EFFECT OF HYDROALCOHOLIC EXTRACT OF PURSLANE (*PORTULACA OLERACEA L.*) ON DIABETIC VARIABLES IN D-GALACTOSE INDUCED AGING MOUSE MODEL

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Abstract

Introduction. Purslane (*Portulaca oleracea L.*) has antioxidant effects. The aim of this study was to evaluate the pancreas protective effect of Purslane hydroalcoholic extract in D-galactose induced aging female mouse model.

Methods. In this experimental study, 72 adult female mice (30 – 35 g) were obtained and divided into 6 groups: control, Purslane hydroalcoholic extract, D-galactose, D-galactose + Purslane hydroalcoholic extract, Aging, Aging + Purslane hydroalcoholic extract. The aging model induced by subcutaneous injection of D-galactose for 45 consecutive days, and Purslane hydroalcoholic extract was orally gavaged in the last 21 days. 24 hours after the last drug and extract administrations, serum samples and pancreas tissues were removed for biochemical and histological assessments.

Results. Glucose decreased in the Purslane, D-galactose + Purslane and Aging + Purslane groups ($p < 0.05$). Insulin and HOMA-IR increased in D-galactose and Aging groups ($p < 0.05$). Furthermore, administration of hydroalcoholic extract of Purslane improved these parameters in D-galactose and Aging treated mice ($p < 0.05$). Diameter of pancreatic islets decreased in Aging and D-galactose groups and Purslane hydroalcoholic extract administration improved this variable.

Conclusions. The present results show that Purslane has pancreas protective effects via its hypoglycemic and insulin resistance reducing activity.

Key words: Purslane, D-galactose, pancreas, aging, mouse.

INTRODUCTION

Purslane (*Portulaca oleracea L.*), known as “vegetable for long life” in Chinese folklore, is an

edible plant used as a diuretic, febrifuge, antiseptic, antispasmodic agent. This plant was able to improve abnormal uterine bleeding in Iranian traditional medicine. Also, some of Purslane pharmacological roles including antibacterial, analgesic, spasmolytic and wound-healing ones have been discovered (1, 2). Purslane leaves contain a great amount of catecholamines, phenolic acids, flavonoids, caffeic acid, ferulic acid and luteolin, which were considered as the main bioactive agents of this plant (3-5). A previous study indicated that catecholamines could improve mezenteric blood flow in diabetic rats (6). Further, it was revealed that flavonoids and phenolic acids improved inflammation, diabetes complications via their antioxidants activity (7). In one report polysaccharides of Purslane have been used for the treatment of headaches, stomach, liver ailments, cough and arthritis (8).

Evidence has shown that free radical or reactive oxygen species (ROS) generation can cause damage to macromolecular components and induce cell aging or death (9, 10). Further, aging-related damage is specifically based on the imbalance between oxidant and antioxidant activity (11).

D-galactose is a hexose monosaccharide, which can produce advanced glycosylated protein end-products (AGE) through the reaction with free amino acids. These AGEs can induce cell and tissue senescence, diabetic inflammation, and immune system disorder. Excessive D-galactose administration can convert to hydroperoxide and aldose that result to produce O_2^- and H_2O_2 . Further, this oxidative stress can induce dysfunction of mitochondrial and antioxidant mechanisms. In a previous study, this model of aging

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triggered much damage to the kidneys, liver, neuronal and cardiovascular system (12). Also, it was revealed that Purslane extracts have antioxidant activities through their components including unsaturated fatty acids, gallotannins, quercetin, flavonoids, ascorbic acid, and glutathione. Further, this plant has a therapeutic effect on the cardiovascular system, liver and brain in D-galactose induced aging mice model (12). Thus, Purslane has preventive effect in the treatment of oxidative stress induced diseases (13).

Considering the antioxidant activity of Purslane and the effects of D-galactose for induction of animal's aging model through the overproduction of ROS and impairment of antioxidant defense and the effects of free radicals on pancreas β -cells, the present study was designed to evaluate the pancreas protective effect of Purslane hydroalcoholic extract in D-galactose induced aging female mouse model.

