Evaluation of the Effect of Okra (*Abelmoschus esculentus)* Supplement on Blood Glucose Levels and Antioxidant Biomarkers on Alloxan Induced Diabetic Wistar Rats

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Abstract

The prevalence of diabetes mellitus (DM) continues to rise alarmingly despite years of intensive research. The need to explore alternative remedies such as food supplements phytotherapy has therefore become increasingly important in the management and treatment of DM. The aim of this study is to evaluate the effect of okra (*Abelmoschus esculentus*) supplement on blood glucose and antioxidant biomarkers on alloxan induced diabetic wistar rats. Diabetes was induced by a single intraperitoneal (i.p) injection of Alloxan monohydrate (150 mg/kg body weight) in Wistar rats. The rats were divided into five groups as follows: non-diabetic control fed distilled water, diabetic control fed distilled water, diabetic group treated with glibenclamide (5 mg/kg.body weight) ,diabetic group treated with 33% okra supplement(*Abelmoschus esculentus*) and diabetic group treated with 66% okra supplement for a period of sixteen days. Blood glucose levels were determined at day 0, 4, 8 12 and 16. The animals were sacrificed by being euthanized with overdose halothane, blood samples were removed by cardiac puncture and the serum extracted were used for the assay of antioxidant enzymes and Lipid peroxidation. The results revealed that treatment with 33 and 66% okra supplements significantly reduced blood glucose levels when compared with the control diabetic untreated. Also in relation to the antioxidant biomarkers levels, there was a decrease in superoxide dismutase (SOD) and malonaldehyde (MDA) levels when compared with the diabetic untreated. However, there was a increase in the levels of reduced glutathione (GSH) and catalase (CAT) when compared with the diabetic untreated. In conclusion, the data obtained in this study demonstrated the hypoglycemic and antioxidant effects of okra supplement in alloxan-induced diabetic rats.

Keywords: Oxidative Stress, Alloxan Monohydrate, Blood Glucose, *Abelmoschus esculentus*.

Introduction

The term diabetes mellitus describes metabolic disorders of multiple aetiology characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion or insulin action.[1] The effects of diabetes mellitus include long–term damage, dysfunction and failure of various organs. Diabetes mellitus may present with characteristic symptoms such as thirst, polyuria, blurring of vision, and weight loss. In its most severe forms, ketoacidosis or a non–ketotic hyperosmolar state may develop and lead to stupor, coma and, in absence of effective treatment, death.[2] Often symptoms are not severe, or may be absent, and consequently hyperglycaemia sufficient to cause pathological and functional changes may be present for a long time before the diagnosis is made.[2] The imbalance between the systemic manifestation of reactive oxygen species (ROS) and the system's ability to readily detoxify the reactive intermediates

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or to repair the resulting damage is termed oxidative stress. The disturbances in the normal redox state of cells can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA.[3] Oxidative stress causes not only negative effects such as lipid peroxidation and oxidative DNA damage, but also physiologic adaptation phenomena and regulation of intracellular signal transduction. It is known to be involved in the pathogenesis of lifestyle-related diseases, including atherosclerosis, hypertension, diabetes mellitus, ischemic diseases, and malignancies et cetera.[4,5]

Medicinal plants are important sources of biologically active antioxidants. Natural antioxidants, which are ubiquitous in fruits and vegetables, have also received great attention and have been studied extensively, since they are effective free radical scavengers and are assumed to be less toxic than synthetic antioxidants.[6] Green leafy vegetables provide a high amount of carotene, ascorbic acid, and microelements which play important roles in nutrient metabolism and slowing down of degenerative diseases.[7] *Abelmoschus esculentus* L. (Family: Malvaceae), also known as *Hibiscus esculentus*, is an important vegetable, widely distributed from Africa to Asia, Southern Europe, and America that is more commonly known as ladies finger, okra, or gumbo.[8] The fibres in ladies finger help to stabilise blood sugar by regulating the rate at which sugar is absorbed from the intestinal tract. Previous studies reported that ladies finger polysaccharide possesses hepatoprotective,[9] antiulcer,[10] anticancer,[11,12] anti-inflammatory, laxative, antihyperlipidemic, antifungal, and analgesic activities.[13] Nutritionally, the richest part of the ladies finger plant is the dried seeds. The oil of ladies finger seeds is edible and the residual meal after oil extraction is rich in protein.

