

ORIGINAL ARTICLE

Antioxidant Modulating effect of *Olea europaea* leaf Extract on Superoxide dismutase (SOD) Activity in Streptozotocin induced diabetes

Maher. M. Al-Enazi^{1,2}, Shaik Rahiman³, A. Z. El- Bahrawy⁴, Bilal Ahmad Tantry⁵

¹Vice president of Academic Affairs, Aljouf Univeristy, Saudi Arabia.

²Department of Clinical Laboratory Sciences, College of Applied Medical sciences, Salman Bin Abdulaziz University, Saudi Arabia

³Department of Biochemistry, College of Medicine, Aljouf University, Saudi Arabia

⁴Department of Biology, College of Science, Aljouf University, Arabia

⁵Department of Microbiology, College of Medicine, Aljouf University, Saudi Arabia

ABSTRACT

Oxidative stress concern one of the most common causes of chronic diseases worldwide. Earlier clinical studies reported that oxidative stress plays a major role in the pathogenesis and development of complications of both types of diabetes mellitus (DM). Most of the synthetic diabetic drugs failed to minimize the side effects. Hence, now a days natural antioxidants gaining popularity in the treatment of diabetes to overcome the adverse effects of synthetic diabetic drugs. The purpose of the current work was to investigate the modulating effect of *Olea europaea* leaf extract on antioxidative enzyme (Superoxide dismutase) activity in Streptozotocin (STZ) induced diabetes. Oral administration of *Olea europaea* methanolic leaf extract (OEMLE) at the doses 250, 500 mg/kg, p.o respectively was studied in STZ induced diabetic mice. Oral glucose tolerance, blood glucose level as well as liver and kidney and serum superoxide dismutase activities were studied. After treatment with OEMLE 250, 500 mg/kg, p.o for 15 days there was a significant decrease in blood glucose levels and Oral glucose tolerance test reviled maximum blood glucose levels after oral glucose challenge after 30 min. The activity of SOD in serum, liver and kidney activity was significantly increase ($p < 0.05$) after the oral treatment with OEMLE (250, 500 mg/kg b.w). We concluded that OEMLE (250, 500 mg/kg, b.w) doses are effective in the protection of oxidative damage in diabetes. The effectiveness is does dependent manner. Our study suggest that OEMLE 500mg/kg, b.w was more effective dose.

Keywords: Oxidative stress, Superoxide dismutase, *Olea europaea* leaf, diabetes mellitus.

Received 21/11/2014 Accepted 22/12/2014

©2014 Society of Education, India

How to cite this article:

Maher. M. Al-E, Shaik R, A. Z. El- Bahrawy, Bilal A T. Antioxidant Modulating effect of *Olea europaea* leaf Extract on Superoxide dismutase (SOD) Activity in Streptozotocin induced diabetes. Adv. Biores., Vol 6 [1] January 2015: 19-24. DOI: 10.15515/abr.0976-4585.6.1.1924

INTRODUCTION

Diabetes is one of the most common chronic metabolic disorders. Globally its prevalence has been estimated that, above 400 million peoples were diabetic in 2010 and it will be expected to rise more than 86% in its number till 2030 [1]. It is characterized by hyperglycemic symptom lead to induction of oxidative stress due to protein glycation and relase reactive oxygen species (ROS) which play an important role in lipid peroxidation and cellular protein oxidation results in many diabetic secondary complications [2].

Oxidative stress results from increased reactive nitrogen species (RNS) and/or ROS [3]. In diabetes, liver is the most effective organ by ROS-mediated injury because of its oxidative and detoxifying functions. Liver oxidative stress mainly due to glucose auto oxidation, shifts in redox balances, decreased concentration of low molecular weight antioxidants in tissue as well as impairment of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) defense functions [4]. Amongst all antioxidative enzymes SOD is one of the most ubiquitous specific antioxidant enzymes shows its detoxify function in cells, tissues, and extracellular fluids. Three major isofroms of SOD are cytosolic CuZn-SOD (SOD1), mitochondrial SOD (SOD2), and extracellular SOD. Extracellular SOD is similar in structure to

SOD1. SOD acts as first line antioxidant defense in the disease pathogenesis [5]. Decreased SOD2 causes lower mitochondrial GSH levels results in increased oxidative stress [6]. Earlier studies reported that, diabetic complications improvement accompanied by improvements in redox status can be proved by the increased expression SOD activity in diabetes [7].

