International Journal of Endorsing
HEALTH SCIENCE RESEARCH

IJEHSR

Original Article

Antioxidant effects of *Moringa oleifera* seed oil against oxidative stress induced by alloxan in rats.

Abeer Sajid[1], Lubna Naz[1], Akbar Mughal[2], Ghazala Yasmeen[1], Nazish Iqbal Khan[1] & Sumera Sohail[1]

[1]Department of Physiology, University of Karachi, Karachi-Pakistan
[2]Department of Physiology, Karachi Medical & Dental College (KMDC), Karachi-Pakistan

Doi: 10.29052/IJEHSR.v8.i4.2020.257-264
Corresponding Author Email: lunaz@uok.edu.pk
Received 21/09/2020
Accepted 23/11/2020
First Published 01/12/2020

OPEN ACCESS

Abstract

Background: Type I diabetes mellitus is a metabolic syndrome manifested with chronic hyperglycemia due to insufficient insulin secretion. Hyperglycemia in diabetes mellitus has been associated with the overproduction of reactive oxygen species (ROS) along with inflammatory mediators. There is no allopathic cure for T1DM; therefore, novel strategies for cost-effective plant origin treatments are needed to be formulated with fewer side effects. The research aims to analyze the potential effects of *Moringa oleifera* (MO) seed oil, tracking certain biochemical markers and antioxidant enzymes in Alloxan induced diabetic rats.

Methodology: Eighteen male Wistar rats having weights of 180-220 g were distributed into 3 groups (n=6). Group I: served as Control group, Group II: served as Alloxan treated and Group III: served as Alloxan + MO treated. Diabetes was induced via intraperitoneally administered Alloxan dissolved in 0.9 % NaCl solution (120 mg/kg body weight). In group III, rats were also given 1.5 ml/kg body weight of MO seed oil daily from 4th day of Alloxan administration. The rats were decapitated just after the 21st day for biochemical and antioxidant assessments.

Results: Treatment with MO seed oil has shown a significant decrease ($p<0.05$) in plasma glucose, serum urea, blood urea nitrogen (BUN), creatinine, alkaline phosphatase (ALP) and alanine aminotransferase (ALT) levels. In contrast, aspartate aminotransferase (AST) levels were not-significant ($p>0.05$) as compared with untreated control groups. Insulin and total protein levels had a significant increase ($p<0.05$) in Alloxan+MO treated group compared to the Alloxan treated group. Also, the antioxidant enzyme activities of superoxide dismutase (SOD), glutathione reductase (GSH), and malondialdehyde (MDA) were significantly increased ($p<0.05$), whereas catalase (CAT) activity was not-significant ($p>0.05$) in Alloxan+MO treated group as compared with Alloxan treated group.

Conclusion: Our study showed that MO seed oil is a potent anti-oxidative hypoglycemic agent capable of improving other clinical conditions related to either oxidative stress or diabetes mellitus, such as the hepatic, renal and pancreatic functions.

Keywords

Diabetes Mellitus, Reactive Oxygen Species, *Moringa oleifera*, Alloxan.

Check for updates

Introduction

Type I diabetes mellitus is defined by chronic hyperglycemia developed because of insulin deficiency or inadequate insulin secretion[1]. In diabetes mellitus, hyperglycemia is linked with enhanced production of ROS and inflammatory mediators, which ultimately leads to oxidative stress. One source of ROS is free endogenous radicals formed by pancreatic beta cells in response to some cytokines[2] and facilitates the decimation of cell membranes along with cellular lipids, which in turn damages many biological molecules producing inflammation, degradation of β-cells and ultimately leads to cell death via direct reaction[3]. Unfortunately, overproduction of ROS has resulted in extremely detrimental effects, as it alters the antioxidant mechanism, inducing a series of deleterious events, inactivates many antioxidant enzymes, promoting protein glycation, resulting in diabetic complications[4] such as ketoacidosis, nephropathy, retinopathy, neuropathy, arthritis, coronary artery disease, cardiac disease, cancer, DNA and protein alterations (leading to replication errors), lipid peroxidation, and early aging[5,6].

