

Effects of Ginger Solvent in Alloxan Induced Diabetic Nephropathy in Rats

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ABSTRACT

Aim: To appraise the effects of Ginger solvent extract on Renal corpuscle number and diameter in Alloxan induced diabetic nephropathy of albino rats.

Place of study: study was conducted Federal postgraduate Medical Institute of Shaikh Zayed hospital and National Health Research Complex Lahore

Methods: 45 adult male wistar albino rats having weight between 250-300g were indiscriminately chosen for the study. . Animals were housed in standard cages. They were allowed free access to water and standard diet under controlled conditions of temperature 25 ± 2 and normal photoperiod (12 hours dark and light) throughout the experiment. Intraperitoneal injection of Alloxan (150 mg/kg body weight) was used to induce diabetes mellitus in albino rats of investigational groups B & C.

Results: Number of renal corpuscles per mm² were more decreased and the diameter of renal corpuscle was more increased in investigational group B than group C. Group association between control and investigational groups B & C.

Conclusion: Ginger role was significant in renal diabetic nephropathy It is due to the antioxidants components counteraction by free radicals and it also helps in improvement of increased blood sugar levels in blood with the help of pancreatic and extra pancreatic mechanisms.

Keywords: Diabetes mellitus, Kidney, Diabetic nephropathy, Ginger, Alloxan

INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic disorders. Hyperglycemia is its common feature which can be due to relative insulin deficiency, resistance or both^{1,3}. Pancreatic beta cells are responsible for insulin production which regulates glucose metabolism⁴. Once beta cells are affected in type 1 diabetes the glucose metabolism gets disturbed resulting in hyperglycemia. Uncontrolled elevated blood glucose levels result in diabetic complications. Kidney is one of the organs that is affected as a complication of diabetes^{5,6,7}. Oxidative stress process in renal injury during raised glucose level in blood may be due to the secondary mediators like protein kinase C and cytokine⁸. A drug named Alloxan is used for the induction of diabetes in experimental animals. It is an analogue of glucose⁹. It damages the beta cells of pancreas and results in diabetes similar to type 1.^{10,11} Various routes of administration have been discussed in literature i.e., intravenous, intraperitoneal and subcutaneous¹². Ginger is from Zingiberaceae family¹³. It is cultured mostly in China, Nigeria, Indonesia, India, and Pakistan¹⁴. It is full of antioxidant property which protects body from oxidative stress.

In Ginger chemically active ingredients like Zingeron, Zingiberene, Zingiberol, Paradols Shogaols, Sesquiterpenes Gingerols, and Monoterpenes Neral, Terpineol¹⁵. The study was intended to estimate the effects of Ginger aqueous extract on the renal corpuscle number and diameter in Alloxan induced diabetic nephropathy of albino rats.

MATERIALS AND METHODS

Forty five adult male wistar albino rats having weight between 250-300g were indiscriminately chosen for the study. All Animals were housed in standard cages in the animal house of post graduate Medical Institute Lahore. They were allowed free access to water and standard diet under controlled conditions of temperature 25 ± 2 and normal photoperiod (12 hours dark and light) throughout the experiment¹⁰. Institutional Review Board of Federal postgraduate Medical Institute Lahore, Shaikh Zayed hospital, National Health Research Complex allowed for the research. All rats were fed the marketable brand of rats. All animals were acclimatized with their surroundings for a continuous period of seven days. The animals were alienated into three main groups including control Group A in which the rats received distilled water 20ml/kg body weight with the help of gavage. Second group is Alloxan induced Diabetic Group B in which intraperitoneally injection Alloxan 150 mg/kg body weight¹⁶ was given. In Third diabetic rats group and Ginger treated Group C was included. After confirmed diagnosis of diabetes, diabetic rats was given the ginger aqueous solution 200mg/kg body weight with the help of gavage given daily for a continuous period of 5 weeks which was started on the eighth day Alloxan injection. Ginger aqueous preparation was done in PCSIR, Laboratories Lahore and procedure is followed as, fresh raw and untreated Ginger was brought from local vegetable market from where crushed ice Ginger roots (500g) were peel off into little pieces. Homogenized material in 250 ml ice cold water with 750ml cold and sterile 0.9% Normal saline solution to prepare 1000 ml of total volume. Homogenization was achieved through Blender period of 12 minutes and cloth was used to pass through a filter for three times. Sample for was centrifuged at 2000rpm for duration of 10 minutes. After Supernatant fraction normal saline was used to put together and make its

