COMPARATIVE EVALUATION OF THE EFFICACY OF SOME AYURVEDIC FORMULATIONS IN ATTENUATING THE PROGRESSION OF DIABETIC NEPHROPATHY

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Summary

The present study investigated the effects of the two ayurvedic formulations Diabecon and Madhumehari in long term renal complication of diabetes. The study was conducted on streptozotocin induced diabetic rats. The results indicated the beneficial effects of both the formulations in preventing the progress of kidney damage due to diabetes.

Keywords: Diabecon, Madhumehari, diabetic nephropathy.

Introduction

Despite the great strides that have been made in the management of diabetes, microvascular complications like diabetic nephropathy continue to confront the patients. Oral hypoglycaemic agents and insulin is the mainstay of treatment of diabetes but fail to alter the course of diabetic complications. An alternative strategy can be the use of natural agents or complementary medicine possessing hypoglycaemic effect. These ayurvedic formulations can be used to assess their efficacy in the prevention of long term complications of diabetes. They also offer advantage in terms of better patient compliance and safety.

Materials and Methods

Ayurvedic Formulations: Diabecon was obtained as a gift sample from Himalaya Drug Company, Bangalore and Madhumehari from Shree Baidyanath Ayurved Bhawan Pvt. Ltd., Kolkata.

Animals

Male inbred albino rats weighing between 150-180 g were procured from Animal House of Birla Institute of Technology, Mesra, Ranchi and were housed in polypropylene cages with one animal in each cage. They were kept under controlled environmental condition of 25 ± 2 ^{o} C and 45-55% relative humidity with natural light / dark cycle and allowed free access to food (standard pellet diet, Hindustan Lever Ltd., India) and water and acclimatized for at least a week before the commencement of the experiment (Reg no.621 / 02 / ac / CPCSEA). All experiments were performed subject to prior approval of the Institutional Animal Ethics committee.

Treatment protocol

Overnight fasted animals were rendered diabetic by a single i.p. injection of 40 mg/kg body weight Streptozotocin freshly prepared in 0.1 M citrate buffer (pH 4.5). The STZ injected animals were then given 5% w/v glucose solution for 5-6 hours following the injection to prevent initial drug induced hypoglycaemic mortality[1]. After 72 hours of STZ injection blood was drawn from the tail vein of rats and fasting blood glucose was estimated by a calibrated glucometer [SD Check Gold, Standard Diagnostics]. The animals having fasting blood sugar above 200mg/dl were included in the study[2]. Treatment was started 7 days after the induction of diabetes. The biochemical and pharmacological experiments were carried out from the 0th day (7th day after diabetes induction) till the 40th day of the experiment.

Drugs were given everyday for a period of 40 days by the oral route by oral gavage using a 5ml syringe. The animals were divided into the following groups with 6 rats in each group:

Group –I: Normal control rats which were kept untreated.
Group –II: Diabetic animals received citrate buffer for the entire period of treatment and served as the diabetic control group.
Group-III: Diabetic animals which received Diabecon at a dose of 1g/kg b.w. by the oral route.
Group-IV: Diabetic animals received Madhumehari at a dose of 500 mg/kg b.w. by the oral route.

Assessment of biochemical parameters

Sample collection: All blood samples were collected under the fasting condition. Urine samples were collected by keeping the animals in metabolic cages for 24 hours.

1. **Estimation of blood and urine chemistries :** The following parameters were estimated :

   a. **Fasting blood glucose levels:** Fasting blood glucose levels were estimated by using a glucometer (SD check gold, Standard diagnostics) from 0th to 40th day of the experiment[3].

   b. **Estimation of serum creatinine levels:** The serum creatinine values were estimated using commercially available kit (Crest Biosystems, Goa) based on modified Jaffe's kinetic method[4, 5].

c. **Estimation of urine creatinine and creatinine clearance:** The urine creatinine and creatinine clearance values were estimated using commercially available kit based on modified Jaffe's kinetic method (Crest Biosystems, Goa)[4,5] .

d. **Estimation of total protein in urine:** The total protein content of urine was estimated by the biuret method[6].

e. **Estimation of albumin level in urine:** The level of albumin was estimated using commercially available kit (Siemens Medical Solutions Diagnostics Ltd.) based on the BCG (Bromo Cresol Green) method[7].

f. **Body weight and urine volume:** The weight of the animals and urine volume was taken on each 10^{th} day for 40 days[8].