Various hypoglycemic allopathic medications have been formulated but have been found to display some serious side-effects, prompting the demand for the emergence of indigenous and affordable herbal products for the management and treatment of diabetes mellitus. MO, a multifunctional plant, appertain to the family Moringaceae. It is acknowledged as the "Miracle-Tree" because of its having diverse applications and benefits[7]. Essentially, all parts of the MO plant have been stated to strengthen a broad range of biological functioning, including anticancer, anti-inflammatory, antioxidant, anti-hyperglycemic, hepatoprotective and neuroprotective activities[8-13]. These properties are attributes of proteins, carbohydrates, tannins, glycosides, fatty-acids such as oleic acid, palmitic acid, arachidonic acid, ascorbic acid and naturally occurring antioxidants such as tocopherols, flavonoids such as quercetin, chlorogenic acid and kaempferol, phenolics and carotenoids[14].

However, the seeds are drawn scientific attention as MO seed kernels incorporate appreciable oil content (more than 40 %) with a high-quality fatty-acid composition (oleic acid greater than 70 %) and a noticeable resistivity to oxidative destruction after refining[15]. Anwar et al. reported that the oil content of Moringa produce in Pakistan's temperate region was about 38-42 %[16]. It has a pale yellow color with a pleasant nutty flavor. Its oxidative stability is better than that of other oleic acid-rich oils such as olive, high oleic sunflower, macadamia, meadow-foam, hybrid safflower, and safflower, apricot and almond oils[17]. The oil contains oleic, behenic, stearic, arachidic and palmitic acid along with tocopherols that are accountable for its antioxidant activity.

A very limited work done has been done on MO seed oil in treating DM that's why the present study focuses on the potential of MO seed oil to act as an exogenous source of antioxidants to protect against diabetes mellitus induced by Alloxan in male Wistar rats. The study further examined specific diabetic biochemical parameters in the liver, kidney and pancreas. Whereas, oxidative stress markers were estimated in the liver.

Methodology

Experimental Animals

Eighteen male Wistar rats (180-220 g) were subjected to the acclimatization period of 3-4 days before the start of the experiment. The rats were grouped according to their weights (n=6) and were housed in a room with a moderate temperature of 22-24°C and a 12-hour light-dark cycle with free access to both water and food prepared as per their daily requirements. All the ethical guidelines and animal protocols used in the experiments were according to the universally recognized principles for laboratory use and vigilance in the animal-based study given by the Health Research Extension Act of 1985 and the ethical guidelines of International ERB.

Extraction of MO Seed Oil

Moringa kernels had been procured from a local market in Karachi. The Moringa seeds were dehusked from the MO kernels. Dry seeds of

Moringa oleifera were crushed then passed through a mesh strainer (40-mesh sieve). The seed powder was used to extract the oil[18]. 10 g of Moringa seed powder was weighed for seed oil and laid on filter paper creased carefully. The filter paper which contained the sample was then introduced into the Soxhlet apparatus. The weights of the filter paper, along with the sample, were noted. Then 200 ml solvent (hexane) was assessed using a measuring beaker and then transferred along with the sample into a 500 ml rounded bottom container and heated at 60°C for 5 hours after which the sample (seed powder along with filter paper) was moved to the air oven for 15 minutes to remove moisture at 100°C. This sample was then weighed again, and the difference was measured as sample weight before extraction - sample weight after extraction divided by the preliminary sample weight, multiplied by 100 to give the percent of oil yield. The seed oil was obtained by evaporating solvent through further heating at lower temperatures until all the solvent evaporated, leaving behind the extracted oil[18].