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volume 1000ml. 500g weight of ginger initially and 500 mg/ml is the concentration of the arranged ginger extract. Extract was freeze at -20°C and desiccated in sample tubes uptill the rats were fed¹⁸.After the complete management of rats all the animals were sacrificed and Kidneys were dissected out for the histopathological results. After isolation to wash the kidneys ice cold saline was used and tissuesamples were preserved in 10% formaldehyde for 48 hours. With the help of rotary microtome and stained hematoxylin and eosin PAS 5 µm thick sections were for histopathological examination. Data was analysed with the help of SPSS Ver.22.0. Quantitative analysis and ANOVA was used for association between the groups. Post Hoc Tukey test was performed and < 0.05 P-value was considered as significant with the confidence level 95%.

RESULTS

Bar Chart-1 Renal corpuscle cells per mm² in control Group A and investigational Alloxan induced diabetic group B & Ginger treated group C

Chart -1 showed that With the 4 x magnification of ocular micrometer 1mm² for the histological sections of renal cells taken and the renal corpuscle cells mm² mean number in control group A was 5.47 ± 0.52 and that for investigational groups B and C were 3.53 ± 0.52 and 4.53 ± 0.52 respectively.

Table-1 showed the comparison between the Groups between control Group A and trial groups B & C Groups showed the significant results in which the p-value <0.001 was significant. Trial group B also had significant results with tentative group C Group which also showed the significant results in which p-values <0.001.

The diameter of renal corpuscle cells were measured with three different fields of microscope and its diameter was calculated with the help of ocular micrometer. Two different diameters were selected from which one was selected at the level of maximum transverse diameter and second point was at the midpoint of perpendicular area of first one. The two different Means of diameter was in use and resulting into its transversical diameter of each corpuscle and its average of transversical diameters of three different renal corpuscles cells were selected and calculated with each and every group.

The renal corpuscle diameter in control group A was 78 ± 8.6 µm and Alloxan induced Diabetic group B was 122 ± 8.6 and Ginger treated group C showed the values were 101 ± 9.2. All group association showed the significant results having p-values <0.001.

Table -2 showed the Group wise comparison showed control group A ,Alloxan induced diabetic group B and Ginger treated group C showed the significant results having p-value <0.001.

