General Summary

- Dietary fenugreek (*Trigonella foenum-graecum*) seeds and the Allium spice - onion (*Allium cepa*) are independently known to be effective in the management of diabetes. The fibre-rich fenugreek seeds and onion are now understood to possess significant hypoglycemic as well as hypolipidemic action, and may also possess antioxidant potential in diabetic situation. Both hypolipidemic and antioxidant potential are likely to have far-reaching implication in alleviating secondary complications associated with diabetes. The mechanism of action of these two spices is dissimilar with respect to modulation of glucose homeostasis. Hence the combined use of these two ingredients may have an additive health beneficial influence.

- Hence, this investigation was intended to explore synergy between dietary fenugreek seeds (as a provider of soluble fibre) and onion in deriving health benefits to the maximum with respect to: (a) hyperglycemia and attendant abnormalities in experimental diabetes, (b) beneficial modulation of compromised antioxidant status, and (c) renal lesions, cataract and cardiac disease.

- Beneficial influence of a combination of dietary fenugreek seeds and onion on hyperglycemia and its associated metabolic abnormalities was evaluated in streptozotocin-induced diabetic rats. Diabetes was experimentally induced with streptozotocin and diabetic rats were fed with 10% fenugreek seed or 3% onion (freeze-dried) or their combination for 6 weeks.

- These dietary interventions significantly countered hyperglycemia, partially improved peripheral insulin resistance and impaired insulin secretion, reduced β-cell mass.

- Dietary fenugreek or onion markedly reversed the abnormalities in plasma albumin, urea, creatinine, glycated haemoglobin and advanced glycation end products in diabetic rats. These beneficial effects were highest in fenugreek+onion group.

- Diabetic rats maintained on these dietary interventions excreted lesser glucose, albumin, urea, and creatinine which were accompanied by improved body weights compared to diabetic control.

- These dietary interventions produced ameliorative effects on pancreatic pathology as reflected by near-normal islet cells, restored glycogen and collagen fiber deposition in diabetic rats.

- Thus, this study documented the hypoglycaemic and insulinotropic effects of dietary fenugreek and onion, which were associated with countering of metabolic abnormalities and pancreatic pathology.
Oxidative stress has a crucial role in the progression of diabetes and its complications. The present study evaluated the additive beneficial effect of dietary fenugreek seeds (10%) and onion (3%) on oxidative stress in diabetic rats.

Hyperglycemia derived ROS impairs the endogenous antioxidant defense system in many ways during diabetes. Activities of enzymes of antioxidant defense system (SOD, CAT, GPx, GR, and GST) were increased in plasma in diabetic condition, probably as an adoptive mechanism during oxidative stress.

It was evidenced that all the three dietary interventions restored plasma antioxidant enzymes and molecules, this effect being more with the combination. This observation was consistent with decreased levels of circulating oxidative stress markers.

The combination of fenugreek and onion showed a positive additive effect with regard to restoring the circulating glutathione reductase and ascorbic acid along with lowering of the circulating oxidative stress markers.

It is inferred that an alleviation of oxidative stress contributes further to the antidiabetic influence and this nutraceutical potential of fenugreek seeds and onion was higher when these two were consumed together.

The altered antioxidant status and lipid abnormalities in the liver of diabetic rats were significantly countered by dietary fenugreek seeds / onion or their combination. A higher beneficial effect was achieved by the combination, sometimes amounting to an additive effect. The histopathological observation substantiated that these spices protected the oxidative damage of hepatocytes.

Hyperglycemia is one of the metabolic and homeostatic abnormalities that increase the cardiovascular mortality in diabetic patients by increased oxidative stress.

In continuation of our observed amelioration of oxidative stress in cardiac tissue by dietary fenugreek seeds and onion in streptozotocin-induced diabetic rats, the mechanistic aspects of the cardio protective influence of dietary fenugreek seeds (10%) and onion (3% powder) on hyperglycemia-mediated myocardium damage was further investigated in this study.

These dietary interventions countered hypercholesterolemia, especially from LDL-associated fraction. The elevated heart cholesterol, triglycerides and phospholipids under diabetic conditions were significantly countered by these dietary interventions, the effect being higher with the combination, amounting to an additive effect.

These findings were also corroborated by restoration of histopathological abnormalities of the heart tissue along with lowered heart weights.
Cardio protective influence of these dietary spices was evidenced by their blocking potential on renin-angiotensin system (RAS). This might be the consequence of reduced activation of Angiotensin-converting enzyme (ACE) and Angiotensin Type 1 receptor (AT₁) in cardiac tissue.

Increased expression of Type IV collagen, Fibronectin, Bax, 4-HNE, iNOS and metabolites of NO (Nitrate/Nitrite) along with disturbed PUFA to SFA ratio and activities of cardiac marker enzymes in blood confirmed the myocardial damage in diabetic condition.

Dietary fenugreek seed, onion, and fenugreek+onion were found to ameliorate these pathological changes, where the beneficial effect on CVD being higher with the combination sometimes amounting to an additive or even synergistic effect in nature.

This investigation evaluated the protective influence of dietary fenugreek seeds (10%) and onion (3%) on erythrocytes of streptozotocin-induced diabetic rats, particularly assessing their potential in modulating reduced haematological indices and antisickling potency.