Diabetes Induction

Diabetes in rats was induced by injecting Alloxan Intraperitoneal (Sigma-Aldrich Chemical co., USA) prepared freshly in 0.9 % NaCl to over-night fasted rats at a dose of 120 mg/kg of bodyweight[19]. Diabetes induction was confirmed on the fourth day by assessing blood glucose levels by tail prick method and observing hyperglycemic states such as polyuria, polyphagia, polydypsia and weight loss. Rats having >200 mg/dl blood glucose levels were considered as diabetic. MO oil administration was commenced from the fourth day, and this was observed as the first day of treatment.

Study Design

The Wistar rats were arranged into three trial groups (n=6). Group I: served as Control group, Group II: served as Alloxan Treated group (120 mg/kg of body weight of Alloxan was once injected for diabetes induction) and Group III: served as Alloxan + MO seed oil Treated group (1.5 ml/kg of body weight). Following 21 days, the rats were sacrificed. Blood was acquired through cardiac puncture and centrifuged at 2500 rev/min (rpm) for 5 min. The serum obtained was carefully stored at −80°C for biochemical analysis, whereas the liver was also isolated from each rat for antioxidant assessments.

Biochemical Analysis

Glucose by glucose oxidase (GOD)- peroxidase (POD) method[20], Insulin by enzyme linked immunosorbent assay (ELISA)[21], Urea and BUN by Urease-Glutamate dehydrogenase (GLDH) kinetic UV method[22], Creatinine by Jaffe's method[23], Total Protein by Biuret method[24], ALT by Kinetic UV method[25], AST[26] and ALP[27] by the colorimetric method were analyzed according to their respective kits manual.

Antioxidant Enzymes Assessments

Assessments of CAT, SOD, GSH were also carried out according to the methods of Sinha, Kono, Carlberg and Mannervik respectively[28-30]. MDA, a lipid peroxidation product, was also assessed by the Ohkawa et al. method[31].

Statistical Analysis

In the current study, the results were proposed as Mean ± SEM (Standard Error of Mean). The statistical analysis was done by independent T-test using SPSS 16.0. One-way ANOVA was done to test the significance of the experimental groups. P-values < 0.05 were referred as statistically significant whereas, p-values > 0.05 were referred as non-significant.

Results

Alloxan treated rats have shown a rapid decline in their body weights whereas, the control and Alloxan+MO treated rats have shown a progressive increase in their body weights throughout the trial period, as shown in Table 1.

Table 1: Assessment of body weights (gm) among the trial groups

Trial Groups	Day 0	Day 07	Day 14	Day 21
Group I (Control)	215.6±3.53	218.46±4.05	223.5±3.88	226.87±3.46
Group II (Alloxan Treated)	184.66±3.84	167.81±3.81	162.5±2.5	159.23±1.2
Group III (Alloxan+MO Treated)	191.66±4.19	174.48±2.82	183.76±2.95	186.66±2.34

Numeric values are proposed as Mean ± SEM.

Prior to the experiment, all rats had normal glucose and insulin levels, but after Alloxan administration, the insulin levels declined because of pancreatic beta-cell death that leads to higher glucose levels in Alloxan treated group. These levels were significantly ($p<0.05$) reversed by the MO seed oil, as shown in Table 2.

Table 2: Effect of MO seed oil on blood glucose and insulin levels among the trial groups

Parameters	Group I Control	Group II Alloxan Treated[1]	Group III Alloxan+MO Treated[1,2]
Glucose (mg/dl) Day 4	105.84±5.75	234.09±2.87*	221.89±1.93NS
Glucose (mg/dl) Day 21	104.18±3.77	201.74±8.28*	118.66±3.17*
Insulin (IU/ml)	2.34±0.10	0.23±0.00*	0.27±0.01*

Numeric values are proposed as Mean ± SEM. 1: Compared with Control; 2: Compared with Alloxan Group; *P-value<0.05; NS: Not-Significant

Treatment with MO seed oil significantly ($p<0.05$) reduced the concentrations of serum urea, creatinine and BUN in the Alloxan+MO treated group, which were found to be elevated in Alloxan treated group because of the kidney damage, whereas the reduction in total protein level in Alloxan treated group was overcome by MO seed oil in Alloxan+MO treated group as shown in Table 3 evaluating the potential of MO seed oil as nephroprotective.

