Journal of Applied Life Sciences International
9(4): 1-8, 2016; Article no.JALSI.29268
ISSN: 2394-1103

SCIENCEDOMAIN *international*
www.sciencedomain.org

Ameliorative Effect of *Allium cepa* (Red Onions) on the Levels of Some Biochemical Markers in Alloxan Induced Diabetic Rats

Lenka Jessica Lapshak[1*], Luka Carol Domkat[1], Lepzem Nankang Gabriel[1] and Jibini Ngozi[1]

[1]Department of Biochemistry, College of Medical Sciences, University of Jos, P.M.B. 2084, Jos, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author LJL designed the study. Author LCD supervised the work. Authors LNG, JN and LJL carried out all laboratory work. Author LNG managed the analyses of the data and author JN took care of the literature searches. Author LJL wrote the first draft of the manuscript. Author LCD edited the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JALSI/2016/29268
Editor(s):
(1) Magdy El-Sharkawy, Professor of Internal Medicine and Nephrology, Ain Shams University, Cairo, Egypt.
(2) J. Rodolfo Rendón Villalobos, Department of Technological Development, National Polytechnic Institute, México.
Reviewers:
(1) César Luiz Da Silva Guimarães, Federal Universy of Rondônia State, Brazil.
(2) Jerzy Bełtowski, Medical University, Poland.
(3) Maha Rizk, National Research Center, Egypt.
Complete Peer review History: http://www.sciencedomain.org/review-history/17260

Original Research Article

Received 31st August 2016
Accepted 6th December 2016
Published 17th December 2016

ABSTRACT

Aims: The present study was carried out to investigate the effects of onion (*Allium cepa*) extract on enzymes activities and some other biochemical parameters in alloxan-induced diabetic rats.
Methodology: Twenty four albino rats were randomized into four groups (A-D) consisting of six rats each and classified as follows; Group A (Normal Control), Group B (Diabetic Control), Group C (Diabetic Treated) and Group D (Normal Treated). Extracts were administered orally at a concentration of 200 mg/kg body weight once a day for 14 days after confirmation of diabetes in the alloxanized groups (B and C). At the end of the experimental period, the rats were sacrificed under diethyl ether anesthesia. Blood was collected from the sacrificed animals and used for the biochemical assays.

**Corresponding author: E-mail: jessicadwankyes@yahoo.com;*

Place and Duration of Study: Department of Biochemistry, University of Jos, between August and December 2015.
Results: The blood glucose of alloxanized rats significantly and progressively reduced in extract treated animals. The significant (P<0.05) increase in the levels of creatinine (144.88 umol/L), urea (180.83 mg/dl), glucose (345.75 mg/dl) in the untreated diabetic rats were reduced significantly in the diabetic treated rats with the values 34.88 umol/L, 35.59 mg/dl, 128.89 mg/dl respectively below the control group. The elevation in the activities of ALT (228 IU/L), AST (350 IU/L), ALP (596 IU/L) were significantly reduced to 126.00 IU/L, 323.90 IU/L, 540.00 IU/L respectively after the administration of the extract of *Allium cepa*. The significant (p<0.05) increase in the total cholesterol (195.97 mmol/L) and triglyceride (195.00 mmol/L) observed in the diabetic animals was drastically reduced to near normal levels, 158.11 mmol/L and 105.30 mmol/L respectively after the administration of the extract. There was slight increase in HDL from 60.77 mmol/L to 63.78 mmol/L.
Conclusion: In conclusion, following the results obtained and observed, the extract can be said to produce hypoglycemic effects and improve biochemical parameters in diabetic rats, justifying its use traditionally as a nutritional supplement in the management of diabetes mellitus.

Keywords: Allium cepa; diabetes mellitus; alloxan; hypoglycemia.

1. INTRODUCTION

Diabetes mellitus is a disorder in which the body is unable to metabolize carbohydrates properly. The disease is characterized by excessive amounts of sugar in the blood and urine; inadequate production and/or utilization of insulin; and by thirst, hunger and loss of weight [1].

In 2006, according to the World Health Organization, at least 171 million people world wide suffer from diabetes. The incidence is increasing rapidly and it is estimated that by the year 2030, this number will double [2].

