ORIGINAL ARTICLE

The Reno-Protective Effect of Ethanolic Extract of Cassia Fistula (Amaltas) Leaves on Streptozotocin Induced Diabetic Nephropathy in Rats

Farhana Adnan, Soban Sadiq, Adnan Jehangir

ABSTRACT

Objective: To determine the Reno-protective effect of ethanolic extract of Cassia fistula (Amaltas) leaves on streptozotocin induced diabetic nephropathy in rats.

Study Design: An animal experimental study

Place of Study: The study was done in Biochemistry department, Islamic International Medical College,Rawalpindi and NIH.Islamabad.

Materials and Methods: Single injection of STZ was given intraperitoneally to rats and those rats that showed fasting blood glucose level over 280mg/dl were included in the study. After induction of diabetes all rats were divided into, normal control group (A), diabetes positive control group (B), and the two groups (C and D) served as experimental groups while group E served as standard as it received glibenclamide. Group C and D diabetic experimental rats received ethanolic extract of Cassia fistula leaves at 400 mg/kg and 500mg/kg of body weight orally for eight weeks on daily basis. On the other hand group E rats received glibenclamide at 0.5 mg/kg body weight orally for eight weeks. Blood samples were collected after eight weeks to find reno-protection against STZ induced diabetic nephropathy.

Results: The diabetic positive group rats showed variable increase in levels of serum glucose, serum urea, serum creatinine, total urinary protein and microalbuminuric levels. Body weight decreased and urine volume increased in diabetic groups. Cassia fistula ethanolic extract of 400mg/kg and 500mg/kg body weight dose and glibenclamide significantly decreased the levels of these parameters in rats. On comparison Cassia fistula ethanolic extract of 500mg/kg dose reduced levels of biochemical parameters more effectively than the 400mg/kg dose of Cassia fistula and glibenclamide. Cassia fistula constituents, especially polyphenols and flavonoids have strong anti-oxidant activity which might be involved in reno-protection.

Conclusion: Cassia fistula ethanolic leaf extract showed reno-protection against STZ induced diabetic nephropathy in rats.

Key words: *Reno-protection, streptozotocin , Cassia fistula, diabetic nephropathy, oxidative stress*

Introduction

Diabetes mellitus (DM) is a syndrome characterized by chronic hyperglycaemia and relative insulin deficiency, resistance or both.[1] Diabetes mellitus is not a single disease but basically a group of disorder which is characterized by hyperglycemia, hyperlipidemia, glycosuria, ketonemia and if prolonged leads to diabetic complications such as nephropathy, neuropathy and retinopathy.[2] Nephropathy is one of the important microangiopathic complications of diabetes mellitus.[3]

Diabetic nephropathy is the leading cause of end stage renal diseases (ESRD) in western societies and accounts for 30 to 35% of patients on renal replacement therapy in North America. Type 1 and type 2 DM affect approximately 0.5 and 4% of the population, respectively.

Nephropathy complicates 30% of cases of type 1 DM and approximately 20% of cases of type 2 DM. However most diabetic patients with ESRD have type 2 DM because of the greater prevalence of type 2 DM worldwide.[4] The level of hyperglycaemia seems to be quantitatively linked to risk of developing renal lesions. Hyperglycaemia

---

Correspondence:
Dr. Farhana Adnan
Assistant Professor
Department of Biochemistry
IIMCT Rawalpindi

enhances the non-enzymatic glycosylation of proteins; hence advanced glycosylation end-products (AGE) are formed. Increased serum levels of AGE seem to predict changes in kidney morphology such as expansion of mesangial cell matrix and glomerular basement membrane thickening.[5] The activation of hyperglycaemia-induced secondary mediators, such as protein kinase C and mitogen-activated protein kinase, and cytokine production are also responsible for oxidative stress induced renal injury in the diabetic condition.[6]

Various experimental animals have been utilized to investigate diabetic nephropathy, however STZ induced hyperglycaemic rats have been used in this model of diabetic nephropathy. STZ is synthesized by streptomycetes achromogenes and is used to induce both type 1 and type 2 DM.[7]