MATERIAL AND METHODS

Animal Preparation

Previous studies on Purslane and aging models were conducted on male mice, while the plant has potentially therapeutic effects on diabetic complications of female (14, 15). In this experimental study, Naval Medical Research Institute (NMRI), female mice including 48 adult three months old mice and 24 aged twenty months old mice (30 – 35 g) were obtained from the animal facility of Ahvaz Jundishapur University of Medical Sciences (AJUMS). The three months aged mice were chosen at this study because they reached full maturity at this age and, the aim of aging mice administration was to reveal the similarity between aging model induction and natural aging. The animals were treated in accordance with the principles and guidelines on animal care of AJUMS as reviewed by an ethics committee (IR.AJUMS.REC.1394.521). The laboratory temperature was kept at $20 \pm 4^\circ\text{C}$ with a 12 h light/ 12 h dark cycle and animals had free access to commercial chow and tap water (16).

After 7 days initial acclimatization, the mice were divided into 6 groups (n=12): control (the mice received subcutaneously (s.c.) injection of normal saline 0.9% injection (0.1 mL) for 45 consecutive days and gavaged by 0.2mL of normal saline in the last 21 days), Purslane hydroalcoholic extract (the mice were received s.c. injected with 0.1mL of normal saline 0.9% for 45 consecutive days and gavaged by 0.2 mL of Purslane hydroalcoholic extract 200 mg/kg in the last 21 days), D-galactose (as aging mouse model that

received s.c. injection of 0.1 mL D-galactose 500 mg/kg for 45 consecutive days and gavaged by 0.1mL of normal saline 0.9% in the last 21 days), D-galactose + Purslane hydroalcoholic extract (the mice received s.c. injection of 0.1mL of D-galactose 500 mg/kg for 45 consecutive days and gavaged by 0.2mL of Purslane hydroalcoholic extract 200 mg/kg in the last 21 days), Aging (0.1mL of normal saline 0.9% was injected s.c. for 45 consecutive days and 0.2mL of this solution gavaged in the last 21 days), Aging + Purslane hydroalcoholic extract (the mice were injected s.c. with 0.1mL of normal saline 0.9% for 45 consecutive days and gavaged by 0.2 mL of hydroalcoholic extract of Purslane 200 mg/kg in the last 21 days). Natural aging mice have used to confirm that D-galactose induced aging model, and produce similar stress condition in all animals (17-19).

Plant Extraction

The leaves of Purslane were obtained from Ahvaz city of Iran, and authenticated by the Botany Department proficient of Ahvaz Jundishapur University, Ahvaz, Iran. Desiccated Purslane leaves were powdered by an electric grinder. Then, 100g of the leaves powder were soaked in 1000 mL mixture of ethanol and distilled water (70: 30) and maintained at room temperature for 3 days. The obtained mixture was filtered with Whatman No. 1 filter paper and centrifuged at 3500 rpm for 20 min. Finally, supernatant was dried at 37°C , and the semi solid mass of acquired extract was stored at 4°C until being used (20).

Experimental Assessment

After an initial acclimatization for 1 week, one experimental method was used to produce same estrus cycle in female mice. Hence, estradiol valerate 100 μg dissolved in 0.2 mL olive oil was injected intramuscularly and 42 h later, 50 μg progesterone was injected. Then, 6 h after progesterone injection vaginal smears were prepared stained with methylene blue (1%) and examined by optical microscope (21, 22).

The mice were sacrificed under deep ketamine-xylazine (10-16 mg/kg intraperitoneally) anesthesia 24h after the last D-galactose and hydroalcoholic extract administrations. The blood samples (1-1.5 mL) were collected by cardiac puncture and centrifuged at 3000 rpm for 15 min. Then, all serum samples were transferred to microtubes and maintained at -80°C (21). Ultimately, glucose was assessed by biochemical assay kits (Pars Azmoon, Iran) and serum insulin levels were evaluated by ELISA assays kits (Monobind,

USA) (The sensitivity of hormone detection per assay tube was 0.182 μ IU/mL). Further, homeostasis model assessment-estimated insulin resistance (HOMA-IR) index was calculated according to the following formula: fasting insulin (μ IU/dL) $\times$ fasting glucose (mg/dL) / 405 (23).

Histopathological Assessment

After mice were killed, their pancreas was removed and fixed in 10% formalin solution. Then tissues were dehydrated in graded alcohols concentrations and stored in paraffin. Pancreas sections (5-7 μ m) were prepared and stained with hematoxylin and eosin (H&E). The evaluation of tissue was performed by light microscopy (Olympus PX 50 F3 model, Japan) (24). Mean diameter of the pancreatic islet was calculated according to the following formula: Mean diameter = $\sqrt{l \times b \times \text{magnification}}$, where l is the length and b the breadth of the islets. This variable was measured through the administration of an ocular micrometer in 6 islets for each section and in total 12 islets for each group (25). Finally, a “blind” method has been used for slides reading.