3. **Renal hypertrophy and glycosylated haemoglobin:** On the 40^{th} day, animals were sacrificed and the degree of renal hypertrophy was expressed as the ratio of the weight of the two kidneys to total body weight[9]. Blood was collected from the heart and was used for the estimation of glycosylated haemoglobin (HbA_1c) by a modified colorimetric method of Fluckiger and Winterhalter[10].

4. **Renal histopathology:** Biochemical findings for kidney damage were further substantiated by histopathological studies of the kidneys using Haematoxylin &Eosin method[11].

Results and Discussion

Diabetic nephropathy is one of the serious complications of diabetes mellitus and also its main cause of mortality. Large amounts of data have shown that early intervention could effectively alleviate or retard the evolvement to late stage nephropathy [12]. The basic pathologic change is kidney hemodynamic disturbances and glomerulosclerosis. The early characteristics of Diabetic Nephropathy are glomerulus hyper filtration and urinary trace albumin. Effective drug intervention at this time could reverse the lesion, and therefore, it is very important to lay emphasis on early treatment of Diabetic Nephropathy. Streptozotocin induced diabetes in rodents results in the development of nephropathy similar to early stage clinical diabetic nephropathy. It has also been reported that streptozotocin has no long term effects on the kidney other than those mediated by diabetes mellitus [13] .

The Diabetic Control and Complications Trial (DCCT) was a major clinical study conducted from 1983 to 1993 which showed that intensive control of blood glucose slows the progression of retinopathy, nephropathy in patients with diabetes mellitus [14]. In the present study Diabecon and Madhumehari was found to significantly lower the blood glucose levels in treatment group as compared to the diabetic control rats (Fig.1). This was found to be in accordance with the earlier reports on hypoglycaemic effects of Diabecon and Madhumehari[15,16].

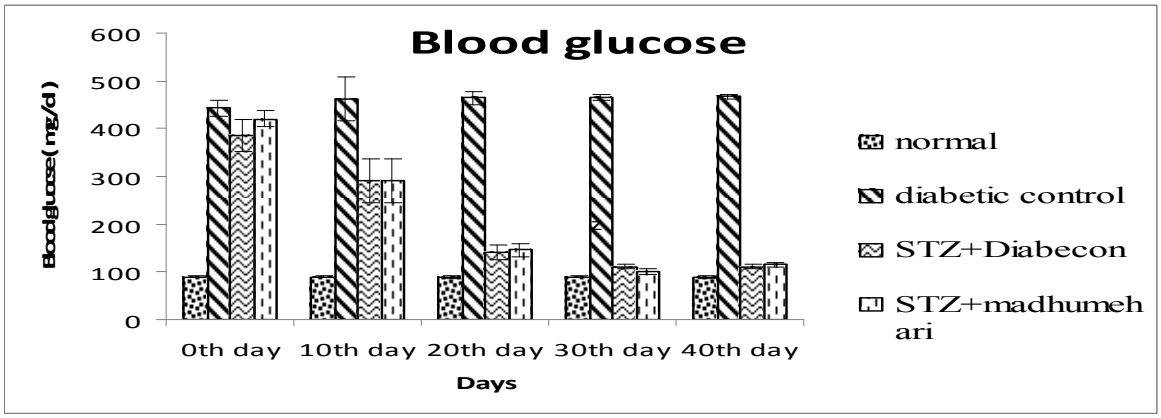

Fig.1: Effect of 40 days treatment with selected doses of the two ayurvedic formulations Diabecon and Madhumehari on blood glucose level (mg / dl) in STZ induced diabetic rats.

Diabecon and Madhumehari were also found to significantly reduce the kidney damage which was evident from the various biochemical parameters studied to assess kidney function. There was a significant increase in creatinine levels in the diabetic control group as compared to normal rats. Treatment with both Diabecon and Madhumehari prevented the increase in serum creatinine levels as diabetes progressed (Fig.2).

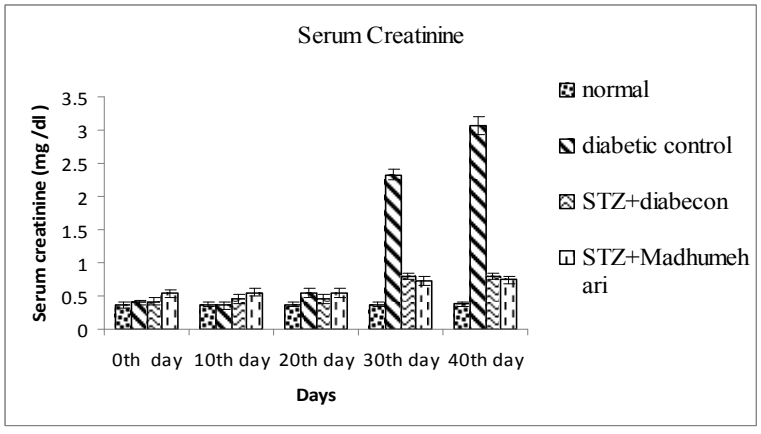

Fig.2: Effect of 40 days treatment with Diabecon and Madhumehari on serum creatinine (mg/dl) in STZ induced diabetic rats.