The aim of this study is to determine the effect of okra supplement on blood glucose levels and antioxidant biomarkers of alloxan induced diabetic wistar rats

Materials and Methods

Animals

A total of twenty-five (25) Wistar rats of both sexes weighing between 120-150 grams were procured at the Department of Human Physiology Animal House, Ahmadu Bello University Zaria, Nigeria. The animals were kept in an aerated laboratory cage in the animal house of the Human Physiology Department of the Ahmadu Bello University. The animals were allowed access to food and water *ad libitum*. The study was conducted in accordance with the guidelines of Ahmadu Bello University in accordance with the rules governing the use of laboratory animals as accepted internationally by National Institute of Health Guide for Care and Use of Laboratory Animals.

Collection, Identification and Preparation of Supplement

Okra fruit was purchased from Samaru market in Zaria, Nigeria in the month of February 2016. It was authenticated at the Herbarium Unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria. A voucher number (00654) was given for future reference. The okra was allowed to dry and then ground into powder using a grinding machine. The feed supplements for the rats were then prepared by mixing 33g of the okra with 67g of normal rat feed and 66g of okra was mixed with 34g of normal rat feed to get the 33% and 66% supplement ratio respectively.

Chemical /Drug Used

Alloxan was purchased from Zayo Company, Jos, Plateau State (Sigma Chemical Company St. Louis U.S.A), Glibenclamide, normal saline, haloethane and all other chemicals that were used were of analytical grade.

Induction of Diabetes Mellitus

The Wistar rats were fasted for about 16-18 h, after which diabetes was induced by a single intraperitoneal injection of alloxan monohydrate dissolved in 0.9% cold normal saline solution at a dose of 150 mg/kg body weight.[14] Alloxan monohydrate produces fatal hypoglycaemia and to prevent this, the rats were treated with 20% glucose solution orally for 6 hours and subsequently placed on 5% glucose solution for 24 hours.[15] Blood was collected from the tail vein of the rats after 72 hours of alloxan injection using a glucometer. Rats having fasting blood glucose level greater than 200 mg/dl were selected for the study.

Experimental Design
Animals were randomly divided into experimental and control groups as follows:

Group 1 (normal control) - Normoglycemic received 5 ml/Kg body weight distilled water for sixteen days.
Group 2 Diabetic control administered 5 ml/Kg body weight distilled water.
Group 3 Diabetic administered glibenclamide (2 mg/kg) orally for sixteen days.
Group 4 Diabetic fed on 33 % okra supplement for sixteen days
Group 5 Diabetic fed on 66 % okra supplement for sixteen days.

Determination of Blood Glucose Level
Determination of fasting blood glucose levels was conducted by collecting blood samples from the animal tail vein at day 0, 4, 8, 12 and 16 days using the glucose oxidase principle [16] using a digital glucometer One Touch (Accu-check Active, Boche Diagnostic Company).

Collection of Serum Sample
All the animals were sacrificed at the end of the experiment. The rats were euthanised with overdose haloethane. Blood was collected via cardiac puncture from each animal. About 5 ml of blood sample was collected from each rat in plain tubes and allowed to clot. Thereafter the serum was separated by centrifugation, using Denley BS400 centrifuge (England) at 3000 *g* for 10 minutes. The supernatant collected were used for the analysis of lipid peroxidation and antioxidant biomarkers.

Biochemical Assay
Determination of Catalase (CAT) Activity
Catalase (CAT) activity was measured using the method of Aebi.[17] Exactly 10 µl of serum was added to a test tube containing 2.80 ml of 50 mM potassium phosphate buffer (pH 7.0). The reaction was initiated by adding 0.1ml of freshly prepared 30mM H_2O_2 and the decomposition rate of H_2O_2 was measured at 240 nm for 5 minute on a spectrophotometer. A molar extinction coefficient (E) of 0.041 mM^{-1} cm^{-1} was used to calculate the catalase activity.

Catalase Conc = Absorbance/E.
Catalase Activity = Catalase Conc /Protein Conc. (mg/ml)

Determination of Superoxide Dismutase (SOD) Activity
Superoxide dismutase activity was analysed using NWLSS™ NWK-SODO2 Superoxide dismutase activity assay kit (North-West Life Science Specialities, Vancouver, WA 98662) following the manufacture's protocol. This is based on the method of Martin et al.[18] with modifications to increase robustness and reliability. Ultrospec Plus Spectrophotometer Model number 4054 at wavelength of 540nm was used.