Statistical Assessment

The obtained results were statistically analyzed by using Statistical Package for the Social Sciences (SPSS) software as mean $\pm$ standard error of mean (SEM) with one-way analysis of variance (ANOVA) and post hoc least significant difference (LSD) tests. Furthermore, differences were statistically considered significant at $p < 0.05$.

RESULT

Effect of hydroalcoholic extract of Purslane on glucose, insulin and HOMA-IR

The results showed that serum glucose levels increased in D-galactose and Aging groups when compared to control ($P < 0.05$). Also, this variable decreased in Purslane ($P < 0.05$), D-galactose + Purslane ($P < 0.05$) and Aging + Purslane ($P < 0.01$) groups in comparison with D-galactose as an aging model. Further, Purslane hydroalcoholic extract administration in normal and Aging treated mice revealed a significant decrease in serum glucose levels compared to natural Aging group ($P < 0.05$ and $P < 0.01$ respectively) (Fig. 1). Serum insulin level increased in D-galactose ($P < 0.05$), Aging ($P < 0.01$) and Aging + Purslane ($P < 0.05$) groups compared with control. Also, a significant reduction of insulin level has occurred in Purslane ($P < 0.05$), D-galactose ($P < 0.05$) and D-galactose + Purslane ($P < 0.01$) groups in comparison with natural Aging mice. Moreover, Purslane administration in natural Aging animals increased the levels of this hormone when compared to Purslane, D-galactose and D-galactose + Purslane groups ($P < 0.05$) (Fig. 2). Insulin resistance index (HOMA-IR) assessment indicated a significant increase in D-galactose ($P < 0.05$), Aging ($P < 0.01$) and Aging + Purslane ($P < 0.05$) groups in comparison with control. Further, administration of Purslane in D-galactose treated mice decreased this resistance index when compared to D-galactose group ($P < 0.05$). Natural Aging animals produced a remarkable increase in HOMA-IR when compared to Purslane

Figure 1. Effect of Purslane hydroalcoholic extract on serum glucose levels. Values are expressed as mean $\pm$ SEM of 12 mice in each group. # $P < 0.05$ compared to control group, * $P < 0.05$, ** $P < 0.01$ compared to D-galactose group, $\Delta P < 0.05$, $\Delta\Delta P < 0.01$ compared to aging group.

Figure 2. Effect of Purslane hydroalcoholic extract on serum insulin levels. Values are expressed as mean $\pm$ SEM of 12 mice in each group. # $P < 0.05$, ## $P < 0.01$ compared to control group, $\Delta P < 0.05$, $\Delta\Delta P < 0.01$ compared to aging group, $\$ P < 0.05$ compared to aging + Purslane group.

Figure 3. Effect of Purslane hydroalcoholic extract on HOMA-IR. Values are expressed as mean ± SEM of 12 mice in each group. #P<0.05, ##P<0.01 compared to control group, *P<0.05 compared to D-galactose group, ΔP<0.05, ΔΔP<0.01 compared to aging group, \$P<0.05 compared to aging + Purslane group.

(P<0.01), D-galactose, D-galactose + Purslane and Aging + Purslane groups (P<0.05). Ultimately, this variable significantly decreased in Purslane (P<0.01), D-galactose and D-galactose + Purslane groups *versus* to Aging + Purslane (P<0.05) (Fig. 3).

Effect of hydroalcoholic extract of Purslane on pancreas histopathology

The sections of pancreas showed a normal appearance in control and hydroalcoholic extract of Purslane groups. Further, natural Aging and D-galactose induced Aging model, showed a decrease in diameter of pancreatic islets (P<0.05). Moreover, Purslane hydroalcoholic extract administration increased this variable in comparison with D-galactose and Aging groups (P<0.05) (Table 1 and Fig. 4).

DISCUSSION

Aging process was associated with impaired glucose tolerance, increased insulin resistance and diminished pancreatic beta cell function that enhanced the incidence of diabetes mellitus (26,27). It was revealed that increase of insulin resistance was associated with lean muscle mass and physical activity reduction during aging process (28). An early hypothesis indicates

Figure 4. Effect of Purslane hydroalcoholic extract on pancreas histopathology. A: Control, B: Purslane hydroalcoholic extract, C: D-galactose, D: D-galactose + Purslane hydroalcoholic extract, E: Aging, F: Aging + Purslane hydroalcoholic extract (H&E stain, ×400).