The creatinine clearance rate is an indicator of glomerular filtration rate which declines relentlessly in diabetic nephropathy[17]. There was a significant decrease in the creatinine clearance value in the diabetic control rats as compared to the normal rats which in turn is indicative of the decrease in the glomerular filtration rate. Treatment with the two formulations

showed an increase in the creatinine clearance values as compared to the diabetic control rats (Fig.3).

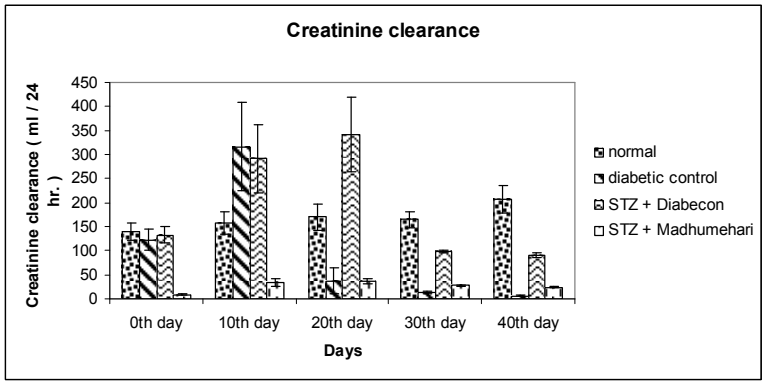

Fig.3: Effect of 40 days treatment with Diabecon and Madhumehari on creatinine clearance (ml/24 hr.) in STZ induced diabetic rats.

Aggressive metabolic intervention made at the onset of microalbuminuria will postpone and may prevent the development of End Stage Renal Disease (ESRD) in both type1 and type2 diabetic patients [18]. When the kidneys are working well, the tiny filters in the kidneys, the glomeruli, keep protein inside the body. High blood glucose damages the filters in the kidney and the protein leaks out of the kidneys into the urine. It was seen in the present study that the total protein excretion significantly increased in diabetic control rats. Treatment with Diabecon and Madhumehari was beneficial in decreasing the excretion of total protein in urine suggesting the reversal of the pathological state (Fig.4).

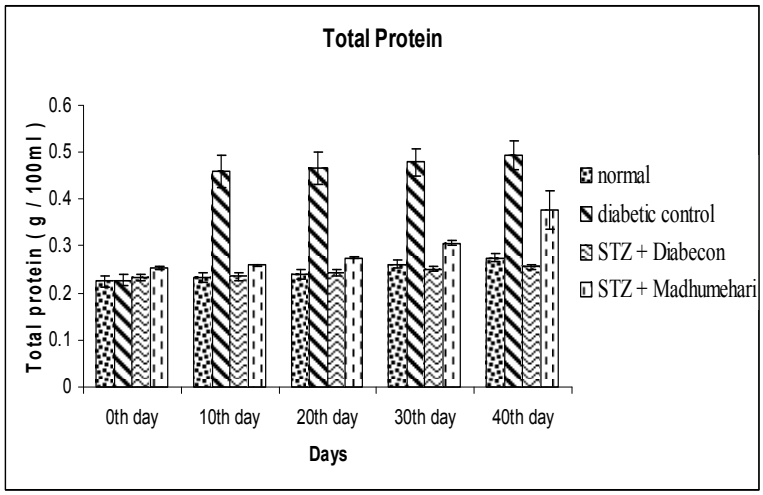

Fig.4: Effect of 40 days treatment with Diabecon and Madhumehari on total protein in urine (g/100ml) in STZ induced diabetic rats.

Urinary albumin excretion levels are a marker of glomerular injury and considered a harbinger of progressive nephropathy [19, 20]. The urine albumin excretion in the diabetic control rats in the present study were found to be markedly elevated in the later stages of the study which shows that there was renal dysfunction in the untreated diabetic rats . The normal rats also showed some albuminuria but it was far less as compared to the diabetic control rats. Treatment with Diabecon and Madhumehari prevented the increase in the excretion of albumin in urine

(Fig.5) from 10th day in Diabecon and 20th day in case of Madhumehari which shows that both the formulations were very efficacious in preventing the renal complications.