Determination of Reduced Glutathione (GSH) concentration
Reduced glutathione (GSH) was determined by the method of Ellman [19]. 1 ml of supernatant (0.5 ml plasma precipitated by 2 ml of 5% TCA) was taken and 0.5 ml of Ellman's reagent (0.0198 % DTNB in 1% sodium citrate) and 3 ml of phosphate buffer (pH 8.0) was added. The colour developed was read at 412 nm.

Determination of Lipid peroxidation (MDA)
The level of thiobarbituric acid reactive substance, malondialdehyde (MDA) as an index of lipid peroxidation was evaluated on the plasma using the method of Draper and Hadley [20]. The principle of the method was based on spectrophotometric measurement of the colour developed during reaction of thiobarbituric acid (TBA) with MDA. The MDA concentration in each sample was calculated by the absorbance coefficient of MDA-TBA complex 1.56×105cm-1M-1 and expressed as nmol mg-1

Statistical Analysis
Data obtained from the present study were expressed as mean ± SEM. The difference between the groups were analysed by one way analysis of variance (ANOVA), followed by Dunnet's *post hoc* test to compare the level of significance between control and experimental groups values of $p < 0.05$ were considered significant.

Results

Table 1: Effect of Okra (*Abelmoschus esculentus*) Supplement on Blood Glucose Levels (mg/dl) on Alloxan Induced Diabetic Wistar Rats.

Experimental Group/ Treatments	Blood glucose Day 0	Blood glucose Day 4	Blood glucose Day 8	Blood glucose Day 12	Blood glucose Day 16
Group 1: Normoglycaemic	72.2 ± 5.12	67.4 ± 2.20	71.0 ± 5.03	64.40 ± 1.27	70.70 ± 1.34
Group 2: Diabetic control	410.2 ± 3.24	414.6 ± 3.37	440 ± 2.23	324.2 ± 3.06	292.4 ± 2.13
Group 3: Diabetic + 2 mg/kg Glibenclamide	434.6 ± 2..22	456.8 ± 2..31	322 ± 3.21	253.0 ± 3.19	128.8 ± 3.50 [a]
Group 4: Diabetic + 33% okra	404.8 ± 3.20	133.6 ± 4.27 [a]	146.6 ± 2.91 [a]	133.2 ± 1.11 [a]	79.6 ± 1.01 [a]
Group 5: Diabetic + 66% okra	420.1 ± 1..33	153.8 ± 2.44 [a]	106.2 ± 2.70 [a]	113.8 ± 2.11 [a]	81.8 ± 5.43. [a]

[a] Statistically Significant At p< 0.05 when Compared with Untreated Diabetic Control Group.

There was a significant ($p< 0.05$) reduction in blood glucose levels in the groups fed with 33 and 66% okra supplement when compared with the diabetic untreated control group . Furthermore, there was a significant increase when compared with the normoglycaemic. Also as regards to the reference drug glibenclamide 5 mg/kg there was a significant decrease at day 12 and 16 respectively, when compared with diabetic control group as shown in Table 1.

Table 2: Effect of Okra Supplement on Antioxidant Enzymes on Diabetic Wistar Rats

Group/Treatment	MDA (nmol/mg)	CAT (U/mg)	GSH (µg/ml)	SOD (U/ml)
Group 1: Normoglycemic control	45.70 ± 0.24	56.26 ± 0.12	68.75 ± 2.32	49.11 ± 1.25
Group 2: Diabetic control	70.60 ± 0.34	10.23 ± 0.21	34.66 ± 1.21	96.52 ± 1.12
Group 3: Diabetic + 2mg/kg (GLB)	52.00 ± 1.31[a]	19.12 ± 0.38 [a]	65.63 ± 2.15 [a]	75.13 ± 2.02 [a]
Group 4: Diabetic + 33% of Okra	34.41 ± 0.22 [a]	38.20 ± 0.37 [a]	61.38 ±2.23 [a]	55.05 ± 1.21 [a]
Group 5: Diabetic Rats + 66% of Okra	44.21 ± 0.11 [a]	45.40 ± 0.51 [a]	60.15 ± 1.12 [a]	44.20 ± 1.01 [a]

