J. Cell. Mol. Med. Vol 7, No 3, 2003 pp 322-329

Effect of chromium picolinate on histopathological alterations in STZ and neonatal STZ diabetic rats

Urmila A. Shinde, R. K. Goyal*

Department of Pharmacology, L. M. College of Pharmacy, Ahmedabad, India

Received: June 27, 2003; Accepted: September 18, 2003

Abstract

Earlier studies from our laboratory have indicated insulin sensitizing action of chromium picolinate as the mechanism of its anti-diabetic activity in experimental models of type I and type II diabetes. In the present investigation, we have evaluated the effects of chronic administration of chromium picolinate on the functional and histological alterations of streptozotocin (STZ)-induced diabetes in rats. Type I diabetes was induced by intravenous injection of STZ (40 mg/kg) in adult rats, whereas, type II diabetes was induced by intraperitoneal injection of STZ (90 mg/kg) in 2-day old rat pups which in adulthood develop abnormalities resembling type II diabetes. Chromium picolinate was administered at 8 μg/ml in drinking water for 6 weeks and was found to improve glucose tolerance and increase insulin sensitivity of STZ-diabetic rats. This treatment decrease elevated serum creatinine and urea levels as well as elevated serum levels of hepatic enzymes of both groups of diabetic rats. Histopathological studies of kidney and liver show decrease in the intensity and incidence of vacuolations, cellular infiltration and hypertrophy of STZ and nSTZ (neonatal STZ) diabetic rats. Chronic treatment with chromium picolinate however, did not alter the normal function or morphology of control rats. Chronic chromium picolinate at the therapeutic doses that improved glucose tolerance, was observed to have no hepatotoxic or nephrotoxic potential. It was rather found to improve renal and hepatic function and to reduce abnormalities associated with STZ-diabetes. Chromium picolinate could play an important role in the long term management of diabetes mellitus.

Keywords: chromium picolinate • streptozotocin • diabetes mellitus • renal function, hepatic function • histopathology

Introduction

Diabetes mellitus is a heterogeneous disease characterized by microvascular pathology leading to chronic complications clinically manifested principally in the kidney and retina [1]. A therapy that normalizes the metabolic disfunctions in diabetes should be expected to prevent, delay or substantially reduce the severity of these long term microvascular complications improving the quality of life [2]. Chromium, a group VIb transition element, is essential for normal carbohydrate and lipid metabolism [3]. Deficiency of chromium has been implicated as one of the causes of diabetes mellitus [4, 5]. In our earlier studies chromium picolinate was found to improve impaired glucose tolerance

* Correspondence to: Dr. Ramesh K. GOYAL,
Department of Pharmacology, L.M. College of Pharmacy
P.O. Box 4011, Navrangpura, Ahmedabad 380 009, India.
Tel.: 91-79-6302746, Fax: 91-79-6304865
E-mail: goyalrk@rediffmail.com

and near normalize the lipid metabolism of streptozotocin (STZ)-induced type I and type II diabetic rats [6]. The mechanism of anti-diabetic action of chromium picolinate was found to be its insulin sensitizing action in vitro at the insulin target organs, mainly skeletal muscle and adipocytes [7].

STZ-induced diabetes in rats had been shown to be associated with functional and/or morphological changes in the kidney and liver [8–11]. It is possible that chromium picolinate would improve associated metabolic disturbances. Furthermore, chromium being a transition element, the toxic effects of chromium picolinate on these organs are investigated. The study of histopathological and functional alterations of control rats treated chronically with chromium picolinate may provide evidence of long term safety of the compound. Therefore, in the present investigation, we carried out studies on the liver and kidney of STZ-induced type I and neonatal STZ-induced type II diabetic rats as well as non-diabetic control rats chronically treated with chromium picolinate.

Material and methods

Animals

Wistar rats were bred under well-controlled conditions of temperature (22 ± 2°C), humidity (55 ± 5%) and a 12/12 h light/dark cycle. Conventional pellet food and tap water were provided ad libitum. The experimental protocol was approved by Institutional Animal Ethical Committee as per the guidance of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Induction of diabetes and treatment

Fifteen adult male Wistar rats (200-250 g) were intravenously injected with 40 mg/kg of streptozotocin (STZ, Sigma Chemicals, USA) in 0.9% sodium chloride solution. Animals from the control group (16 rats) were injected with an equivalent volume of 0.9% sodium chloride solution alone by the same route. 48 h after the injection of STZ, animals exhibiting overt glucosuria were considered as STZ-diabetic resembling type I diabetes in humans. These animals were divided into four groups as control [8], control treated with chromium picolinate [8], STZ-diabetic control [7], STZ-diabetic treated with chromium picolinate [8] (Table 1).

