Abstract
Recently there is increasing evidence that micronutrient status is altered in diabetes mellitus and micronutrient intake may be important in promoting optimum health of diabetic patients. Vanadium, a group Va transition element is reported to produce insulin-like actions in several \textit{in vitro} as well as \textit{in vivo} models of type I and type II diabetes. However, the important drawback of vanadate therapy is that it is poorly absorbed from the gastrointestinal tract and the doses of vanadate required for its anti-diabetic effect are close to the toxic level. Vanadyl sulphate is less toxic than vanadate but is also not well absorbed from the gastrointestinal tract. Complexation of vanadyl ion within an organic matrix has been used to increase the potency of vanadium with respect to the insulin mimetic activity. Bis(maltolato)oxovanadium (IV) (BMOV), a coordination complex of vanadyl and maltol, synthesized with the same objective, has been reported to be two to three times more potent than inorganic vanadium. However, attempts have been going on all over the world to synthesize newer complexes with higher potency and lower toxic potential than the existing complexes. In a similar attempt a new vanadium complex was synthesized in our laboratory. The objective of the present investigation was to (1) \textit{to study the effect of newly synthesized vanadium complex in various experimental models of diabetes mellitus and compare its efficacy with BMOV and (2) to investigate its mechanism of action.}

Chromium, a group VIb transition element, next in position to vanadium in periodic table, is reported to be an essential elements for normal carbohydrate and lipid metabolism. Deficiency of chromium has been implicated as one of the causes of diabetes mellitus and supplementation with chromium has been found to improve glucose tolerance in such patients. However, these reports are controversial as there are few well-controlled clinical trials reporting no or minimal beneficial effects of chromium. Moreover, studies reporting beneficial effects of chromium claim that chromium is a nutrient and not a drug and thus will benefit only those patients where chromium deficiency is the cause of diabetes mellitus. Although the essentiality of chromium in normal glucose homeostasis has been established, its usefulness as a
therapeutic agent for the treatment of diabetes mellitus of variable etiology (other than chromium deficiency) remains questionable. Hence, we have also undertaken an investigation (1) to study the effectiveness of chromium compounds as therapeutic agents in various experimental models of diabetes mellitus and (2) to investigate into their mechanism of action.

The anti-diabetic potential of vanadium and chromium compounds was assessed experimentally using animal models of type I and type II diabetes mellitus. Type I diabetes was induced by intravenous injection of 40mg/kg STZ in adult male Wistar rats whereas type II diabetes was induced by intraperitoneal injection of 90mg/kg STZ in 2-day old Wistar rat neonates which during the adulthood develop abnormalities resembling type II diabetes in humans. Vanadium compounds, BMOV and VUR1 (0.75 mg/ml) and chromium compounds, chromium chloride (10 μg/ml) and chromium picolinate (8 μg/ml) were given for 6 weeks in drinking water. At the end of 6 weeks treatment, blood samples were collected from 8h fasted rats and analyzed for serum glucose, cholesterol, triglyceride, creatinine, urea, glutamate pyruvate transaminase (GPT) and glutamate oxaloacetate transaminase (GOT) using colorimetric analysis. Serum insulin was analyzed using radioimmunoassay technique. At the end of treatment schedule, the animals were also subjected to oral glucose tolerance test and insulin tolerance test. The morphological studies of kidney and liver were carried out using histopathological techniques. To investigate their mechanism of action, the effect of vanadium and chromium compounds on radiolabeled glucose uptake was studied in C2C12 myoblasts, which is a skeletal muscle cell line. The effect of these compounds on the lipogenesis was studied in 3T3-L1 preadipocyte cell line.

Treatment with BMOV and VUR1 significantly decreased elevated fasting serum glucose levels and AUCg of both type I and type II diabetic rats without any significant change in basal serum insulin levels or glucose stimulated insulin response indicating insulin sensitizing action of vanadium compounds in vivo. Treatment with BMOV and VUR1 significantly increased the decreased Kitt values of both type I and type II
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diabetic rats which further substantiates the insulin sensitizing action of vanadium complexes. Since this test assesses peripheral insulin sensitivity, improvement in the insulin sensitivity of diabetic animals with vanadium therapy could be due to increase in the cellular sensitivity at the hepatic level or at the level of target organs like adipose tissue and skeletal muscle.

Further, it is reported that the peripheral insulin resistance reflects mainly a reduced uptake by muscle after exposure to exo or endogenous insulin. Because of the important interaction between free fatty acid and glucose metabolism, insulin resistance in adipocytes with respect to free fatty acid metabolism can lead to the development of insulin resistance. Hence, to investigate the site of insulin sensitizing action of vanadium compounds more closely, effect of vanadium compounds was studied at the cellular level using C2C12 myoblasts and 3T3-L1 adipocytes. BMOV and VUR1 when incubated alone significantly increased the uptake of radiolabeled glucose by C2C12 myoblasts. However, when co-incubated with insulin, these compounds did not potentiate the insulin action indicating that vanadium acts as an insulin mimik rather than insulin enhancer in vitro. Similar results were obtained in 3T3-L1 adipocytes. Vanadium compounds alone significantly increased intracellular triglyceride synthesis in 3T3-L1 adipocytes. However, the triglyceride synthesis in response to vanadium treatment alone was not significantly different from that of co-incubation of vanadium complexes with insulin, indicating that vanadium acts as an insulin mimik in vitro as opposed to insulin enhancer in vivo.