Table 3: Effect of MO seed oil on kidney functions among the trial groups

Parameters	Group I Control	Group II Alloxan Treated[1]	Group III Alloxan+MO Treated[1,2]
Urea (mg/dl)	4.91±0.21	23.95±3.69NS	6.34±1.67*
Creatinine (mg/dl)	0.25±0.09	0.52±0.00*	0.31±0.05*
BUN (mg/dl)	2.29±0.10	11.19±1.72NS	2.96±0.78*
Total Protein (mg/dl)	7.14±0.28	2.53±0.29*	4.17±0.58*

Numeric values are proposed as Mean ± SEM. 1: Compared with Control; 2: Compared with Alloxan Group; *P-value<0.05; NS: Not-Significant; BUN: Blood Urea Nitrogen

Alloxan treated group showed high levels of ALT, AST and ALP enzymes, indicating liver damage. Treatment with MO seed oil significantly ($p<0.05$) reduced ALT, ALP and non-significantly ($p>0.05$) reduced AST levels in Alloxan+MO treated group as shown in Table 4, evaluating the potential of MO seed oil as hepatoprotective.

Table 4: Effect of MO seed oil on liver enzymes among the trial groups

Parameters	Group I Control	Group II Alloxan Treated[1]	Group III Alloxan+MO Treated[1,2]
ALT (U/L)	20.82±0.36	35.71±1.59*	25.61±3.71*
AST (U/L)	50.66±5.23	70.5±18.50NS	51.66±7.86NS
ALP (U/L)	191.55±2.13	491.2±46.92*	295.32±35.44*

Numeric values are proposed as Mean ± SEM. 1: Compared with Control; 2: Compared with Alloxan Group; *P-value<0.05; NS: Not-Significant; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; ALP: Alkaline Phosphatase

Alloxan administration leads to oxidative stress resulting in significantly ($p<0.05$) high MDA and reduced SOD and GSH levels, CAT was also reduced but non-significantly ($p>0.05$) in Alloxan treated group, which were overcome by MO seed oil in Alloxan+MO treated group as shown in Table 5 evaluating the potential of MO seed oil as anti-oxidative.

Table 5: Assessments of hepatic antioxidant enzymes among the trial groups

Parameters	Group I Control	Group II Alloxan Treated[1]	Group III Alloxan+MO Treated[1,2]
CAT (µmol/g tissue)	16.49±1.44	5.44±3.10NS	12.37±2.02NS
SOD (U/g tissue)	1.16±0.13	0.232±0.09*	0.75±0.10*
GSH (U/g tissue)	74.53±2.04	23.12±1.20*	84.69±15.41*
MDA (µmol/g tissue)	0.94±0.13	3.02±0.00*	1.36±0.21*

Numeric values are proposed as Mean ± SEM. 1: Compared with Control; 2: Compared with Alloxan Group; *P-value<0.05; NS: Not-Significant; CAT: Catalase; SOD: Superoxide Dismutase; GSH: Glutathione Reductase; MDA: Malondialdehyde

Discussion

Diabetes is a deliberating condition that affects the overall body resulting in massive health issues. Therefore, its management is necessary to avoid its associated complications. Alternative medicines have shown limitations in their multiple side effects; thus, novel treatment strategies are required, which are more productive, have fewer side effects and are cost-effective. MO is well known because of its antioxidant, anti-hyperglycemic, anti-cancer and anti-inflammatory activities.