This study also evaluated the altered lipid profile in erythrocyte membrane resulting from diabetic hyperlipidemia and further insights were made into the beneficial countering of increased lipid peroxidation, osmotic fragility, along with reduced ATPase activity, reduced membrane fluidity and deformability, nitric oxide production and echinocyte formation.

The present investigation demonstrated that fibre-rich fenugreek seeds and sulfur compounds-rich onion elicit the haemato-protective influence in experimental diabetes. Dietary fenugreek seeds and onion appeared to counter the deformity and fragility of erythrocytes partially in diabetic rats by their antioxidant potential and hypocholesterolemic property.

The antisickling potency of these spices was accomplished by a substantial decrease in echinocyte population, nitric oxide metabolites and AGEs in diabetic rats. The beneficial protective effect to the red blood cells by these spices being higher with fenugreek+onion.

Further insight into the factors that might have reduced the fluidity of erythrocytes in diabetic rats revealed changes in the cholesterol: phospholipid ratio, fatty acid profile, and activities of membrane-bound enzymes.

Oxidative stress plays a major role in the progression of diabetes and pathogenesis of diabetic nephropathy. In this study, the beneficial influence of dietary fenugreek
seeds and onion on oxidative stress-mediated renal injury was evaluated in streptozotocin-induced diabetic rats.

- Animals maintained on these dietary interventions countered nephromegaly, increase in glomerular filtration rate, and oxidative stress in the renal tissue. The up-regulation of the receptor for advanced glycation end products, inflammatory cytokines and oxidative stress markers in the renal tissue of diabetic rats was effectively countered.

- Renal 8-hydroxy-2-deoxyguanosine (marker of oxidative DNA damage), its excretion, DNA fragmentation and mitochondrial DNA deletion were significantly annulled in diabetic rats by these dietary interventions.

- In continuation of the observed alleviation of oxidative stress-mediated renal injury by dietary fenugreek seeds and onion in streptozotocin-induced diabetic rats, the mechanistic aspects of the nephroprotective influence of dietary fenugreek seeds (10%) and onion (3% powder) on hyperglycemia-mediated renal lesions was further investigated in diabetic rats.

- Renal damage was assessed by the extent of proteinuria and enzymuria. Renal integrity was assessed by measuring the activities of enzymes of carbohydrate metabolism, polyol pathway, transaminases, phosphatases, Angiotensin converting enzyme (ACE) and ATPases.

- Diabetic condition resulted in the up-regulation of renal glucose transporters, which was significantly countered by the dietary interventions. These interventions significantly reduced metabolites of polyol pathway, nitric oxide, inducible nitric oxide synthase and N-acetyl-β-D-glucosaminidase activity.

- Furthermore, diabetic condition results in increased activity of renal ACE and mRNA/protein expression of ACE, which was down-regulated by the dietary interventions.

- Markers of podocyte damage including nephrin, podocin, podocalyxin and their subsequent loss in urine were notably normalized with down-regulated KIM-1 expression by these dietary interventions.

- These dietary interventions effectively countered the diabetes-induced structural and functional abnormalities of renal tissue.

- Generally, the beneficial influence of dietary fenugreek and onion on oxidative stress-mediated renal injury was higher when consumed together and this may be strategic to ameliorate the diabetic nephropathy.
Hyperglycemia induced osmotic and oxidative stress in the eye lens is thought to be involved in the pathogenesis of diabetes related secondary complication— retinopathy.

In continuation of our previous observation of the ameliorative influence of these spices on hyperglycemia, attendant metabolic abnormalities, and oxidative stress in tissues of diabetic rats, beneficial influence of dietary fenugreek seeds, onion or their combination was investigated on diabetes-induced alteration in the eye lens in experimental diabetic rats.

Animals maintained on these spices showed significantly countered oxidative stress markers (ROS, MDA and PCO), AGEs and expression of their receptor (RAGE) in the eye lens.

Increased activity of polyol pathway enzymes (Aldose reductase and Sorbitol dehydrogenase), protein and mRNA expression was significantly countered in the cataractogenic lens as a result of these dietary interventions.

Altered crystallin (α-A and α-B) distributional profile, their expression, activity of carbohydrate metabolizing enzymes and antioxidant status was significantly annulled by these dietary treatment.

Ophthalmological examination of treated rat’s eye lens indicated that these dietary interventions resulted in a significantly delayed cataractogenesis in diabetic rats. This investigation evidenced for the first time a beneficial modulation of the progression of cataractogenesis, implicating their potential in ameliorating cataract resulting from diabetics.

On the whole, this investigation provided more insights into the mechanism of action of dietary fenugreek seeds and onion in alleviating metabolic abnormalities associated with diabetes and the secondary complications of the same.

In general, the antidiabetic influence of dietary fenugreek seeds and onion were higher when these two interventions were combined. The basic information generated in this investigation is likely to lead to an effective dietary strategy in the management of metabolic abnormalities and the secondary complications of diabetes.

It may be strategic to derive maximum nutraceutical antidiabetic benefits from these functional food ingredients by consuming them together.