Recently, natural antioxidants gaining popularity in the treatment of diabetes to overcome the adverse effects of synthetic diabetic drugs. Nowadays most of the food substances were identified as potential active in the treatment of diabetes complications [8-11]. These physiological active substance play a vital role for alleviating the oxidative stress in diabetic condition. *Olea europaea* belong to *Oleaceae* family has been widely identified as natural antioxidant source due to the presence of some antioxidants and phenolic compounds in its fruits, leaves and oil. *Olea europaea* gained special value in Mediterranean diet and high-added value products [12]. The main phenolic compounds such as oleuropein, oleuropein aglycone, caffeic acid, catechin, luteolin and hydroxytyrosol presented in olive leaves possess the highest antioxidant activity [13]. Many studies reported that, oleuropein potential pharmacological activities and antioxidant properties in many disease conditions [14,15]. Earlier studies showed that, olive leaf has anti hyperglycemic activity [16], cardioprotective effect oleuropein [17] antinociceptive activities [18], lowered blood cholesterol [18] concentrations, anti atherosclerotic activity [19] antimicrobial activity [20] and antioxidant activity [21]. The purpose of the current work was to investigate the modulating effect of *Olea europaea* leaf extract on antioxidative enzyme (Superoxide dismutase) activity in Streptozotocin (STZ) induced diabetes.

MATERIALS AND METHODS

Preparation of Plant Extract:

The collected leaves of *Olea europaea* were shade dried and reduced to coarse powder using a mechanical grinder. The powdered material of the leaves was exhaustively extracted with methanol under the maceration process. The macerated mixture was filtered by using Whatman No1 filter paper and evaporated at room temperature to yield a solid extract. This extract was kept in refrigerator until the analysis.

Animals

Adult male swiss albino mice (20-25 g) were obtained from animal house college of medicine, Al Jouf university. All animals protocols were approved by the college ethical committee, Al Jouf university. All mice were fed *ad libitum* with standard laboratory pellet diet and free access to tap water. The experimental mice were maintained under a constant 12 hr light and dark cycle at room temperature. Animals were acclimatized to the new experiment environments for 3 days before the study.

Drugs and Chemicals

All the drugs and biochemicals used in this experiment were purchased from Sigma Chemical Company Inc., St Louis, Mo, USA. Bioassay kits such as Glucose assay kit (Biovision U.S.A) Superoxide dismutase assay kit (Cell Biolabs, U.S.A) purchased from respective companies. All other chemicals used in this study were of analytical grade. Streptozotocin (STZ) procured from Biovision, USA.

Acute toxicity

The method described by Lorke (1983) [22] with slight modification was used to determine the safety of the *Olea europaea* methanolic leaf extract (OEMLE) Briefly, normal healthy male mice were divided into groups of five mice in each cage. OEMLE (250 and 500 mg/kg) or vehicle were orally administered to animals 10 in number. Access free to food and water, toxic symptoms and the general behavior of mice were observed continuously for 1 hr after the treatment, intermittently for 4 hr, and thereafter over a period of 24 hr. The mice were further observed for up to 14 days following treatment for any signs of toxicity and mortality.

Induction of diabetes

Induction of diabetes was carried by single dose of intraperitoneal injection of streptozotocin at 45 mg/kg freshly prepared by dissolved in 0.01 M citrate buffer (pH 4.5) injected to the animals after overnight fasting [23]. Control mice received only 0.01 M citrate buffer. The hyperglycemia was confirmed with the measurement of fasting blood glucose level higher than 250 -300 mg/dl after one week of STZ injection and considered as diabetic experimental model for this study.