that beta cell function decreases results to alterations of glucose oxidation, potassium efflux and calcium ions levels through the aging. However, subsequent studies indicated that the old rats could increase the rate of both glucose oxidation and insulin secretion, hence suggesting an attributed potential mechanism that induced beta cells attempting to overcome on age-related peripheral insulin resistance (29). The oxidative stress can induce redox homeostasis alterations (30). Further, antioxidant enzymes are able to eliminate free radicals during aging (31). Previous study indicates that the mice and rats injection with D-galactose can induce animal's model of aging and increase oxidative stress by the generation of oxygen-derived free radicals and decreases antioxidant enzyme activity (31). Also, there is a great deal of evidence that suggests free radicals play an important role in increases of serum glucose levels, insulin resistance incidence and β-cell dysfunction (32). Oxidative stress increased in many tissues such as β-cell through the aging, and it is known that β-cell mass and regenerative capacity decrease via its apoptosis (33). Therefore, administration of D-galactose in the present study demonstrated a significant increase in serum insulin levels and HOMA-IR. Hence, this data implied that insulin resistance has occurred in aging animals model and it may be through the enhancement of free

Table 1. Effect of Purslane hydroalcoholic extract on islets diameter

Variable	Experimental Groups					
	Control	Purslane	D-galactose	D-galactose + Purslane	Aging	Aging + Purslane
Diameter of islets (μm)	365.8±32.3	305.2±20.3*Δ	163.4±15.4#\$	257.6±18.7*Δ	188.7±14.5#	287.4±11.3*Δ

Values are expressed as mean ± SEM of 12 mice in each group. #P<0.05 compared to control group, *P<0.05 compared to D-galactose group, ΔP<0.05 compared to aging group, \$P<0.05 compared to aging + Purslane group.

radical generation. Further, according to the present histopathological assessment, it was revealed that D-galactose-induced aging model affects the beta cells and causes the decrease in diameter of pancreatic islets and this event may occur via increase of antioxidant enzyme activity destruction, however, future study is required to clarify the exact mechanism of this event.

Glucose toxicity can increase peroxide levels in the islets and administration of antioxidants improves glucose levels through reducing apoptosis, improving insulin gene expression and secretion (29). Epidemiological studies demonstrated that plants flavonoids can reduce both the production and progression of age-associated diseases (33). One *in vitro* study showed that isolated islets exposure to flavonoids and polyphenols improves beta cell functions and increases insulin secretion. The main mechanisms of these insulintropic effects of flavonoids and polyphenols were included the enhancement of Ca^{+2} fluxes and oxidative stress improvement (33). Many types of antioxidant compounds including unsaturated fatty acids, coumarins, flavonoids and polyphenols are present in Purslane (34). El-Sayed MI *et al.* study demonstrated that Purslane is a powerful hypoglycemic, insulin resistance reducer and a hepatoprotective plant, whose effects have occurred through its contents such as poly-unsaturated fatty acids, glutathione, flavonoids and antioxidants (35). In the present study, it was demonstrated that hydroalcoholic extract of Purslane can reduce glucose and HOMA-IR in aging model treated and natural aged mice. Further, this extract increases the diameter of pancreatic islet in normal and aging animals. Therefore, the results of the present study can suggest that Purslane induce hypoglycemic and insulin sensitivity effects in aging mice through its components such as flavonoids, polyphenols and antioxidants. However, future studies are required to clarify the exact antidiabetic components of this plant.

Finally, besides the limitations noted for any indirect method of antioxidant enzymes measurement, they are particularly prone to artifacts that lead to overestimate the antioxidant activity. For example, the main limit of superoxide dismutase (SOD) measurement is the inability to provide actual rate data for the catalytic activity of SOD. Furthermore, the presence of artifacts can reduce nitro blue tetrazolium or inhibit xanthine oxidase by antioxidant activity, thereby induce misestimating antioxidant performance (36).

In conclusion, the present study confirms that hydroalcoholic extract of Purslane has a pancreas protective effect on natural aging and aging model of

animals through the increased diameter of pancreatic islet, improves blood glucose and insulin resistance. Further, these effects might be related to the components of Purslane such as phenolic acids, flavonoids and antioxidants.

Conflict of interest

The authors declare that they have no conflict of interest.