Fifteen two-day-old male Wistar rat pups were injected intraperitoneally with 90 mg/kg STZ in 0.9% sodium chloride solution. Control pups [16] received equivalent volume of 0.9% sodium chloride solution alone. Twelve weeks after the injection of STZ, animals exhibiting fasting glucose levels > 140 mg/dl were considered as neonatal-STZ (nSTZ)-diabetic resembling type II diabetes in humans. These animals were divided into four groups as explained above. The non-diabetic control and diabetic control rats received tap water, while the treated control and treated diabetic rats received chromium picolinate in drinking water (8 μg/ml) for six weeks.

Serum analyses

Blood samples were collected after six weeks of drug treatment by nicking the tip of tail after 8hr fast. Serum was separated and analyzed for creatinine, urea, glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) using enzymatic colorimetric assay kits (Bayer Diagnostics, India).

Oral glucose tolerance test (OGTT)

At the end of 6 weeks of treatment, 1.5 g/kg glucose as a 40% solution was administered to 8 h fasted rats. Blood samples were collected from the tail vein at 0, 15, 30, 60 and 120 min after oral glucose administration. Serum was analyzed for glucose using enzymatic colorimetric assay kits (Bayer Diagnostics, India) and for insulin by radioimmunoassay technique using kits obtained from Board of Radiation and Isotope Technology, Mumbai, India. Estimation of insulin sensitivity made from OGTT data was performed using the composite insulin sensitivity index (CISI) proposed by Matsuda and DeFronzo [12]. Calculation of the index was made according to the following equation:

$$CISI = \frac{10,000}{\sqrt{(FSG.FSI)\,(MG.MI)}}$$

where FSG and FSI are fasting serum glucose and insulin concentrations respectively, and MG and MI are the mean glucose and insulin concentrations respectively over the course of OGTT.

Histopathological studies

Following laparotomy, the kidney and liver of each rat were examined grossly. Thereafter, the kidneys and two pieces of liver tissue were removed for histological study. The tissues were washed with normal saline and immersion fixed in 10% buffered formalin immediately upon removal. They were gradually dehydrated, embedded in paraffin, cut into 5µm sections and stained with hematoxylin and eosin for histological examination according to standard procedure [13].

Statistics

All the values in the test are presented as means ± S.E.M. Statistical differences between the means of the various groups were evaluated using one-way analysis of variance (ANOVA) followed by Tukey test. A p value of less than 5% was considered to be statistically significant ($p < 0.05$).

Results

Serum glucose and insulin during OGTT

Fasting serum glucose levels as well as glucose levels after oral glucose load in both STZ and nSTZ-diabetic control rats were significantly higher compared to their respective non-STZ control rats (Fig. 1A, 2A). Fasting serum insulin as well as glucose-stimulated insulin response of STZ-diabetic control rats were significantly lower compared to their non-diabetic counterparts (Fig. 1B). However, serum insulin levels and glucose-stimulated insulin response of nSTZ-diabetic rats was not significantly different from their non-diabetic controls (Fig. 2B). Chronic chromium picolinate treatment significantly reduced fasting serum glucose levels of nSTZ-diabetic rats, but failed to do so in STZ-diabetic rats (Fig. 1A, 2A). The treatment, however, significantly decreased the area under curve for glucose (AUCg) of both STZ and nSTZ diabetic rats without any significant change in fasting serum insulin as well as AUCi of the two as compared to their respective diabetic controls (Fig. 1, 2).

The composite insulin sensitivity index (CISI) of both STZ and nSTZ- diabetic rats was found to be significantly lower compared to respective control groups (Table 1&2). Treatment with chromium picolinate significantly increased the CISI of nSTZ-diabetic rats (Table 2). Treatment also increased the CISI of STZ-diabetic rats, however the difference was not statistically significant when compared to its diabetic control (Table 1).

Serum creatinine and urea

Both STZ and nSTZ-diabetic rats exhibited significantly higher serum creatinine and urea levels compared to their respective non-diabetic control groups (Table 1, 2). Serum creatinine and urea levels of both diabetic groups chronically treated with chromium picolinate were significantly lower compared to their respective controls (Table 1, 2). Serum creatinine and urea levels of healthy rats treated with chromium picolinate were not significantly different from their untreated controls.