Experimental design

Total 50 diabetic mice were randomly divided in to five groups of 10 each. Group1 animals received only normal saline solution. Mice in Group 2 were considered as diabetic control (STZ, 45 mg/kg, *i.p*). Group 3 & 4 mice were treated with OEMLE of 250, 500 mg/kg, *p.o* respectively. A standard drug glibenclamide (10 mg/kg/day, *p.o*) received by Group 5 animals. Mice in Group 1,3, 4 were treated by oral gavage once a day for a period of 15 days. At the end of the experiment day 15 all the mice were overnight fasted and

followed by retro-orbital blood collection. Animals were sacrificed under diethyl ether anesthesia and liver and kidney were excised. The blood was centrifuging at 4000 rpm for 10 min to obtaine serum. Samples were stored at -20°C until used.

Determination of Fasting blood glucose

Blood samples were collected from retro-orbital route of the mice and the blood glucose level was determined by by commercially available glucometer (Accu chek, USA).

Oral glucose tolerance test (OGTT)

OGTT test was performed on the end of the experiment day after overnight fasting. Baseline levels of glucose (0min) was determined from tail vein blood and followed by oral administration of 1.5 g/kg B.W glucose to all group animals and additional blood samples were collected at 30, 60, 90, and 120 min intervals [24]. Blood glucose was measured by commercially available glucometer (Accu chek, USA).

Determination of SOD activity in serum and tissue sample

Serum SOD activity was measured by the commercially available Oxi Select™ SOD Activity Assay Kit ((Cell Biolabs).

SOD activity in liver and kidney were estimated as described by Sarawoot Palipoch and Chuchard Punsawad [25] with little modifications. In brief, liver and kidney were homogenized at 50 mg/ml in cold 1X lysis buffer (10mM Tris, 150mM NaCl pH 7.5, , 0.1 mM EDTA) and centrifuged at 12000 rpm for 10 min and the supernatant was used for SOD activity analysis. As per Oxi Select™ SOD Assay Kit manufacture protocol the semiautomatic analyzer (Chemwell analyzer, U.S.A) program was prepared (20 µL of tissue supernatant followed by the addition of µL of xanthine solution, 5µL of chromagen solution, 5 µL of 10X SOD assay buffer, 50µL of deionized water and finally 10 µL of pre-diluted 1X xanthine oxidase solution to a 96-well microplate). The absorbance was set as 490 nm.

Statistical analyses

Statistical analysis was performed using SPSS software version 17.0. All data are showed as means ± SD. One-way ANOVA test was used to compare experimental animals. Differences in mean values among the four groups were tested using Duncan's multiple tests. A *p*-value of <0.05 was considered statistically significant

RESULTS AND DISCUSSION

Now a days evaluation of plant therapeutetic potential bioactive constituents in the DM treatment gaining intrest all around the world. In recent years, the modulating effect of unbalanced redox status related to DM has been studies in many medicinal plants. Increase blood glucose levels in DM causes the elevated levels of free radicals followed by release of reactive oxygen species (ROS), which casuse lipid peroxidation and alter antioxidant defense system and further glucose metabolism imparment [26].

