World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

Check for updates

Nescafé coffee caused changes in hematological parameters of alloxan-induced diabetic Wistar rats

Nonso Odikpo [1], Francis Oguwike [1], Cornelius Nwozor [1, *], Onyekachukwu Oguekwe [1], Kosisochukwu Ifemenam [2], Henry Enwelum [2] and Okechukwu Peter Nwabuokei [3]

[1] Department of Physiology, Faculty of Basic Medical Sciences, Chukwuemeka Odumegwu Ojukwu University, Uli campus, Anambra State, Nigeria.
[2] Department of Obstetrics and Gynecology, Faculty of Clinical Sciences, Chukwuemeka Odumegwu Ojukwu University Teaching Hospital, Amaku, Awka, Anambra State, Nigeria.
[3] Department of Medicine and Surgery, Faculty of Medical Sciences, International University of Health Sciences, St. Kitts and Nevis State, West Indies.

World Journal of Advanced Research and Reviews, 2024, 22(03), 923–930

Publication history: Received on 05 May 2024; revised on 11 June 2024; accepted on 14 June 2024

Article DOI: https://doi.org/10.30574/wjarr.2024.22.3.1794

Abstract

This study investigated the effect of Nescafe coffee on the haematological parameters in alloxan-induced diabetic male Wistar rats. Thirty male Wistar rats of body weight between 150 and 260 g were used for the study. They were divided randomly into five groups of six rats each. The 24 rats in groups 2, 3, 4, and 5 were alloxanized with 120 mg/kg of alloxan (intraperitoneally) and were confirmed diabetic before the commencement of the experiment. Group 1 rats (control) received feed and water, group 2 (diabetic rats) received 1.5 ml of 0.9% normal saline, group 3 received 500 mg/kg of Nescafe coffee extract, group 4 received 800 mg/kg of Nescafe coffee extract, while group 5 received 5 mg/kg of glibenclamide. After 28 days of experiment, the animals were sacrificed and blood samples were collected from the rats via cardiac puncture for haematological tests. Fasting blood glucose level was determined weekly via tail puncture using a glucometer and readings were recorded. The result for RBC showed a significant decrease ($P < 0.05$) in groups 2 and 4 compared to group 5. PCV result showed a significant decrease ($P < 0.05$) in group 4 compared to group 5. The MCH and MCHC result showed a significant increase ($P < 0.001$) in group 3, compared with group 5.There was also a significant decrease ($P < 0.001$) in group 2, group 4 and group 5, compared with group 1 (control). The haemoglobin, MCV, WBC count, lymphocyte, neutrophil, monocyte counts, MPV, and ESR, result showed no significant difference ($P > 0.05$) in all other groups when compared with group 1 and group 5. There was a significant decrease ($P < 0.05$) in the platelet count of the animals in groups 2 and 4 when compared with the control group. Furthermore, a significant decrease ($P < 0.001$) was noted in the eosinophil count and basophil count ($P < 0.01$) of the animals in groups 3, 4, and 5 when compared to the control group. On the contrary, a significant increase ($P < 0.001$) was noted in the eosinophil count and basophil count ($P < 0.01$) of the animals in group 2 when compared to group 5. In conclusion, the results showed that Nescafe coffee (especially when consumed in high quantity) significantly decreased some of the blood parameters of the diabetic animals.

Keywords: Nescafe coffee; Haemoglobin; Alloxan-induced diabetes; Wistar rats

1. Introduction

Diabetes mellitus is a metabolic disorder of global health concern[1]. The disease is characterized by a high glucose level in the blood (hyperglycemia), in urine (glycosuria), and inadequate production and utilization of insulin. The symptoms include polyuria, polydipsia, polyphagia and loss of weight[1].

* Corresponding author: Cornelius Nwozor

Caffeine is the most widely consumed stimulant worldwide, and coffee is the main dietary source[2]. The caffeine content varies between 65-150 mg of coffee cup, depending on the type of coffee and its preparation method. One cup of Nescafé instant coffee containing a teaspoon of powder may contain 30–90 mg of caffeine, while one cup of regular coffee contains about 70–140 mg of caffeine[3].

Caffeinated coffee are consumed for different reasons, such as, for weight reduction, as a means of food especially breakfast in homes, and as stimulant to remain active during exercise or strenuous activities[4].

The commonly grown coffee bean types are *C. arabica* and *C. robusta.* Coffee plants are grown in the Americas, Asia, and Africa[5].

