SUMMARY
&
CONCLUSION
The metabolic syndrome (MS) is characterized by a cluster of common pathologies: abdominal obesity linked to an excess visceral fat, insulin resistance, dyslipidemia with hypertriglyceridemia and hypertension. Insulin resistance is postulated to be the common underlying pathogenic link between the various components of the MS (Ferrannini et al., 1991). The syndrome is occurring at epidemic rates, with dramatic consequences for human health worldwide and appears to have emerged largely from changes in our diet and reduced physical activity. In general excessive intake of calories, specifically refined carbohydrates, fats and cholesterol are considered to the dietary risk factors for the MS (Dario Giugliano et al., 2008). An important but not well appreciated dietary change has been the substantial increase in the fructose intake, which appears to be an important causative factor in the MS. The MS has become a subject of great interest because of its association with type 2 diabetes mellitus (T2DM) and atherosclerotic cardiovascular disease (Jacobs et al., 2004).

A recent estimate of the prevalence of the MS suggests that 25% of adults in the United States have this condition (Ford et al., 2002: Park et al., 2003). It increases with age in both sexes. Looking at various studies around the world, which included population samples aged from 20 to 25 and upwards, the prevalence varies from 8% (India) to 24% (USA) in men and from 7% (France) to 46% (India) in women (Cameran et al., 2004). The prevalence of diabetes mellitus (DM) and the MS are rapidly increasing in Asia including India. The MS prevalence is much higher in DM patients and insulin resistance (IR) is believed to be the underlying cause for the both T2DM and MS (Meigs et al., 2007).

Experimental animals fed a high fructose diet (>60% of total calories) provide a useful animal model of metabolic syndrome/insulin resistance (Thorburn et al., 1989). The sites of fructose induced insulin resistance are documented to be the liver, skeletal muscle and adipose tissue. The rats also develop a cluster of abnormalities, which include hypertension, hypertriglyceridemia, hyperglycemia and glucose intolerance in addition to hyperinsulinemia. It has been documented that hypertension develops when normal rats are fed a fructose-enriched diet, as early as two weeks after initiation of diet (Lee et al., 1994). Fructose has also been shown to have pro-oxidant effects. Both enhanced oxidative damage to cellular
constituents and diminished antioxidative capacity have been reported in fructose fed rats (Faure et al., 1997). Fructose is a highly reactive reducing sugar, considerably greater than other reducing sugars (e.g., glucose and lactose) and promotes the formation of advanced glycation end products (AGEs), which appear to accelerate the aging process and to play a role in the pathogenesis of diabetes complications (McPherson et al., 1988).

Nature has been a source of medicinal treatment for thousands of years, and plant based systems continue to play an essential role in the primary health care. In the last few years there has been an exponential growth in the field of herbal medicine and these drugs are gaining popularity both in developing and developed countries because of their natural origin and less side effects. Ocimum sanctum (OS; Lamiaceae) is a widely grown, sacred plant of India. It is commonly known as Tulsi in Hindi, Tulasi in Telugu and Holibasil in English. The ancient systems of medicine including Ayurveda, Greek, Roman, Siddha, and Unani, have mentioned its therapeutic applications in cardiovascular disorders, diabetes and asthma without any adverse effects or toxicity (Sethi et al., 2003). Traditionally OS is used to treat malaria, cholera, gastric disorders, hepatic infections, cutaneous diseases, earache and many other ailments. Ocimum sanctum leaves are abundant in tannins like gallic acid, chlorogenic acid etc., and also contain alkaloids, glycosides and saponins along with volatile oil (Gupta et al., 2002). The leaves of OS have been traditionally used in the treatment of DM. Intake of OS leaves also led to significant increase in antioxidant property and reduction in peroxidized lipid levels (Jyothi Sethi et al., 2004). Although anti hyperglycemic and hypoglycemic activities are of this plant were well documented, studies regarding its efficacy in management of hyperinsulinemia and its role in prevention of metabolic syndrome/insulin resistance have not been undertaken. Therefore, the present study was designed to investigate whether aqueous extract of OS whole plant has any effect in the prevention of fructose induced metabolic syndrome/insulin resistance in rats, by studying the efficacy of this plant in preventing the development of hyperglycemia, hypertriglyceridemia, glucose intolerance and insulin resistance.
Earlier studies on antihyperglycemic and antihyperlipidemic activity of *O. sanctum* are fragmentary and no studies are available on the efficacy of *O. sanctum* in preventing metabolic syndrome. However, very little information is available on antioxidant activity of *O. sanctum*. So, the present study was undertaken to explore possible beneficial effects of *O. sanctum* whole plant in prevention of metabolic syndrome.