The doubts about the efficacy and safety of the oral hypoglycaemic agents have prompted a search for safer and more effective drugs in the treatment of diabetes [3] and therefore medicinal plants continue to provide valuable therapeutic agents, in both modern medicine and in traditional systems. In spite of the fact that insulin has become one of the most important therapeutic agents known to medicine, researchers have been making efforts to find insulin substitutes from synthetic or plant sources for the treatment of diabetes as alternatives to conventional therapy because the cost of administering modern anti diabetic drugs is beyond the reach of most people in the low income group and those living in rural areas where insulin is not readily available [4].

The onion (*Allium cepa*) is the most widely cultivated species of the genus *Allium* [5]. Onions are members of the lily family, they are perennials that are cultivated for food worldwide. There are many varieties. Most onion bulbs are white, yellow, or red. The green stems and leaves are hollow and can reach 3 ft. (1 m) in height. The plants bear small flowers that are usually white or purple. The fleshy bulb that grows below the ground is used medicinally as well as for food [6]. Onions are found in a large number of recipes and preparations spanning almost the totality of the world's cultures. The whole plant is edible and is used as food in some form or the other. Onion has been found to contain quercetin, fructose, quercetin-3-glucoside, isorhamnetin-4-glucoside, xylose, galactose, glucose, mannose, organosulfur compounds, allylsulfides, flavonoids, flavenols, S-alk(en)yl cysteine sulfoxides, cycloalliin, selenium, thiosulfinates, and sulfur and seleno compounds [7].

Wide-ranging claims have been made for the effectiveness of onions against conditions ranging from the common cold to heart disease, diabetes, osteoporosis, and other diseases. They contain chemical compounds believed to have anti-inflammatory, anticancer, and antioxidant properties, such as quercetin. Preliminary studies have shown increased consumption of onions reduces the risk of head and neck cancers [8].

Onions has also been shown to lower blood pressure [9], it also has antiseptic [10] hypoglycaemic and hypocholesteremic properties [11].

The type of onion used for this research is the red onions. Red onions have purplish red skin and white flesh tinged in red. These onions tend to be medium to large in size and can have a mild to sweet flavour, but after being stored for

short time can become quite pungent. They are often consumed raw, grilled, or lightly cooked with other foods, or added as colour to salads. They tend to lose their redness when cooked.

This present study was designed to determine the effects of *Allium cepa* (Onions) aqueous extract on alloxan induced diabetic and normal rats in the control of hyperglycaemia and hyperlipidaemia characteristic of diabetes mellitus in line with the World Health Organisation 1980 expert committees recommendation [12] that traditional methods of management of diabetes mellitus be investigated.

2. MATERIALS AND METHODS

2.1 Plant Collection

The fresh bulbs of red onions (*Allium cepa*) used for this study were obtained from the Angwa Rukuba market, Jos, Nigeria and was authenticated by Mr. Agyeno Otuse at the Department of Plant Science and Technology, University of Jos, Jos, Nigeria.

2.2 Experimental Animals

Male adult Wistar strain rats weighing between 180-250 g were obtained from the National Veterinary Research Institute, Vom, Jos, Nigeria. The animals were housed in aluminium cages under standard conditions. They were maintained on standard animal pellets (containing ingredients such as protein-14.50%, fat -7.00%, crude fiber-7.20%, calcium-0.80%, phosphorus-0.40%, metabolizable energy-2500kCal/Kg obtained from Grand Cereal and Oil Mills Limited, a subsidiary of UACN) and water *ad libitum*. The animals were acclimatized for 7 days prior to commencement of the experiment.

The study was carried out after approval from the institutional animal care and use (IAUC) ethical Committee of the University of Jos.

2.3 Preparation of Plant Extract

Aqueous extract of *Allium cepa* bulbs was obtained using a slight modification of the method described by [13]. The fresh bulbs of *Allium cepa* were cleaned, cut into small pieces, dried and ground into powder. About 250 ml of distilled water per 100 g of dried onion was added and blended in a blender. The resultant slurry was squeezed and filtered with Whatman No. 1 filter paper and then concentrated on a steam bath. The extract is freshly reconstituted in distilled water daily to give the required dose of 200 mg/kg body weight as used in this study. The reconstituted aqueous extract was administered orally to normal and diabetic rats using cannula.

2.4 Induction of Diabetes and Blood Glucose Determination

The animals were fasted overnight. Thereafter diabetes was induced by a single intraperitoneal injection of a freshly prepared Alloxan monohydrate (150 mg/kg body weight) in ice cold 0.9% NaCl solution. The animals were allowed 5% glucose solution overnight *ad libitum* to overcome the drug-induced hypoglycemia. Control (normal) rats were not injected with alloxan and were placed on normal saline alone. Blood samples were drawn from the tail vein and glucose levels were determined to confirm the induction of diabetes using the Bayer Contour™ test strips and Glucometer according to the instructions outlined in the User Guide. Only rats with blood glucose level higher than 7.0 mmol/L were considered diabetic and used for the experiment. Feeding was stopped 12 hours before blood sampling.