Coffee can stimulate the central nervous system, increasing a person's mood, vigilance and mental alertness. It also decreases fatigue and depression[6].

Very high doses of about 100 cups of coffee containing 10 grams of caffeine have been reported to induce cardiac arrhythmias, lethargy, increased secretion of gastric acid and irritability[7].

Hematological parameters include platelet count, red blood cell count (RBC), white blood cell count (WBC) and differentials, hemoglobin, packed cell volume (PCV), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC). They are clinical indicators in health and disease and they give a good report on the physiological status of an individual[8]. This study aimed to evaluate the effect of Nescafé coffee on the hematological parameters in alloxan-induced male diabetic Wistar rats.

2. Methodology

2.1. Study area

The research was done in the animal house, Department of Human Physiology, Faculty of Basic Medical Sciences, Chukwuemeka Odumegwu Ojukwu University Uli Campus, Anambra State, Nigeria.

2.2. Experimental animals

Thirty (30) male Wistar weighing 150-260 g were used for the study. The animals were housed in wooden cages with stainless-steel wire lids on sawdust bedding and maintained at room temperature and 12-hour cycles of light and darkness. Animals were allowed access to feed and clean water *ad libitum* throughout the experiment. They were allowed to acclimatize for two weeks.

2.3. Experimental design

After acclimatization of the animals, the weight of the rats was recorded. At the commencement of the experiment, they were divided into five (5) groups. Each group consisted of six rats. Group 1 (control), group 2 (120 mg/kg alloxan i.p), group 3 (alloxan +500 mg/kg Nescafe coffee), group 4 (alloxan +800 mg/kg Nescafe coffee, group 5 (standard drug: glibenclamide, 5 mg/kg).

2.4. Induction of diabetes mellitus

One of the very active methods to induce experimental type 1 diabetes mellitus is through chemical induction by the diabetogenic substance, alloxan[9].

Experimental diabetes mellitus was induced on all the rats (except the rats in the control group) by the use of alloxan (Sigma St. Louis, Mo, USA). It was purchased in a powdered form at Pyrex I.G Chemical Company, Benin Nigeria. The rats were fasted overnight and the fasting blood glucose level and body weight were recorded before induction of diabetes. Twenty four rats were administered with alloxan (120 mg/kg of alloxan intraperitoneally according to the individual weight of rats). The rats were held in a tactfully while the drug was administered. After three days of induction, blood was obtained through the tail vein of the rats via tail puncture, and glucose level was determined using a glucometer. The rats were marked as diabetic and were used for the study. Their blood glucose level ranged between 250-550 mg/dl. The diabetic rats were weighed and divided into four experimental groups (groups 2, 3, 4, 5,) and the experiment lasted for 28 days post induction of diabetes.

2.5. Preparation of coffee extract

50 g of Nescafe coffee extract was measured and then dissolved in 500ml of distilled water to give a stock solution of 100 mg/ml.500 mg/kg and 800 mg/kg were used as low dose and high dose respectively.

2.6. Administration of Nescafe coffee extract to the rats

The aqueous extract of Nescafe coffee was given to the rats once daily (orally) for 28 days using a 2ml syringe (with the needle removed). Administration was done in the early hours of the morning before the animals were fed.

2.7. Administration of the standard drug

The animals in group 5 were given glibenclamide once daily (orally) for a period of 28 days.

2.8. Monitoring of the animals

There was constant monitoring of the rats to ensure a well-conducive environment for the animals. The rats were provided with sufficient feed and clean water to ensure adequate growth and good health. Also, good hygienic practices were maintained by constant removal of waste products from the cages. This was done on a daily basis. The rats were weighed weekly and were closely monitored for weight changes. The blood sugar level was checked weekly throughout the treatment to ensure any fluctuation in blood sugar level is detected easily.

2.9. Sample collection and analysis

After twenty-eight (28) days of treatment, the rats were starved for the next 24 hours. The animals were anaesthetized using chloroform and cotton wool in a chamber and then sacrificed. Blood was collected via cardiac puncture using a syringe and then emptied into labeled EDTA bottles for hematological assays.

2.10. Statistical analysis

The results of this study were expressed as mean ± standard deviation (mean ± SD). The statistical significance between the groups was assessed by one way analysis of variance (ANOVA) followed by least significant difference (LSD) test. The level of significance was $P < 0.05$.