In our study, intraperitoneal administration of Alloxan established type 1 diabetes mellitus in about 3 days. As Alloxan is toxic, it initially destroys some beta cells of the pancreas that may extend to damage kidneys and liver. It also generates ROS, which further exaggerates the destruction of beta cells by which insulin secretion ceases leading to a hyperglycemic state confirmed by elevated glucose levels in the rats of groups II & III. Then MO oil was administered in group 3 using oral gavage for a period of consecutive 17 days. Rats treated with MO seed oil had significantly lower circulating levels of glucose and elevated insulin levels compared to Alloxan treated diabetic group (Table 2). MO treated diabetic rats (Group III) also showed an increase in body weight, which was decreased after diabetes induction compared with Alloxan treated group. The body weights were continued to decrease throughout the trial period (Table 1). These results were analogous with the previous studies[32].

Serum biochemical parameters such as creatinine, urea, ALT, AST, etc., were also determined using commercial kits. Any increase in urea, creatinine and BUN levels indicates impairment of renal function. Elevation in urea and creatinine, along

with elevated blood glucose levels, damages the kidney because protein excretes in urine result in lower serum levels of total protein[33]. In this study, levels of urea, BUN and creatinine were reduced significantly ($p<0.05$) after treating with MO seed oil in the Alloxan + MO Treated group, which were elevated after diabetes induction as compared with the Alloxan treated group (Table 3) which indicates impairment in renal function. Consequently, the kidney function gets badly affected because protein excretes in urine result in lower serum levels of total protein[33]. Also, a reduction in total protein was noted in the Alloxan treated group, which might be due to the kidney damage caused by Alloxan's reactive species. While in the Alloxan + MO Treated group, total protein was found to be increased, indicating that MO oil restores kidney function, thereby decrease protein excretion in urine (Table 3).

The ALT, AST and ALP are liver enzymes. Elevated levels of these enzymes result in hepatic injuries[34]. Alloxan treated group showed high AST levels, ALT, and ALP enzymes that might because of their leakage from distorted hepatocytes or bile ducts compared to Alloxan +MO treated group (Table 4). These results are parallel with the previous studies[35].

MO seed oil is also found to be efficacious against oxidative stress induced by Alloxan in rats. CAT activity was found significantly decreased ($p<0.05$) in Alloxan-induced diabetic rats when compared with normal controls (Table 5) whereas non-significantly increased ($p>0.05$) in Alloxan+MO treated rats when compared with Alloxan, treated diabetic group (Table 5). The levels of SOD and GSH were decreased in the Alloxan treated group compared with the control group (Table 5). In contrast, SOD and GSH concentrations were significantly increased ($p<0.05$) in the Alloxan+MO treated group when compared with the Alloxan treated diabetic group (Table 5). Increased levels of CAT, GSH and SOD against ROS produced by Alloxan induction is owing to the presence of large amounts of antioxidants such as tocopherols, phenolic compounds, and flavonoids, specifically quercetin, kaempferol and chlorogenic acid in MO seed oil that have scavenging potential against ROS. It was also found that Alloxan induction in group II rats markedly increased hepatic MDA levels, which is indicative of increased oxidative stress: these findings correlate with the previous studies[36]. Group treated with the Alloxan+MO seed oil showed significantly lower MDA levels than the Alloxan treated diabetic group (Table 5). In the present study, Moringa seed oil acts as a nutritional (exogenous) antioxidant source that tends to assist the antioxidant enzymes in maintaining hepatocytes' integrity. Our study confirms that MO seed oil may be of greater medical benefit if used as a dietary supplement for diabetic patients[11].

Conclusion

This study's experimental evidence based on biochemical assessments and antioxidant levels strongly suggests that MO seed oil is a potent dietary supplement that lowers ROS and may be very much effective against elevated glucose levels. Its prolonged use might have beneficial effects on the cure and management of diabetes mellitus and its associated complications. But still, there is a need for further studies on the maximum extraction of MO oil, isolating its polyphenols, flavonoids and other compounds in the absolute form to determine the exact mechanisms and possible pathways through which moringa seed oil exerts its anti-diabetic and anti-oxidative activity on a cellular level. A clinical trial is highly recommended, considering the complexity of diabetes.