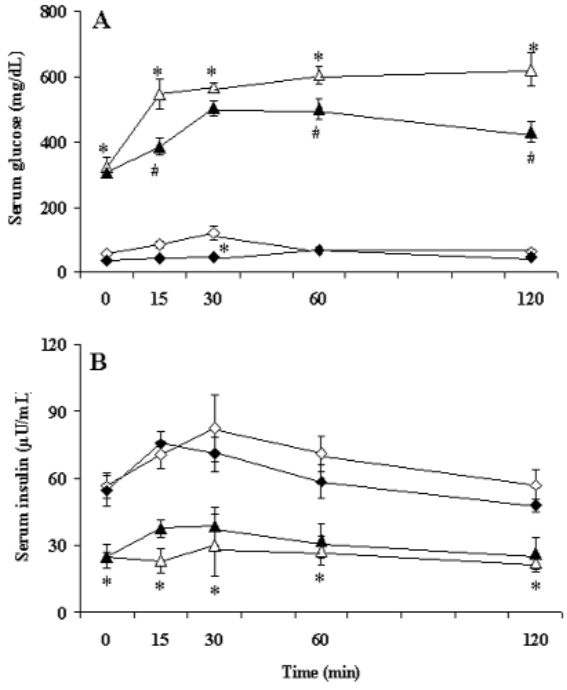

Fig. 1 Effect of chromium picolinate on serum glucose (A) and insulin (B) responses of STZ-diabetic rats to an oral glucose load (1.5 g/kg) during OGTT at the end of six weeks of treatment with chromium picolinate in control (◊), control treated (◆), STZ-diabetic control (△) and STZ-diabetic treated (▲) rats. Values are mean ± SEM. * significantly different from control; # significantly different from STZ-diabetic control, p<0.05.

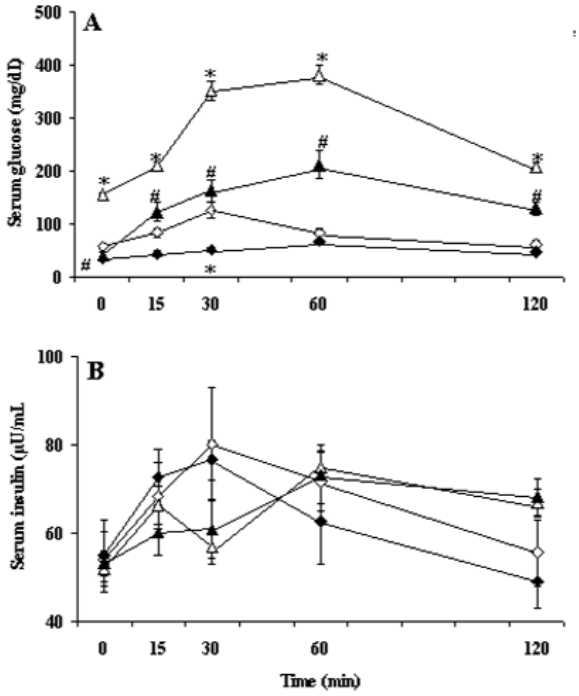

Fig. 2 Effect of chromium picolinate on serum glucose (A) and insulin (B) responses of nSTZ-diabetic rats to an oral glucose load (1.5g/kg) during OGTT at the end of six weeks of treatment with chromium picolinate in control (◊), control treated (◆), nSTZ-diabetic control (△) and nSTZ-diabetic treated (▲) rats. Values are mean ± SEM. * significantly different from control; # significantly different from nSTZ-diabetic control, $p<0.05$.

Serum GOT and GPT

Serum GOT and GPT levels of STZ as well as nSTZ-diabetic rats were significantly higher than those of the non-diabetic control groups. Serum GOT and GPT levels of healthy rats treated with chromium picolinate were not significantly different from those of the non-diabetic controls. Chronic treatment with chromium picolinate significantly decreased the elevated serum GOT and GPT levels of both treated diabetic groups compared to their respective controls (Table 1, 2).

Kidney morphology

Histological examination of the sections of kidneys from STZ-diabetic rats showed marked microscopic changes like multifocal areas of moderate cortical tubular vacuolations and interstitial mononuclear cell infiltration (Fig. 3A). Dilatation of cortical tubules especially at the corticomedullary junction was also observed. However, the classical signs of diabetic nephropathy such as nodular lesions of glomerulus were not evident. The incidence and intensity of tubular vacuolations and interstitial cellular infiltration in STZ-diabetic rats treated with chromium picolinate was much lower compared to the diabetic control kidneys (Fig. 3B).