[a] Statistically Significant at *p*< 0.05 when Compared with Untreated Diabetic Control

As regards the superoxide dismutase (SOD) levels, there was a significant ($p< 0.05$) reduction in the groups given 33% and 66% okra supplement as compared to the diabetic control. In relation to the catalase (CAT) levels, there was a significant increase ($p< 0.05$) in the groups given 33% and 66 % okra supplement when compared with the diabetic control. Also, there was significant increase ($p< 0.05$) in the reduced glutathione concentration (GSH) in the groups fed with 33% and 66 % okra supplement when compared with the diabetic control group. Furthermore, there was a significant reduction ($p< 0.05$) in the malondialdehyde (MDA) of the group fed with 33% and 66% okra supplement when compared with the diabetic control . However there was no significant change in all the tested parameter when compared with the normoglycaemic control group as shown in Table 2.

Discussion

Alloxan, a beta cytotoxin, destroys pancreatic β-cells of islets of Langerhans resulting in a decrease in endogenous insulin secretion and paves way for the decreased utilization of glucose by body tissues leading to elevation of blood glucose level, decreased protein content, increased levels of cholesterol and triglycerides.[21] On day 0, there was no significant change in the blood glucose levels before treatment. However, on day 4, 8, 12 and 16 of feeding, there was significant decrease in the blood glucose levels in the groups that were fed with 33 and 66% okra supplement when compared with the diabetic control, but when compared with the normoglycaemic control there was a significant increase in the blood glucose levels as compared with the other groups. In relation the standard drug (glibenclimide) there was a significant ($p < 0.05$) decrease in blood glucose levels on Day 12 and 16 respectively when compared with the untreated diabetic group. it was reported that infusion of okra roots when used as stomachic and infusion of roasted okra seed help in the therapy of diabetes mellitus.[22]

Furthermore reported the seed of okra which helps to lower blood glucose level.[23] This result is found to be in agreement with that of [24] who reported the presence of high fiber, vitamin C and folate content in okra which helps to stabilise blood glucose by regulating the rate at which sugar is absorbed from the intestinal tract. However, the hypoglycaemic activity observed could be due to the polysaccharide content that has been found in *Abelmoschus esculentus* (okra) supplement.[25] In addition Vitamin B_1 (Thiamine pyrophosphate) (TPP) is an essential vitamin for glucoses metabolism [26] that Vitamin B_1 is a constituents of okra supplement which function in blood glucose level.

The imbalance between the systemic manifestation of reactive oxygen species (ROS) and the system's ability to readily detoxify the reactive intermediates or to repair the resulting damage is termed oxidative stress. The disturbances in the normal redox state of cells can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA.[3] In relation to the superoxide dismutase when compared with the diabetic control untreated group there was a significant decrease in the group that was fed with 33 and 66 % okra supplement. However, when compared with the normoglyceamic control group, there was no significant change in the groups that were fed with the 33 and 66% okra supplement. As regards to the catalase enzymes there was a significant increase in the levels when compared with the diabetic untreated group.

Also when compared with the normoglycaemic group there was no significant change in the groups that were fed with the supplement of okra at the tested percentage. The decreased activity of catalase could also be due to decreased protein expression levels in the diabetic condition.[27] It could also be due to the inactivation of the enzymes by cross-linking or due to the exhaustion of the enzymes by increased lipid peroxidation.[28] In reduced glutathione level, there was a significant increase in the groups fed with the okra supplements when compared with the control untreated group. But when compared with the normoglyceamic group there was no significant change. This could be due to the impaired scavenging of H_2O_2 and lipid hydroperoxides[29] or impaired reduced glutathione synthesis and thiol transport making the cells become susceptible to oxidative damage.[30] In diabetes, a positive correlation between carbonyl

proteins and reduced glutathione suggests enhanced oxidative stress which may result in increased protein gluthathionylation, having an adverse effect on cellular glutathione levels and thus further explaining the reduced glutathione level in this study[31] Reduced glutathione deficiency in diabetes mellitus is due to decreased availability of precursor aminoacids cysteine and glycine due to the fact that deficiency of protein content in the diets of healthy humans has shown to result in suppression of reduced glutathione turnover *in vivo* [32] In this study, administration of okra supplement to the diabetic rats led to significant increase in the reduced glutathione level as compared with the diabetic control group. This action may suggest the free radical scavenging property of okra in diabetic rats.