All animals were 6 weeks of age, weighing about 200 g at the time of dietary manipulation. The animals were randomly assigned into four groups of eight each: 1) Group-C animals received control diet 2) Group-F animals received fructose diet 3) Group-F+OSAE animals received fructose diet and treated with OSAE (400 mg/kg/day) by gastric intubation. 4) Group-C+OSAE animals received control diet and treated with OSAE (400 mg/kg/day) by gastric intubation. The animals were maintained in their respective groups for 60 days. The dose of OSAE used in the current study was based on the earlier report on the antihyperglycemic effect of this plant on experimental diabetic rats (Grover *et al.*, 2000) and our previous dose fixation studies (unpublished observation) for anti-hyperglycemic effect of OS in alloxan-induced diabetic rats.

Group-F animals showed a gradual and significant increase in plasma glucose, insulin, triglycerides and body weight during experimental period along with glucose tolerance. The glucose-insulin index of group-F is significantly higher indicating the development of insulin resistance. Co-administration of OSAE along with fructose diet (group-F+OSAE) completely prevented fructose diet induced gain in body weight, hyperglycemia, hypertriglyceridemia, glucose intolerance and insulin resistance with partially prevented hyperinsulinemia. Group-C+OSAE showed normoglycemia with significantly lower insulin levels suggesting the beneficiary role of OSAE in increasing insulin sensitivity in normal treated rats. There is no difference in gain in body weight, plasma triglycerides, glucose tolerance and glucose insulin index between group-C+OSAE and group-C.

Decreased activities of key glycolytic enzymes (HK and PFK in liver and muscle) seen before the entry of fructose metabolites into glycolytic pathway with enhanced PK enzyme activity was observed in liver and muscle of group-F animals. Group-F showed enhanced
operation of gluconeogenesis in liver and kidney as evident by enhanced activities of gluconeogenic enzymes (fructose-1, 6-bisphosphatase and glucose-6-phosphatase). Group-F exhibited enhanced glycogen stores of liver and muscle with decreased hepatic glycogen phosphorylase activity and enhanced operation of HMP shunt (G6PDH). The increased activity of G6PDH in liver provides large amounts of NADPH needed for lipid synthesis and it may be an adaptive mechanism to combat the oxidative stress as NADPH is need for regenerating GSH from GSSG. All these abnormalities were completely prevented except hepatic glycogen content in group-F+OSAE.

Increased hepatic fructokinase activity was observed in group-F. The fructokinase activity of group-F+OSAE was significantly lower than that of group-F but still significantly higher than that of group-C. The increased activity of hepatic fructokinase in group-F and F+OSAE was due to fructose overload on liver. No change in liver hepatic fructokinase activity was observed in group-C+OSAE when compared to group-C.

Thus the enhanced peripheral utilization of glucose by enhanced activities of glycolytic enzymes and decreased production of glucose by decreasing the gluconeogenic and glycogenolytic enzyme activities of group-F+OSAE compared to group-F may be responsible for the observed antihyperglycemic activity of OSAE which indicated that the above changes may be a sequel to improved insulin sensitivity in group-F+OSAE compared to group-F.

Enhanced activities of intestinal disaccharidases (maltase, sucrose and lactase) were observed in group-F compared to group-C indicating the increased rate of digestion of disaccharides in insulin resistant condition. Therefore, they may play an important role in aggravating postprandial hyperglycemia. OSAE treatment significantly reduced than activities of intestinal disaccharidases in group-F+OSAE which indicate preventive effect of OSAE treatment against postprandial hyperglycemia by delaying the absorption of disaccharides from intestine.

The observed elevation in the activities of hepatic and renal transaminases of group-F is an indication of increased protein degradation and amino acid catabolism in the fructose fed condition, thus providing precursors for gluconeogenesis. Administration of OSAE for 60 days prevented the increased transaminase activities.
The hepatic and cardiac tissue lipids (total lipid content, cholesterol, triglycerides, phospholipids and free fatty acids) are markedly elevated in group-F compared to group-C. It is well known that the process of lipid accumulation interferes with utilization of glucose through principles of Randle cycle (Randle, 1998). The lipogenic character of fructose was evident with increased activity of FAS and malic enzyme in liver and decreased activity of lipoprotein lipase in adipose tissue of fructose fed animals. The enhanced tissue lipid content was completely prevented by OSAE treatment in group F+OSAE. There was no deviation in tissue lipids and lipogenic enzymes of group-C+OSAE when compared with group-C.