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References

1. Daniel M. Medicinal Plants: Chemistry and Properties. Enfield NH: Science Publishers, 2006: 184.
2. Lee AS, Lee YJ, Lee SM, Yoon JJ, Kim JS, Kang DG, Lee HS. Portulaca oleracea Ameliorates Diabetic Vascular Inflammation and Endothelial Dysfunction in db/dbMice. J Evid Based Complementary Altern Med 2012; 741824: 1-9.
3. Ziegler MG, Elayan H, Milic M, Gharaibeh M. Epinephrine and the metabolic syndrome. Current Hypertension Reports 2012; 14(1): 1-7.
4. Strack D, Vogt T, Schliemann W. Recent advances in betalain research. Phytochemistry 2003; 62(3): 247-269
5. Xiang L, Xing DM, Wang W, Wang Rf, Ding Y, Du LJ. Alkaloids from Portulaca oleracea L. Phytochemistry 2005; 66(21): 2595-2601.
6. del Campo L, Blanco-Rivero J, Balfagon G. Fenofibrate increases neuronal vasoconstrictor response in mesenteric arteries from diabetic rats: role of noradrenaline, neuronal nitric oxide and calcitonin gene-related peptide. Eur J Pharmacol 2011; 666(1-3): 142-149.
7. Gu JF, Zheng ZY, Yuan JR, Zhao BJ, Wang CF, Zhang L, Xu QY, Yin GW, Feng L, Jia XB. Comparison on hypoglycemic and antioxidant activities of the fresh and dried Portulaca oleracea L. in insulin-resistant HepG2 cells and streptozotocin-induced C57BL/6J diabetic mice. J Ethnopharmacol 2015; 161: 214-223.
8. YouGuo C, ZongJi S, XiaoPing C. Evaluation of free radicals scavenging and immunity-modulatory activities of Purslane polysaccharides. Int J Biol Macromol 2009; 45(5): 448-452.
9. Kumar D, Rizvi SI. Plasma paraoxonase 1 arylesterase activity in D-galactose-induced aged rat model: correlation with LDL oxidation and redox status. Aging Clin Exp Res 2014; 26(3): 261-267.
10. Ghanbari S, Yonessi M, Mohammadirad A, Gholami M, Bacceri M, Khorram-Khorshid HR, Gharibdoost F, Abdollahi M. Effects of IMOD™ and Angipars™ on mouse D-galactose-induced model of aging. Daru 2012; 20(1): 68.
11. Karimi G, Aghasizadeh M, Razavi M, Taghiabadi E. Protective effects of aqueous and ethanolic extracts of Nigella sativa L. and Portulaca oleracea L. on free radical induced hemolysis of RBCs. Daru 2011; 19(4): 295-300.
12. Hongxing Z, Nancai Y, Guofu H, Jianbo S, Yanxia W, Hanju H, Qian L, Wei M, Yandong Y, Hao H. Neuroprotective effects of Purslane herb aqueous extracts against D-galactose induced neurotoxicity. Chem Biol Interact 2007; 170(3): 145-152.
13. Behravan J, Mosafa F, Soudmand N, Taghiabadi E, Razavi BM, Karimi G. Protective effects of aqueous and ethanolic extract of