There was also a significant increase in the malondialdehyde (MDA) concentration of the diabetic control group as compared with the normoglycaemic control group. This is due to the fact that during diabetes, an increased oxidative stress in certain tissues may lead to a rise in the rate of lipid peroxidation and lipid peroxidation is an important factor determining the level and composition of tissue lipids associated with cellular membranes.[33,29] The increase in the level of the malondialdehyde (MDA) is associated with hyperglycemia, reason being that the auto-oxidation of glucose, leads to the generation of free radicals. The continual, increase in blood glucose in the animals with diabetes possibly led to the generation of high levels of ROS/MDA.[34,30] However, in groups that were fed with the okra supplements at the 33 and 66 % there was a significant decrease in the malondialdehyde level when compared with the control. The decrease in MDA level suggests anti-lipid peroxidation action of okra in diabetic rats.

Conclusion

The results of this study indicate that okra (*Abelmoschus esculentus*) supplement possesses anti diabetic and antioxidant activities on alloxan induced diabetic wistar rats.

References

1. Patel V, Sharma V. Diabetes mellitus and its classes. *Research Journal of Pharmaceutical Sciences*, 2014; **3**(4):1-6.

2. Piero MN, Nzaro GM, Njagi JM. Diabetes mellitus a devastating metabolic disorder. *Asian Journal of Biomedical and Pharmaceutical Sciences*. 2015; **4**(40): 1-7.

3. Kala C, Ali SA, Mohd A, Rajpoot S, Khan NA. Protection against FCA Induced Oxidative Stress Induced DNA Damage as a Model of Arthritis and In vitro Anti-arthritic Potential of Costus speciosus Rhizome Extract. *International Journal of Pharmacognosy and Phytochemical Research*, 2015;**7**(2): 383–389.

4. Shinde A, Ganu J, Naik P. Effect of free radicals antioxidants on oxidative stress. *Journal of Dental and Allied Sciences*, 2015; **1**(2): 63-66.

5. Rani V, Deep G, Singh RK, Palle K, Yadav UCS. Oxidative stress and metabolic disorders: Pathogenesis and therapeutic strategies. *Life Sciences,* 2015; **148**(1): 183–193

6. Gul MZ, Bhakshu LM, Ahmad F, Kondapi AKI, Qureshi A, Ghazi IA. Evaluation of *Abelmoschusmoschatus* extracts for antioxidant, free radical scavenging, antimicrobial and antiproliferative activities using in vitro assays *BMC Complementary and Alternative Medicine*, 2011; 11,64.

7. Olaiya C, Adebisi J. Phytoevaluation of the nutritional values of ten green leafy vegetables in South-Western Nigeria," *The Internet Journal of Nutrition and Wellness*, 2010; 9,2

8. Panadda K, Walairorn T, Noppakun P, Maitree S, Piyanete C. Antioxidative activities and phenolic content of extracts from Okra (*Abelmoschus esculentus* L.),

Research Journal of Biological Sciences, 2010; **5**(4)310–313.

9. Hu L, Yu W, Li Y, Prasad N, Tang Z. Antioxidant activity of extract and its major constituents from okra seed on rat hepatocytes injured by carbon tetrachloride," *BioMed Research International*, 2014; 9 Article ID 341291.

10. Olorunnipa, T.A. Igbokwe, C.C. Lawal,T.O. Adeniyi, B.A & Mahady, B.A (2013) "Anti-helicobacter pylori activity of *Abelmoschus esculentus* L. moench (okra): an *in vitro* study," *Clinical Microbiology*, **2**; 14- 19

11. Chu YF, Sun J, Wu X, Liu RH Antioxidant and antiproliferative activities of common vegetables, *Journal of Agricultural and Food Chemistry*, 2002; **50**(23):6910–6916

12. Shah BN, Seth AK, Maheshwari KM, Desai RV. Screening of abelmoschus esculentus fruits for its analgesic activity," *Pharmacology Online*, 2002; (2) 17–21.

13. Shui G, Peng LL. An improved method for the analysis of major antioxidants of *Hibiscus esculentus* Linn, *Journal of Chromatography A*, 2004;**1048**(1)17–24

14. Katsumata KY, Katsumata TO, Katsumata T. Potentiating effects of combined usage of three sulfonylurea drugs on the occurrence of alloxan-induced diabetes in rats. *Horm Metab Res,* 1999; **25**: 125-126.