Histological examination of kidney sections of nSTZ-diabetic rats showed multiple areas of tubular vacuolations with tubular epithelial hypertrophy (Fig. 4A). Multifocal tubules also revealed degeneration of epithelium characterized by eosinophilic appearance

Table 1. Effect of chronic treatment with chromium picolinate on biochemical parameters of the experimental animals resembling type I diabetes

Parameters	*Groups*			
	Control (n=8)	Control treated with chromium picolinate (n=8)	Type I diabetic control (n=7)	Diabetic treated with chromium picolinate (n=8)
CISI	2.47 ± 0.43	3.32 ± 0.28	0.95 ± 0.09*	1.24 ± 0.09
Serum creatinine (mg/dl)	0.39 ± 0.04	0.43 ± 0.02	0.64 ± 0.09*	0.41 ± 0.01#
Serum urea (mg/dl)	28.0 ± 2.2	34.1 ± 1.6	75.4 ± 11.8*	44.2 ± 2.7#
Serum GOT (U/ml)	63.3 ± 4.6	60.1 ± 8.1	171.4 ± 10.2*	72.1 ± 7.9#
Serum GPT (U/ml)	57.1 ± 7.08	57.4 ± 5.15	89.7 ± 9.8*	51.1 ± 10.2#

Each value is mean ± SEM for number of rats in parentheses. * $p<0.05$ vs non-diabetic control group, # $p<0.05$ vs STZ-diabetic control group

Table 2. Effect of chronic treatment with chromium picolinate on biochemical parameters of the experimental animals resembling type II diabetes

Parameters	*Groups*			
	Control (n=8)	Control treated with chromium picolinate (n=8)	Type I diabetic control (n=7)	Diabetic treated with chromium picolinate (n=8)
CISI	2.50 ± 0.38	3.41 ± .033	0.88 ± 0.08*	2.37 ± 0.22*
Creatinine (mg/dl)	0.38 ± 0.05	0.42 ± 0.04	0.58 ± 0.04*	0.37 ± 0.05#
Urea (mg/dl)	26.8 ± 4.2	34.0 ± 6.4	60.4 ± 4.8*	35.6 ± 3.2#
GOT (U/ml)	61.5 ± 4.5	62.3 ± 9.2	127.8 ± 8.2*	78.9 ± 9.3#
GPT (U/ml)	55.3 ± 5.06	59.8 ± 5.00	80.44 ± 1.19*	47.00 ± 2.21#

Each value is mean ± SEM for number of rats in parentheses. * $p<0.05$ vs non-diabetic control group, # $p<0.05$ vs nSTZ-diabetic control group

with pyknosis of nuclei. However, these changes of epithelial hypertrophy and degeneration were significantly lower in the kidney sections of nSTZ-diabetic rats treated with chromium picolinate (Fig. 4B). Kidney sections of healthy rats treated with chromium picolinate showed no pathological changes and were comparable to those of control rats.

Liver morphology

Livers of all groups except nSTZ-diabetic control group appeared normal, both macroscopic and microscopic. Histopathological examination of liver in control animals showed normal hepatic lobules. The central venule with radiating columns of liver cells of normal shape and size were seen. There were no signs of congestion, inflammation, cellular necrosis or cholestasis in control liver sections. Liver sections of STZ-diabetic control rats showed no appreciable histological changes compared to controls. However, those of nSTZ-diabetic control rats showed multifocal areas of hepatocellular vacuolations and hypertrophy (Fig. 5A). Focal areas of chronic inflammation with eosinophil infiltration were also seen. Livers of nSTZ-diabetic rats treated with chromium picolinate showed similar pathological changes as those of nSTZ-diabetic control rats, however their intensity was much lower (Fig. 5B). Liver sections of STZ-diabetic rats and control rats treated with chromium picolinate were comparable to those of untreated control rats.