3. Results

There was a significant decrease in RBC in groups 2 (diabetic) (7.28±0.52) and 4 (7.25±0.81) when compared to group 5 (glanil) (8.42 ± 0.26) ($p<0.05$).

There was no significant difference in hemoglobin level in groups 2 (diabetic), 3, and 4 when compared to groups 1 and 5 ($p>0.05$).

There was a significant decrease in hematocrit ($P < 0.05$) in group 4 (42.20±3.76a) when compared to group 5 (glanil) (49.20±3.91) (see Table 1).

Table 1 The effect of Nescafe coffee on red blood count (RBC), hemoglobin (HB) and packed cell volume (PCV)

GROUPS	RBC (X10^9/L) ± S.D	HB (g/dL)± S.D	PCV (%)± S.D
Group 1 (Control). n= 6	7.42±0.36	15.52±1.90	43.02±2.01
Group 2 (Diabetic Control). n = 6	7.28±0.52 a	14.10±0.92	43.14±2.81
Group 3 (Low Dose). n = 6	7.55±0.39	15.45±1.04	43.85±2.21
Group 4 (High Dose). n = 6	7.25±0.81 a	13.93±1.53	42.20±3.76 a
Group 5 (Glanil). n = 6	8.42±0.26	15.58±0.61	49.20±3.91
p-value	0.021	0.073	0.025

Data were expressed as mean ± SD, (a) MCH and MCHC significantly different in comparison with the glanil group, and (*) in comparison with the control group.

There was a significant decrease (P < 0.001) in MCH in group 2 (diabetic) (19.34±0.72*), group 4 (19.15±0.37*), and group 5 (glanil) (18.43±0.62*), when compared to group 1 (control) (20.88±0.98), and there was also a significant increase (P < 0.001) in group 3 (20.40±0.64a), when compared with group 5 (18.43±0.62).

MCHC result showed a significant decrease (P < 0.001) in group 2 (diabetic) (32.66±1.12*), group 4 (32.93±1.11*), and group 5 (glanil) (31.70±1.26*), when compared to group 1 (control) (36.00±1.55), and there was also a significant increase (P < 0.001) in group 3 (35.18±1.24a), when compared with group 5 (31.70±1.26).

Regarding MCV, there was no significant difference (P < 0.05) in groups 2, 3, and 4 when compared with groups 1 (control) and 5 (see Table 2).

Table 2 The effect of Nescafé coffee on the mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and means corpuscular volume

GROUPS	MCH (pg)± S.D	MCHC (g/dL) ± S.D	MCV (fL)± S.D
Group 1 (Control). n = 6	20.88±0.98	36.00±1.55	58.08±2.08
Group 2 (Diabetic Control). n = 6	19.34±0.72 *	32.66±1.12 *	59.38±1.26
Group 3 (Low Dose). n = 6	20.40±0.64 a	35.18±1.24 a	58.38±1.15
Group 4 (High Dose). n = 6	19.15±0.37 *	32.93±1.11 *	58.38±1.80
Group 5 (Glanil) n = 6	18.43±0.62 *	31.70±1.26 *	58.08±2.08
p-value	<0.001	<0.001	0.883

Data represented as mean ± SD; (a) (P < 0.05) in total WBC count, neutrophil, monocyte, and lymphocyte count, (P < 0.001) in eosinophil count and (P < 0.01) in basophil count, significantly different in comparison with the glanil group and (*) in comparison with the control group.

For the WBC count, neutrophil, monocyte, and lymphocyte count, there was no significant difference (P < 0.05) in groups 2, 3, and 4, when compared to groups 1 (control) and 5 (see Table 3).

There was a significant decrease (P < 0.001) in the eosinophil count in groups 3(0.30 ± 0.20*), 4 (0.68 ± 0.30*) and 5 (0.58 ± 0.21*) compared to group 1(2.70 ± 1.61) and also a significant increase (P < 0.001) in the eosinophil count in groups 2(2.43 ± 0.95a) when compared to group 5 (0.58 ±0.21*) (see Table 4).

There was a significant decrease (P < 0.01) in the basophil count in groups 3 (0.03 ± 0.02*), 4 (0.05 ± 0.03*) and 5 (0.08 ± 0.05*) compared to group 1(0.48 ± 0.26). There was a significant increase (P < 0.01) in the basophil count in group 2 (0.33 ± 0.22a) when compared to group 5 (0.08 ± 0.05*) (see Table 4).

