mg/kg and 100 mg/kg body weight). Extracts of AMSME were

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administered orally to the rats for 28 days, after which the rats were sacrificed through ocular puncture and blood was collected for biochemical tests and examination. In comparison to the positive control (160.2±0.22 mg/dl), the results demonstrated a significantly lower blood glucose level ($p < 0.05$) in all the groups that received AMSME at a dose of 100 mg/kg body weight (99.7±0.03 mg/dl). The erythropoietic impact of AMSME at all dosages was shown by the activity of all the erythropoietic marker enzymes (Hb, WBC, RBC, and PCV) showing a substantial rise in all the groups treated with AMSME as compared with the untreated negative control, which exhibited no trace of inflammatory damage. The results also show a significant ($P < 0.05$) increase in high-density lipoprotein (HDL), while total cholesterol (TC), triacylglycerides (TG), and low-density lipoprotein (LDL) were significantly ($p > 0.05$) reduced compared to the diabetic untreated group. The study's findings revealed that AMSME's ability to prevent diabetes may be attributed to its protective and haematological effects on pancreatic beta-cells, which in turn improve the body's reaction to glucose.

Keywords: Alloxan; Annona muricata; diabetes; haematology; hypolipidemic.

1. INTRODUCTION

A moderate to medium insulin deficit causes diabetes mellitus, a metabolic disorder that affects how carbs, lipids, and proteins are broken down [1,2]. High blood glucose levels brought on by unbalanced insulin production are a distinctive sign of the clinical condition known as diabetes [1]. “Diabetes is associated with an increased risk of dying from cardiovascular disease (CVD) because atherogenic dyslipidemia is characterized by an increase in total cholesterol, triglycerides, low-density lipoproteins (LDL), and very-low-density lipoproteins (VLDL), and a decrease in high-density lipoprotein (HDL) particles” [3,4]. “Additionally, it has been proposed that hyperglycemia, which is associated with an increase in non-enzymatic glycosylation of red blood cell (RBC) membrane proteins, causes anaemia in people with diabetes mellitus” [5]. Every diabetes mellitus treatment strategy calls for bringing blood sugar levels to a healthy range while decreasing cardiovascular risk, particularly by managing hypertension and treating dyslipidemia [6]. “Several medicinal plants are utilized as traditional treatments for diabetes because they are efficient, have fewer side effects, and are reasonably inexpensive” [2]. One of these herbs, *Annona muricata* (family Annonaceae), is utilized in Nigerian folk medicine to treat diabetes mellitus.

“Annona muricata (sour-sop) seeds are high in nutrients and provide many compounds with acetogenins” [7]. These include bulatacin, asimisin, and squamosin. Annona muricata leaf extract included secondary metabolites such as tannins, steroids, and cardiac glycosides, as shown by phytochemical analysis [8]. “Soursop leaf contains a number of plant compounds with antioxidant effects, including luteolin, quercetin, and tangeretin. Sour-sop leaves are good for controlling and treating diabetes because the minerals in them bring blood sugar levels back down to a healthy range” [8]. “Parts of the Annona muricata plant have been emphasised for their anti-inflammatory [9], anti-arthritic [10], anti-diabetic [11] and anti-cancer [12] effects”.

Plants have long been an integral part of human life, whether in the form of food, medicine, or other items [13]. Plants have always been essential to human survival, not simply as a source of food but also as the foundational creatures upon which the rest of the food web rests. As a result of these and other factors, it is now ingrained in the human character to discover which plants are useful for food, medicine, and other human endeavours [13]. Furthermore, plants are playing uncontrolled roles in human health maintenance due to their therapeutic properties. In rural regions, people often use a decoction made from the plant's stem, seed, roots, and leaves to cure ringworm, itching, eczema, helminthiasis, cut wounds, boils, nasty ulcers, diabetes, and other digestive problems [13]. This research aimed to evaluate the potential hypoglycemic, haemolytic, and hypolipidemic effects of a methanol extract of *Annona muricata* seed on alloxan-induced Wistar rats due to the extensive medicinal and pharmacological abilities of *Annona muricata* for the treatment of diseases.