2.5 Experimental Design

The rats were divided into four groups of six animals each and were allowed to acclimatize for seven days before the commencement of the study. The experimental groupings are as follows:

Group A: Diabetic Control
Group B: Diabetic Treated
Group C: Normal Control
Group D: Normal Treated

2.6 Collection of Blood Sample and Preparation of Serum

The methods described by Yakubu et al. [14] were used for the collection of blood samples and preparation of serum. In brief, with the animal under diethyl ether anaesthesia, the neck area was quickly shaved to expose the jugular veins. The veins after being slightly displaced (to avoid contamination with interstitial fluid) were then sharply cut with a sterile scalpel blade. Blood was collected into clean, sterile sample bottles which were allowed to clot for 30 minutes. This was then centrifuged at 33.5 g for 15 minutes using a Uniscope Laboratory Centrifuge.

The sera were aspirated with Pasteur pipettes and stored frozen until required for the biochemical analysis.

2.7 Biochemical Assays

The biochemical parameters were determined in the serum using standard methods described for activities of Aspartate transaminase (AST) and Alanine transaminase (ALT) [15], activity of Alkaline phosphatase (ALP) [16], Creatinine [17] and Urea [18]. Total protein, and albumin were estimated using Randox kits (Randox Laboratories Ltd, UK). Serum concentrations of Triglyceride (TG), Total Cholesterol (TC), High-Density Lipoprotein (HDL), and Low-Density Lipoprotein (LDL) were determined using commercially available kits (BIOSINO Biotechnology and Science INC, China).

All spectrophotometric measurements were done using Spectronic 21 spectrophotometer (Bausch and Lomb, NY).

2.8 Statistical Analysis

Data were expressed as mean ± standard deviation (SD). Comparison of the data from test control groups of animals were analyzed by One Way Analysis of Variance (ANOVA) at the confidence limit of 95% and where applicable, Least Significant Difference (LSD) was used to determine significant results, differences between groups were considered statistically significant at P≤0.05.

3. RESULTS AND DISCUSSION

The cytotoxic action of alloxan is mediated via reactive oxygen species, with simultaneous massive increase in cytosolic calcium concentration, resulting to a rapid destruction of the β-cell [19,20]. Therefore, the determination of blood glucose concentration among others is a useful quantitative parameter for diabetes.

Table 1. Effect of aqueous extract of *Allium cepa* on blood glucose, protein and albumin in alloxan-induced diabetic rats

Treatment group	Glucose (mg/dL)	Protein (g/L)	Albumin (g/L)
Normal Control	101.50±0.04	8.73±0.02	4.00±0.01
Diabetic Control	345.75±1.33[a]	7.38±0.02[a]	3.94±0.02
Diabetic Treated	128.89±0.15[ab]	7.97±0.02[ab]	4.02±0.02
Normal Treated	80.25±0.64[ab]	8.71±0.02[b]	3.99±0.03

Values are expressed as mean ± SD, n= 6 for each group, [a] values are significantly different from normal control (p<0.05), [b] values are significantly different from the diabetic control group (p<0.05)

Table 2. Effect of aqueous extract *of Allium cepa on* urea and creatinine in alloxan induced diabetic rats

Groups	Urea (mg/dL)	Creatinine (µmol/L)
Normal control	51.03±0.01	37.98±0.82
Diabetic control	180.83±0.01[a]	144.88±0.85[a]
Diabetic treated	35.59±0.01[ab]	34.88±0.85[ab]
Normal treated	46.28±0.02[ab]	35.88±0.85[ab]

Values are expressed as mean ± SD, n= 6 for each group, [a] values are significantly different from the normal control (p<0.05), [b] values are significantly different from the diabetic control group (p<0.05)

Table 3. Effect of aqueous extract of *Allium cepa* on serum lipid profile of alloxan induced diabetic rats

Treatment group	Lipid profile (mmol/L)		HDL	LDL
	TC	TG		
Normal control	159.22±0.02	103.00±1.83	84.38±0.02	61.02±0.02
Diabetic control	195.97±0.02[a]	195.00±1.83[a]	60.77±0.02[a]	89.34±0.02[a]
Diabetic treated	158.11±0.01[ab]	105.30±0.18[b]	63.78±0.02[ab]	80.43±0.02[ab]
Normal treated	144.75±0.01[ab]	100.80±0.18[ab]	97.67±0.02[ab]	60.62±0.02[ab]

