# Current Research Areas

Drug discovery and development has undergone a paradigm shift with increasing stress on 'compound optimization' in the preclinical stage, to reduce the attrition rate in the later stages of drug development. Genomics and Proteomics techniques are to be involved in predicting the toxicity of new chemical entities and also in identifying new drug targets. Future of drug discovery lies in appropriate utilization of these states of art techniques. Thus my endeavor is to utilize genomics and proteomics techniques in discovering new drug targets as well as identifying disease specific proteomic and genomic markers. *Furthermore, involvement of histone modifying enzymes in altering chromatin structure at the promoter region of specific genes will help in understanding pathophysiology of disease like cancer and diabetes.*

## Epigenetics and Disease

***Diabetes:***

- Devolopment of Type1 and Type 2 diabetic nephropathy model
- Molecular mechanism in the development of diabetic nephropathy
- Genomic and proteomic changes during diabetic nephropathy
- Insulin and high glucose induced changes in chromatin structure

***Cancer:***

- Development of chemical induced colon cancer and mammary cancer models.
- Changes in histone modifications in colon and mammary cancer
- Inhibitors for DNA methylation and histone acetylation in cancer

***Nanotoxicology:***

- Toxicity of nanoparticles involved in drug delivery

nature INDIA

# Cracking the histone code

Subhra Priyadarshini

Kulbhushan Tikoo (sitting) with co-researchers

Curcumin, the main ingredient of Indian spice turmeric, provides protection against diabetic nephropathy, a kidney abnormality triggered by diabetes. New research has now pinpointed how this works.

Curcumin plays a role in modifying the histones (proteins that wrap up DNA to form chromatin), in turn bolstering the anti-oxidant defense system of the body, an epigenetic study on rats has concluded[1].

A research team from the National Institute of Pharmaceutical Education and Research (NIPER), Punjab, designed a series of experiments to unravel this protective effect of curcumin. "Curcumin has been used to treat cancer, diabetes and other pathologies. However, little was known regarding its role in bringing about change in histone H3," says lead researcher Kulbhushan Tikoo.

In diabetes, there are changes in the 'histone code' which result in modifying gene expression and lead to the major microvascular complication — diabetic nephropathy. Curcumin was found to decrease the oxidative stress by boosting the anti-oxidant defense system of the body and improving biochemical alterations that resulted in better health.

The team underpinned the role of histone code acting as a prelude to the disease progression and development. The finding could have implications in drug design and developmental programmes targeting diabetes and its complications.

- **References**

1. Tikoo, K. *et al.* Change in post-translational modifications of histone H3, heat-shock protein-27 and MAP kinase p38 expression by curcumin in streptozotocin-induced type I diabetic nephropathy. Brit. J. Pharmacol. 153, 1225-1231 (2008) |

natureINDIA

# Fast cure for diabetes

Fasting could be an effective way to control diabetes, says Kulbhushan Tikoo.


Kulbhushan Tikoo.

Fasting is a well established way of dietary restriction. It involves limiting food and calorie intake below normal levels without reaching malnutrition and can extend the lifespan in most, if not all species, including humans.

Based on this assumption, dietary restriction should prolong not only life span, but also youthfulness and keep at bay diseases associated with lifestyle disorders. In fact, in both rodents and humans, limiting the caloric intake delays many age-associated traits and diseases, including cognitive deterioration and cancer. In addition, dietary restriction can reduce body weight and normalise blood glucose, insulin, and leptin levels in obese animals and humans.

Research[1] carried out at the National Institute of Pharmaceutical Education and Research, Mohali explored the role of intermittent fasting in the progression of diabetic nephropathy and the different underlying mechanisms involved. The observations suggest that alternate day fasting in diabetic animals prevents progression of renal disease. Dietary restrictions also helped reduce tumor formation and increased resistance of neurons to dysfunction and degeneration in experimental models of Alzheimer's, Parkinson's disease and stroke.