2. MATERIALS AND METHODS

2.1 Plant Materials/Extraction

Annona muricata fruits were bought from Orie-Ugba market in Umuahia North LGA, Abia State. The plant was authenticated by a Taxonomist (Dr

Ibe K. Ndukwe) from the forestry department, College of Natural Resources and Environment Management (CNREM), Michael Okpara University of Agriculture Umudike where a voucher specimen (IHF 26123) was deposited in the departmental herbarium. The leaves were collected, washed, and dried under shade at room temperature 25^{o}C, then weighed and milled into powder 250g. The leaves powder were soaked in 80:20 v:v of methanol: distilled water for three days with occasional shaking, filtered by using Whatman filter paper No.1, the solvent was evaporated by rotary evaporator under reduced pressure at 40^{o}C.

2.2 Experimental Animals

The investigation was conducted using twenty-four (24) healthy male Wistar rats, weighing 100–120g, that were procured from the Ogive Integrated Farm, located in Aba, Abia State. The animals were weighed when they first arrived to determine their starting weight, and they were acclimated for 14 days at the animal house of the Biochemistry Department in the College of Natural Sciences at Michael Okpara University of Agriculture, Umudike. The animals were exposed to sunlight for 12 hours each day in typical tropical weather while receiving access to regular food and water up to the conclusion of the 28-day research study. All of the rats were kept in sterile metal cages at a constant temperature of 25°C in a normally humid daytime environment. The rats were freely fed pellets, given tap water, and made available throughout the experiment as approved by the departmental committee on animal use guidelines at the Michael Okpara University of Agriculture, Umudike, on handling experimental animals.

2.3 Induction of Diabetes

Animals were given 120 mg/kg intraperitoneal injections of alloxan monohydrate dissolved in normal saline to produce type 2 diabetes. 72 hours after the drugs were administered, the animals' fasting blood glucose levels were measured to verify induction. Rats were diagnosed with diabetes if their fasting blood sugar levels were above 140 mg/dl.

2.4 Experimental Design and Animal Grouping

Rats were divided into five groups of four rats each respectively.

2.5 Sacrifice and Sample Collection

After the experiment, Blood samples were collected through cardiac puncture under anaesthesia into an EDTA bottle. Pooled blood sample (1 ml per rat, 9 ml per treatment) was used for biochemical analysis.

2.6 Determination of Biochemical Parameters

Total cholesterol was evaluated using the enzymatic colourimetric chod-pap test method [14]; “Triglycerides were also determined spectrophotometrically using the method of Tietz” [15]; “High-density lipoproteins (HDL) were evaluated by the method of Grove” [16]. “Low-density lipoprotein (LDL) was determined as the difference between total cholesterol and cholesterol content of the supernatant after precipitation of the LDL fraction by polyvinyl sulphate (PVS) in the presence of polyethene-glycol monomethyl ether” [17].

2.7 Determination of Haematological Parameters

“Haematological parameters were analyzed using a haematology analyzer (Mindray Auto Hematology Analyzer, BC-5200, USA.) following the methods of Chhabra” [18]. The parameters assayed were as follows: white blood cell count (WBC), red blood cell count (RBC), haemoglobin (Hb), and packed cell volume (PCV).

2.8 Statistical Analysis

Turkey's multiple comparison post hoc tests were used to examine the degree of significance between the test groups after the data were statistically analysed using one-way analysis of variance (ANOVA) and mean standard deviation (SD) to describe the data. P values of 0.05 were regarded as significant.

3. RESULTS

According to Fig. 1, treatment with Annona muricata (seed) extract at dosages of 50 mg/kg and 100 mg/kg, respectively, considerably ($P>0.05$) lowered the level of blood glucose in comparison to the control. However, there was a substantial ($P>0.05$) drop in blood glucose levels when compared to the positive control drug, glibenclamide. Nevertheless, in comparison to the control, the dosage of 100 mg/kg exhibited the greatest glucose-reducing impact.

