International Journal of Food Science and Nutrition
ISSN: 2455-4898
Impact Factor: RJIF 5.14
www.foodsciencejournal.com
Volume 3; Issue 4; July 2018; Page No. 148-152

The effect of *Cyperus esculentus* (Tigernut) oil on liver, kidney and hematological biomarkers in low dose streptozocin and high fat diet exposed male wistar rats

Nwawuba Stanley U[1*] Okechukwu Francis C[2]
[1] Nutritional and Industrial Biochemistry Unit, Department of Biochemistry, College of Medicine, University of Ibadan, Ibadan, Oyo State, Nigeria
[2] Department of Biochemistry, School of Life Science, Hemwati Nandan Bahuguna Garhwal University Shrinager, Uttarakhand, India

Abstract
The purpose of this study is to investigate the effect of *Cyperus esculentus* (tiger nut) tuber oil on liver, kidney and hematological biomarkers in low dose Streptozocin (35mg/kg) and high fat diet exposed male wistar rats. *Cyperus esculentus* tuber oil was extracted from the tuber using a soxhlet extractor with n-hexane as solvent and the proximate and mineral analyses of high fat diet were done according to Standard procedure. A total of 40 rats between the weights of 88-115g were divided into five group's n=8, and some selected groups were subsequently fed with a high fat diet for 30days and then intraperitoneal injected with 35mg/kg Streptozotocin. Analysis of liver, kidney function biomarkers (AST, ALT and ALP), (Creatinine, Urea, Sodium and Potassium) and hematological parameters (PCV, Hb, RBC and WBC) were conducted using standard procedures. The result findings showed that *Cyperus esculentus* oil significantly lowered $p<0.05$ levels of liver, kidney biomarkers and maintained the levels of hematological parameters. We concluded that *Cyperus esculentus* oil exhibited hepatoprotective ability, enhances renal function and maintains hematological status thus do not cause anemia and it is non-toxic.

Keywords: *Cyperus esculentus* (tigernut), liver function, kidney function, hematology and streptozotocin

1. Introduction
Non-communicable diseases (NCDs) are among the most severe threats to global economic development, probably more detrimental than fiscal crisis, as underlined by the World Economic Forum's 2009 report and NCDs are the leading cause of death in the world [1]. Liver disease and chronic kidney disease are enlisted amongst NCDs [2]. Furthermore, liver disease occurs throughout the world irrespective of age, sex, region or race and it has been found to contribute markedly to the global burden of mortality and morbidity [3]. In the 2010 Global Burden of Disease (GDB) study, more than one million deaths and 31,027,000 Disability Adjusted Life Years (DALYs) were due to liver cirrhosis [4] and kidney disease has also been reported to be a worldwide health crisis according to the World Health Organization [5]. Also, a study has identified a mechanisms common to both diseases linked through an inter-organ communication orchestrated by fetuin-A and adiponectin. In liver and kidney, the energy sensor 5′-AMP activated protein kinase (AMPK) is pivotal to directing podocytes and hepatocytes to compensatory and potentially deleterious pathways, leading to inflammatory and profibrotic cascades culminating in end-organ damage [6].
The liver transaminases aspartate transaminase (AST or SGOT), alanine transaminase (ALT or SGPT), alkaline phosphatase (ALP) and bilirubin are useful biomarkers of liver disease [7] meanwhile creatinine; urea and electrolytes are markers of kidney function [8] and the present study examined useful biomarkers of liver and kidney function in male wistar rats in order to ascertain the nutraceutical power of *Cyperus esculentus* oil. Metabolic risk factor for non-communicable disease (NCDs) includes overweight and hyperglycemia [9] and this study induced overweight by placing the rats on a high fat diet and hyperglycemia with a known diabetogenic agent streptozocin which has also be shown exerts some levels of liver/kidney dysfunction [10, 11, 12].
Cyperus esculentus (tiger nut) oil contains high monounsaturated fatty acids, similar to olive, avocado and hazelnut oil [13]. This monounstaturated oil has high unsaponifiable matter, phospholipids and other bioactive compounds such as tocopherols, phytosterols and polyphenols [14, 13]. The small round tubers found along the roots have a slightly almond flavor and are eaten raw or cooked, or made into a traditional chufa drink called *orxata*. These tubers contain high levels of protein, carbohydrate and oleic acid [15, 16]. Previous study has also demonstrated it contains phytosterol, vitamin E and β-carotene [17] and such substances, together with the unsaturated fatty acids of tigernut oil may be responsible for the overall antioxidant activity.
Quite a numbers of researches have been carried out using medicinal plants to mitigate various disease conditions and the results have demonstrated that it provides efficient and cost-effective therapeutic solution and as such *Cyperus esculentus* oil a potent nutraceutical though not totally new still remains largely unexploited and underutilized. Thus the aim of the present study is to investigate the ability of *Cyperus escuentus* oil on liver, kidney and hematological biomarkers in low dose streptozotocin and high fat diet exposed male wistar rats the first of its kind.