Table 3 The effect of Nescafé coffee on the total white blood cell count, neutrophils, lymphocytes, and monocyte count

GROUPS	**WBC (X10^9/L)± S.D**	**LYMPHOCYTES (%)± S.D**	**NEUTROPHILS (%)± S.D**	**MONOCYTES (%)± S.D**
Group 1 (Control). n = 6	5.02±1.68	40.36±6.72	41.44±11.70	18.20±7.32
Group 2 (Diabetic Control). n = 6	7.64±2.82	46.94±8.08	29.98±10.50	23.08±3.45
Group 3 (Low Dose). n = 6	5.95±2.59	48.18±4.35	33.23±1.35	18.60±3.98
Group 4 (High Dose). n = 6	7.60±0.65	50.50±7.44	28.45±5.00	21.05±5.53
Group 5 (Glanil) n = 6	5.90±1.60	50.90±10.54	30.18±10.11	18.93±1.98
p-value	0.257	0.267	0.221	0.526

Table 4 The effect of Nescafe coffee on eosinophils and basophils

GROUPS	EOSINOPHILS (%) ± S.D	BASOPHILS (%) ± S.D
Group 1 (Control). n = 6	±1.61	0.48 ±0.26
Group 2 (Diabetic). n = 6	2.43 ± 0.95a	0.33 ± 0.22a
Group 3 (Low Dose). n = 6	0.30 ±0.20*	0.03 ± 0.02*
Group 4 (High Dose). n = 6	0.68 ±0.30*	0.05 ± 0.03*
Group 5 (Glanil) n = 6	0.58 ±0.21*	0.08 ± 0.05*
P- value	0.0006	0.0024

Data represented as Mean ± SD; (a) (P < 0.05) significantly different in comparison with the glanil group and (*) in comparison with the positive control group.

Considering platelet count, there was a significant decrease (P < 0.05) in group 2 (379.00±67.34*) and group 4 (390.25±131.48*) when compared with group 1 (control) (584.20±126.00).

For MPV and ESR there was no significant difference (P < 0.05) in groups 2, 3, and 4 when compared to groups 1 (control) and 5 (glanil) (see Table 5 below).

Table 5 The effect of Nescafé coffee on the platelet count, mean platelet volume, and erythrocyte sedimentation rate

GROUPS	PLATELET (X10^3/uL)± S.D	MPV (fL)± S.D	ESR (mm/hr)± S.D
Group 1 (Control) n = 6	584.20±126.00	7.38±0.34	2.21±0.84
Group 2 (Diabetic Control) n = 6	379.00±67.34*	7.24±0.13	1.60±0.55
Group 3 (Low Dose) n = 6	575.25±180.80	7.60±0.22	1.50±0.57
Group 4 (High Dose) n = 6	390.25±131.48*	7.58±0.21	2.00±1.16
Group 5 (Glanil) n = 6	569.00±85.90	7.50±0.26	1.25±0.50
p-value	0.035	0.193	0.372

4. Discussion

Hematological assessment is crucial for the evaluation of the health status of the body. It estimates the effect of a given extract on the hematological functions of the body[10]. An elevation or reduction in one or more of the hematological parameters could be an indication of a disease condition or an injury to the blood producing organs.

There was a significant decrease in the RBC count of the diabetic rats in group 2 (diabetic rats) and group 4 when compared with group 5 (treated with glibenclamide) suggesting that untreated diabetes and high consumption of Nescafé coffee caused a reduction in the erythrocyte count in the animals. Low RBC count indicates anemia..

There was an insignificant difference in the hemoglobin concentration of the diabetic rats in group 2 (diabetic rats), group 3 and group 4, when compared to the control group and group 5 (treated with glibenclamide).

There was a significant decrease in the volume of the packed cells (hematocrit) of the diabetic rats in group 4 given high dose of Nescafé coffee, when compared with the diabetic rats in group 5 treated with glibenclamide. This suggests that a high consumption of Nescafé coffee in the male diabetic rats caused a significant decrease in the hematocrit level. A decrease in the PCV level suggests anemia (a reduction in the total amount of RBCs) which could be due to an inability of the bone marrow to produce erythrocyte. Hematological indices indicate the red cell size (MCV), level of hemoglobin content (MCH), hemoglobin concentration (MCHC), and are important markers for diagnosing the causes of anaemia[11].

