Toxic effects of chemicals and drugs in experimental animals have been evaluated using a number of approaches such as clinical assessment, nephrotoxicity, hepatotoxicity and neurotoxicity. Histopathological assessments comprised morphological and immunohistochemical changes and has long been employed to enhance the understanding of chemical injury to functional systems.

**Diabetes and histological alterations**


Prolonged diabetes is shown to accelerate of neuronal damage, increase infarct volume and induce post-ischemic seizures (Muranyi *et al.*, 2003). According to Nahla and Refat, (2012) long duration diabetes onset resulted in complications including congestion of meningeal and cerebral blood vessels,
encephalomalacia, satellitosis, neuronophagia as well as gliosis and in the white matter demyelination. Similar results were found in studies of Baydas et al. (2002) and Navaratna et al. (2011) and authors demonstrated gliosis and neurodegeneration in long duration onset diabetic rats.

**Fluoride and histological alterations**

*Liver histopathology:*

Several authors demonstrated to show NaF caused impairments in liver function (Chinoy et al., 1991; Mello et al., 1963). Dąbrowska et al. (2006) reported dose dependent effects of fluoride and results inferred lack of distinct morphological changes in the liver at lower dose (10.6mg NaF/dm³) while high doses of NaF (32mg NaF/dm³) showed vacuolar degeneration, damage or blurring of cell or nuclear membrane and vessel dilation. Besides, prolonged exposure to high dose NaF (32mg NaF/dm³) led to liver fibrosis characterised by collagen bundles and necrosis in sinusal lumen of liver. Zonal necrosis and pyknosis of hepatocyte nuclei and disintegration in the arrangement of hepatic cords were reported by Chinoy et al. (1991). Like wise, Mello et al. (1963) showed degenerative lesions in the liver of rats upon 1ppm fluoride intoxication. Bogdanffy et al. (1995) indicated hepatocellular adenoma, hepatic foci of clear cells and basophilic alterations and sinusoidal dilation in the liver of rats and mice upon 2,500ppm vinyl fluoride exposure. Studies of Chattopadhyay et al. (2011) have reported vacuolar degeneration, micronecrotic foci in the hepatocytes, and hepatocellular hypertrophy were found in the mice exposed to 15mg NaF/L for 30 days while 90 days treatment resulted in sinusoidal dilation with enlarged central vein surrounded by deep-blue erythrocytes.

*Kidney histopathology:*

Conflicting reports are available regarding fluoride induced toxicity in kidney. For instance, Bosworth and McCay, (1962) found no significant changes in kidney histology of rats upon 10ppm of NaF administration through drinking water. Similar results were also observed by DeCamargo and Merzel, (1980) in rats receiving 1, 10 and 100mg NaF/kg in drinking water for 180 days. Studies of
Machalinska et al. (2002) reported no changes in the kidney and liver of fluoride intoxicated Balb-C mice. On the contrary, experiments of Ogilvie, (1953) have indicated changes in the tubular structures of the kidney. Fluoride in high doses (100 and 250mg fluoride/kg) are shown to cause renal histological changes such as necrosis of glomeruli and tubules, atrophic glomeruli, dilations of glomerular capsule and tubules dilatation (Zhan et al., 2006a). Studies of Ramseyer et al. (1957) revealed hypertrophy and hyperplasia in the renal tubules of rats treated with 1, 5 and 100ppm of fluoride for 500 days through drinking water. Shrunken kidney structure, atrophy of glomeruli, degeneration of tubular cells and dilation of convoluted tubules were some of the changes observed in fluoride exposed mice (Kour and Singh, 1980). In rabbits, NaF exposure for 15-weeks led to significant necrotic and degenerative changes in kidney and liver (Shashi and Thapar, 2000; Shashi et al., 2002). Recent studies of Chattopadhyay et al. (2011) have showed blood filled spaces, disintegration of tubular epithelium, and atrophy of glomeruli in the kidney of mice exposed to 15mg NaF/l for 90 days.

**Brain histopathology**

Histological studies conducted on fluoride intoxicated rats exhibited changes such as decrease in size and number of hippocampal neurons and cerebellar neurons, signs of chromatolysis and gliosis in the motor cortex (Shivarajashankara et al., 2002). Developing rats fed with high fluoride and low iodine showed neuronal pyknosis in cerebral cortex and reduction in the number of Nissil substance with elongated dendrites (Ge et al., 2005). Likewise, Shah and Chinoy, (2004) showed vacuolisation and pynosis of neuronal cells in fluoride intoxicated mice. Studies of Bhatnagar et al. (2002) have found neurodegenerative changes viz., cytoplasmic eosinophilia, dark cells, condensed nucleus and reduction in the number of cells in female mice upon fluoride intoxication. Observations of El-Dien et al. (2010) indicated shrunken and deeply stained Purkinje cells in rat cerebellar cortex upon fluoride intoxication. Varner et al. (1998) reported chromatin clumping, pyknotic nuclei, vacuolisation in neuronal tissues upon fluoride intoxication to rats.
Phytoextracts and amelioration