2. Material and methods

2.1 Plant collection

Cyperus esculentus (tiger nut) was harvested freshly from a local farm in Maiduguri, Borno State, Nigeria and identified at Botany Department, University of Ibadan. The tubers were inspected; spoilt ones were removed by hand picking. Matured healthy tubers were washed, air dried at room temperature for 2 weeks, ground using Hammer mill into coarse powder and stored in sealed cellophane bags prior to extraction.

2.2 Oil extraction

4kg of the powdered tubers was transferred into a glass container and 7.5 L of redistilled commercial grade n-hexane as added for 72 hours, the filtrate was removed using muslin cloth. To the residue was added another 5litres of pure n-hexane and macerated in the cold for 3 days, thus the process was repeated. The combined n-hexane extract was then further filtered using filter paper and was concentrated using Büchi rotary evaporator (France) model at 30°C to remove the solvent and was dried further using a vacuum oven model VF-220 set at 30°C and with a pressure of 700 mg/Hg. The obtained oil was stored in a bottle till needed for analysis.

2.3 Analysis of feed

The proximate and mineral analyses of high fat diet were done according to Standard procedure [18]. All the chemicals used in this study were of analytical grade, unless stated otherwise.

Table 1: Feed composition

Parameters	Normal Chow	Formulated Chow
Protein	21%	29.90%
Fat(Butter)	3.5%	40%
Fibre	6.0%	3.70%
Calcium	0.8%	0.115%
Phosphorus	0.8	0.32
Cholesterol		2%
Dry Matter		88.91%

2.4 Animals used

A total of 40 male wistar rats between the weights of 88-115g were procured from the central animal house, College of Medicine, University of Ibadan, Nigeria and were allowed to acclimatize for two weeks and some selected groups were subsequently fed with a high fat diet for another 30days. They male wistar rats were kept in well kempt and ventilated cages and their beddings changed every three days and they were allowed free access to clean drinking water. All the processes involved in the handling and experiment were carried out according to standard protocols approved by the animal ethics committee of the department.

2.5 Exposure to low dose Streptozocin and high fat diet

The rats were divided into two dietary regimens consisting of 16 rats fed normal chow and 24 rats fed with a high fat diet for a period of 30days. After the period of 30days, the experimental groups were injected intraperitoneally (i.p.) with low dose of Streptozotocin (STZ) (35 mg/kg) dissolved in a citrate buffer (0.1 M, pH 4.5) as described by [19] and treatment followed for a period of 21days.

2.6 Experimental design

The male Wistar rats were divided into five (5) groups (n=8).

Group 1: Rats fed with normal rat chow for and received water (Normal control). (Represented by NPD)

Group 2: Rats fed with a high fat diet + Streptozotocin (35 mg kg−1) (Negative control). (Represented by HFD+STZ).