The alterations observed in the activities of enzymes of carbohydrate and lipid metabolism in group-F rats were significantly prevented by OSAE treatment in group-F+OSAE.

The development of oxidative stress, an imbalance between pro- and anti-oxidant status, has been shown to play an important role in mediating insulin resistance, and therefore we studied the oxidative status and antioxidant potential of OSAE. Hyperglycemia, advanced glycation end products, autooxidation of glucose, polyol pathway and intracellular accumulation of lipids and metabolic alterations all leads to the increased formation of oxygen-derived reactive oxygen species, which cause damage either directly affecting a specific molecule or indirectly by forming numerous toxic derivatives, High ROS production and decreased protection by low amounts of antioxidants by dietary fructose had been suggested as a possible mechanism for the detrimental effects of fructose (Busserolles et al., 2002).

The enhancement of tissue LPO and protein oxidation (liver, pancreas and heart) in group-F indicates the existence of oxidative stress in fructose fed condition. In addition, defective antioxidant status was evident in group-F from the decreased GSH content and significantly lowered activities of GSH dependent (GR,GST, GPx) and independent enzymes (SOD and CAT). These abnormalities were prevented with the treatment of OSAE in group- F+OSAE.

Enhanced operation of polyol pathway of group-F was evident by the observed elevated activities of aldose reductase and sorbitol dehydrogenase in liver, pancreas and heart compared
to group-C. Treatment with OSAE prevented the alterations observed in polyol pathway enzymes. Further, our results of the XRF analysis of OSAE showed the presence of elements (K,Cr,Cu,Zn, Mn and Se) that posses beneficiary role in improving glucose tolerance.

It appears that apart from acting on carbohydrate metabolic targets, compounds present in medicinal plants both alone or in combination, possess a variety of beneficial activities and have the potential to impart therapeutic effect holistically in complicated disorders like metabolic syndrome.

The histopathological changes in various organs were studied in four different groups of animals. The following changes were observed in histopathological studies of group-F animals:

- Wending sinusoid spaces and mild hemorrhages were observed in hepatocytes
- Pancreas showed degenerated islets of langerhans
- Small intestine showed degenerated intestinal mucosa
- Degenerated myocardial fibers and blood clumps were seen in heart
- Degenerated bowman’s capsule and damaged renal cells were seen in kidneys
- Degenerative changes were observed in adipose tissue

Co administration of OSAE along with fructose diet (group-F+OSAE) significantly prevented all the histological alterations caused by fructose diet. Normal histological features were seen in group-C and C+OSAE animals.

It is known that high fructose feeding leads to increased oxidative stress which may be responsible for the degenerative changes in the various organs of group-F animals. OSAE treatment significantly prevented the degeneration of organs, suggesting that it attenuates excessive formation of ROS. Our biochemical studies were also revealed the antioxidant property of OSAE.

No toxic effects of OSAE treatment was observed when normal animals were treated with OSAE during the experimental period. No visible side effects and variation in animal behavior (respiratory distress, abnormal locomotion and catalepsy) were observed in group-C+OSAE indicating the non-toxic nature of OSAE. OS has been used for long periods in Ayurveda, Siddha, Folkloric, Unani and other systems of medicine for wide range of diseases.
without any evidence of adverse effects of toxicity. Approximate LD50 of Ocimum sanctum was found to be 4505±80 mg/kg body weight on administration by oral route and 3241±71 mg/kg, body weight by intra-peritoneal routes. In Ayurveda, OS is considered as safe drug (Bhargava and Singh., 1981).

The multiple beneficiary properties like antihyperglycemic activities by manipulating carbohydrate and lipid metabolisms respectively, with antioxidant potential of OSAE offers an exciting opportunity to develop this into a novel therapeutic approach for insulin resistance. The present study provides additional evidence in support of use of OS in Ayurveda for hyperlipidemia, diabetes mellitus and other related metabolic disorders. In conclusion, aqueous extract of Ocimum sanctum is useful as an adjuvant for the prevention and/or management of metabolic syndrome.