**Molecular mechanism**

The merits of fasting to cure diabetes find mention in ancient Indian Ayurvedic texts such as the Caraka Samhita Sutra. Several ayurvedic practioners in the country still use fasting as one of the treatment protocols in diabetes. As one starves, digestive enzymes inactivate different toxin as well as all patho-physiological factors responsible for progression of the disease. This is how Ayurveda explains the mechanism. However, the different molecular and cellular mechanisms involved in fasting are not completely known.

More recently, several possible molecular mechanisms have been proposed that might explain the beneficial effects of intermittent fasting on aging and disease including reduction in mitochondrial oxyradical production, induction of a cytoprotective cellular stress response, and stimulation of the production of growth factors.

In addition, to these mechanisms, the NIPER study demonstrated that alternate day fasting prevents the decrease in sirtuins (Sir 2) expression in the kidney of diabetic animals during the nephropathy progression. Several reports have shown involvement of sirtuins (a longevity protein) in extending the life span of wide variety of organisms[2].

Two different paradigms to extend life span are widely employed in rat and mice — calorie restriction (CR), in which 30 to 40% less than normal food is allowed for consumption; and intermittent fasting (IF), in which food is given every other day. Out of several possible molecular mechanisms that might explain the beneficial effect of CR or IF, one is increase in expression of Sirtuins. Several reports also show involvement of sirtuins in inflammation, which is involved in number of pathological conditions, including diabetes, cancer, arthritis, asthma, heart disease and neurodegeneratation.

Sirtuins target many proteins that are not histones, they have been demonstrated to bind and deacetylate p53 in vitro and in vivo. The expression of p53 protein in the kidneys of diabetic animals increased as compared to their respective controls. However, the expression of proapoptotic p53 reduced significantly in the kidneys of diabetic rats on IF regimen.

The expression as well as activation of p53 is thought to be mediated by Sir2 dependent deacetylation. They both share an inverse relationship as is evident from the results where the Sir2 expression is decreased and at the same time p53 is upregulated. Further IF decreases the level of caspases-3 and p38 which are involved in apoptosis as compared to diabetic animal kidney and shows its antiapoptotic effect. Although the mechanism by which IF exerts its anti apoptotic effects is yet to be understood in detail but these results suggest a cross talk between Sir2, p53, p38 and caspase-3.

These and other findings may have unique clinical efficacy in preventing the development and progression of diabetic complications in diabetes. In the present scenario in India, there has been a marked change in lifestyle and eating habits of people rendering them more prone to develop different lifestyle disorders like diabetes and metabolic syndrome. Fasting could be a useful intervention for enhancing life span as well as minimising the risk of metabolic disorders.

**Past work**

A clinical study aimed at increasing exercise combined with diet is able to decrease the incidence of type 2 diabetes[3]. The Indian Diabetes Prevention Programme demonstrated the lifestyle modification and metformin prevent type 2 diabetes in Asian Indian subjects with impaired glucose tolerance[4].

As per World Health Organization (WHO), across the world the total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030. The maximum absolute increase in the number of people with diabetes will be in India. In 2000, the number of diabetic patients was 31 million and is predicted to go up to 79 million till 2030[5]. India is the capital of the lifestyle disease diabetes.

Of all the abnormalities associated with diabetes, nephropathy or renal failure has became the world's leading cause of chronic and end-stage renal disease[6]. It is associated with structural changes in the glomerulus such as thickening of the glomerular basement membrane and eventually causes progressive hyperfiltration and albuminuria. As the disease progresses, glomerular filtration rate (GFR) declines and leads to end-stage renal disease.

To treat diabetic nephropathy, a variety of therapeutic approaches or treatment are available. While they can slow down the development of the disease, they do not stop the progression of end stage renal failure.

The author is an associate professor in the department of pharmacology and toxicology at the National Institute of Pharmaceutical Education and Research, Punjab, India.