15. Dhandapani S, Ramasamy SV, Rajagopal S, Namasivayam N. Hypolipidemic effect of *Cuminum cyminum* L. on alloxan-induced diabetic rats. *Pharmacol. Res***,** 2002; **46**(3)251-255.

16. Beach EF,TurnerJJ. An enzymatic method for glucose determination in body fluids.*Clinical Chemistry*, 1958; **4**:462-465.

17. Aebi, H (1984). Catalase. *Methods in Enzymology*. **105**:125-126

18. Martin JJP, Dailey M, Sugarman E. Negative and Positive Assays of Superoxide Dismutase Based on Hematoxylin Autoxidation, *Archives of Biochemistry and Physics*, 1987; **255**: 329-336.

19. Ellman GL. Arch. *Biochem.* 1959; 82, 70-77

20. Draper H, Hadley M. Malondialdehyde determination as index of lipid peroxidation. *Methods in Enzymology,* 1990; **186**: 421–431.

21. Lenzen S. Alloxan and streptozotocin diabetes. *Advances research Institutes of Diabetes Animals*, 2010; **6**(4):113-122.

22. Lim TK. Edible Medicinal and Non-Medicinal Plants: , Fruits, *Springer Science Business Media B.V*. 2012; **3**: 160

23. Smit R, Neeraj K, Preeti K. Traditional MedicinalPlants Used for the Treatment of Diabetes, *International Journal of Pharmaceutical and Phytopharmacological Research*, 2013; **3**(3): 171-175.

24. Zaharuddin ND, Noordin MI, Kadivar Ali. The Use of Hibiscus esculentus (Okra) Gum in Sustaining the Release of Propranolol Hydrochloride in a Solid Oral Dosage Form, *Biomedical Research International.* 2014; Article ID 735891, 8

25. Tomoda M, Shimizu N, Gonda R, Kanari M, Yamada H, Hikino Anticomplementary and hypoglycemic activity of okra and hibiscus mucilages. *Carbohydrate Research,* 1989; **190**(2), 323-328.

26. Marcus R, Coulston AM. Water-soluble vitamins. The vitamin B complex and Ascorbic acid: *Goodman and Gilman's the Pharmacological Basis of Therapeutic*

(Hardman JG, Limbird LE, eds). New York: McGraw-Hill 3. 2001

27. Sindhu RK, Koo JR, Roberts CK, Vaziri, ND. Dysregulation of hepatic superoxide dismutase, catalase and glutathione peroxidase in diabetes response to insulin and antioxidant therapies. *Clinical and Experimental Hypertension*. 2004; **26**: 43–53

28. Okoduwa SI, Umar IA, Ibrahim S, Bello F, Habila N. Age-dependent alteration of antioxidant defense system in hypertensive and type-2 diabetes patients. Journal of *Diabetes and Metabolic Disorders* 2015; DOI: 10.1186/s40200-015-0164-

29. Shanmugam KR, Mallikarjuna K, Nishanth K, Kuo CH, Reddy KS. Protective effect of dietary ginger on antioxidant enzymes and oxidative damage in experimental diabetic rat tissues. *Food Chemistry.* 2011; **124**(4):1436–1442.

30. Yoshida K, Hirokawa J, Tagami S, Kawakami Y, Urata Y, Kondo T. Weakened cellular scavenging activity against oxidative stress in diabetes mellitus: regulation of glutathione synthesis and efflux. *Diabetologia* 1995; **38**:201–210.

31. Livingstone C, Davis J. Targeting against gluthathione depletion in diabetes and its complications. *British Journal of Diabetics and Vascular Disease,* 2007; **7**(6); 258-265

32. Rajagopal VS. Glutathione synthesis is diminished in patients with uncontrolled diabetes mellitus and restored by dietary supplementation with cysteine and glycine. *Diabetes Care*, 2011; **34**(1):162-167

33. Quine SD, Raghu PS. Effects of epicatechin, a flavonoid on lipid peroxidation and antioxidants in streptozotocin-induced diabetic liver, kidney and heart *Pharmacological Reports*. 2005; **57**: 610–615

34. Salem M, Kholoussi S, Kholoussi N, Fawzy R. Malondialdehyde and trace element levels in patients with type 2 diabetes mellitus. *Arch Hellenic Med.* 2011; **28**(1):83–8.