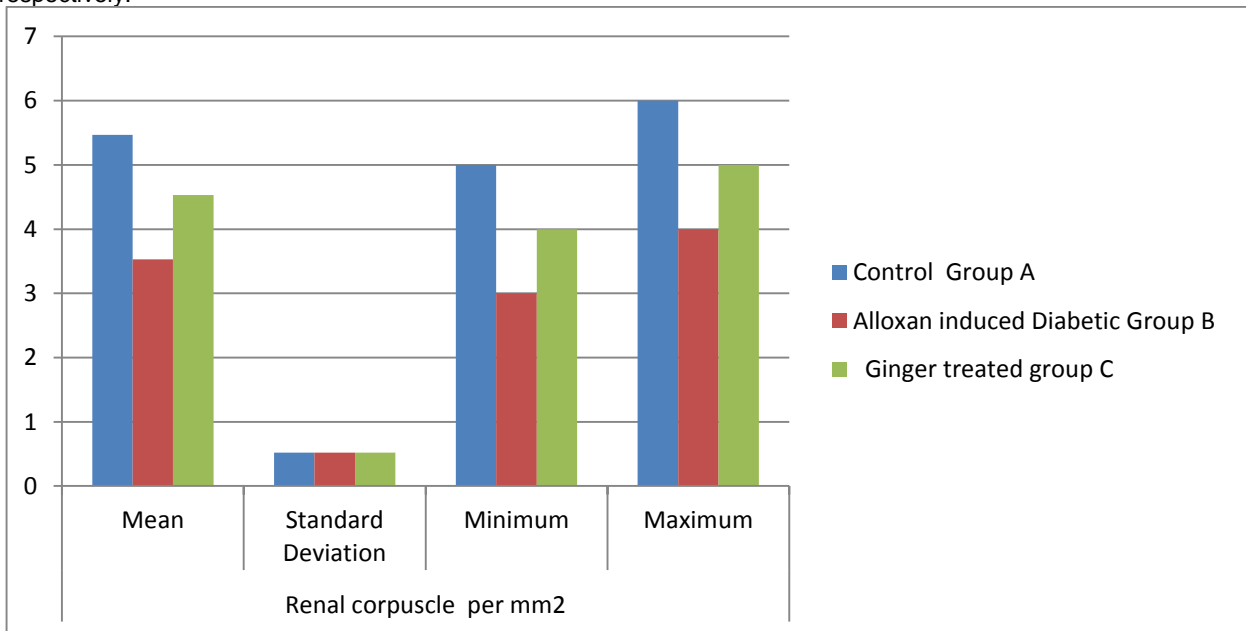


Table 1: Comparison of control Group A and investigational Alloxan induced diabetic group B & Ginger treated group C for number of renal corpuscle per mm²

| (I) Groups | (J) Groups | Mean Difference (I_J) | Std. Error | P-value |
|----------------------------------|----------------------------------|-----------------------|------------|-----------|
| Control Group A | Alloxan induced Diabetic Group B | 1.93* | 0.189 | < 0.001** |
| | Ginger treated group C | 0.93* | 0.189 | < 0.001** |
| Alloxan induced Diabetic Group B | Ginger treated group C | -1.00* | 0.189 | < 0.001** |

Bar Chart-2 Diameter of renal corpuscle in control Group A and investigational Alloxan induced diabetic group B & Ginger treated group C

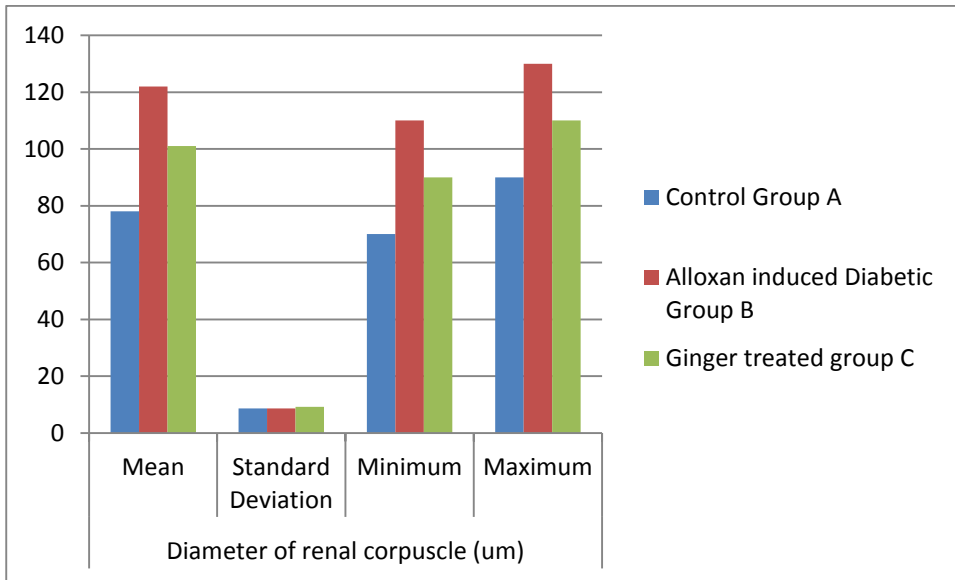


Table-2 Group wise comparison among control Group A and investigational Alloxan induced diabetic group B & Ginger treated group C for diameter of renal corpuscle

| (I) Groups | (J) Groups | Mean Difference (I_J) | Std. Error | P-value |
|----------------------------------|----------------------------------|-----------------------|------------|-----------------------|
| Control Group A | Alloxan induced Diabetic Group B | -44.00 [*] | 3.21 | < 0.001 ^{**} |
| | Ginger treated group C | -23.33 [*] | 3.21 | < 0.001 ^{**} |
| Alloxan induced Diabetic Group B | Ginger treated group C | 20.67 [*] | 3.21 | < 0.001 ^{**} |