Treatment with BMOV and VUR1 significantly decreased the elevated cholesterol levels of both type I and type II diabetic rats and elevated triglyceride levels of type I diabetic rats. However, they did not produce any significant effect on the triglyceride levels of non-diabetic and type II diabetic rats. Though, both, BMOV and VUR1 normalized lipid profile of diabetic rats, the effect was more pronounced in case of VUR1. The improvement in lipid homeostasis is reported to be independent of glucose homeostasis.
Treatment with BMOV and VUR1 significantly improved the impaired kidney and liver functions without any significant effect on the normal functions of control rats indicating no nephrotoxic and hepatotoxic effect of vanadium therapy under the conditions of this experiment. Treatment did not have any significant effect on the body weights, food and water intakes of control as well as type II diabetic rats suggesting that all the observed effects of vanadium therapy are independent of their effect on body weight and food-water intake. The treatment, however, significantly decreased elevated food and water intake of type I diabetic rats without any significant effect on their body weights. The improvement of kidney and liver functions and food and water intake could be due to the alleviation of diabetic condition of diabetic rats.

It can be concluded that BMOV and the newly synthesized vanadium complex, VUR1, significantly improved deranged glucose and lipid metabolism and kidney and liver functions of both type I and type II diabetic rats. Improvement in glucose metabolism was not associated with any change in insulin homeostasis indicating insulin sensitizing action of vanadium compounds. Vanadium compounds alone increased triglyceride synthesis in 3T3-L1 adipocytes and glucose transport in C2C12 myoblasts. These compounds did not potentiate the effect of insulin at these target organs indicating that vanadium compounds act as insulin mimics in vitro as opposed to their insulin sensitizing action in vitro. In all these studies the efficacy of VUR1 was found to be slightly higher than, if not comparable to that of BMOV. The compound VUR1 also lacked the major side effect of vanadium therapy i.e. diarrhoea which was observed with BMOV treatment. Thus, both BMOV and VUR1 can be considered as adjunct therapy if not a monotherapy, in the treatment of diabetes mellitus.

Chronic treatment with chromium chloride and chromium picolinate significantly reduced the elevated glucose levels of non-diabetic and type II diabetic rats, however failed to do so in STZ-induced type I diabetic rats. Treatment with chromium chloride and chromium picolinate also significantly decreased elevated AUCg of both type I and
type II diabetic rats indicating improvement in glucose tolerance of treated rats. Decrease in the fasting glucose levels or AUCg of diabetic animals was not accompanied by any change in basal insulin levels or glucose stimulated insulin response during OGTT indicating insulin sensitizing action of chromium. Treatment with chromium compounds also significantly increased the K_{irr} values of both type I and type II diabetic rats, further substantiating the insulin sensitizing action of chromium compounds. The insulin sensitizing effect was more pronounced in type II diabetic rats having normal insulin levels and insulin response to glucose load as compared to type I diabetic rats, which are insulinopenic. To further investigate into the mechanism of insulin sensitizing action of chromium, the effect of chromium compounds was studied at the insulin target organs using 3T3-L1 adipocyte cell line and C2C12 myoblasts, a skeletal muscle cell line. When incubated alone, chromium compounds did not produce any effect on the intracellular triglyceride synthesis, however, co-incubation with insulin significantly increased the intracellular triglyceride synthesis in 3T3-L1 adipocytes. Similarly, incubation of C2C12 myoblasts with chromium compounds alone did not produce any change in basal glucose uptake. However, when incubated together with insulin, chromium compounds resulted in increased uptake by the cells and the effect was significantly higher than that of insulin alone indicating insulin sensitizing action of chromium in vitro.

Treatment with chromium chloride and chromium picolinate significantly decreased the elevated cholesterol levels of both type I and type II diabetic rats. Treatment also significantly decreased the triglyceride levels of control as well as both type I and type II diabetic rats, indicating improved lipid homeostasis. Treatment with chromium compounds significantly improved the impaired kidney and liver functions without any significant effect on the normal functions of control rats. These data also indicate that chromium therapy does not produce nephrotoxic and hepatotoxic effect under the conditions of this experiment. Treatment did not produce any significant effect on the body weights and food and water intakes of control as well as type I and type II
diabetic rats suggesting that all the observed effects of vanadium therapy were independent of their effect on body weight and food-water intake. The improvement of kidney and liver functions and food and water intake could be due to the alleviation of diabetic condition of diabetic rats.

In conclusion, our data suggest that chromium compounds possess significant anti-diabetic activity in various experimental models of diabetes that were not chromium deficient. The anti-diabetic activity of chromium is mainly due to its insulin enhancing effects particularly at the peripheral tissues including skeletal muscle and adipocytes. They did not exert any toxic effects under the conditions of the present investigation, rather improve the impaired kidney and liver functions that are subsequent to diabetes mellitus. Chromium picolinate appears to be more effective in all these actions compared to chromium chloride.