Antioxidant abilities of ginseng as well as banaba are well documented (Kim et al., 2011; Kitts and Hu, 2000; Shareef et al., 2012). Pertaining to histopathological observations, Conflicting reports are available on the efficacy of ginseng phytoextract. Findings of Sawiress, (2011) indicated that ginseng administration ameliorated the histological alterations in kidney tissues of diabetic rats. Khalid et al. (2009) reported Panax Ginseng offered amelioration of toxic effects in developing rat liver characterised by malformed hepatocytes with ill-defined margins, nuclear fragmentation and condensation. No study is so far reported on the efficacy of banaba extract in normalising the histopathological alterations. Besides, there is a dearth in literature on the histological alterations imposed by fluoride on diabetics; hence the presence study was undertaken.

MATERIALS AND METHODS

In the present study, Group I comprises control animals given normal tap water and Group II animals consists of STZ (50mg/kgbw, ip) those were found diabetic. Group III animals treated with 600-ppm NaF in drinking water. Group IV serves as positive control where on confirmation of induced diabetes (>200 mg/dl), these diabetic animals were treated with 600ppm NaF through drinking water for 30-days. They were segregated into groups to check the efficacy of plant extracts, GE and BLE, exposed alone and together for a period of 15-days. While continuing the F exposure, from 31st day onwards, Group V, VI and VII animals were given intragastrically GE, BLE and co-exposure of GE and BLE at doses of 150mg/kgbw/day, respectively. At the end of 45th day, all test animals along with control group were sacrificed by spinal dislocation and tissues were dissected out and immediately used for histological analysis.

Histopathology

The tissues were fixed in Bouin’s fluid (saturated picric acid: formalin: glacial acetic acid mixed in the ratio 15: 15: 1) for 24hrs. The next day, they were washed with distilled water (5-6 times) and upgraded through a series of alcohol grades (70%, 80%, 90% and absolute alcohol for 15min each). The tissues were
then placed in a mixture of absolute alcohol and xylene (1:1) for 15min, and cleared in xylene for 30min. Thereafter, they were placed in a mixture of xylene and paraffin wax (1:1) for 15min and then in paraffin wax for 15min each (3 changes of paraffin wax). ‘L’ blocks smeared with glycerine were placed on a glass plate where the melted paraffin wax was poured and the tissues were finally embedded in paraffin wax.

The paraffin blocks containing the tissues then serially sectioned at 5 microns and placed on a clean glass slide pre-smeared with egg albumin white and processed further for the haematoxylin-eosin staining as follows:

The slides containing the serial sections of tissues were cleared in xylene for 30min and then downgraded through a series of alcohol (absolute alcohol, 90%, 80%, 70% for 5-10min each). The slides were washed in distilled water for 5-10min, and thereafter stained in haematoxylin stain for 45min. The slides were dipped in distilled water, and placed in 70% and 80% alcohol for 5-10 min each. The slides were further counter stained with eosin for 30secs, then dipped in 90% alcohol and placed in absolute alcohol for 1min. The slides were finally cleared in xylene for 5-10min, mounted in DPX and analysed using light microscope.

RESULTS

Changes in liver

The liver of control mice histologically appeared normal with lobular structures and cord like arrangement of hepatocytes around the central canal (Table 6.1 & Fig 6.1), while mild to severe alterations in the histoarchitecture of liver was observed in fluoride intoxicated mice. Furthermore, STZ induced diabetic mice showed pronounced hepatocellular injury by loss of normal architecture in the liver tissues. The hepatocytes appeared to be having mild cloudy swelling or ballooning with marked cytoplasmic vacuolations, fatty infiltration, nuclei of most cells revealed clear signs of pyknosis and karyolysis. The changes observed in the liver of fluoride exposed and fluoride intoxicated diabetic mice include hepatocellular hypertrophy, cytoplasmic vacuolization and hepatic sinusoidal
dilation etc. In this study, the co-supplementation of GE and BLE at a dose of 150mg/kgbw caused improvement by enriching the status of liver where the hepatocytes exhibited less degree of cell degeneration, less sinusoidal dilation and less necrotic hepatocytes.

