MATERIALS/CHEMICALS

All Chemicals of AR grade were obtained from M/S Merck Ltd. and M/S Rankem Ltd. 100bp DNA marker was purchased from M/S SRL Ltd and other enzymes from M/S Sigma-Aldrich Ltd. Fluoride standards and TISAB were procured from M/S Thermo Orion. Polypropylene labwares for fluoride estimation and tissue homogenate storage vials were brought from M/S Tarsons and M/S Polylab.

PLANT MATERIAL

Standardized aqueous stem and leaf extract of Panax ginseng (Asian Ginseng, GE HPLC grade having 80% ginsenosides) (Batch No: GPE80-061108) and leaf extract of Lagerstroemia speciosa (Banaba, BLE having 1% corosolic acid (Spectrophotometric analysis) (Batch No: BLP01-051608) were procured from Changsha Botaniex Inc, China.

(i) ANIMALS : ETHICAL GUIDELINES AND ANIMAL MAINTENANCE

Adult albino mice, Mus musculus, (males) three months old, weighing 25 ± 5g were procured from Sri Raghavendra Enterprises, Bangalore and acclimatized to laboratory conditions (12:12hr dark/light, 25 ± 2°C) for one week before commencement of the experiment. They were maintained on standard rodent pellet and tap water ad libitum; in accordance with the guidelines of the National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, and experimental protocol was approved by the Institutional Animal Ethical Committee, Bangalore University, Bangalore (CPCSEA registration no. 402, file no. 25/525/2009 and dt 23.03.2011).

(ii) DIABETOGENIC ACTIONS OF STREPTOZOTOCIN

Experimentally induced diabetes is advantageous for the analysis of biochemical, hormonal and morphological changes that occur during the development of diabetes and even in diabetic state (Andrade-Cetto and Heinrich, 2000). Streptozotocin (STZ, 2 – deoxy – 2 - ( 3 – methyl – 3 – nitrosourea)-1-D-glucopyranose), a diabetogenic agent having the ability to induce pancreatic β-cell toxicity, is one of the chemical used worldwide to induce diabetes in a simple and convenient method. It is an antibiotic synthesized by Streptomyces achromogenes (M.W. 265 and empirical formula – C₁₄H₂₄N₅O₁₂) (Dorr and Fritz, 1980).
Streptozotocin is commonly used to induce insulin-dependent diabetes mellitus in experimental animals (Punithavathi et al., 2008). According to Szkudelski (2001), STZ enters the insulin secreting pancreatic β-cells through glucose transporter-2 thereby cause DNA damage and finally triggers pancreatic β-cell necrosis. Streptozotocin has the potential to increase pancreatic islet protein glycation resulting in β-cell necrosis (Konrad et al., 2000). Besides, STZ acts on O-GlcNAcase (O-linked-β-N-Acetylglucosaminidase), an enzyme responsible for the removal of O-GlcNAc from protein especially in pancreatic β-cells, leading to irreversible β-cell toxicity (Konrad et al., 2001). Several studies have been made to unravel the mechanisms of oxidative damage caused by acute doses of STZ and its impact on pancreatic islet cells where it produces ROS or stimulate H$_2$O$_2$ generation (Friesen et al., 2004) and impairs antioxidant system (Coskun et al., 2005). Chemically, STZ impairs glucose oxidation (Bedoya et al., 1996) and thereby decrease the insulin biosynthesis and its secretion (Bolaffi et al., 1986; Nukatsuka et al., 1990). However, the action of STZ on β-cells is accompanied by characteristic alterations in blood insulin and glucose concentrations, which reflects the abnormalities of β-cell function.