- **References**


1. Tikoo, K. *et al.* Intermittent fasting prevents the progression of type I diabetic nephropathy in rats and changes the expression of Sir2 and p53. FEBS Lett. 581, 1071-1078 (2007) |
2. Cohen, H. Y. *et al.* Calorie restriction promotes mammalian cell survival by inducing the SIRT1 deacetylase. Science 305, 390-392 (2004) |
3. Orozco, L. J. *et al.* Exercise or exercise and diet for preventing type 2 diabetes mellitus. Cochrane Db. Syst. Rev. 3, CD003054 (2008)
4. Ramachandran, A. *et al.* The Indian Diabetes Prevention Programme shows that lifestyle modification and metformin prevent type 2 diabetes in Asian Indian subjects with impaired glucose tolerance (IDPP-1). Diabetologia 49, 289-297 (2006) |
5. Wild, S. *et al.* Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care 27, 1047-1053 (2004)
6. Zimmet, P. *et al.* Global and societal implications of the diabetes epidemic. Nature 414, 782-787 (2001)


**Grants and Projects (Project(s) carried out as Principal Investigator):**

- **Extramural funding by government agencies**

| Sr. No. | Title of Project | Funding Agency | Amount (Cr) | Date of sanction and duration |
|---|---|---|---|---|
| 1 | Epigenetic changes during hyperglycemia induced oxidative stress and its role in modulating expression of genes (diabetogenes) involved in pathogenesis of diabetes. | Department of Biotechnology, India. | 0.45 | 2010-2013 |
| 2 | Toxicity screening of nanoparticles used for drug delivery | Department of Science and Technology, India | 3.2 | 2008-2011 |
| 3 | Regulatory Toxicology-development of GLP-certified facility for toxicological screening of new chemical entities | Department of Science and Technology, India | 1.3 | 2006-2008 |
| 4 | Role of Histone Modifications and its Inhibitors in Reactive Oxygen species (ROS) –Induced Cell-Death | Department of Biotechnology, India. | 0.25 | 2003-2007 |