**Changes in kidney**

The gross histological examinations showed no change in the colour on the external surface of the kidneys taken from the experimental groups (Table 6.2 & fig 6.2). The kidney of control group showed the normal architecture with no signs of pathology in cortex and medulla. STZ induced kidney exhibited distorted and slightly expanded glomeruli with slightly thickened glomerular basement membranes (GBMs) while least necrosis was noticed in some sections of convoluted tubules. Lobulated and hypertrophied glomeruli, tubular necrosis and loss of brush border leading to degeneration of convoluted tubules were seen in fluoride intoxicated animals. In fluoride intoxicated diabetic mice, besides the extensive tubular necrosis and widening of lumen, presence of lobulated, hypertrophied glomeruli, accelerated mesangial expansion and glomeruli K-W (Kimmelstiel Wilson) nodules were observed. Combined treatment of GE and BLE at dose of 150mg/kgbw was found to cause reduction in the glomerular lesions /nodules, reduced thickening of GBM and increased mesangial matrix content and tubular dilatation.

**Changes in discrete brain regions**

**Cerebrum**

Cerebral cortex of control mice showed normal morphology with intact layer of neurons and cytoplasm (Table 6.3 & Fig 6.3). Sections of cerebral cortex from diabetic mice showed degenerative changes in neurons and vacuolar spaces. High fluoride exposure to diabetic mice caused severe alterations in the cytoarchitecture of cerebral cortex. The changes observed include vacuolar spaces around the pyramidal cells and the nuclei of some neuron were pyknotic and absent. In phytoextract, GE and BLE, supplemented groups, appreciable
improvement in the organization of cellular layers was observed and a reduction in vacuolar spaces near the pyramidal cells was observed.

**Cerebellum**

Normal histoarchitecture of cerebellum comprising intact molecular layer, purkinje cell layer and granular layer were observed in control mice (Table 6.4 & Fig 6.4), while differentiated cellular layers along with an altered characteristic axonal networks of purkinje’s cell layer were observed in diabetic mice. Disorganisation of purkinje cell layer and presence of eosinophilic purkinje cells were observed in fluoride toxicated diabetic mice. A prominent space/gap in between the granular layer and purkinje cells was observed in the fluoride toxicated diabetic mice. However, GE and BLE supplementation proved advantageous where the molecular, granular as well as purkinje cell layers were quite intact.

**Hippocampus**

Fig 6.5 shows the normal morphology of the hippocampus of the control mice. Almost normal hippocampal neurons were observed in the hippocampus of STZ induced mice. Presence of degenerated neurons in CA1 and CA3 subregions was evident and distorted nerve cells were prominently observed in fluoride toxicated diabetic group. The severity of degenerative changes in CA1 and CA3 subregions were more apparent in hippocampus of positive control mice. However, GE and BLE supplementations were found to be beneficial and offered considerable improvement in the nerve network of CA1 and CA3 subregions (Table 6.5).
CV – Central vein, nh – necrotic hepatocytes
Arrows (→) represent degenerated hepatocytes;
arrows (↑) represent vacuolization of hepatic cells;
star (☆) represent enhanced sinusoidal space

Fig 6.1 Histopathological alterations in liver upon GE and BLE supplementation in fluoride toxicated diabetic mice (400X) (a) Control (b) STZ induced (c) F toxicated (d) STZ+F exposed (e) STZ+F+ GE 150mg/kgbw (f) STZ +F+BLE 150mg/kgbw (g) STZ+F+GE+BLE 150mg/kgbw
**Table 6.1** Summary of histopathological alterations observed in the liver of fluoride intoxicated diabetic mice and influence of phytoextracts (GE and BLE at 150mg/kg bw dose) in the mitigation of toxicity

<table>
<thead>
<tr>
<th>No</th>
<th>Lesions</th>
<th>Control</th>
<th>STZ</th>
<th>F</th>
<th>STZ + F</th>
<th>STZ+F + GE 150</th>
<th>STZ+F+BLE 150</th>
<th>STZ+F+ GE +BLE 150</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hepatocellular degeneration</td>
<td>--</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Hypertrophy of kupffer cells</td>
<td>--</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Ballooning of hepatocytes</td>
<td>--</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Necrosis</td>
<td>--</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Karyolysis</td>
<td>--</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Hepatocellular vacuolisation</td>
<td>--</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

n = 6; Symbols represent: -, nil (0); +, minimal (<12%); ++, mild (<22%); ++++, moderate (<45%); and ++++, severe (>45%).
Fig 6.2 Histopathological alterations in kidney upon GE and BLE supplementation on fluoride intoxicated diabetic mice (400X) (A) Control (B) STZ induced (C) F intoxicated (D) STZ+F exposed (E) STZ+F+GE 150mg/kgbw (F) STZ+F+BLE 150mg/kgbw (G) STZ+F+GE +BLE 150mg/kgbw
Table 6.2  Summary of histopathological alterations observed in the kidney of fluoride toxicated diabetic mice and influence of phytoextracts (GE and BLE at 150mg/kgbw dose) in the mitigation of toxicity