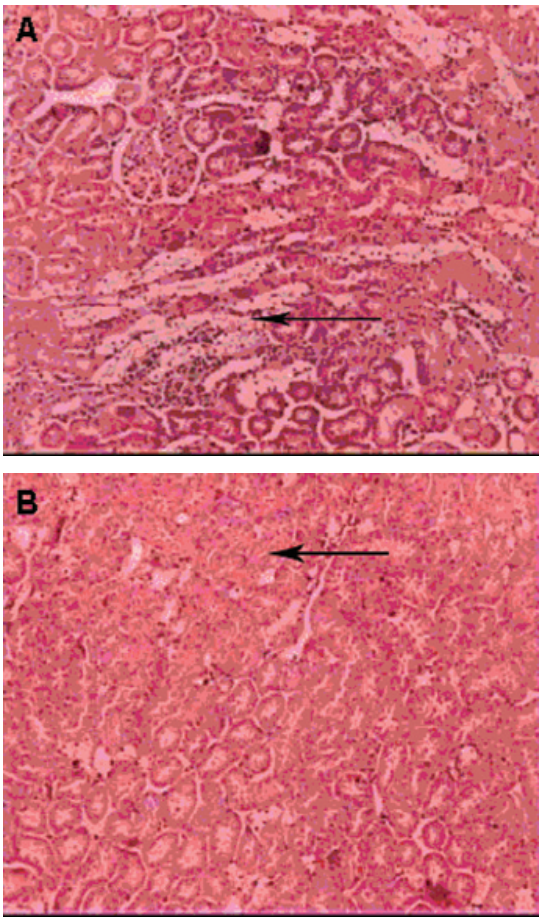

Fig. 3 Kidney sections of STZ-diabetic control and diabetic rats treated with chromium picolinate (X100). (A) Extensive vacuolations of the epithelial cells in cortical renal tubules with mononuclear cell infiltration (arrow) in untreated STZ-diabetic rat. (B) Minimal degree of vacuolation of epithelial cells of renal tubules and cellular infiltration in STZ-diabetic rats treated with chromium picolinate (arrow).

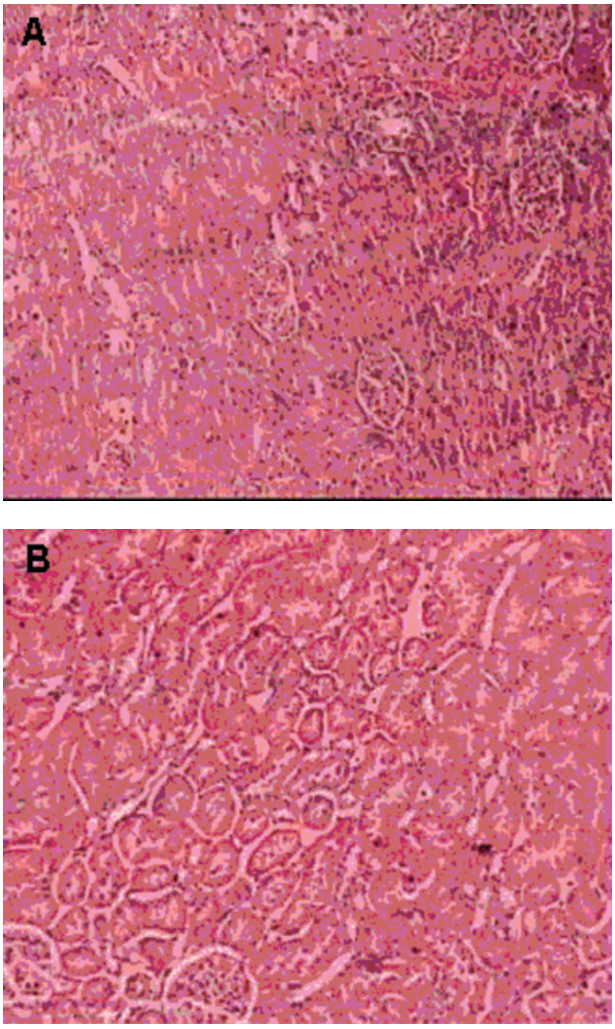

Fig. 4 Kidney sections of nSTZ-diabetic control and diabetic rats treated with chromium picolinate (X100). (A) Generalized hypertrophy of tubules in untreated nSTZ-diabetic rat. (B) Moderate hypertrophy of tubules in chromium picolinate treated nSTZ-diabetic rat.