Conflicts of Interest

The authors clearly declared that there is no conflict of interest among them.

Acknowledgement

We would like to thank Ms. Iqra and Ms. Javeria, for their support in carrying out lab work and Mr. Tariq (laboratory assistant) for animal handling.

Funding

None.

References

1. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes care. 2012; 35(Suppl 1), S64–S71.
2. Collier JJ, Burke SJ, Eisenhauer ME, Lu D, Sapp RC, Frydman CJ, Campagna SR. Pancreatic β-cell death in response to pro-inflammatory cytokines is distinct from genuine apoptosis. PLoS One. 2011;6(7):e22485.
3. Kowluru RA, Chan PS. Oxidative stress and diabetic retinopathy. Exp. Diabetes Res. 2007.
4. Singh VP, Bali A, Singh N, Jaggi AS. Advanced glycation end products and diabetic complications. Korean J Physiol Pharmacol. 2014;18(1):1-4.
5. Omodanisi EI, Aboua YG, Oguntibeju OO. Assessment of the anti-hyperglycaemic, anti-inflammatory and antioxidant activities of the methanol extract of Moringa oleifera in diabetes-induced nephrotoxic male wistar rats. Molecules. 2017; 22(4):439.
6. Halliwell B. Oxidative stress and neurodegeneration: where are we now? J. Neurochem. 2006;97(6):1634-1658.
7. Nouman W, Basra SM, Siddiqui MT, Yasmeen A, Gull T, Alcayde MA. Potential of Moringa oleifera L. as livestock fodder crop: a review. Turk J Agric For. 2014;38(1):1-4.
8. Al-Asmari AK, Albalawi SM, Athar MT, Khan AQ, Al-Shahrani H, Islam M. Moringa oleifera as an anti-cancer agent against breast and colorectal cancer cell lines. PloS one. 2015;10(8):e0135814.
9. Araújo LC, Aguiar JS, Napoleão TH, Mota FV, Barros AL, Moura MC, Coriolano MC, Coelho LC, Silva TG, Paiva PM. Evaluation of cytotoxic and anti-inflammatory activities of extracts and lectins from Moringa oleifera seeds. PloS one. 2013;8(12):e81973.
10. Verma AR, Vijayakumar M, Mathela CS, Rao CV. In vitro and in vivo antioxidant properties of different fractions of Moringa oleifera leaves. Food Chem. Toxicol. 2009;47(9):2196-2201.
11. Al-Malki AL, El Rabey HA. The antidiabetic effect of low doses of Moringa oleifera Lam. seeds on streptozotocin induced diabetes and diabetic nephropathy in male rats. Biomed Res. Int. 2015; Article ID 381040.
12. Singh D, Arya PV, Aggarwal VP, Gupta RS. Evaluation of antioxidant and hepatoprotective activities of Moringa oleifera Lam. leaves in carbon tetrachloride-intoxicated rats. Antioxidants. 2014;3(3):569-591.
13. Sutalangka C, Wattanathorn J, Muchimapura S, Thukham-mee W. Moringa oleifera mitigates memory impairment and neurodegeneration in animal model of age-related dementia. Oxid. Med. Cell. Longev. 2013; Article ID 695936.
14. Toma A, Deyno S. Phytochemistry and pharmacological activities of Moringa oleifera. Int. J. Pharmacol. 2014; 1(4): 222-231.
15. Anwar F, Ashraf M, Bhanger MI. Interprovenance variation in the composition of Moringa oleifera oilseeds from Pakistan. J. Am. Oil Chem.' Soc. 2005;82(1):45-51.
16. Anwar F, Bhanger MI. Analytical characterization of Moringa oleifera seed oil grown in temperate regions of Pakistan. J. Agric. Food Chem. 2003;51(22):6558–6563.
17. Leone A, Spada A, Battezzati A, Schiraldi A, Aristil J, Bertoli S. Moringa oleifera seeds and oil: Characteristics and uses for human health. Int. J. Mol. Sci. 2016;17:E2141.
18. Nadro MS, Audu AS, Glen E. Anti-diabetic Effects of Aqueous Extract and Oil of Moringa oleifera Seed on Liver and Kidney Functions in Streptozotocin-induced Diabetes in Rats. Am J Biochem Biotechnol. 2018; 8(4): 69-74.
19. Jarald E, Joshi SB, Jain DC. Biochemical study on the hypoglycaemic effects of extract and fraction of Acacia catechu willd in alloxan-induced diabetic rats. Int J Diabetes Metab. 2009; 17:63-69.
20. Kaplan LA. Glucose. Clin Chem. 1984; 30: 1032-1036.
21. Ashby J, Frier B. Circulating C-Peptide: Measurement and clinical applications. Ann Clin Biochem. 1981;18:125.
22. Kaplan A, Chaney A L, Lynch R L, Meites S. Urea nitrogen and urinary ammonia. Stand methods Clin Chem. 1965; 5:245-256.
23. Allen LC, Michalko K, Coons C. More on cephalosporin interference with creatinine determinations. Clin Chem. 1982; 28(3):555-6.
24. Henry RJ, Charles, Sobel, Berkman S. Interferences with biuret methods for serum proteins use of Benedicts qualitative glucose reagent as a biuret reagent. Anal Chemi, 1957;29(10):1491-1495.
25. Moss DW, Henderson AR, Clinical enzymology, In: Burtis CA, Ashwood, editors. Tietz. Textbook of Clinical Chemistry. Third Edition. Philadelphia: W. B. Saunders Company, 1999: 617-721.
26. Reitman S, Frankel S. A colorimetric method fot the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Am. J Clin. Pathol. 1957; 28(1):56-63.