Acute toxicity test results showed that dose of 250 mg/kg b.w, 500 mg/kg b.w were suitable for oral route administration. Our study results revealed the methanolic extract of *O.europaea* exhibit the significantnt hypoglycemic effect in STZ-induced-diabetic mice as compared to diabetic control in dose depended manner (Table 1). Our results were similar to earlier studies reported *O.europaea* leaves anti hyperglycemic activity [27,28]. The blood glucose lowering effect of OEMLE (250, 500 mg/kg) is assumed may be due to decrease in the intestinal glucose absorption rate causes increase pancreatic action such as enhance the uptake of peripheral glucose [29] or stimulation of glycogenic and glycolytic mechanisams leads to decrease in glyconeogenesis and glycogenolysis [30]. The hypoglycemic effect *O.europaea* may be due to the presence of insulin like substance that stimulates of B-cells to produce more insulin [28]. The high level of fiber content in plant also effect the absorption mechanisam of carbohydrate absorption or on pancreatic tissue regenerative mechanism. Oral glucose tolerance test results reviled that the maximum blood glucose levels in all experimental groups were occurred after oral glucose challenge after 30 min (Figure 1). OEMLE 250, 500mg/kg b.w showed significantly suppressed the high level of blood glucose level after 30 min and at 120min it reached a higher level than its initial blood glucose level as compared to control. The effect of glucose tolerance is dose dependent manner. This blood glucose suppression effect level would persist until the blood glucose level reached the initial level (Figure 1).

SOD is one of the antioxidant enzymes involved in the first line antioxidant dense system against oxidative stress and cellular defense mechanisms protects tissues against oxidative damage due to ROS. SOD defense involved the scavenging of superoxide radical by converting it into molecular oxygen and hydrogen peroxide [31].

In this study, STZ administration in mice resulted in markedly diminished SOD activity in serum, liver and kidney as compared to normal mice control group. Earlier studies also reported that decreased activities of antioxidant enzymes in serum, kidney and liver of diabetic mice [32]. After the oral treatment with

OEMLE (250, 500 mg/kg b.w) (10 mg/kg/day) for 14 days significantly increase ($p < 0.05$) the activity of SOD in serum, liver and kidney activity ($P < 0.05$) as compared to diabetic control mice group (Figure 2, 3). The results of elevated SOD activities after treatment suggest that OEMLE has a free radical scavenging activity and which could exert potential protective effect in oxidative damage caused by ROS in DM. Madar et al. [33] also reported that the modulatory effect of olive leaf extract on the expression of SOD enzyme in response to oxidative stress. The increase activities of antioxidant enzymes in OEMLE treated group might be due to presence of phenolic compound in the olive leaf such as oleuropein and hydroxytyrosol, shown to be superoxide anions scavengers [13]. These two bioactive constituents in olive leaf are also reported to be effective in scavenging of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical [34]. We concluded that OEMLE (250, 500 mg/kg, b.w) doses are effective in the protection of oxidative damage in diabetes. The effectiveness is dose dependent manner. Our study showed OEMLE 500mg/kg, b.w was more effective than other dose. The use of OEMLE effective dose could help in the modulation the diabetes by enhance the SOD active as a first line antioxidant defense mechanism and also aid in the suppression of diabetic complication.

Table 1: Effect of OEMLE on blood glucose level (mg/dl) of normal and STZ-induced diabetic mice.

Group	0 day	7 th day	14 th day
Control	92.5±2.11	94.6±2.06	94.1±1.9
Diabetic control	220.52±2.42*	246±2.31*	299±2.04*
Standard	206±3.11*	166±2.97*	122±2.53*
250mg/kg	216.9±1.92*	198±1.56*	171±1.92*
500mg/kg	214.2±1.81*	182±1.66*	139±1.81*

*Significantly difference at $p < 0.05$.

Figure 1: Effect of OEMLE on oral glucose tolerance of experimental mice.