MCV shows the average sizes of the erythrocytes and it helps to monitor and diagnose certain blood disorders. From the results, there was an insignificant difference in the MCV of the diabetic rats in group 2 (diabetic rats), group 3 and group 4, administered with low and high doses of Nescafé coffee when compared to the control group and group 5

(treated with glibenclamide). This suggests that the MCV was normal and consumption of Nescafé coffee had no effect on the average size of the red blood cells of the diabetic animals (RBCs are said to be normocytic).

There was a significant decrease in the MCH and MCHC of the diabetic rats in group 2 (diabetic rats), group 4 and group 5, when compared to the control group and there was also a significant increase in group 3 when compared with group 5 (glibenclamide). MCHC as a diagnostic test helps to identify the cause of anemia. The low level of MCHC shows an insufficiency of haemoglobin content indicating anemia. From the result, high consumption of Nescafé coffee caused a decrease in MCH and MCHC leading to anemic conditions. RBCs are hypochromic and the type of anemia indicated is known as the hypochromic microcytic anemia which occurs when the RBCs are too small and fragile to accommodate sufficient hemoglobin.

The reduction in the levels of these blood indices could be due to dietary problems (lack of iron or inability of the body to absorb iron leading to iron deficiency anemia), lack of vitamins C, B12 and folic acid supplements, haemolysis and chemical or substance poisoning.

Acrylamide present in coffee products has been indicated to significantly decrease hematocrit values after ingestion[12]. Reduction in the PCV value in acrylamide exposure or consumption could be as a result of low production of RBCs [12]. Acrylamide has also been found to destroy the cell membrane of erythrocytes and cause a change in the parameters of blood viscosity; hence it causes a reduction in the erythrocytes count[13]. Acrylamide is converted to glycidamide, and both compounds can bind to hemoglobin and cause an imbalance in some of the blood parameters.

It is common for diabetics to develop anemia. Diabetes often leads to kidney dysfunction and further renal damage. Healthy kidneys release a hormone called the erythropoietin hormone (EPO) when the body needs new red blood cells, this hormone signals the bone marrow to make more red blood cells [14]. The hormone becomes deficient when the kidneys are damaged. Also, in diabetes the blood vessels are likely to be inflamed preventing the bone marrow from getting signals to produce more erythrocytes [14].

This research study is in agreement with Tarskikh [15], who reported that acrylamide leads to a reduction in erythrocytes count due to its attack on the cell membrane. Also, [16] and [17] reported reduction in the haematocrit level and platelet count. This research also supports the work of [18], who reported a non-significant difference in the WBC count in acrylamide in coffee intake. Caffeine is very much present in coffee and this research also agrees with [19] who also reported a reduction in the RBC and PCV of animals administered with caffeine.

There was an insignificant difference in the total WBC count of the diabetic rats in group 2 (diabetic rats), group 3 and group 4, when compared to the control group and group 5 (glibenclamide) suggesting that Nescafé coffee had no significant effect on the total leukocyte count.

In the white cell differential count, there was an insignificant difference on the lymphocyte, neutrophils, and monocyte count in the animals in groups 2, 3, and 4 when compared to the control group and group 5 (glibenclamide) suggesting that Nescafé coffee had no significant effect on these parameters.

Though, caffeine present in coffee can sometimes change immune responses and may induce lymphocytosis and neutrophilia [18].

There was a significant decrease in the eosinophil and basophil counts of the animals in groups 3, 4, and 5. Eosinopenia could be linked to the oxidative stress from the diabetes while basopenia could be linked to hypersensitivity or allergic reactions from drugs or substance.

There was an insignificant difference in the MPV and ESR of the diabetic rats in group 2 , group 3 and group 4, administered with low and high doses of Nescafé coffee when compared to the control group and group 5 (glibenclamide) suggesting that Nescafé coffee had no effect on both parameters.

There was a significant decrease in the platelet count of the animals in groups 2 and 4 given the high dose of Nescafé coffee when compared to group 1 (control).

5. Conclusion

From this study, it can be deduced that, although Nescafé coffee may have many health benefits, it also has some negative effects on some of the hematological parameters of diabetic rats, especially when consumed in large quantities. Therefore, we recommend a reduction in the consumption of Nescafe coffee by diabetic individuals to minimize its negative effect on some blood parameters.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

Ethical approval was obtained from the Ethical Committee of the Faculty of Basic Medical Sciences, Chukwuemeka Odumegwu Ojukwu University, Uli campus, Anambra State, Nigeria.