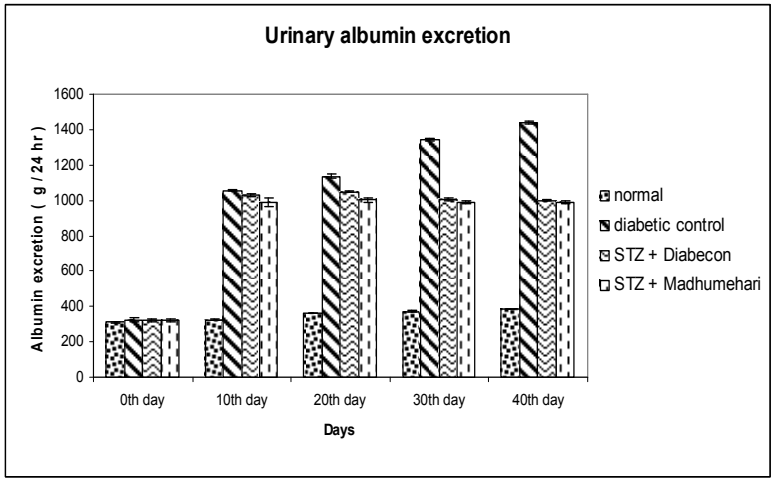

Fig.5: Effect of 40 days treatment with Diabecon and Madhumehari on urinary albumin excretion in STZ induced diabetic rats.

Treatment with the formulations Diabecon and Madhumehari did not produce any significant difference with respect to the body weight changes (Fig.6) suggesting that probably these formulations do not have much effect on the catabolic changes during diabetes.

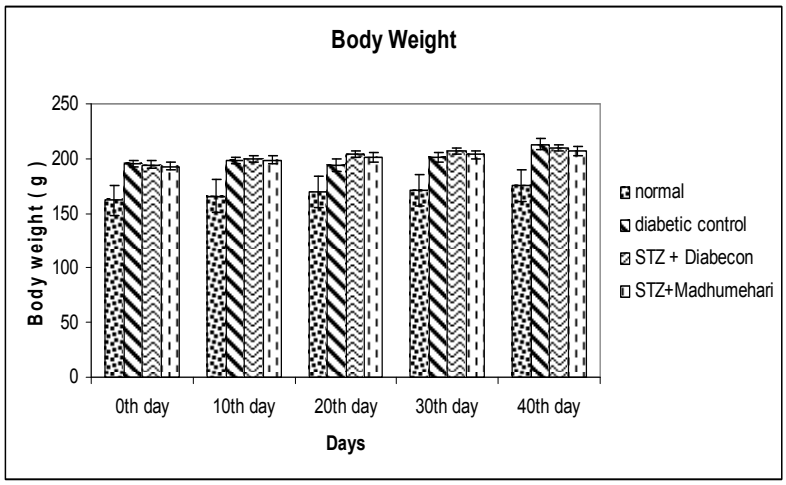

Fig.6: Effect of 40 days treatment with Diabecon and Madhumehari on body weight (g) in STZ induced diabetic rats.

Polyuria is a characteristic symptom of diabetes. Twenty-four hour urine volume was significantly increased in diabetic control group with respect to the normal control. Treatment with Diabecon and Madhumehari showed a significant decrease in the urine volume with respect to the diabetic control group (Fig.7).

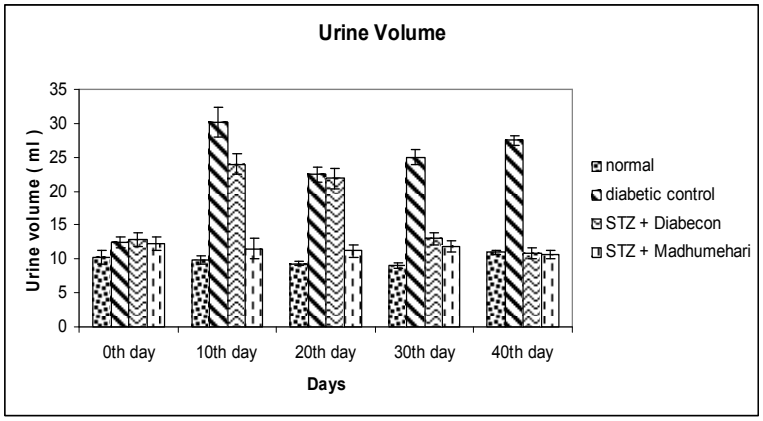

Fig.7: Effect of 40 days treatment with Diabecon and Madhumehari on urine volume (ml) in STZ induced diabetic rats.