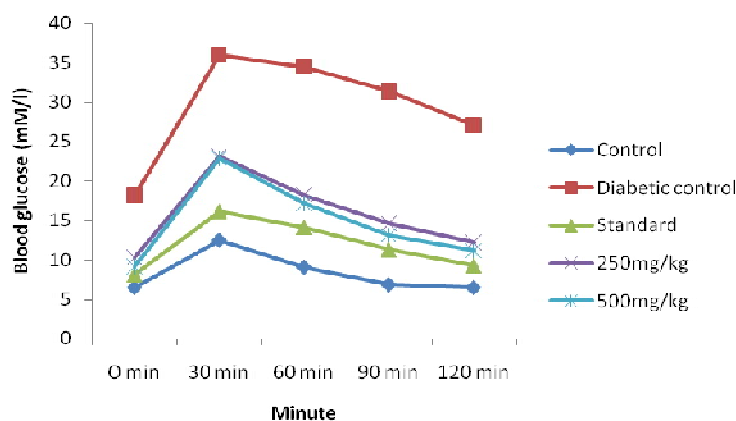
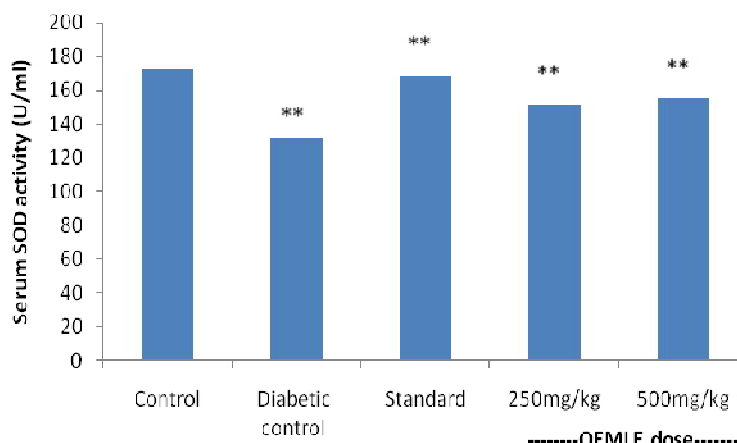
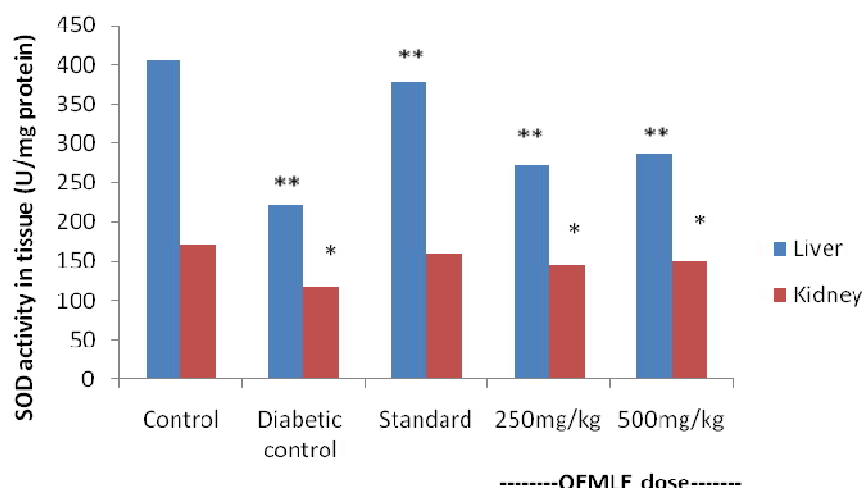


Figure 2: Effect of OEMLE on serum SOD enzyme activity



** Significantly difference at $p < 0.05$

Figure 3: Effect of OEMLE on serum SOD enzyme activity in liver and kidney of experimental animals

*, ** Significantly difference at $p < 0.05$

ACKNOWLEDGEMENTS

This work was made possible by the financial support of Aljouf University, Saudi Arabia,