Table 1. Grouping and treatment of animals

Groups		Treatment
Group 1	Normal control	Feed + H_2O ad libitum
Group 2	Negative control	Alloxan + Feed + H_2O ad libitum
Group 3	Positive control	Alloxan+ Standard drug (Glibenclamide) + Feed + H_2O
Group 4	Annona muricata	Alloxan + 50mg/kg extract + Feed + H_2O ad libitum
Group 5	Annona muricata	Alloxan + 100mg/kg extract + Feed + H_2O ad libitum

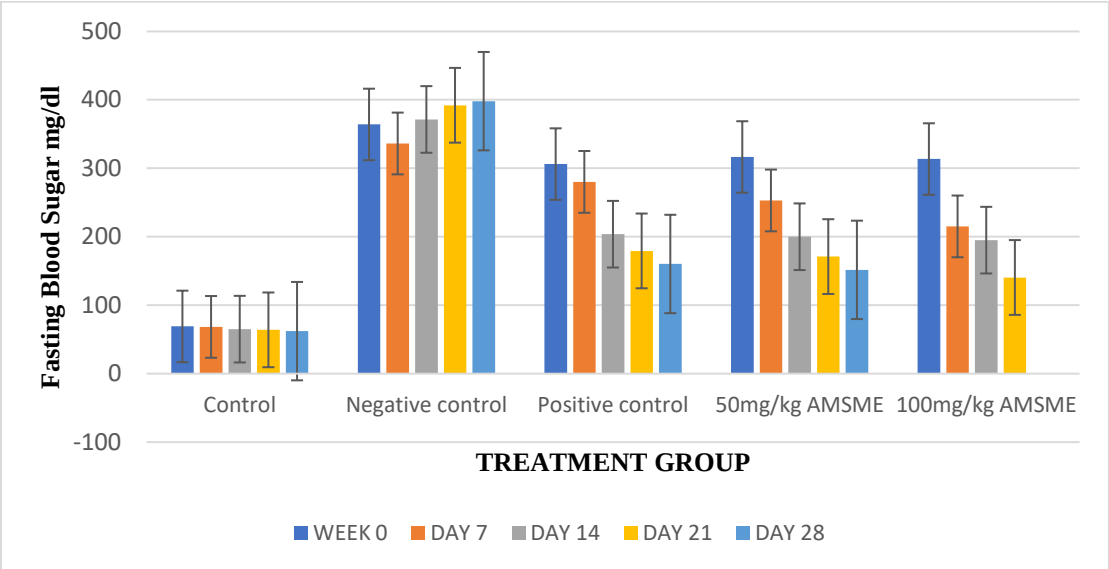

Fig. 1. Comparison of mean glucose level in the *Annona muricata seed* extract in normal control, positive and negative control for 28 days

Result expressed as Values are mean ± SD; n=5

Our findings showed a significant (P< 0.05) reduction in TWBC, HB, RBC and PCV in the diabetic animals. Treatment with AMSE extracts significantly (P< 0.05) improves the levels of these indices in diabetic animals.

The triacylglycerol level in the diabetic control animals was significantly ($p < 0.05$) higher than that of the healthy animals. Treatment with AMSE in all the administered doses significantly lowered triacylglycerol levels in the diabetic animal to levels comparable to that of the normal control animals. This was similar to the effect of Glibenclamide, which was also able to lower significantly ($p < 0.05$) the triacylglycerol level in diabetic animals. Our results also showed a significant elevation in the level of cholesterol in the diabetic animal compared to the healthy control animals. The AMSE - treated group showed a significant ($p < 0.05$) decrease in cholesterol level compared to the diabetic control animals. Glibenclamide also significantly ($p < 0.05$) decreased cholesterol levels in diabetic animals. The concentration of HDL in the diabetic animals was significantly ($p < 0.05$) lower than that of the healthy animals. The different doses of AMSE (50 and 100 mg/kg) were able to significantly ($p < 0.05$, respectively) elevate the reduced HDL in the diabetic animals. Glibenclamide also significantly ($p < 0.05$) elevated HDL levels in diabetic animals. Induction of diabetes elevated the LDL levels of the animals. However, AMSE at different doses (50 and 100 mg/kg) were able to significantly ($p < 0.05$, respectively) elevate the reduced LDL in the diabetic animals.

4. DISCUSSION

Results from studies on Annona muricata seed extract (AMSE) indicated that AMSE extract is a generic drug for maintaining normal glucose levels [19]. Significant antihyperglycemic action at 100 mg/kg was seen and compared well to glibenclamide, suggesting that the results may be useful in the management of diabetes. (the standard drug). Different flavonoid components in AMSE have various biological and pharmacological effects [20]. Flavonoids have a wide range of biological functions in plants, including serving as UV filters, signal molecules, allelopathic substances, phytoalexins, detoxifying

agents, and antimicrobial defence compounds [20]. Based on the results of these experiments, it seems that the flavonoids present in the *A. muricata* methanolic seed extract are responsible for its anti-diabetic and anti-hyperglycemic properties.