Values are expressed as mean ± SD, n= 6 for each group, [a] values are significantly different from normal control (p<0.05), [b] values are significantly different from the diabetic control group (p<0.05)

Table 4. Effect of aqueous extract of *Allium cepa* on selected marker enzymes in alloxan induced diabetic rats

Groups	Marker enzymes (IU/L)		
	ALT	AST	ALP
Normal control	32.47±0.02	142.86±0.02	579.60±0.18
Diabetic control	228.00±0.83[a]	350.00±0.83[a]	596.00±1.82[a]
Diabetic treated	126.00±0.02[ab]	323.90±0.02[ab]	540.00±1.83[ab]
Normal treated	32.44±0.02[ab]	140.80±0.18[ab]	570.40±0.18[ab]

Values are expressed as mean ± SD, n= 6 for each group, [a] values are significantly different from the normal control (p<0.05), [b] values are significantly different from the diabetic control group (p<0.05)

Table 5. Effect of aqueous extract of *Allium cepa* on bilirubin concentrations of alloxan induced diabetic rats

Groups	Bilirubin concentration (mg/l)	
	Total bilirubin	Direct bilirubin
Normal control	0.24±0.01	0.12±0.01
Diabetic control	0.56±0.02[a]	0.14±0.01[a]
Diabetic treated	0.16±0.02[ab]	0.09±0.02[ab]
Normal treated	0.31±0.01[ab]	0.13±0.02

Values are expressed as mean ± SD, n= 6 for each group, [a] values are significantly different from the normal control (p<0.05), [b] values are significantly different from the diabetic control group (p<0.05)

The increase in the blood glucose levels of the alloxan induced rats to over three times the normal value indicates the effect of the alloxan in inducing the diabetic condition. The subsequent reduction by the administration of aqueous extract of *Allium cepa* in this study suggests hypoglycemic effect of the plant extract. This observation is similar to that of the previous work of Sheela et al. [21] that onions possess a hypoglycaemic effect and allyl propyl disulfide is implicated to be the active principle; it lowers blood sugar levels by increasing the amount of free insulin available. Insulin plays critical role in the maintenance of protein balance, since in addition to stimulating the uptake of amino acids and protein synthesis, it also inhibits protein degradation [22]. Extract administration significantly increased protein levels in both diabetic and normal rats to near normal levels when compared to their respective controls.

Diabetic hyperglycaemia induces elevation of plasma levels of urea and creatinine which are considered as significant markers of renal dysfunction [23] as observed also in this study. These results were also similar to previous report by Uladimir [24] who stated that hyperglycemia is associated with long-term damage, dysfunction and failure of various organs, like the kidneys. There was significant reduction in the plasma protein in both diabetic treated and untreated animals therefore showing that the increased urea and creatinine in diabetic rats may be attributed to enhanced catabolism of both liver and plasma proteins that accompany glyconeogenesis [25]. Administration of plant extract to the diabetic rats reverses the renal function indices to near normal levels.

The prevalence of atherosclerosis and hyperlipidaemia among diabetics is on the increase worldwide. Alterations in serum lipid profile are known in diabetes, which is related to significant changes in lipid metabolism and structure in the diseased state and are likely to increase the risk of coronary heart disease [26,27,28]. The abnormalities in serum cholesterol metabolism could be partly responsible for the changes in the serum cholesterol levels in diabetes which have been reported to increase the accumulation of lipids in cells. Hypercholesterolemia has been reported to occur in alloxan induced diabetic rats [29,30]. Lipid profile which is altered in serum of diabetic patients [31,32] appeared to be a significant factor in the development of premature atherosclerosis through increase in serum triglyceride and total cholesterol levels. The marked hyperlipidaemia that characterizes the diabetic state may be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat depots [33]. The significant reduction in serum cholesterol and total lipids in a dose dependent manner as observed in this experiment were in agreement with previous reports [34]. The hypolipidaemic effect of onions may be connected to its active ingredient allyl propyl disulfide. A reduction in lipid profile could be beneficial in preventing diabetic complications as well as improving lipid metabolism in diabetics [35]. Considering onion

extracts effects on lipid components, it can be assumed a potential hypolipidaemic agent which will be a great advantage both in diabetic conditions as well as the associated atherosclerosis or hyperlipidaemic conditions.