Group 3: Rats fed with a high fat diet + Streptozotocin (35 mg kg−1) and treated with glimepiride (2mg/kg). (Represented by HFD+STZ+GLI)

Group 4: Rats fed with a normal rat chow + Streptozotocin (35 mg kg−1). (Represented by NPD+STZ)

Group 5: Rats fed with a high fat diet Streptozotocin (35 mg kg−1) and treated with 0.5ml *Cyperus esculentus* oil. (Represented by HFD+STZ+CEO)

2.7 Biochemical analysis

Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) were determined by colorimetric method using RANDOX kit (AL 100) and (AS 101) as described by [20]. Alkaline phosphatase was determined by colorimetric method using RANDOX kit (AP 542) by standard method according to the recommendation of [21]. Creatinine level was determined by colorimetric method using RANDOX kit (CR 510) as described by [22]. Bilirubin level was determined by colorimetric method using RANDOX kit (BR 411) as described by [23]. Urea and calcium were determined using colorimetric method using RANDOX kit (UR 1068) and (CA 590). Potassium and sodium were determined by colorimetric method using TECO DIAGNOSTICS kit according to the method of [24, 25].

2.8 Relative organ weight

After 21days of treatment, the body weight of all the animals were weighed in grams using standard laboratory weighing balance after which there were euthanized by exsanguination under chloroform anesthesia. The organs namely the liver and kidneys were carefully dissected out and also weighed in grams (absolute organ weight). The relative organ weight of each animal was then calculated as follows:

$$\text{Relative Organ weight} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of rat (g)}} \text{ X } 100$$

2.9 Determination of hematological parameters

The blood pooled from all the animals in each group was collected into bottles containing ethylenediamine tetra acetic acid (EDTA) as anticoagulant for hematological analysis. RBC and WBC counts were estimated by using the neubauer counting chamber as described by [26]. Packed cell Volume (PCV) with Win trobe hematocrit tubes, Hemoglobin (Hb) by Sahli's method [27].

Statistical analysis

Data were treated by ANOVA (analysis of variance) and mean separation was done using Duncan multiple range test. $p<0.05$ were considered significant. Data was expressed as means ± standard deviation. All statistical analysis was done using IBM SPSS Version 22 and Microsoft Excel.

3. Results

3.1 Liver function

Table 2 demonstrates that high fat feeding and low dose of STZ (35mg/kg) altered liver function biomarkers (ALT, AST, ALP, & Bilirubin). As seen the levels of serum (AST, ALP, & Total Bilirubin) significantly increased (P<0.05) in untreated group HFD+STZ (54.00±27.8, 144.82±11.5, & 0.717±0.1) relative to the normal control (22.20±13.2, 99.40±14.2, & 0.120±0.4) which was unexposed to neither HFD nor STZ and treatment with 0.5ml *Cyperus esculentus* (tigernut) oil (HFD+STZ+CEO) restored the liver integrity by significantly lowering (P<0.05) the levels of liver function biomarkers in relation to the negative standard control (HFD+STZ) in this study.

Table 2: Effect of *Cyperus esculentus* (tigernut) oil on liver function

Groups	ALT	AST	ALP	Total Bilirubin
Normal Control	22.40±6.5^{a}	22.20±13.2^{a}	99.40±14.2^{a}	0.120±0.4^{a}
HFD+STZ	42.50±17.2^{b}	54.00±27.8^{b}	141.82±11.5^{b}	0.717±0.1^{c}
HFD+STZ+GLI	25.29±3.3^{ab}	33.86±10.0^{ab}	112.00±9.4^{a}	0.414±0.13^{b}
NPD+STZ	36.86±16.1^{ab}	46.00±22.5^{ab}	135.14±11.8^{b}	0.657±0.11^{c}
HFD+STZ+CEO	26.71±2.9^{ab}	28.14±1.6^{a}	110.43±12.9^{a}	0.329±0.16^{b}

Data are expressed as means ± SD: Means with same alphabet as superscript within each column variable are non-significantly (p>0.05) different from each other. The abbreviations denote HFD: high fat diet, NPD: normal pellet diet, CEO: *Cyperus esculentus* oil, GLI: glimepiride, STZ: Streptozotocin, ALT: alanine transferase, AST: aspartate transaminase, ALP: alkaline phosphatase

3.2 Kidney Activity

Table 3 shows ameliorative potential of tigernut oil on kidney dysfunction. Following i.p. injection of 35mg/kg of STZ after 30days of high fat feeding, the rats were treated for 21days with tigernut oil and at the end of the treatment, serum levels of kidney function biomarkers (Creatinine, Urea, Sodium, & potassium) were determined. *Cyperus esculentus* (tigernut) oil treated group (HFD+STZ+CEO) significantly lowered (p<0.05) the levels of kidney function biomarkers when compared to the untreated group (HFD+STZ). However, there was no significant difference P>0.05 when compared to the normal control which remained unexposed to neither HFD nor STZ.