The average net weight of both kidneys of diabetic control group was significantly higher than normal controls. Treatment with both the formulations showed prevention of hypertrophy significantly (Fig.8).

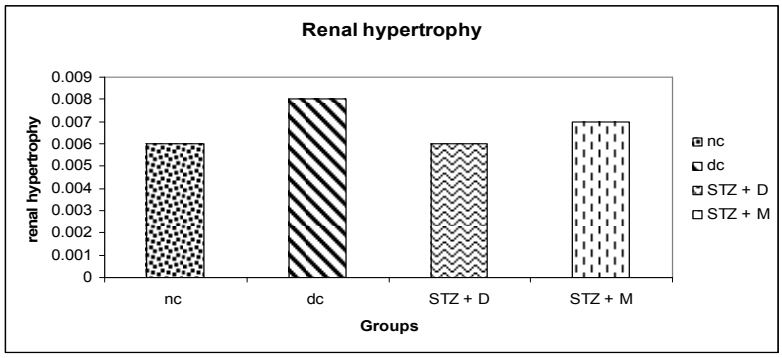

Fig.8: Effect of 40 days treatment with Diabecon and Madhumehari on renal hypertrophy in STZ induced diabetic rats.

Glucose combines with many proteins in circulation and in tissues via a nonenzymatic, irreversible process to form advanced glycosylation end products (AGEs). The best known of these is glycosylated hemoglobin. Any reduction in HbA_{1c} is likely to reduce the risk of complications, lowest risk being in those with HbA_{1c} values in the normal range (< 6.0 %) [21]. Glycosylated haemoglobin level in diabetic control rats was found to be significantly higher as compared to normal controls. Treatment with Diabecon and Madhumehari significantly decreased the level of glycosylated haemoglobin indicating good glycemic control and reduced risk of complications (Fig.9).

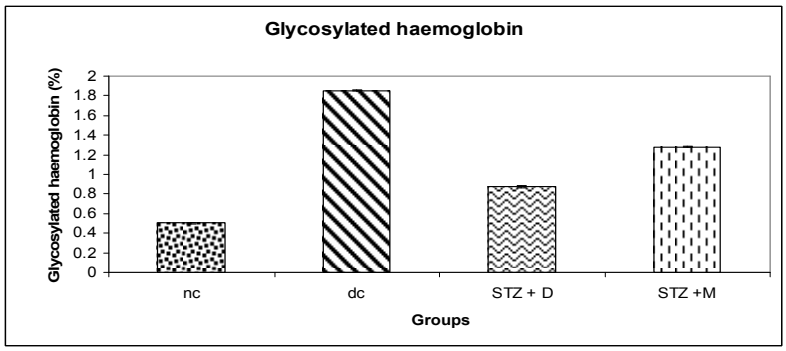

Fig.9: Effect of 40 days treatment with Diabecon and Madhumehari on glycosylated haemoglobin (%) in STZ induced diabetic rats.

The histopathological changes in diabetic rats showed diabetes induced enlargement of glomeruli, dilatation of subcapsular space and presence of maculae densae. Similar findings were reported earlier [22]. The diabetes-induced enlargement of many glomeruli, capillary tufts and of the subcapsular urinary space was found to be less expressed in the Diabecon and Madhumehari treated diabetic groups in this study.

Hyperglycemia may be toxic either by non – enzymatic reaction of glucose with proteins and subsequent formation of advanced glycosylation end products or by increased metabolism leading to increased oxidative stress.

The results of the present study can be explained on the basis of glycemic theory as treatment with Diabecon and Madhumehari was able to achieve a euglycemic state and so halted the progression of diabetic nephropathy.