DISCUSSION

Diabetes mellitus (DM) is a worldwide syndrome.1 About 346 million people are suffering from this.26. Many pathogenic processes cause destruction of pancreatic beta cells which results in decreased production of insulin. Diabetes mellitus is a syndrome with disordered metabolism and inappropriate hyperglycemia due to either a deficiency of insulin secretion or to a combination of insulin resistance and inadequate insulin secretion to compensate for the resistance. Etiological classification of diabetes has been suggested by American Diabetes association⁴ including Type-I and Type-II, Chemical or drug induced like steroids, types of DM including pancreatic diseases of exocrine nature like cystic fibrosis, pancreatitis, other types are endocrinopathy its example is Cushing syndrome and Gestational diabetes mellitus. Renal failure is one of the early complications of uncontrolled diabetes. Renal structural abnormalities in diabetic nephropathy include enlargement of glomerulus, atrophy of tubules and vascular hyalinosis²⁷. Researches have proved the various physiological mechanisms are responsible for the DM but oxidative stress may be the one of them.7 Oxidative stress can be prevented by use of antioxidants²⁸. Our Study revealed that number of renal corpuscles per mm² were more decreased in investigational Alloxan induced group B than Ginger treated group C and the diameter of renal corpuscle was more increased Alloxan induced group B than Ginger treated group C. Similar results were also found in different study

of M. Maeda et al²⁹. Kidney swelling is may be caused by definite factors like, lipogenesis, glycogen accumulation and protein synthesis in diabetic renal patients³⁰. Role of Ginger radically increases with the activities of enzymes like Succinate dehydrogenase, Glucose 6 Phosphatase Dehydrogenase and Glutamate dehydrogenase which mainly reduces the fatty infiltration among the kidneys.31 Ginger helps in improving the increased blood sugar level in blood with pancreatic and extra pancreatic mechanisms, which also reduces the oxidative stress and leading to reverses the effects and resultant in kidney hypertrophy^{14,18}. Ginger role in protecting the tissues from lipid per oxidation and helps to significantly decrease all lipid profile parameters. Its effects in Antiobesity may be partially because of inhibition of dietary fat of intestinal absorption³¹.

CONCLUSION

After getting the results of my study it is concluded that progression of diabetic nephropathy induced by Alloxan can be reduced with the treatment of Ginger aqueous extract in male Albino Rats. Histopathological results of ginger extract showed the significant effects. It is also concluded that ginger role was significant in renal diabetic nephropathy may be because of a antioxidants components counteraction by free radicals and it also helps in improvement of increased blood sugar levels in

blood with the help of pancreatic and extra pancreatic mechanisms.