REFERENCES

- Azmi MB, Qureshi S A. (2012). Methanolic root extract of *Rauwolfia serpentina* Benth improves the glycemic, antiatherogenic, and cardioprotective indices in alloxan-induced diabetic mice. *Adv Pharmacol Sci*.
- Chueh WH, Lin JY. Berberine, (2011). An isoquinoline alkaloid in herbal plants, protects pancreatic islets and serum lipids in nonobese diabetic mice. *J Agric Food Chem*, 59, 8021-7
- Joseph LE, Ira DG, Betty AM, Gerold MG. (2003). Are oxidative stress activated signaling pathways mediators of insulin resistance and cell dysfunction? *Diabetes* ; 52:1-8.
- Haskins K, Bradley B, Powers K.(2003). Oxidative stress in type 1 diabetes. *Ann N Y Acad Sci*; 1005:43-54
- P. Pacher, J. S. Beckman, and L. Liaudet, (2007). "Nitric oxide and peroxynitrite in health and disease," *Physiological Reviews*, vol. 87, no. 1, pp. 315-424.
- Xie C, Lovell MA, Xiong S, Kindy MS, Guo J, Xie J, Amaranth V, Montine TJ, Markesbery WR (2001). Expression of glutathione-S-transferase isozyme in the SY5Y neuroblastoma cell line increases resistance to oxidative stress. *Free Radic Biol Med* 31:73-81
- Peixoto. E. B.M. I, B. S. Pessoa, S. K. Biswas, and J. B. Lopes De Faria, (2009). "Antioxidant SOD mimetic prevents NADPH oxidase-induced oxidative stress and renal damage in the early stage of experimental diabetes and hypertension," *American Journal of Nephrology*, vol. 29, no. 4, pp. 309-318.
- Arya A, Cheah SC, Looi CY, Taha H, Mustafa MR, Mohd MA. (2012). The methanolic fraction of *Centratherrum anthelminticum* seed downregulates pro-inflammatory cytokines, oxidative stress, and hyperglycemia in STZ-nicotinamide-induced type 2 diabetic rats. *Food Chem Toxicol* ;50:4209-20.
- Sunil C, Ignacimuthu S, Agastian P. (2011). Antidiabetic effect of *Symplocos cochinchinensis* (Lour.) S. Moore. in type 2 diabetic rats. *Journal of ethnopharmacology* ;134:298-304.
- Yang KM, Shin SR, Jang JH. (2009). Effect of combined extract of safflower seed with herbs on blood glucose level and biochemical parameters in Streptozotocin-induced Diabetic Rats. *J Glucose and Lipid Metabolism in Streptozotocin-induced Diabetic Rats. Korean J Food and Nutr*;21:48-56.
- Briante, R.; Patumi, M.; Terenziani, S.; Bismuto, E.; Febbraio, F.; Nucci, R.(2002). *Olea europaea* L. leaf extract and derivatives: antioxidant properties. *J. Agric. Food Chem*. 17, 4934-4940.
- Omar SH. (2010). Oleuropein in olive and its pharmacological effects. *Sci Pharm*;78:133-54.
- Visioli, F.; Poli, A.; Galli, C. Antioxidant and other biological activities of phenols from olives and olive oil. *Med. Res. Rev.* 2002, 22, 65-75
- Visioli, F.; Bellasta, S.; Galli, C. Oleuropein, the bitter principle of olives, enhances nitric oxide production by mouse macrophages. *Life Sci*. 1998, 62, 541-546
- Jemai H, El Feki A, Sayadi S. Antidiabetic and antioxidant effects of hydroxytyrosol and oleuropein from olive leaves in alloxan-diabetic rats. *J Agric Food Chem* 2009;57:8798-804.
- Covas MI, Konstantinidou V, Fito M. (2009). Olive oil and cardiovascular health. *J Cardiovasc Pharmacol*;54:477-82.
- Chebbi Mahjoub R, Khemiss M, Dhidah M, Dellai A, Bouraoui A, Khemiss F. (2011). Chloroformic and Methanolic Extracts of *Olea europaea* L. Leaves Present Anti- Inflammatory and Analgesic Activities. *ISRN pharmacology* :564972.