The increase in the activities of plasma AST, ALT and ALP indicate that diabetes may induce hepatic dysfunction. Supporting this finding it has been found by Larcan et al. [36] that liver was necrotized in diabetic patients. Therefore the increment of the activities of AST, ALP and ALP in plasma may be due to the leakage of these enzymes from the cytosol into the blood stream [37], which gives an indication on the hepatotoxic effect of alloxan. Treatment of the diabetic rats with onions caused a reduction in the activity of these enzymes in plasma compared to the mean values of the diabetic groups, though only ALT was significantly reduced. These results are in agreement with those obtained by Ohaeri [38] in rats. A possible explanation for the reduction in the activities of these enzymes by the effect of onions is that the treatment may inhibit the liver damage induced by alloxan.

The elevation in plasma bilirubin indicates liver damage as confirmed by the changes in the activities of plasma liver enzymes. The improvement of the liver damage by oral administration of onions could be confirmed through studying their effect on the level of plasma bilirubin. The intake of onions extract produced significant ($p<0.05$) decrease in plasma bilirubin.

4. CONCLUSION

It can be concluded from this study that onions are able to normalize the blood glucose level. The levels of total serum cholesterol, liver enzymes (ALP, ALT and AST), total serum lipids and blood glucose levels which were actually raised in alloxan diabetic rats are lowered after the administration of onions aqueous extracts. The hypoglycaemic and hypolipidaemic effects are thus protective mechanisms against the development of atherosclerosis, hyperlipidaemia and hyperglycaemia common in diabetes mellitus. This may provide a basis for dietary supplementation of onions compounds in diabetics to reduce over dependence on drug.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Rang HP, Dale MM, Moore JM, Ritter PK. The endocrine pancreas and the control of blood glucose, 5th ed, Livingston Publication, London. 1999;1:380-393.
2. American Diabetes Association. Total prevalence of diabetes and pre-diabetes. 2005;15.
3. Reaven E, Wright D, Mondon CE, Solomon R, Ho H, Reaven GM. Effect of age and diet on insulin secretion and insulin action in the rat. Diabetes. 1983;32:175-180.
4. Sanchez FD, Game MJ, Jimenez I, Zarzuelo A. Hypoglycaemic activity of Juniperus berries. Plant Medicine. 1994;60:197-200.
5. Brewster James L. Onions and other vegetable alliums (1st ed.). Wallingford, UK: CAB International. 1994:16.
   ISBN: 0-85198-753-2
6. Davidson T, Frey R. *Allium cepa*. Gale Encyclopedia of Alternative Medicine; 2005.
   Available:http://www.encyclopedia.com
   (Accessed 28 Jan. 2016)
7. Sigma-Aldrich Co. LLC; Plant profiler, Onion (*Allium cepa*); 2016.
8. World's Healthiest Foods; 2006.
   Available:http://www.whfoods.com/genpage.php?tname=foodspice&dbid=45
9. Ikram MA. A review on chemical and medicinal aspects of *Allium cepa*. Pak. J. Sci. Ind. Res. 1971;14(5):395-398.
10. Jain RC. Onion and garlic in experimental cholesterol induced atherosclerosis in rabbit I. Indian J. Med. Res. 1976;64(10): 1509-1515.
11. Mathew PT, Augusti KT. Hypoglycaemic effects of onion, *Allium cepa* linn on diabetes mellitus. A prelimnary report. Indian J. Physiol. Pharmacol. 1975;19(4): 212-217.
12. The World Health Organization Committee on diabetes mellitus: Second Report. Technical Report Series 646. World Health Organization, Geneva; 1980.
13. Azu NC, Onyeagba RA, Nworie O, Kalu J. Antibacterial activity of *Allium cepa* (Onions) and *Zingiber officinale* (Ginger) on *Staphylococcus aureus* and

Pseudomonas aeruginosa isolated from high vaginal swab. Internet J. Trop. Med. 2007;3(2):234-237.