Cassia fistula belonging to the family Leguminosae Casesalpinaceae is commonly called as Amaltas, an Indian Labernum and is native to India, Sri Lanka and is extensively diffused in various countries.[8]

Its main property being that of a mild laxative suitable for children and pregnant women. It has been used to treat many intestinal disorders like ulcers.[9-10] The plant has a high therapeutic value and it exerts antipyretic and analgesic effects.[11] Cassia fistula leaf extract is also used for its anti-tussive and wound healing properties. [12-13] It has been concluded that plant parts could be used as a therapeutic agent in the treatment of hypercholesterolemia partially due to their fibre and mucilage content.[14] It has been reported to possess antitumor[15] ,hepatoprotective,[16] antifertility and antioxidant properties.[17] The Cassia fistula leaves are known to be an important source of secondary metabolites, notably phenolic compounds.

The purpose of the present experimental model was to observe the effect of Cassia fistula on streptozotocin induced diabetic nephropathy when given together to wistar rats.

Material and Methods

The study was done in Biochemistry department, Islamic International Medical College,Rawalpindi and NIH.Islamabad.

Animals:

The present study was carried out on 50 male wistar rats, each weighing 200 250g and were divided into 5 groups (10 each). Each group was kept in a separate cage, in the same room and under similar physiological conditions in animal house of National Institute of Health (NIH) Islamabad.

Initially all groups were fed on normal rat chow (consisting of wed, starch, choline, methionine, vitamin, mineral mixture and fat) and water for a period of one week for acclimatization before starting the experiment The care and handling of rats were in accordance with the internationally accepted standard guidelines for use of experimental animals.

Chemicals/Instruments:

Commercially available kits (Randox) for biochemical analysis of glucose, urea, creatinine, total urinary protein, microalbuminuria, 95% ethanol, pre-coated TLC(Thin Layer Chromatography) plate silica gel GF254 and toluene. The standard compounds used are ellagic acid, gallic acid and protocatechuic acid. The instruments used were soxhlet and rotary evaporator, centrifuge (Germany), clinical chemistry

analyzer and TLC scanner III (Camag, Switzerland) with win CATS software.

Plant materials and preparation of the extract

Leaves of Cassia fistula were collected from Jinnah Park, Rawalpindi and authenticated from a botanist. Ethanolic leaf extract (ELE) was prepared from Cassia fistula. Freshly collected leaves were washed in tap water and then distilled water and were shade dried for about 1 week. The dried leaves were crushed into a coarse powder. One hundred gram of the powder was soaked in 1 liter of ethanol for 30 days with occasional shaking. After 30 days this ELE was filtered and evaporated to dryness over a water bath at 60°C [18].The yield of ethanolic leaf extract was 19-20%.This (ELE) of Cassia fistula was got standardized from NIH, Islamabad.

Standardization of plant extract:

TLC (Thin Layer Chromatography) was used for standardization. TLC was performed on a pre-coated TLC plate silica gel GF254. Sample was applied on the plate as 8 mm wide bands with an automatic TLC sampler. The development was carried out in trough chamber (20 cm × 10 cm),which was pre-saturated with mobile phase(solvent system, toluene-ethyl acetate-formic acid-methanol (30:30:8:2), for 20 min at room temperature (25 ± 2°Cand 40% relative humidity). Subsequent to the development, TLC plates were dried under stream of hot air and then subjected to densitometric scanning using a TLC scanner III (Camag, Switzerland) with win CATS software (version 1.4.1) in the absorbance-reflectance scan mode. Quantitative evaluation of the plate was performed in absorption-reflection mode at 338 nm. The standard compounds used are ellagic acid, gallic acid and protocatechuic acid.