18. Wang L, Geng C, Jiang L, Gong D, Liu D, Yoshimura H, et al. (2008). The anti-atherosclerotic effect of olive leaf extract is related to suppress inflammatory response in rabbits with experimental atherosclerosis. *Eur J Nutr* ;47:235-43.
19. Jemai H, Bouaziz M, Fki I, El Feki A, Sayadi S. (2008). Hypolipidemic and antioxidant activities of oleuropein and its hydrolysis derivative-rich extracts from Chemlali olive leaves. *Chem Biol Interact.* 176:88–98.
20. Lee OH, Lee BY. (2010). Antioxidant and antimicrobial activities of individual and combined phenolics in *Olea europaea* leaf extract. *Bioresource technology*; 101:3751-4.
21. Cumaoglu A, Rackova L, Stefek M, Kartal M, Maechler P, Karasu C. (2011). Effects of olive leaf polyphenols against H(2)O(2) toxicity in insulin secreting beta-cells. *Acta Biochim Pol* ;58:45-50.
22. Lorke, D. (1983). A new approach to practical acute toxicity testing. *Archives of Toxicology*; 54, 275–287
23. O. Siddique, Y. Sun, J.C. Lin, Y.W. Chien, (187). Facilité de transport transdermique de l'insuline, *J. Pharm. Sci.* 76 341–345.
24. Chih-Chiang Wang, Wen-Sheng Liu, Fu-Hsin Chang , Pei-Yi Tsai , Ming-Kai Tsai , Jeng-Chuan Shiangand Zhi-Hong Wen. (2013). *Rhodobacter sphaeroides* Extract Improves Glucose Homeostasis in Streptozotocin-Induced Diabetic Mice. *J Microb Biochem Technol* , 6(1): 038-042
25. Sarawoot Palipoch and Chuchard Punsawad. Biochemical and Histological Study of Rat Liver and Kidney Injury Induced by Cisplatin. *J Toxicol Pathol* 2013; 26: 293–299.
26. Balashubashini MS, Rukkumani R, Viswanathan P, Venugopal PM. Ferulic acid alleviates lipid peroxidation in diabetic rats. *Phytother Res*, 2004; 18:310-4.
27. Eidi, A., Eidi, M. and Darzi, R. (2009). Antidiabetic effect of *olea europaea* L. in normaland diabetic rats. *Phytother. Res.* 23:347-350.
28. Sally Badawi, Saleh Ahmed, Nabeel Al –Ani. (2013). Effect of Ethanolic Olive leaf and its Callus Ethanol Extracts in Alloxan- Induced Diabetic mice (Blood glucose and lipid profiles). *Journal of Biotechnology Research Center*, 7 (2), 62-66.
29. Gray, A.M., Flatt, P.R., (1998). Actions of the traditional antidiabetic plant, *Agrimony eupatoria* (agrimony): effects on hyperglycaemia, cellular glucose metabolism and insulin secretion. *British Journal of Nutrition* 80, 109–114.
30. Porchezian, E., Ansari, S.H., Shreedharan, N.K.K., (2000). Antihyperglycemic activity of *Euphrasia officinale* leaves. *Fitoterapia* 71, 522–526.
31. P. Pacher, J. S. Beckman, and L. Liaudet, (2007). “Nitric oxide and peroxy nitrite in health and disease,” *Physiological Reviews*, vol. 87, no. 1, pp. 315–424.
32. Yuntao Liu, Jun Sun, Shengqi Rao, Yujie Su, Yanjun Yang, (2013). Antihyperglycemic, antihyperlipidemic and antioxidant activities of polysaccharides from *Catathelasma ventricosum* in streptozotocin-induced diabetic mice, *Food and Chemical Toxicology*, , Volume 57,p 39-45
33. Madar, Z., Maayan, N., Sarit, O., Eliraz, A., (2004). Antioxidants modulate the nitric oxide system and SOD activity and expression in rat epithelial lung cells. *Asia Pacific Journal of Clinical Nutrition* 13, S101.
34. Gordon, M. H., F. Paiva-Martins and M Almeida. (2001). Antioxidant activity of hydroxytyrosol acetate compared with that of other olive oil polyphenols *J. Agric. Food Chem* 49:2480-2485.