**RECENT PUBLICATIONS:**

| Sr. No | Publication | Impact Factor 2008 |
|---|---|---|
| 30 | **Tikoo K**, Surse VM, Gupta J, Esculetin induced changes in Mmp-13 and Bmp-6 gene expression and histone H3 modifications attenuate development of glomerulosclerosis in diabetic rats, **Journal of Molecular Endocrinology 2011 [Epub ahead of print]** | **3.221** |
| 29 | **Tikoo K**, Sane MS, Gupta C, Tannic acid ameliorates doxorubicin-induced cardiotoxicity and potentiates its anti-cancer activity: Potential role of tannins in cancer chemotherapy, **Toxicology and Applied Pharmacology 2010 Dec 29. [Epub ahead of print]** | **3.359** |
| 28 | Gaikwad AB, Gupta J, **Tikoo K** (2010).Epigenetic changes and alteration of Fbn-1 and Col3A1 gene expression under hyperglycemic and hyperinsulinemic conditions. **Biochemical Journal. 2010 Nov 12;432(2):333-41** | **5.1** |
| 27 | Gupta J, Gaikwad AB, **Tikoo K** (2010).Hepatic expression profiling shows involvement of PKC epsilon, DGK eta, Tnfiap and Rho kinase in type 2 diabetic nephropathy rats. **Journal of Cellular Biochemistry. Nov 1;111(4):944-54.** | **3.2** |
| 26 | Sayyed SG, Gaikwad AB, Lichtnekert J, Kulkarni O, Eulberg D, Klussmann S, **Tikoo K,** Anders HJ. Progressive glomerulosclerosis in type 2 diabetes is associated with renal histone H3K9 and H3K23 acetylation, H3K4 dimethylation and phosphorylation at serine 10. **Nephrology Dialysis Transplantation,** 2010, January 12. | **3.4** |
| 25 | Mulay SR, Gaikwad AB, **Tikoo K.** Combination of Aspirin with Telmisartan suppress the augmented TGF-beta/smad signaling during the development of Streptozotocin induced type I diabetic nephropathy. **Chemico-biological Interactions**, 2010, March 16. | **3.1** |
| 24 | Gaikwad AB, Sayyed SG, Lichtnekert J, **Tikoo K,** Anders HJ (2010). Renal Failure Increases Cardiac Histone H3 Acetylation, Dimethylation, and Phosphorylation and the Induction of Cardiomyopathy-Related Genes in Type 2 Diabetes. **American Journal of Pathology** 176(3): 1079. | **6.1** |
| 23 | **Tikoo K**, Ali IY, Gupta J, Gupta C. 5-Azacytidine prevents cisplatin induced nephrotoxicity and potentiates anticancer activity of cisplatin by involving inhibition of metallothionein, pAKT and DNMT1 expression in chemical induced cancer rats. **Toxicology Letters**. 2009, article in press | **3.2** |
| 22 | **Tikoo K.,** Fast Cure for Diabetes. **Nature India**, Doi:10.1038/nindia.2009.155. | |
| 21 | Kabra DG, Gupta J, **Tikoo K.,** Insulin induced alteration in post-translational modifications of histone H3 under a hyperglycemic condition in L6 skeletal muscle myoblasts. **Biochim Biophys Acta**. 2009 Jun; 1792(6):574-83. | **4.579** |
| 20 | **Tikoo K,** Kumar P, Gupta J. Rosiglitazone synergizes anticancer activity of cisplatin and reduces its nephrotoxicity in 7, 12-dimethyl benz{a}anthracene (DMBA) induced breast cancer rats. **BMC Cancer**. 2009 Apr 8; 9:107. | **3.09** |
| 19 | Chandak P.G., Gaikwad, A.B. & **Tikoo, K.,** Gallotannin, Ameliorates the Development of Streptozotocin Induced Diabetic Nephropathy by Preventing the Activation of PARP. **Phytotherapy Research.** 2009 Jan;23(1):72-7 | **1.772** |
| 18 | **Tikoo K.,** Cracking the histone code. **Nature India**, Doi:10.1038/nindia.2008.159. | |
| 17 | **Tikoo**. **K**, Roshan Lal Meena, Dhiraj Kabra and Anil Bhanudas Gaikwad **(2008)** Change in post-translational modifications of histone H3, HSP-27 and p38 expression by curcumin in diabetic nephropathy. **British Journal of Pharmacology.** 2008 153(6):1225-31 | **4.902** |
| 16 | **Tikoo**. **K**, Anupama Tamta and Anil Bhanudas Gaikwad **(2008)** Tannic acid prevents Azidothymidine (AZT) induced hepatotoxicity and genotoxicity along with change in expression of PARG and histone H3 acetylation **Toxicology Letters.** 2008;177(2):90-6 | **3.2** |
| 15 | **Tikoo, K**., Singh K., Kabra, D.G., Sharma, V. & Gaikwad, A.B. 2008. Change in histone H3 phosphorylation, MAP kinase p38, SIR 2 and p53 expression by resveratrol in preventing streptozotocin induced type I diabetic nephropathy. **Free Radical Research** 2008 Apr;42(4):397-404. | **2.826** |
| 14 | **Tikoo, K.,** Tripathi, D.N., Kabra, D.G., Sharma, V. & Gaikwad, A.B. **(2007).** Intermittent fasting prevents the progression of type I diabetic nephropathy in rats and changes the expression of Sir2 and p53. **FEBS Lett**, 581, 1071-8. | **3.264** |
| 13 | **Tikoo, K.** Bhatt, D.K., Gaikwad, A.B., Sharma, V. & Kabra, D.G. **(2007).** Differential effects of tannic acid on cisplatin induced nephrotoxicity. **FEBS Lett**, May15;581(10):2027-35 | **3.264** |
| 12 | Sharma, G., Italia, J.L., Sonaje, K., **Tikoo, K**. & Ravi Kumar, M.N. **(2007).** Biodegradable in situ gelling system for subcutaneous administration of ellagic acid and ellagic acid loaded nanoparticles: Evaluation of their antioxidant potential against cyclosporine induced nephrotoxicity in rats. **J Control Release**, 118, 27-37. | **3.696** |
| 11 | Garkhal, K., Verma, S., **Tikoo, K**. & Kumar, N. **(2007)**. Surface modified poly(L- | **2.743** |

