Abstract

Type 2 diabetes (T2D) is the most prevalent and serious metabolic disease all over the world. Increased oxidative stress plays an important role in the progression and onset of diabetes and its complications therefore, strategies meant to reduce the oxidative stress are needed which can mitigate these deleterious process. The present study aims to investigate the in vitro ameliorative potential of four antioxidant molecules in L6 myotubes under oxidative stress condition induced by 4-hydroxy-2-nonenal (HNE) and also to comprehend the gene expression patterns of oxidative stress genes, insulin signalling genes, MAPK signalling genes and PPAR signalling gene upon the supplementation of different antioxidants in induced stress condition. The study results demonstrated a marked increase in the level of malondialdehyde levels, protein carbonyl content levels, LDH levels and caspase-3 activity levels with a subsequent increase in the free radicals that was reversed by the pretreatment of different dietary antioxidant. From the expression analysis of all the pathway genes, it is evident that the expression of these genes is modulated by the presence of antioxidants. The highest expression was found in the cells treated with Insulin in conjugation with an antioxidant. Resveratrol is the most potent modulator followed by Baicalein, Curcumin, and Carvacrol. The present study results ascertains the potency of Resveratrol along with Insulin in scavenging the reactive oxygen species (ROS) generated under induced stress conditions through antioxidant defense mechanism against excessive ROS production, contributing to the prevention of oxidative damage in L6 myotubes.

Further, in vivo experiments were done, for the two best antioxidant molecules obtained from the results of in vitro experiments. In the present study, experimental diabetes was induced by a single intraperitoneal (i.p.) injection of STZ (55 mg /kg b.wt), 15 min after the i.p. administration of NA. The findings of the in vivo study showed that oral administration of Resveratrol and Baicalein to STZ-NA induced diabetic rats significantly
lowers the blood glucose level and has a similar effect as the oral hypoglycemic drug Glibenclamide in reducing the alterations in various biochemical parameters such as TC, TG, HDL, LDL, AST, ALT, plasma insulin, urea and uric acid. Resveratrol and Baicalein showed potential oxidative stress amelioration in STZ-NA induced diabetic rats which were ascertained by improved glycemic levels and reduced lipid peroxidation levels along with improved antioxidant enzymatic activities. Moreover, both the antioxidant Resveratrol and Baicalein increased the mRNA levels of SOD, CAT, GPx and GST in the liver tissues, as well as enhanced the mRNA levels of PPARγ and FALDH in adipose tissue of STZ-NA induced diabetic rats. These results suggest that Resveratrol and Bacalein supplementation may reduce diabetes. In the comparative analysis, Resveratrol was found to be more potent antioxidant, antihyperglycemic and antidyslipidimic efficacies as compared to the other three tested antioxidant molecules, suggesting that Resveratrol holds enormous potential in controlling oxidative stress mediated hyperglycemia in in vitro and in vivo models.

Another important aspect of T2D is a genome-wide association study (GWAS). GWAS studies have revolutionized diabetes genomics research worldwide and have illuminated single nucleotide polymorphism in several genes including, FTO, KCNJ11, and SLC30A8. To investigate the involvement of these polymorphisms in conferring susceptibility to T2D in the North East Indian population, this pilot study was attempted. DNA was extracted from blood samples of 155 patients with diabetes and 100 controls. Genotyping was performed by PCR-RFLP and DNA sequencing. To confirm the association between inheritance of SNP’s and T2D development, logistic regression analysis was performed. The “p values” obtained were compared and interpreted. For the rs9939609 variant, of FTO gene, the dominant model AA/(AT+TT) revealed a statistically significant association with T2D [OR=2.03, p=0.02], but was non-significant post correction for multiple testing (p=0.002). For the rs13266634 variant (SLC30A8), there was a considerable
difference in the distribution pattern of genotypic polymorphisms between cases and controls (p = 0.004). A significant association was observed in case of the recessive model (CC+CT)/TT [OR=4.56 p=0.001], after adjusting for age, gender, and Body Mass Index. Similarly, a significant association (p=0.001) of BMI>26 kg/m\(^2\) could be established with the \textit{SLC30A8} polymorphism assuming dominant model. The current study demonstrated a modest but statistically significant effect of \textit{SLC30A8} (rs13266634) polymorphisms with BMI levels. Considering the burgeoning prevalence of T2D in the Indian population, the contribution of these genetic variants studied, to the ever-increasing number of T2D cases, appears to be relatively low. Nevertheless, this study could serve as a foundation for performing future GWAS involving larger populations.