Table 3: Effect of *Cyperus esculentus* (tigernut) oil on kidney function

Groups	Creatinine	Urea	Sodium	Potassium
Normal Control	0.420±0.08^{a}	11.28±1.58^{a}	141.67±1.15^{a}	5.37±0.29^{a}
HFD+STZ	0.783±0.09^{b}	23.52±4.34^{c}	148.00±1.00^{c}	6.67±0.51^{c}
HFD+STZ+GLI	0.543±0.09^{a}	17.43±2.91^{bc}	143.67±1.53^{ab}	5.73±0.15^{ab}
NPD+STZ	0.614±0.13^{ab}	20.09±3.09^{bc}	146.00±1.00^{bc}	6.47±0.35^{bc}
HFD+STZ+CEO	0.571±0.16^{a}	17.09±5.20^{ab}	144.33±1.53^{ab}	5.97±0.23^{ab}

Data are expressed as means ± SD: Means with same alphabet as superscript within each column variable are non-significantly (p>0.05) different from each other. The abbreviations denote HFD: high fat diet, NPD: normal pellet diet, CEO: *Cyperus esculentus* oil, GLI: glimepiride, STZ: Streptozotocin.

3.3 Relative organ weights

They was a significant increase P<0.05 in the relative weight of the rat liver in high fat diet fed group (HFD+STZ) relative to only STZ exposed group (NPD+STZ) and the same was seen for the kidney although it was not statistically significant. However, *Cyperus esculentus* (tigernut) oil treated group (HFD+STZ+CEO) significantly reduced P<0.05 the relative Weights of the liver and kidney relative to the negative control (HFD+STZ).

Table 4: Effects of *Cyperus esculentus* (tigernut) oil on the relative organ weights

Groups	Relative liver weight	Relative kidney weight
Normal Control	2.48±0.18^{a}	0.29±0.02^{a}
HFD+STZ	3.60±0.21^{c}	0.38±0.02^{b}
HFD+STZ+GLI	3.07±0.39^{bc}	0.33±0.04^{ab}
NPD+STZ	2.70±0.42^{ab}	0.34±0.06^{ab}
HFD+STZ+CEO	2.78±0.32^{ab}	0.30±0.03^{a}

Data are expressed as means ± SD: Means with same alphabet as superscript within each column variable are non-significantly (p>0.05) different from each other. The abbreviations denote HFD: high fat diet, NPD: normal pellet diet, CEO: *Cyperus esculentus* oil, GLI: glimepiride, STZ: Streptozotocin.

3.4 *Cyperus esculentus* (tigernut) oil on hematological parameters

The estimation of hematological parameters in male wistar rats were carried using standard procedures. Packed cell volume (PCV) expressed in percentage (%) and red blood cell (RBC) expressed in microliter (µl) were significantly reduced P<0.05 in the negative control (HFD+STZ) in relation to the normal control and the group treated with *Cyperus esculentus* (tigernut) oil (HFD+STZ+CEO) which was exposed to the same toxicant as the negative control showed an increase the levels of PCV and RBC relative to the negative control although not statistically significant. Hemoglobin (Hb) level expressed in (g/dl) was significantly elevated P<0.05 in tigernut oil treated group (HFD+STZ+CEO) when compared to the negative controls. However, they were no significant differences P>0.05 in the levels of white blood cells (WBC) table 5.