The sensitivity of STZ varies with species, strain, sex and nutritional status (Balamurugan et al., 2003). Intravenous or intraperitoneal administration of STZ to laboratory mice in multiple sub-diabetogenic doses induce(s) pancreatic insulinitis with gradual destruction of insulin-secreting β-cells (Zafar and Naqvi, 2010). Single intravenous administration of STZ at dose of 40-68mg/kgbw to rats was found to be effective in inducing IDDM (Ganda et al., 1976). Lopes et al. (2004) induced diabetes in rats by intravenous administration of STZ at a dose of 65mg/kgbw and 2 days later, rats were found to have blood glucose levels more than 300mg/dl. Intraperitoneal administration of STZ was also efficacious at 40-60mg/kgbw or higher doses (Katsumata et al., 1992). Similar results were also observed in studies of Jayasri et al. (2008) and Zafar et al. (2009).
INDUCTION OF DIABETES BY STREPTOZOTOCIN ADMINISTRATION

To laboratory mice (12 hr fasted), diabetes was induced by a single intraperitoneal dose of 50 mg/kg bw of STZ dissolved in freshly prepared 0.1 mol/l citrate buffer (pH 4.5). On the fifth day of STZ injection (Katakam et al., 2005), the blood sample was obtained by sequential snipping of the tail (Fluttert et al., 2000). A glucometer (One-touch Horizon Glucometer, LifeScan, Johnson & Johnson Company, LifeScan, Milpitas, CA USA 95035) was used to measure the blood glucose levels. Mice with diabetes having hyperglycemia (>200 mg/dl) were taken for the experiment.

(iii) RATIONALE FOR THE SELECTION OF 600 ppm NaF (270 ppm F) DOSE OF FLUORIDE

Research on fluoride toxicity has sometimes been criticized for repeated high dose regimens that are commonly used. It is true that many of toxic regimens are not clinically designed but are intended to maximize the toxicity of fluoride to better understand the mechanisms and consequences. Moreover, humans encounter higher doses of fluoride due to secondary sources. For instance, osteoporosis treatments use more than 600 ppm fluoride (Demos et al., 2001) and certain dental formulations possess more than 1,000 ppm fluoride (Twetman et al., 2003; Davies et al., 2003; Curnow et al., 2002). In certain parts of Andhra Pradesh and Rajasthan (India), human and animal populations are exposed to more than 36 ppm fluoride through drinking water (Brindha and Elango 2011; Garg et al., 2009). Several studies used 600 ppm NaF as a dose to induce toxicity for variety of assessments ranging from reproductive, renal, and neurotoxicity (Nabavi et al., 2012b; Sinha et al., 2007). Thereby 600 ppm NaF (having 270 ppm fluoride) dose was used in this study to induce toxicity. Furthermore, the NOAEL (no observable adverse effect level) of fluoride for rats and mice is between 150 and 200 ppm fluoride (~18 mg/kg bw/day) in drinking water and no mortality was observed in the present study with 270 ppm fluoride treatment.

Preparation of fluoride water

A stock of 1000 ppm fluoride solution was prepared by dissolving 2.21 g of sodium fluoride in 1 l of tap water (The concentration of fluoride in tap water
during experimentation was 0.06 mg/l. To prepare 270ppm fluoride water (600ppm NaF), 270ml of the stock solution was taken and made to 1l with tap water.

**(iv) DATA INTERPRETATION AND STATISTICAL ANALYSIS**

The results of this study are expressed as mean ± standard error of mean. Values represented in parenthesis in respective chapters and tables represent the % recovery with regard to efficacy of phytoextracts used (viz Ginseng and Banaba) upon fluoride intoxication to diabetic mice. ‘-‘ sign indicates a decrease, ‘+‘ sign indicates an increase over positive controls. The % recovery was calculated as follows:

\[
Recovery = \frac{(A - B)}{A} \times 100
\]

A – Positive control
B – Herbal extracts supplemented groups

**Statistical analysis**

To represent statistics of *in vitro* studies, the linear regression analysis was done for calculating IC\textsubscript{50} values.

To determine the optimum duration of antioxidant supplementation, a pilot study for a period of 60-days was conducted and the data was analyzed statistically using both one-way analysis of variance and two-way analysis of variance (ANOVA).

Statistical analysis for all biochemical parameters was done by one-way analysis of variance (ANOVA) with Bonferroni and Duncan’s multiple range test (DMRT) post hoc at P < 0.05 level of significance by using SPSS software (15.0 version).

Data analysis for the genotoxicity studies were performed by Students t-test (P<0.05) using SPSS software (15.0 version).

The graphical preparations were made using OriginPro software 7.0.