Experimental Procedure

After acclimatization, 10 rats were labeled as control. All other rats were starved for 16 hours and diabetes was induced using a single intraperitoneal injection of freshly dissolved streptozotocin (60 mg/kg) in 0.01M citrate buffer (pH 4.5). One week after the streptozotocin injection, rats were assessed for diabetes and those with fasting blood glucose over 280 mg/dl were included in this study.[19] Thereafter, all rats were divided into five groups each having 10 animals. The control rats (Group A) were fed on standard diet with tap water and received no drug. Group B i.e. diabetic control rats received 60 mg/kg of STZ as a single intraperitoneal injection and were fed on standard diet and tap water.

Group C i.e. experimental group rats received 60 mg/kg of STZ as a single intraperitoneal injection and ELE in a daily oral dose of 400 mg/kg for a period of sixty days. Group D i.e. experimental group rats received 60 mg/kg of STZ as a single intraperitoneal injection and ELE using a dose of 500 mg/kg body weight daily (orally) for a period of sixty days.16, 18 Group E i.e. standard group rats received 60 mg/kg of STZ as a single intraperitoneal injection and glibenclamide 0.5mg/kg body weight orally for a period of sixty days.

Sample collection:

Timings: (day 0, day 60th). Blood samples were taken at day 0 to establish that all rats had normal glucose and renal profile. Twenty four hour after administration of the last dose of extract i.e. on 60th day and after overnight fasting, the animals were weighed and anaesthetized under ether vapours. A

sample of 2ml blood was drawn from tail vein from all animals. Blood was transferred to the sterile vacuotainers with gel and allowed to clot at room temperature for one hour. It was then centrifuged for ten minutes at a speed of 3000 rpm. Serum was separated and stored in sterile eppendorf tubes at -20°C for analysis of biochemical parameters.[20]

Twenty four hour urine samples were collected using metabolic cages and analyzed. The animals were kept individually in metabolic cages, they were given only water. The urine was then collected in 24 hours and measured. Body weight also measured initially and at the end of experiment.

Biochemical Analysis

Glucose levels were estimated using commercially available kit (Randox, UK) based on glucose oxidase method.[21] Serum urea was estimated by commercially available kits (Randox, UK), based on enzymatic colorimetric method[22] while serum creatinine using Jaffe alkaline picrate method (Randox, UK). [23]Total urinary protein was estimated by colorimetric method and microalbumin level based on immunoturbimetric assay (Randox, UK).[24-25]

Statistical Analysis

The data was entered and analyzed using SPSS 17.0 (Statistical Package for Social Sciences). All data are shown as mean ± S.E.M. One way ANOVA was applied to observe group mean differences. Post Hoc Tukey test was applied to observe mean differences among the groups. A p-value of <0.05 was considered as statistically significant.

Results

The biochemical parameters showed that the injection of STZ caused a significantly ($p<0.05$) increased serum glucose, serum creatinine and total urinary protein levels in the rats of group B, C, D and E as compared to control group. On the other hand, simultaneous administration of ethanolic extract of Cassia fistula to group C and D and glibenclamide to group E resulted in a significant ($p<0.01$) decrease in the serum glucose, serum creatinine and total urinary protein levels in groups C ,D and E when compared with that of group B.

The urea levels showed that the injection of STZ caused a significantly ($p<0.05$) increased urea levels in the rats of group B, C, D and E as compared to the control. On the other hand, the simultaneous administration of ethanolic extract of Cassia fistula resulted in a significant ($p<0.01$) decrease in urea levels in the rats of group C and D when compared with that of group B. When group B was compared with group E, no significant difference was observed in urea levels ($p = 0.05$).

The microalbuminuric levels showed that the injection of STZ caused a significantly ($p<0.05$) increased microalbuminuric levels in the rats of group B, C, D and E as compared to the control. On the other hand, the simultaneous administration of ethanolic extract of Cassia fistula resulted in a significant ($p<0.01$) decrease in the microalbuminuria in the rats in group D when compared with that of group B. When group B was compared with group C ($p=0.07$) and E ($p=0.06$), no significant difference was observed in microalbuminuric levels.