Table 5: the effect of *Cyperus esculentus* (tigernut) oil on hematological parameters

Groups	PCV (%)	Hb (g/dl)	RBC(x106µl)	WBC(x103 µl)
Normal Control	49.75±1.50^{c}	16.07±0.30^{d}	7.89±0.43^{c}	6062.50±1161.5^{a}
HFD+STZ	39.75±3.30^{a}	11.50±.35^{a}	6.39±0.73^{a}	5075.00±2666.6^{a}
HFD+STZ+GLI	44.50±1.29^{bc}	14.62±0.69^{cd}	7.53±0.42^{bc}	5500±771.4^{a}
NPD+STZ	41.25±0.96^{ab}	12.55±0.82^{ab}	6.59±0.55^{ab}	5112.50±1566.5^{a}
HFD+STZ+CEO	43.00±0.82^{abc}	13.22±0.92^{bc}	7.25±1.13^{abc}	5250±1161.9^{a}

Data are expressed as means ± SD: Means with same alphabet as superscript within each column variable are non-significantly ($p>0.05$) different from each other. The abbreviations denote HFD: high fat diet, NPD: normal pellet diet, CEO: *Cyperus esculentus* oil, GLI: glimepiride, STZ: Streptozotocin.

4. Discussion

Enzyme activities in the tissues are often used as 'marker' to ascertain early toxic effects of administered foreign compounds to experimental animals [28]. ALP is a membrane bound enzyme while ALT and AST are cytosolic enzymes and high levels of ALP, ALT and AST respectively in the serum are indicators of cell membrane permeability and consequent degree of damage to the liver [29]. The observed significant $p<0.05$ increase in the serum levels of liver enzymes (ALP,ALT,AST and total bilirubin) Table 2 in the group exposed to high fat diet and low dose streptozotocin 35mg/kg body weight (HFD+STZ) relative to the normal control indicates that high fat diet and STZ caused a hepatocellular damage. Intraperitoneal injection of STZ has been reported to be associated with degree of liver damage [30] and Fat could increase the body's metabolism burden via fatty depositions in the endothelial vascular system and can reduce the endothelial production of antioxidant enzymes, increased production of free radicals leading to an oxidative stress which damages the body's biological macromolecules, tissue and organ function disorders then ensues [31]. Treatment with *C. esculentus* (tigernut) oil restored the liver integrity to almost normal as the was a significant decrease $p<0.05$ in the levels of liver enzymes (ALT, AST and ALP) and relative liver weight Table 4 in *C. esculentus* oil treated group (HFD+STZ+CEO) relative to the negative control (HFD+STZ). interestingly, no significant difference $p>0.05$ was observed relative to the normal control indicating the hepatoprotective ability of *C. esculentus* oil and this may be pointed to presence of dietary antioxidant levels and oleic acid in *C. esculentus* oil [13, 17] also According to the Xinhua Outline of Material Medical records, *C. esculentus* is an acrid, sweet, warm and effective treatment for the liver, spleen and stomach [32].

Creatinine, urea and electrolytes are one of the markers of kidney function [8]. The result of this study table 3 revealed that *C. esculentus* oil plays a beneficial role in mitigating renal dysfunction induced by exposure of the rats to high fat diet and low dose STZ and an indication of kidney dysfunction is observed as the levels of markers of kidney function (Creatinine, Urea, Sodium and Potassium) was significantly elevated $p<0.05$ in negative control (HFD+STZ) relative to the normal control and the treatment groups which were exposed to the same toxicant. However, *C. esculentus* oil (HFD+STZ+CEO) treated group significantly lowered the levels of markers of kidney function as well as reducing the relative kidney weight table 4. *C. esculentus* oil has been shown to contain unsaturated fatty acid [32, 13, 33] and as reported by [34, 35] this effect may be attributed to the positive role of unsaturated fatty acids in preservation of glomerular filtration rate and effective renal plasma flow. Regarding the result on hematological parameters table 5, it was found that the levels of hematological parameters examined in this study (PCV, Hb, RBC and WBC) were maintained in all the experimental groups however the mean value of the group treated with *C. esculentus* oil was higher although not statistically significant relative to the negative control and this is an indication of it nontoxic nature which is consistent with the finding of [34, 36] and nutritional profiles and unique functional properties have made tiger nut as unique food [37].