14. Yakubu MT, Akanji MA, Oladeji AT. Aphrodisiac potentials of aqueous extract of *Fadogia agrestis* (Schweinf. Ex Heirn) stem in male albino rats. Asian J Androl. 2005;7(4):399-404.
15. Schmidt E, Schmidt FW. Determination of serum GOT and GPT activities. Enzyme Biology Chemistry. 1963;3:1-5.
16. Wright PJ, Leathwood AO, Plummer DT. Enzymes in rat urine: Alkaline phosphatase. Enzymologia. 1972;42:317-327.
17. Tietz NW. Fundermental of clinical chemistry. W.B. Saunders Company, London. 2000;1020-1038.
18. Veniamin MP, Varkirtzi LC. Chemical bases of the carbamidodiacetyl micro. Method for estimation of urea, citruline and carbamyl derivatives. Clin Chem. 1970;16: 3-6.
19. Szkudelski T. The mechanism of alloxan and streptozotocin action in beta cells of rat pancrease. Physiol. Res. 2001;50:536-546.
20. Yakubu MT, Akanji MA, Nafiu MO. Anti-diabetic activity of aqueous extract of *Cochlospermum planchonii* root in alloxan-induced diabetic rats. Camaroon Journal of Expeerimental Biology. 2010;6(2): 91-100.
21. Sheela C, Kumud K, Augusti K. Anti-diabetic effects of onion and garlic sulfoxide amino acids in rats. Planta Medica. 1995;61:356-357.
22. Pathak A, Dhawan D. Effects of lithium on the levels of blood urea and creatinine in diabetic rats. Med. Sci. Sports Exerc. 1988;36:807-813.
23. Almdal TP, Vilstrup H. Strict insulin treatment normalizes the organic nitrogen contents and the capacity of urea-N synthesis in experimental diabetes in rats. Diabetologica. 1988;31:114-118.
24. Uladimir OM. Coronary risk factor. J. Diabet. Assoc. India. 2003;29:3-8.
25. Blessing OI, Oloruntoba F, Olarewaju MO. Plasma glucose, creatinine and urea levels in type 2 diabetic patients attending A Nigerian Teaching Hospital. Research Journal of Medical Sciences. 2011;5(1): 1-3.
26. Laakso M. Lipids and lipoproteins as risk factors for coronary heart disease in non insulin dependent diabetes mellitus. Ann. Med. 1996;28:341-345.
27. Steiner DF, Ohagi S, Nagamatsu S, Bell GI, Nishi M. Is islet amyloid polypeptide a significant factor in pathogenesis or pathphysiology of diabetes. Diabetes. 1991;40(3):305-309.
28. Massing MW, Sueta CA, Chowdhury M, Biggs DP, Simpson RJ. Lipid management among coronary artery disease patients in diabetes mellitus or advanced age. Am. J. Cardiol. 2001;87:646-664.
29. Sharma SR, Dwivedi SK, Swarup D. Hypoglycaemic and hypolipidaemic effects of *Cinnamon tomala* nees leaves. Indian J. Exp. Biol. 1996;34:372-374.
30. Pushparaj P, Tan CH, Tan BK. Effect of *Averrhoa bilimli* leaf extract on blood glucose and lipids in streptozotocin diabetic rats. J. Ethnopharmacol. 2000; 72:69-76.
31. Orchard TJ. Dyslipoproteinemia and diabetes: Endocrinol. Metab. Clin. North Am. 1990;19:361-379.
32. Betteridge DJ. Diabetic dyslipidaemia. Am. J. Med. 1994;96:25-31.
33. Hardman JG, Limberd LE. Insulin, oral hypoglycaemic agents and the pharmacology of the endocrine pancreas. *In*: Goodman and Gilman s. The Pharmacological Basis of Therapeutics (10^{th} edition). McGraw Hill Company limited, U.S.A. 2001;1383-1399.
34. Blumenthal M. The complete German commission E monographs: Therapeutic guide to herbal medicines. Am. Botanical Assoc., Austin, USA; 1998.
35. Cho SK, Park JY, Park EM, Choi MS, Lee MY, Jeon SM, Jang MK, Kim MJ, Park YB. Alteration of hepatic antioxidant enzyme activities and lipid profile in streptozotocin induced diabetic rats by supplementation of dandelion water extracts. Clinical Chem. Acta. 2002;317: 109-117.
36. Larcan A, Lambert H, Laprevote-Heully MC, Delorme N. Light and electron microscopic study of hepatic lesions in the course of hyperlactatemia in diabetic patients. Diabetes Metabolism. 1979;5: 103-112.

37. Navarro CM, Montilla, PM, Martin A, Jimenez J, Utrilla PM. Free radicals scavenger and antihepatotoxic activity of Rosmarinus. Plant Medicine. 1993;59: 312-314.

38. Ohaeri OC. Effect of garlic oil on the levels of various enzymes in the serum and tissue of streptozotocin diabetic rats. Bioscience and Reproduction. 2001;21: 19-24.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/17260