References

[1] Evert AB, Dennison M, Gardner CD, Garvey WT, Lau KH (2019). Nutrition Therapy for Adults with Diabetes or Prediabetes. *Diabetes Care.* 42 (5): 731-754.

[2] Bravi F, Bosetti C, Tavani A, Gallus S, (2018). Coffee reduces the risk of hepatocellular carcinoma; an updated meta-analysis. *Clinical gastroenterology and hepatology.* 11(11), 1413-1421.

[3] Chen S, Teoh N, C, Chitturi S, Farrell G. C (2019). Coffee and non-alcoholic fatty liver disease. Brewing evidence for hepatoprotection. *Journal of gastroenterology and hepatology*. 29 (3), 435-441.

[4] Jang, E.S., Jeong S.H, Hwang S.H, Kim H.Y., Ahn, S.Y. Lee, J & Kim,N (2015). Effects of coffee smoking, and alcohol on liver function tests: a comprehensive cross-sectional study. *Gastroenterology,* 12(1) 145.

[5] Mussatto, Solange I.; Machado, Ercília M. S.; Martins, Silvia; Teixeira, José A. (2014). "Production, Composition, and Application of Coffee and Its Industrial Residues". *Food and Bioprocess Technology*. 4 (5): 661–72.

[6] Elmaadawy A. A, Saad H. H, Ismail M. S (2015). The impact of different preparation of coffee on body weight, serum uric acid and liver enzymes in Experimental Rats. *Merit Res J* 3(7); 292-300.

[7] Shim S. G, Jun D.W, Kim E. K, Saeed W.K, Lee K. N (2017). Caffeine attenuates liver fibrosis via defective adhesion of hepatic stellate cells in the cirrhotic model. *Journal of gastroenterology and hepatology.* 28(12): 1877-1884.

[8] Mooney, C; Byrne, M; Kapuya, P; Pentony, L; De la Salle, B; Cambridge, T; Foley, D (2019). "Point of care testing in general haematology". *British Journal of Haematology.*187 (3): 296–306.

[9] Etuk EU. (2010). Animals models for studying diabetes mellitus. *Agric Biol J N Am;* 1:130-134.

[10] Akomas, S.C, Okafor A.I, Ijioma S.N. (2014). Glucose level, Hematological parameters and lipid profile in Ficus sur treated diabetic rats. *Compr J. Agric. Biol. Sci.* 2, 5-11.

[11] Holy B, Kenanaga B, Onwuli, D. O (2015). Hemato-pathological effects of dichlorovos on the blood profile and liver cells of albino rats. *J Toxicol. Environ. Health Sci*. 7, 18-23.

[12] Rawi S.M, Marie A.S, Fahmy S.R (2012). Hazardous effects of acrylamide on immature male and female rats. *African J Pharm Pharmcol*. 6: 1367-1386.

[13] Barber D. S, Hunt J. R, Ehrich E.J, Lehning and R. M LoPachin (2001). Metabolism , toxicokinetics and haemoglobin adduct formation in rats following sub-acute and sub-chronic acrylamide dosing. *Neurotoxicology.* 22: 341-353.

[14] McGill J.B and Bell D.S.H (2006). Anaemia and the role of erythropoietin in diabetes. *Journal of Diabetes and its complications.* Vol 20, pp. 262-272.

[15] Tarskikh M.M (2006). Damage to the erythrocyte membrane as the mechanism for acrylate toxicity. *Bull Experim Book Med*. 142: 690-692.

[16] Ali M.A, Elawady A.I, Aly E.M (2014). Effectiveness of selenium on acrylamide toxicity to retina. *Int J Ophthalmol*. 7: 614-620.

[17] Osman M.A, Romeilah R.M, Eman S, Hasan R.S (2016). Subchronic toxicity of acrylamide in fried rice and preventive effects of grape leaves. *Asian J Biochem.* 11: 68-81

[18] Jin F, Liang C.L, Jia X.O, Ning L (2014). Immunotoxicity of acrylamide in female Barb mice. *Booked Environ Sci*. 27:401-409.

[19] Agomuo E, Amadi P, Duru M, Amadi B., Ugwokaegbe P (2017). Effect of Caffeine on Some Selected Biochemical Parameters Using Rat Model. *Advances in Biology*. Volume 2017, Article ID 9303276, 8 pages https: //doi.org/10.1155/2017/9303276.