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References

1. Frode, T. S., Medeiros, Y. S., 2008. Animal models to test drugs with potential antidiabetic activity. Journal of Ethnopharmacology.115, 173-183.
2. Mehranjani, M.S., Shariatzadeh, M.A., Desfulian, A.R., Noori, M., Abnosi, M.H., Moghadam, Z.H., 2007. Effect of *Medicago sativa* on nephropathy in diabetic rats. Indian Journal of Pharmaceutical Sciences. 69,768-772.
3. Grover, J. K., Vats, V., Rathi, S. S., Dawar, R., 2001. Traditional Indian anti-diabetic plants attenuate progression of renal damage in streptozotocin induced diabetic mice. Journal of Ethnopharmacology. 76, 233-238.
4. Sharma, S., Anjaneyulu, M., Kulkarni, S. K., Chopra, K., 2006. Resveratrol, a polyphenolic phytoalexin, attenuates diabetic nephropathy in rats. Pharmacology. 76, 69-75.
5. Jaffe, M., 1886. The colorimetric determination of creatinine by the Jaffe procedure. Journal of Biological Chemistry. 158, 581-91.
6. Sperlingova, I., Dabrowsky, L., Tichy, M., Kucera, J., 1998. Reference material total protein in human urine. Fresenius Journal of Analytical Chemistry. 361, 756-60.
7. Bengershom, E., 1975. Screening for Albuminuria: A case for estimation of albumin in urine. Clinical Chemistry. 21(2), 1795-98.

8. Grover, J. K., Yadav, S. P., Vats, V., 2003. Effect of feeding *Murraya koeingii* and *Brassica juncea* diet on kidney function in streptozotocin diabetic mice. Journal of Ethnopharmacology. 85, 1-5.

9. Grover, J. K., Vats, V., Rathi, S. S., Dawar, R., 2001. Traditional Indian anti-diabetic plants attenuate progression of renal damage in streptozotocin induced diabetic mice. Journal of Ethnopharmacology. 76, 233-38.

10. Kareem, I., Jaweed, S. A., Bardapurkar, J. S., Patil, V. P., 2004. Study of magnesium, glycosylated haemoglobin and lipid profile in diabetic retinopathy. Indian Journal of Clinical Biochemistry. 19(2), 124-27.

11. Pattanayak, S., Mazumder, P. M., 2008. Histopathological approach to rat liver tissue. Protocol online. (http:// www.protocol.online.org).

12. Jie, L. U., Hai, H. E., 2005. Clinical observation of *Gingko biloba* extracts injection in treating early diabetic nephropathy. Chinese Journal of Integrated Medicine. 11 (3), 226 -228.

13. Yokogawa, T., Nakagawa, T., Wakaki, K., Koizumi, F., 2001. Animal model of diabetic nephropathy. Experiments in Toxicology and Pathology. 53, 359 – 363.

14. The Diabetes Control and Complications Trial Research Group. 1993. The effect of intensive treatment of diabetes on the development and progression of long term complications in Insulin – Dependent Diabetes mellitus. New England Journal of Medicine. 392, 977 – 86.

15. Mitra, S. K., Gopumadhavan, S., Muralidhar, T. S., Anturlikar, S. D., Sujatha, M. D., 1996. Effect of a herbomineral preparation D- 400 in streptozotocin-induced diabetic rats. Journal of Ethnopharmacology. 54:41- 46.

16. Ahmed, F., Sairam, S., Urooj, A., 2009. Effect of various Ayurvedic formulations and medicinal plants on carbohydrate hydrolyzing enzymes and glucose uptake by yeast cells-an *in vitro* study. Journal of Pharmacy Research. 2 (3), 563-568.

17. Mogensen, C.E., 2000. Diabetic nephropathy: evidence for renoprotection and practice. Heart. 84, i26-i28.

18. Campbell, R. C., Ruggenenti, P., Remuzzi, G., 2003. Proteinuria in diabetic nephropathy: treatment and evolution. Current Diabetes Reports. 3, 497 – 504.

19. Viswanathan, V., Snehalatha, C., Kumutha, R., Jayaraman, M., Ramchandran, A., 2004. Serum albumin levels in different stages of type 2 diabetic nephropathy patients. Indian Journal of Nephrology. 14, 89 – 92.

20. Deckert, T., Rasmussen, B. F., Johnson, K. B., Jensen, T., Enevoldsen, A. K. , 1989. Albuminuria reflects widespread vascular damage. Diabetologia. 32 (4), 219 – 226.

21. Stratton, M. I ., Adler, A. I., Neil, H. A. W., Matthews, D. R., Manley, S. E., Cull , C. A., Hadden, D., Turner, R. C., Holman, R. R., 2000. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. British Medical Journal. 321, 405 – 412.

22. Welt, K., Weiss, J., Martin, R., Hermsdorf, T., Drews, S., Fitzl, G., 2007. *Ginkgo biloba* extract protects rat kidney from diabetic and hypoxic damage. Phytomedicine. 14, 196 – 203.