5. Conclusion

C. esculentus oil meets the recommendations of medical associations that have recognized the benefits of edible oils containing more than 80% unsaturated fatty acids [32, 38]. The results of the present study indicate that *C. esculentus* oil exhibited hepatoprotective ability, enhances renal function and maintains hematological status in high fat diet and low dose streptozotocin exposed male wistar rat. However, further studies are required to elucidate the mechanism of its renal and liver protection ability and further highlight the potentials of *C. esculentus* oil for wellness.

6. References

1. Norberto P, Giuseppe R. Chronic kidney disease: a research and public health priority. Nephrology Dialysis Transplantation. 2012; 27(3):319-326.
2. Charles P, Jayadeep P, Jürgen R. Alcohol consumption and non-communicable diseases: epidemiology and policy implications. PMC. 2011; 106(10):1718-1724.
3. Lozano RM, Naghavi K, Foreman S, Lim K, Shibuya V, *et al*. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet. 2012; 380:2095-2128.
4. Rehm J, Andriy VS, Kevin DS. Global burden of alcoholic liver diseases. Journal of Hepatology. 2013; 59(1):160-168.
5. Levey AS, Atkins R, Coresh J. Chronic kidney disease as a global public health problem: approaches and initiatives - a position statement from Kidney Disease Improving Global Outcomes. Kidney Int. 2007; 72(3):247-259.
6. Joachim HI, Kumar S. Mechanisms Linking Obesity, Chronic Kidney Disease, and Fatty Liver Disease: The Roles of Fetuin-A, Adiponectin, and AMPK. J Am Soc Nephrol. 2010; 21(3):406-412.
7. Mengel MB, Schwiebert LP. Family medicine: ambulatory care & prevention, McGraw-Hill Professional. 2005, 268.
8. Shivaraj G, Prakash BD, Shruthi SK, Vinayak VH,