The urine volume showed that the injection of STZ caused a significantly ($p<0.05$)

increased levels of urine volume in the rats of group B, C, D and E as compared to the control group. On the other hand, the simultaneous administration of ethanolic extract of Cassia fistula resulted in a significant ($p<0.01$) decrease in the levels of urine volume of the rats in group C and D when compared with that of group B. When group B was compared with group E, no significant difference was observed in urine volume levels ($p=0.08$).

The body weight showed that the injection of STZ caused a significant ($p<0.05$) decrease in body weight in the rats of group B, C, D and E as compared to the control.

On the other hand, the simultaneous administration of ethanolic extract of Cassia fistula resulted in an increase in the body weight of the rats in group D. When group B was compared with group C ($p= 0.63$) and E ($p=0.55$), no significant difference was observed in body weight.

Table I: Mean± SEM values of different biochemical parameters in all groups (A, B, C, D and E)

Parameter	Group A	Group B	Group C	Group D	Group E
Serum Glucose mg/dl	130.54±5.18	298.49±1.95*	200.23±6.41**	148.80±4.72**	209.70±4.87**
Serum Urea (mg/dl)	25.18±2.48	63.43±2.38*	51.19±2.84**	43.50±1.86**	53.44±2.61
Serum Creatinine (mg/dl)	0.62±0.03	1.81±0.06*	1.26±0.10**	0.80±0.04**	1.35±0.09**
Total Urinary Protein (mg/24 hr)	90.70±5.87	236.60±15.51*	184.70±6.86**	153.50±7.38**	189.20±5.28**
Microalbuminuria (mg/24 hr)	4.28±0.60	49.91±3.00*	41.20±2.88	29.01±1.77**	40.92±2.37
Urine volume (ml/24 hr)	6.50±0.34	13.00±0.71*	9.70±0.65**	7.80±0.44**	10.90±0.57
Body weight (g)	214.20±3.10	171±1.97*	176±2.48	195±2.64**	176±2.85

* p<0.05 when compared with group A (control)

** p<0.05 when compared with group B (diabetic)

Discussion

The earliest clinical evidence of nephropathy is the appearance of low but abnormal levels (=30mg/day or 20mcg/min) of albumin in the urine referred as microalbuminuria and patients with microalbuminuria are referred to as nephropathy.[26] Oxidative stress is considered to be the major pathophysiological mechanisms involved in the development of diabetic nephropathy. High glucose directly increases hydrogen peroxide production by mesangial cells and also causes lipid peroxidation of glomeruli and glomerular mesangial cells.[27] Effective control of blood glucose level is a key step in reversing diabetic complications and improving the quality of life in diabetic patients. Currently available drugs for the treatment of DM have a number of limitations including adverse effects and high rate of secondary failure. A number of plants are being assessed for their therapeutic potential as there is a growing trend towards the use of natural remedies as adjuncts to conventional therapy.[28]

The present study showed a significant elevation observed in the levels of serum glucose, urea, creatinine, total urinary protein and urinary albumin excretion in group B diabetic rats as compared to group A normal rats.[29] Urine volume was also elevated in group B rats while body weight reduced in group B rats as compared to group A rats. Elevated levels of these parameters in serum and urine are presumptive markers of diabetes associated lesions in kidneys of rats. Co-administration of ethanolic extract of Cassia fistula leaves brought the levels of these diagnostic parameters in the serum and urine of group C, D and E animals towards normal as compared to group B rats (Table I).