Hyperglycemia and an elevated lipid profile describe diabetes mellitus, a metabolic disease. This condition may be caused by insulin's failure to initiate the cellular absorption of glucose after digestion. However, it is crucial to create more potent and affordable medications to treat and control the condition, given the rise in mortality caused by it. Numerous studies have documented a strong effect of *A. muricata* on variables related to the development of diabetes mellitus. According to studies, antihyperglycemic effects, a rise in body weight, and improved serum lipid profile by lowering TCHO, TRIG and LDL, VLDL, and increasing TCHO, HDL, and the percentage of the anti-atherogenic index (AAI), are all recorded [3].

The most frequent consequences of diabetes mellitus are changes in lipid metabolism, which present as hyperlipidemia. According to studies, lipid profile changes in diabetes patients are a risk factor for cardiovascular illnesses [6]. Compared to the control rat in the research, the alloxan-induced diabetic rats showed hypertriglyceridemia, decreased HDL levels, hypercholesterolemia, and a modest rise in LDL levels. In addition, Alaebo et al. [4] observed that alloxan-induced diabetic rats had higher plasma cholesterol, TAG, LDL-c, VLDL-c, and lower HDL cholesterol. The elevated levels of triglycerides and cholesterol seen in this study may be caused by hormone-sensitive lipase being activated due to insulin insufficiency or sensitivity, which causes a rise in the mobilization of free fatty acids from peripheral stores. MEAM therapy improved HDL levels and decreased triglyceride, cholesterol and LDL levels in diabetic rats. AMSE contains a phenolic substance that contributes to the normalization of the lipid profile, which may explain its capacity to alleviate the changes in lipid metabolism in diabetic animals.

The importance of erythrocytes in transporting oxygen to tissues throughout the body's circulatory system is well established [21]. It has been shown that the hyperglycemia that occurs in diabetes mellitus reduces the capacity of red blood cells to deform without rupturing when they endure continuous flow conditions in tight capillaries [22]. The production of reactive oxygen species is a hallmark of diabetes mellitus and a major contributor to this impairment. This is in line with the study's conclusion that diabetic animals had significantly lower levels of TWBC, HB, RBC, and PCV. Alloxan administration may have decreased WBC, Hb, RBC, and PCV levels because of aberrant haemoglobin synthesis, poor blood osmoregulation, and high plasma osmolarity [22]. As the extract was administered, the RBC level and associated indices significantly improved. This supports the claim that the AMSE extract can promote the production or release of erythropoietin, which prompts bone marrow stem cells to create red blood cells [23].

Table 2. Effect of Haematological indices of alloxan-induced diabetic albino rats treated with methanol extract of Sour-sop (*Annona muricata)* seed

Groups	Treatment	RBC (g/dl)	TWBC (g/dl)	Hb (g/dl)	PCV (g/dl)
1	Normal Control (Feed + H_2O ad libitium)	165.13 ± 0.30	75.14 ± 1.20	12.24 ± 2.20	53.24 ± 2.05
2	Negative Control (Alloxan + Feed + H_2O ad libitium)	131.32 ± 1.40	43.20 ± 2.10	8.13 ± 2.00	31.16 ± 1.20
3	Positive Control (Alloxan + Standard drug, Glibenclamide + Feed + H_2O ad libitium)	160.20 ± 1.10*	73.05 ± 3.01*	11.04 ± 1.30*	53.07 ± 2.30*
4	AMSE50mg/kg extract (Alloxan + Feed + H_2O ad libitium)	170.14 ± 3.04*	78.04 ± 2.00*	14.17 ± 0.40*	55.04 ± 0.20*
5	AMSE100mg/kg extract (Alloxan + Feed + H_2O ad libitium)	173.25 ± 5.01*	82.34 ± 1.05*	15.01 ± 0.01*	58.24 ± 0.10*

The table is expressed as Values are mean ± SD; n=5, p<0.05 significant difference compared to the diabetic untreated (group 2). Values are expressed as mean ± SD (n = 5). *p<0.05 when compared with the negative control.*
Abbreviation: TWBC: Total White Blood Cells; Hb: Hemoglobin; RBC: Red Blood Cells; PCV: Packed Cell Volume. AMSE: Annona muricata seed methanol ext