| | | |
|---|---|---|
| | lactide-co-epsilon-caprolactone) microspheres as scaffold for tissue engineering. **J Biomed Mater Res** A, 82(3):747-56. | |
| 10 | Italia, J.L., Bhatt, D. k., Bhardwaj, V., **Tikoo, K.** & Ravi kumar, M.N.V. **(2007).** PLGA nanoparticles for oral delivery of cylcosporine: Nephrotoxicity and pharmacokinetic studies in comparison to Sandimmune Neoral. **J Control Release**, Jun 4;119(2):197-206. | **3.696** |
| 9 | Sonaje, K., Italia, J.L., Sharma, G., Bhardwaj, V., **Tikoo, K.** & Ravi kumar, M.N.V. **(2007).** Development of biodegradable nanoparticles for oral delivery of ellagic acid and evaluation of their antioxidant efficacy against cyclosporine A-induced nephrotoxicity in rats. **Pharm Res,** May;24(5):899-908. | **2.752** |
| 8 | Kalra S, Jena G, **Tikoo K**, Mukhopadhyay AK Preferential inhibition of xanthine oxidase by 2-amino-6-hydroxy-8-mercaptopurine and 2-amino-6-purine thiol.**BMC Biochem**. 2007 May 18; 8:8 | **2.04** |
| 7 | Monks, T.J., Xie, R., **Tikoo, K.** & Lau, S.S. **(2006).** Ros-induced histone modifications and their role in cell survival and cell death. **Drug Metab Rev**, 38, 755-67. | **5.153** |
| 6 | Jain, M., Vangapandu, S., Sachdeva, S., Singh, S., Singh, P.P., Jena, G.B., **Tikoo, K.,** Ramarao, P., Kaul, C.L. & Jain, R. **(2004).** Discovery of a bulky 2-tert-butyl group containing primaquine analogue that exhibits potent blood-schizontocidal antimalarial activities and complete elimination of methemoglobin toxicity. **J Med Chem**, 47, 285-7. | **4.926** |
| 5 | Dong, J., Ramachandiran, S., **Tikoo, K.,** Jia, Z., Lau, S.S. & Monks, T.J. **(2004).** EGFR-independent activation of p38 MAPK and EGFR-dependent activation of ERK1/2 are required for ROS-induced renal cell death. **Am J Physiol Renal Physiol**, 287, F1049-58. | **4.263** |
| 4 | **Tikoo, K.,** Lau, S.S. & Monks, T.J. **(2001)**. Histone H3 phosphorylation is coupled to poly-(ADP-ribosylation) during reactive oxygen species-induced cell death in renal proximal tubular epithelial cells. **Mol Pharmacol**, 60, 394-402. | **4.711** |
| 3 | **Tikoo, K.,** Gupta, S., Hamid, Q.A., Shah, V., Chatterjee, B. & Ali, Z. **(1997).** Structure of active chromatin: isolation and characterization of transcriptionally active chromatin from rat liver. **Biochem J**, 322 ( Pt 1), 273-9 | **4.371** |
| 2 | **Tikoo, K.** & Ali, Z. **(1997).** Structure of active chromatin: covalent modifications of histones in active and inactive genes of control and hypothyroid rat liver. **Biochem J**, 322 (Pt 1), 281-7. | **4.371** |
| 1 | **Tikoo, K.,** Hamid, Q.A. & Ali, Z. **(1997)**. Structure of active chromatin: higher-order folding of transcriptionally active chromatin in control and hypothyroid rat liver. **Biochem J,** 322 (Pt 1), 289-96. | **4.371** |

**NUMBER OF STUDENTS SUPERVISED:**

**PhD Students:** Guided 3 students and currently 4 are registered.

**MS Students:** Guided 16 students and currently 16 are registered.

**PATENT: 6**