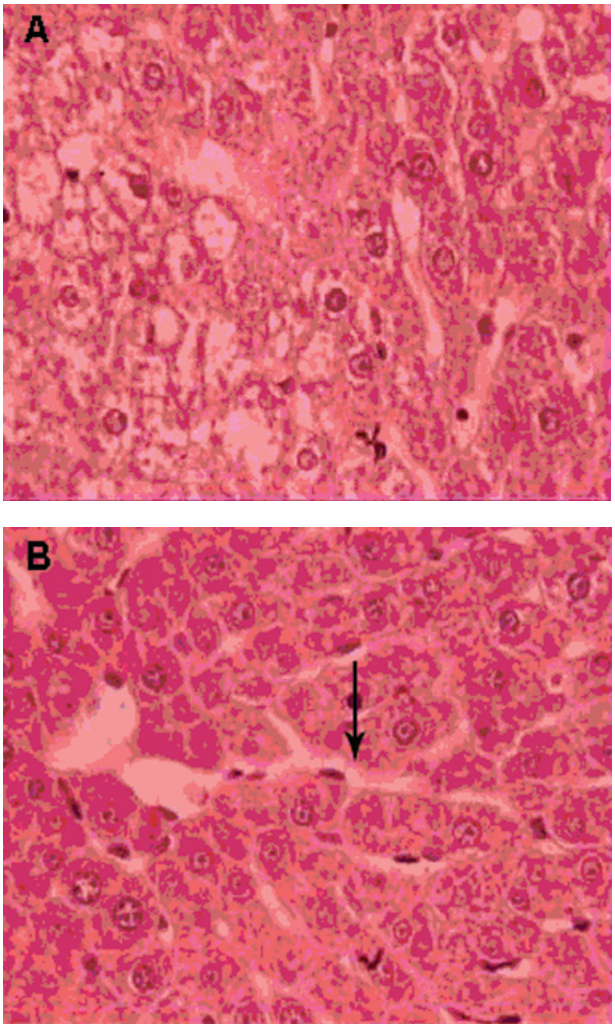

Fig. 5 Liver sections of nSTZ-diabetic control and diabetic rats treated with chromium picolinate (X100). (A) Extensive hepatocellular vacuolation & hypertrophy (arrow) in nSTZ-diabetic control rats. (B) Moderate hepatocellular vacuolation and hypertrophy (arrow) in chromium picolinate treated nSTZ-diabetic rats.

Discussion

The present study demonstrates the efficacy of chromium picolinate in preventing/reverting diabetes-induced functional and histological alterations in kidney and liver of STZ-diabetic rats. The study also emphasizes the lack of toxicity of chronic administration of chromium at the dose used in the treatment of STZ-diabetes.

Chronic chromium picolinate treatment, at doses varying from 3.57-11.19 μM/day as calculated from their daily water intake and body weights, was found to significantly decrease the elevated glucose levels and improve impaired glucose tolerance and insulin sensitivity of STZ as well as nSTZ- rats, indicating the effectiveness of chromium picolinate in improving disturbed glucose metabolism.

Histologically the major finding of diabetic kidney is confined to the glomerulus, which includes nodular changes [14]. In the present investigation, histological examination of both STZ and nSTZ-diabetic rats did not show any such changes. However, STZ-diabetic rats presented multifocal areas of cortical tubular vacuolations with dilatation of tubules especially at the corticomedullary junction. Patches of interstitial mononuclear cell infiltration were observed, suggest-

ing presence of moderate degree of chronic inflammatory changes in these animals. nSTZ-diabetic rats showed tubular vacuolations with tubular epithelial hypertrophy. Tubular degeneration expressed by the eosinophilic appearance with nuclear pyknosis was also observed. The morphological changes in STZ and nSTZ diabetic rats in the present investigation were associated with a significant elevation in serum creatinine and urea levels indicating impaired renal function of diabetic animals. These observations are consistent with those reported earlier by Jensen *et al.* [8], Bleasel & Yong [9], Cam *et al.* [15] and Dai *et al.* [16]. Treatment with chromium picolinate produced considerable reduction in the intensity and incidence of these changes. This improvement in the renal morphology could be correlated with a significant decrease in the elevated serum urea and creatinine levels in both groups of diabetic rats indicating improvement in kidney function.