When we compared mean values of group C with group D, although both decrease glucose and renal parameters levels, but group D reduced the levels more as compared to group C. When we compare mean values of group C and D with group E, although glibenclamide decrease levels, but cassia fistula ethanolic leaf extract reduced the levels more as compared to glibenclamide. Cassia fistula also improved the body weight as compared to glibenclamide (Table I), showing better effectiveness of cassia fistula ethanolic leaf extract over glibenclamide. Our results are in accordance with the reports by others who used chemical antioxidants and diet of natural antioxidant plants. [30-31]

The main constituents in Cassia fistula are polyphenols (quercetin), anthraquinones, flavonoids and flavan-3-ol derivatives. The proposed mechanism of Cassia fistula in renoprotection could be due to the antioxidant mechanism. Anjaneyulu and Chopra observed that quercetin is a strong antioxidant.[27] It is likely that the scavenging effects of quercetin on free radicals and the inhibition of lipid peroxidation may play a role in improving renal dysfunction in diabetes.[32] Robards and Antolovich in 1997 have critically reviewed the analytical chemistry of bioflavonoid and it was found that flavonoids possess antioxidant activity, they are potent free radical scavengers and metal chelators and they also inhibit lipid oxidation [33].Therefore, in our study flavonoids and quercetin in Cassia fistula might have a role in the recovery in STZ induced diabetic nephropathy in rats.

Further studies are needed to observe histopathological examination and also to see effect of higher doses and variable routes of administration for its protective effect on the kidneys of diabetic nephropathy subjects.

Conclusion

The results of the present study show that the co-treatment of Cassia fistula ethanolic leaf extract prevents STZ induced diabetic nephropathy in rats. The high dose (500mg/kg) Cassia fistula leaf extract, showed better results as compared to low dose (400mg/kg) Cassia fistula leaf extract and glibenclamide.

Acknowledgments

The authors would like to thank the Principal IIMC Maj.Gen(Retd) Dr. Masood Anwar for his continuous supervision and NIH animal house incharge Mr.Hussain Ali for helping in instrument handling and biochemical analysis.

References:

1. Gale EA, Anderson JV. Diabetes mellitus and

other disorder of metabolism. In:Kumar P, Clark M, Eds. Kumar and Clark clinical medicine (Fifth edition). Philadelphia: W.B.Saunders, 2002:1069-1104.
2. Silawat N, Jarald EE, Jain N, Yadav A, Deshmukh PT. The mechanism of hypoglycemic and antidiabetic action of hydroalcoholic extracts of Cassia fistula in rats. The Pharma Research 2009; 1:82-92.
3. Baynes JW. Role of oxidative stress in the development of complications in diabetes. Diabetes 1991; 40:405-12.
4. Brady HR, O'Meara YM, Brenner BM. Glomerular diseases. In: Kasper DL, Braunwald E, Fauci AS, Hauser SL, Longo DL, Jameson JL, editors. Harrison's principles of internal medicine.16th ed. New York: McGraw Hill; 2005:1688.
5. Berg TJ, Bangstad HJ, Torjesen PA, Osterby R, Bucala R, Hanssen KF.Advanced glycation end products in serum predicts changes in the kidney morphology of patients with insulin-dependent diabetes mellitus. Metabolism1997; 46:661-5.
6. Cooper ME. Interaction of metabolic and haemodynamic factors in mediating experimental diabetic nephropathy. Diabetologia 2001; 44:1957-72.
7. Yotsumoto T, Naitoh T, Shikada K, Tanaka S. Effects of specific antagonist of angiotensin II receptors and captopril on diabetic nephropathy in mice. Jpn J Pharmacol.1997;75:59-64.
8. Bahorun T., Neergheen VS., Aruoma OI. Phytochemical constituents of Cassia fistula. Afr J Biotechnol., 2005:1530-40.
9. Biswas K., Ghosh AB., Banawasadhi. Calcutta University Advancement of Learning, Vol. 2, Calcutta, 1973.
10. Kirtikar KR., Basu B. In: Singh B.,Singh M P. Indian Medicinal Plants, Vol. 2, Dehradun, 1975: 858.
11. Patel D G., Karbhari S S., Gulati OD., Gokhale SD. Antipyretic and analgesic activities of Aconitum spicatum and Cassia fistula. Arch In Pharmacodyn. 1965: 22-27.
12. Bhatka T., Mukherjee P K., Pal M., Saha B P. Studies on antitussive activity of Cassia fistula leaf extract. J Ethnopharmacol 1998:140-3.
13. Bhatka T., Mukherjee PK., Pal M., Saha B P. Studies on vivo wound healing activity of Cassia fistula Linn. leaves in rats. Nat Prod Sci 1998: 84-87.
14. El-Saadany S S., El-Massry, Labib SM., Sitohy M Z. The biochemical role and hypercholesterolaemic rats. Nahrung 1991: 807-15.
15. Gupta M., Mazumder UK., Rath N., MukhopadhyayD K. Antitumor activity of methanolic extract of Cassia fistula L. Seed against Ehrlich ascites carcinoma. J Ethnopharmacol 2000: 151-6.
16. Bhatka T., Mukherjee P K.,Mukherjee K., Banerjee S., Mandal S C., Miaty TK., et al.Evaluation of hepatoprotective activity of Cassia fistula leaf extract. J Ethnopharmacol 1999: 277-82.
17. Yadav R., Jain GC. Antifertility effect of aqueous extract of seeds of Cassia fistula in female rats. Adv Contracept 1999: 293-301.
18. Pradeep K., Mohan CV., Gobianand K., Karthikeyan S. Effect of Cassia fistula Linn. Leaf extract on diethylnitosmine induced hepatic injury in rats. Chem Biol Interact 2007; 167: 8-12.
19. Tesch GH, Allen TJ.Rodent models of streptozotocin induced diabetic nephropathy. Nephrology 2007; 12:261-6.
20. Shad MA, Iqbal T, Shah MH, Mahmood RK, Tayyab M. Vegetable oils and dyslipidemia. Pak J Med Sci 2003; 19: 45-51.
21. Barham D,Trinder P. An improved color reagent for the determination of blood glucose by the Oxidase system. Analyst 1972; 97:142.
22. Fawcett JK,Scott JE. A rapid and precise method for the determination of urea. J Clin Path 1960; 13:156.
23. Bartels H, Boehmer M. Microdetermination of creatinine. Clin Chim Acta 1971; 32:81.
24. Orsonneau JL, Dovet P, Massoubre C, Lustenberger P, Bernard S. An improved pyrogallol red-molybdate method for determining total urinary protein. Clin Chem 1989; 35:22-33.
25. Bakker AJ. Immunoturbidimetry of urinary albumin: prevention of adsorption of albumin; influence of other urinary constituents. Clin Chem 1988; 34:82-6.
26. American Diabetes Association: Nephropathy in diabetes. Diabetes Care 2004; 27 :79-83.
27. Anjaneyulu M, Chopra K. Quercetin, an antioxidant bioflavonoids, attenuates diabetic nephropathy in rats. Clin Exp Pharmacol Physiol

2004; 31:244-8.
28. Evans JL, Goldfine ID, Maddux BA, Grodsky GM. Oxidative stress and stress-activated signalling pathways: a unifying hypothesis of type 2 diabetes. Endocr Re 2002; 23:599-622.
29. Suradkar SG, Ghodasara DJ, Vihol P, Patel J,Jaiswal V,Prajapati KS.Haemato-biochemical alterations induced by lead acetate toxicity in wistar rats. Veterinary world 2009;2:429-31.
30. Sharma SB, Nasir A, Prabhu KM, Murthy PS, Dev G. Hypoglycaemic and hypolipidemic effect of ethanolic extract of seeds of Eugenia jambolana in alloxan-induced diabetic rabbits.J. Ethnopharmacol 2003; 85:201-6.
31. Akpanabiatu MI, Umoh IB, Udoh AE, Edet EF. Rats serum electrolytes, lipid profile and cardiovascular activity on Nuclea latifolia leaf extract administration. Indian J Clin Biochem 2005; 20: 29-34.
32. Chan EC, Pannangpeth P, Woodman OL. Relaxation to flavones and flavonols in rat isolated thoracic aorta: Mechanism of action and structure activity relationship. Cardiovasc Pharmacol 2000;35:326-33
33. Robards K, Antolovich M. Analytical chemistry of fruit bioflavonoids. A review. Analyst 1997; 122:11-30.