Table 3. Effect of Lipid profile of alloxan-induced diabetic albino rats treated with methanol extract of Sour-sop (*Annona muricata)* seed

Groups	Treatment	TCHOL (mg/dl)	TG (mg/dl)	LDL-C (mg/dl)	HDL-C (mg/dl)
1	Normal Control (Feed + H_2O ad libitium)	75.12 ± 1.230	72.14 ± 4.420	15.24 ± 10.20	83.04 ± 2.105
2	Negative Control (Alloxan + Feed + H_2O ad libitium)	91.02 ± 1.450	163.20 ± 3.170	78.13 ± 2.300	31.16 ± 1.720
3	Positive Control (Alloxan + Standard drug, Glibenclamide + Feed + H_2O ad libitium)	66.21 ± 1.140	90.25 ± 30.31*	13.04 ± 1.430*	73.27± 2.430*
4	AMSE 50mg/kg extract (Alloxan + Feed + H_2O ad libitium)	70.17 ± 3.724	101.4 ± 2.030*	24.17 ± 0.440*	65.84 ± 6.720*
5	AMSE 100mg/kg extract (Alloxan + Feed + H_2O ad libitium)	57.25 ± 2.051	92.34 ± 1.405*	10.41 ± 10.01*	68.24 ± 5.310*

The table is expressed as mean ± SEM n=5, p<0.05 significant difference compared to the diabetic untreated (group 2).*
Abbreviation: TCHOL: Total Cholesterol,
TG: Triacyl glyceride, HDL: High-density lipoprotein; LDL; Low-density lipoprotein; AMSE: Annona muricata seed methanol extract

5. CONCLUSION

At dosages of 50 and 100 mg/Kg body weight, the ingestion of AMSE extracts has been proven to produce hypolipidemic effects. In this study, the ability to lower blood cholesterol, which may be connected to a high concentration of phytonutrients, was associated with the reversal of the effects of diabetes on numerous biochemical and haematological parameters. Therefore, it can be said that AMSE extracts normalize the haematological anomalies connected to diabetes mellitus securely and efficiently. They may thus be recommended as an addition to dietary treatment for diabetes.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors with this declare that principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the College of Natural Sciences, Michael Okpara University of Agriculture (MOUAU) Research and Ethics Committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Prince OA, Chinwe EO, Chiemeziem AO, Chimaraoke O, Peter OE, George CN. Hypoglycemic effect of methanol extract of pawpaw (Carica papaya) leaves in alloxan-induced diabetic rats. International Journal of Innovative Science Research Technology. 2022;7(1):627-631. Available:https://doi.org/10.5281/zenodo.5995940
2. David UI, George CN, Prisca CA, Isabel CN, Mildred CI, Chizurum PC. Effect of Poly-Herbal Formula (PHF5) on hepatoprotective and biochemical parameters of alloxan-induced diabetic Wistar Rats. Asian Journal of Biochemistry, Genetics and Molecular Biology. 2022;10(3):33-41. Available:https://doi.org/10.9734/ajbgmb/2022/v10i330247
3. Prince OA, Chimaraoke O, Chinwe EO, George CN, David UI, Peter OE. Hepato-protective effect and lipid profile of honey on alloxan-induced diabetic Rats. Asian Journal of Research Biochemistry. 2022; 10(1):16-24. Avaailable:https://doi.org/10.9734/ajrb/2022/v10i130212
4. Ogochukwu AP, Chigozie NG, Chukwuma UG, Onyinye UC. Hypoglycemic and Hypolipidemic Effect of Dialum guineense (ICHEKU) Fresh Leaves on Alloxan-induced Diabetes in Male Albino Rats. Asian Journal of Research in Cardiovascular Diseases. 2022;4(4):1–7. Available:https://journalijrrc.com/index.php/AJRCD/article/view/54
5. Alaebo PO, Njoku GC, Oriaku CE, Iloanusi DU, Ezeh CJ, Ugboaja TC, James UA, EkwunohPO, Anyadike NN. Histological assessment and haematological parameters of honey on alloxan induced diabetic male albino rats. International Journal of Biochemistry Research & Review. 2022;31(2):17-26. Available:https://doi.org/10.9734/ijbcrr/2022/v31i230303
6. Alaebo PO, Onyeabo C, Oriaku CE, Njoku GC, Iloanusi DU, Ekwunoh PO. Hepato-protective effect and lipid profile of honey on alloxan-induced diabetic rats. Asian Journal of Research in Biochemistry. 2022;10(1):16–24. Available:https://doi.org/10.9734/ajrb/2022/v10i130212
7. Alaebo PO, Chukwu CN, Nwuke CP, Ezeigwe OC, Ekwunoh PO. Hepatoprotective and antioxidant effects of methanol extract of soursop (*Annona Muricata*) seeds on alloxan-induced diabetic wistar rats. Nigerian Research Journal of Chemical Science. 2020;8 (12):199-210
8. Moghadamtousi S, Fadaeinasab M, Nikzad S, Mohan G, Ali H, Kadir H. *Annona muricata*(Annonaceae): A review of its traditional uses, isolated acetogenins and biological activities. International journal of molecular sciences. 2015;16(7):15625-15658.
9. Moghadamtousi S, Fadaeinasab M, Nikzad S, Mohan G, Ali H, Kadir HJI. *Annona muricata* (Annonaceae): A review of its traditional uses, isolated acetogenins and biological activities. 2015;16(7):15625-15658.
10. Coria-Téllez AV, Montalvo-Gónzalez E, Yahia EM, Obledo-Vázquez ENJA.