Typical pathological (mesangial thickening) and immunohistochemical changes of diabetic renal disease both in rats with experimentally induced diabetes [17] and with spontaneous diabetes [18] have been reported. Such changes are also seen in normal kidneys transplanted in diabetic rats [19]. Islet cell transplantation into diabetic rats which results in normalization of carbohydrate metabolism, also causes reversal of established renal lesions [20]. Vigorous insulin therapy has been shown to prevent development of mesangial and glomerular basement membrane thickening in rats with either experimental [21–24] or spontaneous [18] diabetes. Moreover, it has been reported that STZ does not possess any significant nephrotoxic potential [25]. All the functional and structural changes in kidneys resulting from STZ administration in rats can thus be attributed to the altered metabolism in diabetes. The improvement in the impaired renal morphology and function associated with STZ-diabetes with chromium picolinate treatment in the present investigation could be attributed to its anti-diabetic action resulting in alleviation of the altered metabolic status of diabetic animals. No changes in the renal morphology and serum urea and creatinine levels of non-diabetic control rats treated chronically with chromium picolinate indicated lack of nephrotoxicity of chromium picolinate in the present set up. Oral administration of 0.45-77ppm of trivalent chromium drinking water did not produce any pathological changes in the liver and kidneys of dogs, though these organs had a relatively high chromium content [26]. However, in one case, 1200 to 2400 mg chromium picolinate for 4-5 months was found to produce renal toxicity [27] and in another case 600 mg of chromium daily for 6 weeks was found to produce acute interstitial nephritis [28].

Diabetes mellitus is associated with increased frequency of hepatic histopathologic lesions. The most common lesions seen are an increase in liver glycogen leading to vacuolization in cytoplasm and hepatocyte nuclei [29]. STZ-diabetic rats have been shown to exhibit an elevated plasma ALT level without morphological changes in liver [10, 11, 15, 16]. In the present investigation also a significant elevation in serum levels of liver enzymes of both STZ and nSTZ-diabetic rats was observed. However no appreciable changes were observed in the hepatic morphology of STZ-diabetic rats compared to that of control rats. Whereas, nSTZ-diabetic rats showed multifocal areas of hepatocellular vacuolations indicating glycogen accumulation and hypertrophy. No correlation was observed between the functional and structural changes in the livers of diabetic rats. Treatment with chromium picolinate significantly decreased elevated GOT and GPT levels. Treatment also decreased the vacuolization and hypertrophy of hepatic cells observed in nSTZ-diabetic rats. Further, liver being the next organ to kidney in accumulating chromium, the possibility of nephrotoxicity in addition to the effect on altered hepatic function was studied in the present investigation. Treatment with chromium picolinate did not alter serum GPT and GOT levels as well as liver morphology of non-diabetic rats. This indicates that chromium picolinate does not cause hepatoxicity in non-diabetic or diabetic rats under the conditions of the present investigation. Since chromium picolinate was found to possess significant anti-diabetic potential in STZ as well as nSTZ-diabetic rats [5, 6], it is reasonable to believe that improvement in renal and hepatic function as well as morphology could have resulted from the alleviation of the diabetic state of animals.

In summary, chromium picolinate, at doses that improved altered glucose metabolism associated with STZ-diabetes, was found to have no hepatotoxic or nephrotoxic potential. It was rather found to effectively improve the renal and hepatic function and reduce lesions associated with diabetic state in STZ-diabetic rats. This study establishes the efficacy of chromium picolinate in preventing/ reverting long term diabetic complications.

Acknowledgements

We wish to acknowledge Senior Research Fellowship awarded to Ms Urmila A. Shinde by Council of Scientific and Industrial Research, New Delhi (No. 8/261(1)/99-EMR-I-(SPS). The work was also supported by research grant from All India Council of Technical Education, New Delhi. We are grateful to M/s Softcaps Pvt Ltd., Chennai, India for the generous gift sample of chromium picolinate.