27. Englehardt A. Measurement of alkaline phosphatase. Aerztl Labor. 1970; 16:42.
28. Sinha KA. Colorimetric assay of Catalase. Ana Biochem. 1972;47:389-394.
29. Kono Y. Generation of superoxide radical during autoxidation of hydroxylamine and an assay for superoxide dismutase. Arch Biochem Biophys. 1978;186:189-195.
30. Carlberg R, Mannervik. Glutathione Reductase. Methods Enzymol.1985;113:484-490.
31. Ohkawa H, Ohishi N, Yagi. Assay for lipid peroxides in animal tissues by thiobarbituric reaction. Ana Biochem. 1979;95:351-358.
32. Olayaki LA, Irekpita JE, Yakubu MT, Ojo OO. Methanolic extract of Moringa oleifera leaves improves glucose tolerance, glycogen synthesis and lipid metabolism in alloxan-induced diabetic rats. JBCPP. 2015;26(6):585-593.
33. Elgazar AF, Rezq AA, Bukhari HM. Anti-hyperglycemic effect of saffron extract in alloxan-induced diabetic rats. Eur J Biol Sci. 2013;5(1):14-22.
34. Qureshi SA, Asad W, Sultana V. The effect of Phyllantus emblica Linn on type-II diabetes, triglycerides and liver-specific enzyme. Pak J Nutr. 2009;8(2):125-128.
35. Paula PC, Sousa DO, Oliveira JT, Carvalho AF, Alves BG, Pereira ML, Farias DF, Viana MP, Santos FA, Morais TC, Vasconcelos IM. A protein isolate from Moringa oleifera leaves has hypoglycemic and antioxidant effects in alloxan-induced diabetic mice. Molecules. 2017;22(2):271.
36. Dar PA, Ali F, Sheikh IA, Ganie SA, Dar TA. Amelioration of hyperglycaemia and modulation of antioxidant status by Alcea rosea seeds in alloxan-induced diabetic rats. Pharmaceu Biol. 2017;55(1):1849-1855.