- Portulaca oleracea L. aerial parts on H₂O₂-induced DNA damage in lymphocytes by comet assay. J Acupunct Meridian Stud 2011; 4(3): 193-197.
14. Ahangarpour A, Oroojan AA, Badavi M. Exendin-4 protects mice from D-galactose-induced hepatic and pancreatic dysfunction. Pathobiol Aging Age Relat Dis. 2018; 8 (1): 1418593.
15. Wainstein J, Landau Z, Bar Dayan Y, Jakubowicz D, Grothe T, Perrinjaquet-Moccetti T, Boaz M. Purslane extract and glucose homeostasis in adults with Type 2 Diabetes: a double-blind, placebo-controlled clinical trial of efficacy and safety. J Med Food. 2016; 19(2): 133-140.
16. Ahangarpour A, Oroojan AA, Heydari H. Effect of hydro-alcoholic extract of *Dorema aucheri* on serum levels of testosterone, FSH and sperm count in Nicotinamide-Stz- induced diabetic rat models. ZUMS Journal 2013; 21(87): 22-31. (Persian)
17. Karimi G, Khoei A, Omid A, Kalantari M, Babaei J, Taghiabadi E, Razavi BM. Protective effect of aqueous and ethanolic extracts of *Portulaca oleracea* against cisplatin induced nephrotoxicity. Iran Basic Med Sci 2010; 13(2): 31-35.
18. Ho SC, Liu JH, Wu RY. Establishment of the mimetic aging effect in mice caused by D-galactose. Biogerontology 2003; 4(1): 15-18.
19. Wang D, Liu M, Cao J, Cheng Y, Zhuo C, Xu H, Tian S, Zhang Y, Zhang J, Wang F. Effect of Colla corii asini (E'jiao) on D-galactose induced aging mice. Biol Pharm Bull 2012; 35(12): 2128-2132.
20. Ahangarpour A, Oroojan AA, Heidari H, Ghaedi E, Taherkani R. Effects of hydro-alcoholic extract from *Arctium lappa* L. (Burdock) root on gonadotropins, testosterone, and sperm count and viability in male mice with Nicotinamide/ Streptozotocin-induced Type 2 Diabetes. Malays J Med Sci 2015; 22 (2): 25-32
21. Fox JG, Barthold SW, Davisson MT, Newcomer CE, Quimby FW, Smith AL. The mouse in biomedical research. 2nd Edition. California: American College of Laboratory Animal Medicine Series. 2007; 3: 97-103.
22. Hoseini E, Forouzan Far M, Paye Dar A. The effect of hydroalcoholic extract of purslane (*Portulaca oleracea* L) on serum concentration of estrogen, progesterone, prolactin and gonadotropins in mature female rats. J Shahrekord Univ Med Sci 2013; 15 (5): 12-21. (Persian)
23. Ahangarpour A, Oroojan AA, Heidari H, Ehsan G, Rashidi Nooshabadi MR. Effects of hydroalcoholic extract of *Rhus coriaria* (Sumac) seeds on reproductive complications of Nicotinamide-Streptozotocin induced Type-2 Diabetes in male mice. World J Mens Health 2014; 32(3): 151-158.
24. Khorsandi L, Orazizadeh M. Protective effect of *Curcuma longa* extract on acetaminophen induced nephrotoxicity in mice. Daru 2008; 16(3): 155-159.
25. Masjedi F, Gol A, Dabiri S. Preventive effect of garlic (*Allium sativum* L.) on serum biochemical factors and histopathology of pancreas and liver in Streptozotocin- induced diabetic rats. Iran J Pharm Res 2013; 12(3): 325-338.
26. Wang S, Sun QQ, Xiang B, Li XJ. Pancreatic islet cell autophagy during aging in rats. Clin Invest Med 2013; 36(2): E72-80.
27. Szoke E, Shrayyef MZ, Messing S, Woerle HJ, van Haften TW, Meyer C, Mitrakou A, Pimenta W, Gerich JE. Effect of aging on glucose homeostasis: accelerated deterioration of beta-cell function in individuals with impaired glucose tolerance. Diabetes Care 2008; 31(3): 539-543.
28. Scheen AJ. Diabetes mellitus in the elderly: insulin resistance and/or impaired insulin secretion? Diabetes Metab 2005; 31(2): 5S27-5S34.
29. Gunasekaran U, Gannon M. Type 2 diabetes and the aging pancreatic beta cell. Aging (Albany NY) 2011; 3(6): 565-675.
30. Ahangarpour A, Oroojan AA, Ramezani Aliakbari F. Effects of C-peptide and Nicotinamide on serum LH, FSH, testosterone levels and sperm count in nicotinamide/STZ induced diabetic mice. Acta Endocrinologica (Buc). 2014; X(4): 588-594.
31. Park S, Kim CS, Min J, Lee SH, Jung YS. A high-fat diet increases oxidative renal injury and protein glycation in d-galactose-induced aging rats and its prevention by Korea red ginseng. J Nutr Sci Vitaminol (Tokyo) 2014; 60(3): 159-166.
32. Rahman K. Studies on free radicals, antioxidants, and co-factors. Clin Interv Aging 2007; 2(2): 219-236.
33. Zhu M, Hu J, Perez E, Phillips D, Kim W, Ghaedian R, Napora JK, Zou S. Effects of long-term cranberry supplementation on endocrine pancreas in aging rats. J Gerontol A Biol Sci Med Sci 2011; 66(11): 1139-1151.
34. Xiang L, Xing DM, Wang W, Wang RF, Du LJ. Review on chemical constituents of *Portulaca oleracea* L. Asia-Pac Trad Med 2006; 7: 64-68.
35. El-Sayed MI. Effects of *Portulaca oleracea* L. seeds in treatment of type-2 diabetes mellitus patients as adjunctive and alternative therapy. J Ethnopharmacol 2011; 137(1): 643-651.
36. Amorati R, Valgimigli L. Advantages and limitations of common testing methods for antioxidants. Free Radic Res 2015; 49(5): 633-649.