REFERENCES

- 1- Teoh SL, AbdLatiff A, Das S. Histological changes in the Kidneys of experimental diabetic rats fed with Momordicacharantia (bitter gourd) Extract. Rom J MorpholEmbryol.2010;51(1):91-5.
- 2- Kaveeshwar SA, Cornwall J. The current state of diabetes mellitus in India. Australas.Med.J.2014;7(1):45-48.
- 3- Mahar SA, Shahid M. Diabetes Care in Pakistan- A Real Challenge. J.Liaquat.Univ.Med.Health.Sci.2014;13(1).
- 4- American Diabetes Association: Diagnosis and classification of diabetes mellitus. Diabetes care 2006;29Suppl 1:S43-8.
- 5- Fowler MJ. Microvascular and Macrovascular Complications of Diabetes.Clin.Diabetes.2008;26(2):77-82.
- 6- AlsaadKo, Herzenberg AM. Distinguishing diabetic nephropathy from other causes of glomerulosclerosis: an update. J.Clin.Pathol.2007;60:18-26.
- 7- Afshari AT, Shirpoor A, Farshid A, Saadatian R, Rasmi Y, Saboory E, Ilkhnizadeh B, Allameh A. The effect of ginger on diabetic nephropathy, plasma antioxidant capacity and lipid peroxidation in rats. J. Food Chemistry.(2007);101:148-153.
- 8- Cooper ME. Interaction of metabolic and homodynamic factors in mediating experimental diabetic nephropathy. Diabetologia2001;44:1957-72.
- 9- Rohilla A, Ali S. Alloxan Induced Diabetes: Mechanisms and Effects. Int.J.Res.Pharm.Biomed.Sci.2012;3(2):819-22.
- 10- Jafri SA, Abass S, Qasim M. Hypoglycemic effect of ginger (Zingiberofficinale) in Alloxan induced diabetic rats. Pak Vet J 2011; 31(2): 160-62.
- 11- Lenzen S. The mechanism of alloxan- and streptozotocin-induced diabetes. Diabetologia, 51: 236-237.
- 12- Chougale AD, Panaskar SN, Gurao Pm, Arvindekar AU. Optimization of Alloxan dose is essential to induce stable diabetes for prolonged period. Asian J Biochem 2007; 2(6): 402-408.
- 13- Sakr SA. Ameliorative effect of ginger (zingiberOfficinale) on Mancozeb fungicide induced liver injury in albino rats. Aust J Basic ApplSci 2007; 1(4): 650-656.
- 14- Kaejaiye OF, Iwalewa EO, Omobuwajo OR, Oyedapo OO. Hypoglycemic effects of Nigerian ZingiberOfficinale Rhizome on experimental diabetic rats. Nig J Nat Prod and Med 2002; 06: 33-35.
- 15- Butt MS, Sultan MT. Ginger and its health claims: Molecular aspects. Crit Rev Food SciNutr 2011; 51: 383-393.
- 16- El-Kott AF, El-sayad SM, Abdel-Aziz AM. The effects of ginger (zingiberofficinale) on histology and immunohistochemistry of liver and kidney and certain haematological parameters in alloxan induced diabetic rats. Egypt. J.Exp. Biol. (zool).2010;6(1):61-70.
- 17- J Shah, M Patel, K Patel, T Gandhi. Evaluation of Anti-diabetic and Anti-oxidant Activity of Centratherumanthelmintica in STZ – induced Diabetic Rats. Internet.J. Pharmacol 2007; 6(1):16.
- 18- Ramudu SK, Korivi M, Kesireddy N. Lee LC, Cheng IS, Kuo CH, Kesireddy SR. Nephroprotective effects of a ginger extract on cytosolic and mitochondrial enzymes against streptozocin (STZ) induced diabetic complications in rats. Chin J Physiol 2011; 54(2): 79-86.
- 19- Al-kushi AG. Biochemical and Ultrastructure Changes in the Kidney of Streptozotocin-induced Diabetic Rat. Pak.J.nutr.2013; 12(4):313-321.
- 20- A Compendium of Drugs Used for Laboratory Animal Anesthesia, Analgesia, Tranquilization and Restraint. [Cited 2012 Aug 29]. Available from: URL; http://www.lebl.ucr.ac.cr/files/Anestesia/IACUC_drugs.pdf
- 21- Euthanasia Guidelines. [Cited 2012 Aug 30]. Available from: URL;<http://www.ahc.umn.edu/rar/euthanasia.html>
- 22- Gamble M. The Hematoxylins and Eosin. In Theory and Practice of Histological Techniques, 6th, Churchill Livingstone Elsevier, Philadelphia, PA, USA. 2008; 121-134.
- 23- Totty BA. Mucins. In: Bancroft JD, Gamble M, editors. Theory and practice of histologic techniques. 5th ed. London: Churchill Livingstone; 2002:175.
- 24- BanikS.Akhtar F. Diameter of renal corpuscles of Bangladeshi people in different age groups. J. Enam.Med.Col.2014;4(1):36-38.
- 25- Zaman UKS, Khalil M, Rehman MM et al. Histological changes of human kidney with age in Bangladeshi people. Bangladesh Med.J.2011;40(1):13-17.
- 26- Amritanshu K, Kumar A, Anand K, Garg N, Banerjee DP. Clinical profile and factors associated with microalbuminuria in type 1 diabetes mellitus in children and adolescents. Int. J.Res.Med.Sci. 2015;3(5):1247-1251
- 27- Fioretto P, Mauer M. Histopathology of Diabetic Nephropathy. Semin Nephrol.2007;27(2):195-207.
- 28- Evans JL, Goldfine ID, Maddux BA, Grodsky GM. Oxidative stress and stress- activated signaling pathways: a unifying hypothesis of type 2 diabetes. Endocr Rev. 2002;23:599-622.
- 29- Maeda M, Yabuki A, Suzuki S, Matsumoto M, Taniguchi K, Nishinakagawa H. Renal Lesions in Spontaneous Insulin-dependent Diabetes Mellitus in the Nonobese Diabetic Mouse: Acute Phase of Diabetes. Vet. Pathol.2003;40:187-195
- 30- Pourghasem M, Nasiri E, Shafi H. Early Renal Histological Changes in Alloxan- Induced Diabetic Rats. Int. J. Mol. Cell. Med. Winter. 2014; 3(1): 11-15
- 31- Li Y, Tran VH, Duke CC, Roufogalis BD. Preventive and Protective Properties of Zingiberofficinale (Ginger) in Diabetes Mellitus, Diabetic Complications, and associated Lipid and Other Metabolic Disorders: A Brief Review. Evidence-Based Complementary Alter. Med.2012;Article ID 516870: 10 pages.