References

1. **DeFronzo R.A.**, Lilly Lecture 1987: the triumvirate: -cell, muscle, liver: a collusion responsible for NIDDM, *Diabetes,* **37**: 667-687, 1988
2. **Bailey C.J.**, Potential new treatments for type 2 diabetes, *TIPS, **21***: 259-265, 2000
3. **Mertz W.**, Chromium in human nutrition: a review, *J. Nutr.,* **123**: 626-633, 1993
4. **Anderson R.A.**, Chromium, glucose tolerance, diabetes and lipid metabolism, *J. Adv. Med.,* **8**: 37-49, 1995
5. **Anderson R.A.**, Recent advances in the clinical and biochemical manifestation of chromium deficiency in human and animal nutrition, *J. Trace. Elem. Exp. Med.,* **11**: 241-250, 1998
6. **Shinde U.A., Goyal R.K.**, Insulin sensitizing action of chromium in experimental models of diabetes mellitus, *Indian J. Pharmacol.,* **33**: 51, 2001
7. **Shinde U.A., Goyal R.K.**, Mechanism of anti-diabetic action of chromium, *Indian J. Pharmacol.,* **34**: 142, 2002
8. **Jensen P.K., Christiansen J.S., Steven K., Parving H.H.**, Renal function in streptozotocin-diabetic rats, *Diabetologia,* **21**: 409-414, 1981
9. **Bleasel A.F., Yong C.J.**, Streptozotocin induced diabetic nephropathy and renal tumors in the rat, *Experientia,* **38**: 129-130, 1982
10. **Domingo J.L., Gomez M., Llobet J.M., Corbella J., Keen C.L.**, Oral vanadium administration to streptozotocin-diabetic rats has marked negative side-effects which are independent of the form of vanadium used, *Toxicology,* **66**: 279-287, 1991
11. **Domingo J.L., Gomez M., Llobet J.M., Corbella J., Keen C.L.**, Improvement of glucose homeostasis by oral vanadyl or vanadate treatment in diabetic rats is accompanied by negative side effects, *Pharmacol. Toxicol.,* **68**: 249-253, 1991
12. **Matsuda M., DeFronzo R.A.**, Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp, *Diabetes Care,* **22**: 1462-1470, 1999
13. **Ross M.H., Reith E.J., Romrell L.J.**, Histology. A text an atlas (ki sp k), Williams & Wilkins, Baltimore, Maryland, 1989, pp. 1-2
14. **Tisher C.C.**, Anatomy of the kidney. In: Brenner B., Rector F., eds., The Kidney, vol.2, WB Saunders, Philadelphia, 1981, pp. 3-75
15. **Cam M.C., Pederson R.A., Brownsey R.W., McNeill J.H.**, Long-term effectiveness of oral vanadyl sulfate in STZ-diabetic rats, *Diabetologia,* **36**: 218-224, 1993
16. **Dai S., Yuen V.G., Orvig C., McNeill J.H.**, Prevention of diabetes-induced pathology in STZ-diabetic rats by bis(maltolato)oxovanadium (IV), *Pharmacol. Commun.,* **3**: 311-321, 1993
17. **Brown D.M., Andres G.A., Hostetter T.H., Mauer S.M., Price R., Venkatachalam M.A.**, Kidney complications, *Diabetes,* **31**: 71-81, 1982
18. **Cohen A.J., McGill P.D., Rossetti R.G., Guberski D.L., Like A.A.**, Glomerulopathy in spontaneously diabetic rats. Impact of glycemic control, *Diabetes,* **36**: 944-951, 1987
19. **Lee C.S., Mauer S.M., Brown D.M., Sutherland D.E., Michael A.F., Najarian J.S.**, Renal transplantation in diabetes mellitus in rats, *J. Exp. Med.,* **139**: 793-801, 1974
20. **Mauer S.M., Sutherland D.E.R., Steffes M.W., Leonard R.J., Najarian J.S., Michael A.F., Brown D.M.**, Pancreatic islet transplantation. Effects on the glomerular lesions of experimental diabetes in the rat, *Diabetes,* **23**: 748-753, 1974
21. **Fox C.J., Darby S.C., Ireland J.T., Sonkensen P.H.**, Blood glucose control and glomerular capillary basement membrane thickening in experimental diabetes, *Br. Med. J.,* **2**: 605-607, 1977
22. **Rasch P.**, Prevention of diabetic glomerulopathy in streptozotocin diabetic rats by insulin treatment, *Diabetologia,* **16**: 319-324, 1979
23. **Rasch R.**, Prevention of diabetic glomerulopathy in streptozotocin diabetic rats by insulin treatment. The mesangial regions, *Diabetologia,* **17**: 243-248, 1979
24. **Rasch R.**, Prevention of diabetic glomerulopathy in streptozotocin diabetic rats by insulin treatment. Albumin excretion, *Diabetologia,* **18**: 413-416, 1980
25. **Evan A.P., Mong S.A.**, The effect of streptozotocin induced diabetes on kidney, Renal Physiol., 7: 78-89, 1984
26. Mertz W., Biological role of chromium, *Fed. Proc.,* **26**: 186-193, 1967
27. **Cerulli J., Grabe D.W., Gauthier I., Malone M., McGoldrick M.D.**, Chromium picolinate toxicity, *Ann. Pharmacother.,* **32**: 428-431, 1998
28. **Wasser W.G., Feldman N.S., D'Agati V.D.**, Chronic renal failure after ingestion of over-the-counter chromium picolinate, *Ann. Intern. Med.* **126**: 410, 1997
29. **Glick M.E., Hoefs J.C., Meshkinpour H.**, Glucose intolerance and hepatic, biliary tract and pancreatic dysfunction, *Dig. Dis.* **5**: 78-96, 1987