A*nnona muricata*: A comprehensive review on its traditional medicinal uses, phytochemicals, pharmacological activities, mechanisms of action and toxicity. 2018;11(5):662-69.

11. Justino AB, Miranda NC, Franco RR, Martins MM, da Silva NM. *Annona muricata Linn*. leaf as a source of antioxidant compounds with in-vitro antidiabetic and inhibitory potential against α-amylase, α-glucosidase, lipase, non-enzymatic glycation and lipid peroxidation. Espindola Pharmacotherapy. 2018;100:83-92.
12. Rady I, Bloch MB, Chamcheu RCN, BanangMbeumi S, Anwar MR, Mohamed H, El Sayed KA. Anti-cancer properties of Graviola (*Annona muricata*): A comprehensive mechanistic review .Oxidative medicine and cellular longevity; 2018.
13. Kumar M, Faheem M, Singh S, Shahzad A, Bhargava AK. Antifungal activity of the Carica papaya important food and drug plant. Asian Journal of Plant Science and Research. 2013;3(1):83-6.
14. Allain CC, Poon LS, Chan LS, Richmond CSG, FuPC. Enzymatic determination of total serum cholesterol. Clinical Chemistry. 1974;20:470-475.
15. Tietz NW. Clinical guide to laboratory test. 2ndedition. Philadephia, USA: W.B. SaundersCompany. 1990:554-556.
16. Grove TH. Effect of reagent pH on determining high-density lipoprotein cholesterol by precipitation with sodium phosphotungstate-magnesium. Clinical Chemistry. 1979;25:260-264.
17. Bergmenyer HU. Methods of enzymatic analysis. 3rdedition. New York: Academic Press. 1985; 154-160
18. Chhabra G. Automated haematology analyzers: Recent trends and applications. Journal of Laboratory Physics. 2018;10(1): 015–016.
19. Alaebo PO, Chukwu CN, Nwuke CP, Ukpabi-ugo JC, Ezeigwe OC, Ekwunoh PO. Hypoglycaemic and hepato-protective effects of crude extract of averrhoa carambola leaves in alloxan-induced female diabetic rats. Nigerian Research Journal of Chemical Sciences. 2020;8 (2):40-56.
20. Arango TJ, Bacanumenth PA. La adecuación de tierras en el departamento de Antioquia; Revista Facultad Nacional de Agronomía Medellín. 2009;52(1):395-424
21. Waggiallah H, Alzohairy M. The effect of oxidative stress on human red cells glutathione peroxidase, glutathione reductase level, and prevalence of anaemia among diabetics. Northern American Journal of Medical Science. 2011;3(7):344–347.
22. Stookey JD, Burg M, Sellmeyer DE, Greenleaf JE, Arieff A, Van Hove L, Gardner C, King JC. A proposed method for assessing plasma hypertonicity in vivo. European Journal of Clinical Nutrition. 2007;61(1):143–146.
23. Lodish H, Flygare J, Chou S. From stem cell to erythroblast: regulating red cell production at multiple levels by multiple hormones. International Union of Biochemistry and Molecular Biology Life. 2010;62(7):492–496

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