Avinash AK, Sonal NV. Markers of renal function tests. N Am J Med Sci. 2010; 2(4):170-173.
9. Esmailnasab N, Moradi G, Delaveri A. Risk Factors of Non-Communicable Diseases and Metabolic Syndrome. Iran J Public Health. 2012; 41(7):77-85.
10. Afrin RS, Arumugam V, Soetikno RA, Thandavarayan V, Pitchaimani V, Karuppagounder R, *et al*. Curcumin ameliorates streptozotocin-induced liver damage through modulation of endoplasmic reticulum stress-mediated apoptosis in diabetic rats. Free Radic. Res. 2015; 49(3):279-289.
11. Kohl TN, Gehrke AS, Nagel M, Wörns MA, Sprinzl MF, Zimmermann T, *et al*. Diabetic liver injury from streptozotocin is regulated through the caspase-8 homolog cFLIP involving activation of JNK2 and intrahepatic immunocompetent cells. Cell Death Dis. 2013; 4(7):712.
12. Omonkhua AA, Adebayo EA, Saliu JA, Ogunwa TH, Adeyelu TT. Liver function of Streptozotocin- Induced Diabetic Rats Orally Administered Aqueous Root-Bark Extracts of *Tetrapleura tetraptera* (Taub). Nigerian Journal of Basic and Applied Science. 2014; 22(3&4):99-106.
13. Ezeh O, Micheal HG, Niranjan K. Tiger nut oil (*Cyperus esculentus* L.): A review of its composition and physico-chemical properties. Eur. J Lipid Sci. Technol. 2014; 166(7):783-794.
14. Sanchez-Zapata E, Fernández-López J, Angel PJ. Tiger nut (*Cyperus esculentus*) commercialization: health aspects, composition, properties, and food applications. Compr. Rev. Food Sci. Food Saf. 2012; 11(4): 366-377.
15. Warra AA. Quality characteristics of oil from Brown and Yellow *Cyperus esculentus L*. Tubers. Research and Reviews: Journal of Botanical Sciences. 2013; 3(1):23-26.
16. Bamishaiye EI, Bamishaiye OM. Tiger nut: as a plant, its derivatives and benefits. African Journal of Food, Agriculture, Nutrition & Development. 2011; 11(5):5157-5170.
17. Ozcan MM, Gumuscu A, Er F, Arslan D, Ozkalp B. Chemical and fatty acid composition of Cyperus esculentus. Chemistry of Natural Compounds. 2010; 46:276-277.
18. AOAC. Official Methods of Analysis, Association of Official Analytical Chemists, 16th edition, Washington DC, 1995.
19. Srinivasan K, Viswanad B, Asrat L, Kaul CL, Ramarao P. Combination of High-Fat fed and Low-dose streptozotocin-Treated Rat: A Model for Type 2 Diabetes and Pharmacological Screening. Pharmacological Research. 2005; 52:313-320.
20. Reitman S, Frankel S. Amer. J Clin. Path. 1957; 28:56.
21. Englehardt A. Aerztl Labor. 1970; 16:42.
22. Bartels H, Bohmer M. Clin Chem. Acta. 1972; 37:193.
23. Jendrassik L, Grof P. Biochem. Z. 1938; 297:81.
24. Terri AE, Sessin PG. Am. J. Clin. Path. 1958; 29:86.
25. Maruna RFL. Clin. Chem. Acta. 1958; 2:581.
26. Dacie JV, Lewis SM. Practical Haematology, 7th Edn, ELBS with Churchill Livingston, England, 1991, 37-85.
27. Sharma S. Experiments and techniques in Biochemistry, Haematology determination, Galgotia (New Delhi), 2007, 90-100.
28. Adesokan AA, Oyewole OI, Turay MS. Kidney and Liver Function Parameters in Alloxan-Induced Diabetic Rats Treated with Aloe Barbadensis Juice Extract. Sierra Leone Journal of Biomedical Research. 2009; 1(1):33-37.
29. Cotran R, Kumar V, Robins S. Robin's pathological basis of disease, 4th edn, W.B Saunders Co, Harcourt, 1989, 212-217.
30. Prasenjit M, Joydeep D, Jyotirmoy G, Parames CS. Contribution of type 1 diabetes to rat liver dysfunction and cellular damage via activation of NOS, PARP, IκBα/NF-κB, MAPKSs, and mitochondria-dependent pathways: Prophylactic role of arjunolic acid. Free Radical Biology and Medicine. 2010; 48(11):1465-1484.
31. Aneckova, R. The role of leptin in human physiology and pathophysiology. Physiological Research. 2001; 50:443-459.
32. Siqun J, Weiqi O, Zhiyan R, Hengxu X, Zexin M. The Invitro and In vivo Antioxidant Properties of Cyperus Esculentus Oil from Xinjiang, China. J Sci Food Agric 2013; 93:1505-1509.
33. Muhammad N, Bamishaiye E, Bamishaiye O, Usman L, Salawu MO, Nafiu MO, *et al*. Physicochemical properties and fatty acid composition of cyperus esculentus (Tiger Nut) Tuber Oil. Biores. Bull. 2011; 5:51-54.
34. Hanaa A, Hassan. The Potential effects of Tigernut Oil on Some Haemato-Biochemical Blood Indices in Male Albino Rats. The Egyptian Journal of Experimental Biology (Zoology). 2007; 3:49-54.
35. Melhado VE, Bioim MA, Versolato C, Moura LA, Stella SR, Schor N. Effect of eicosapentanoic acid on the progression of chronic renal failure in rats. Nephron. 1992; 62 (4):449-453.
36. Bamishaiye E, Muhammad N, Bamishaiye O. Haematological Parameters of Albino Rats Fed On Tiger Nuts (Cyperus Esculentus) Tuber Oil Meal-Based Diet. The International Journal of Nutrition and Wellness. 2009; 10(1):143-156.
37. Ekeanyanwu RC, Ononogbu CI. Nutritive value of Nigerian tigernut (*Cyperus esculentus* L.). Agric. J. 2010; 5(5):297-302.
38. Ma L. Comparison of camellia oil and olive oil in nutritional value. Agric Eng Technol (Agric Prod Proc). 2007; 9:42-44.