<table>
<thead>
<tr>
<th>No</th>
<th>Lesions</th>
<th>Control</th>
<th>STZ</th>
<th>F</th>
<th>STZ + F</th>
<th>STZ+F+ GE 150</th>
<th>STZ+F+BLE 150</th>
<th>STZ+F+ GE +BLE 150</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hypertrophied glomeruli</td>
<td>--</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>--</td>
</tr>
<tr>
<td>2</td>
<td>Tubular congestion</td>
<td>_</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>+</td>
<td>--</td>
<td>_</td>
</tr>
<tr>
<td>3</td>
<td>Tubular degeneration</td>
<td>_</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>_</td>
</tr>
<tr>
<td>4</td>
<td>Karyolysis</td>
<td>_</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>+</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>5</td>
<td>Necrosis</td>
<td>_</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>_</td>
<td>+</td>
<td>_</td>
</tr>
</tbody>
</table>

n = 6; Symbols represent; -, nil (0); +, minimal (<12%); ++, mild (< 22%); ++++, moderate (<45%); and ++++, severe (>45%).
Fig 6.3 Histological alterations in cerebral cortex upon GE and BLE supplementation in fluoride toxicated diabetic mice (400X) (A) Control (B) STZ induced (C) F toxciated (D) STZ+F exposed (E) STZ +F+GE 150mg/kgbw (F) STZ+F+BLE 150mg/kgbw (G) STZ + F+GE +B LE 150mg/kgbw

- nN – normal neurons,
- Hc – hyperchromasia,
- V – vacuoles
- Arrows (→) represent degenerated neurons;
- arrowheads (▲) cellular necrosis
Table 6.3. Summary of histopathological alterations observed in the cerebral cortex of fluoride intoxicated diabetic mice and influence of phytoextracts (GE and BLE at 150mg/kgbw dose) in the mitigation of toxicity

<table>
<thead>
<tr>
<th>No</th>
<th>Lesions</th>
<th>Control</th>
<th>STZ</th>
<th>F</th>
<th>STZ + F</th>
<th>STZ + F + GE 150</th>
<th>STZ + F + BLE 150</th>
<th>STZ + F + GE + BLE 150</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Neuronal degeneration</td>
<td>--</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>+</td>
<td>++</td>
<td>--</td>
</tr>
<tr>
<td>2</td>
<td>Neuronal vacuolisation</td>
<td>--</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>++</td>
<td>--</td>
</tr>
<tr>
<td>3</td>
<td>Cellular necrosis</td>
<td>--</td>
<td>-</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>++</td>
<td>--</td>
</tr>
<tr>
<td>4</td>
<td>Pyknotic nuclei</td>
<td>--</td>
<td>--</td>
<td>++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>--</td>
</tr>
</tbody>
</table>

n = 6; Symbols represent: -, nil (0); +, minimal (<12%); ++, mild (<22%); ++++, moderate (<45%); and ++++, severe (>45%).
Fig 6.4 Histological alterations in cerebellum upon GE and BLE supplementation in fluoride intoxicated diabetic mice (400X) (A) Control (B) STZ induced (C) F toxicated (D) STZ+F exposed (E) STZ +F+GE 150mg/kgbw (F) STZ+F+BLE 150mg/kgbw (C) STZ + F+CE +B LE 150mg/kgbw
Table 6.4 Summary of histopathological alterations observed in the cerebellum of fluoride intoxicated diabetic mice and influence of phytoextracts (GE and BLE at 150mg/kg bw dose) in the mitigation of toxicity

<table>
<thead>
<tr>
<th>No</th>
<th>Lesions</th>
<th>Control</th>
<th>STZ</th>
<th>F</th>
<th>STZ + F</th>
<th>STZ + F + GE 150</th>
<th>STZ + F + BLE 150</th>
<th>STZ + F + GE + BLE 150</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Disorganised purkinje cell layer</td>
<td>--</td>
<td>--</td>
<td>++</td>
<td>+++</td>
<td>+</td>
<td>++</td>
<td>--</td>
</tr>
<tr>
<td>2</td>
<td>Eosinophilic cells</td>
<td>--</td>
<td>--</td>
<td>++</td>
<td>+++</td>
<td>+</td>
<td>++</td>
<td>--</td>
</tr>
<tr>
<td>3</td>
<td>Neuronal vacuolisation</td>
<td>--</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>++</td>
<td>--</td>
</tr>
<tr>
<td>4</td>
<td>Cellular necrosis</td>
<td>--</td>
<td>-</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>++</td>
<td>--</td>
</tr>
<tr>
<td>5</td>
<td>Pyknotic nuclei</td>
<td>--</td>
<td>--</td>
<td>++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>--</td>
</tr>
</tbody>
</table>

n = 6; Symbols represent: -, nil (0); +, minimal (<12%); ++, mild (<22%); ++++, moderate (<45%); and ++++, severe (>45%).
Fig 6.5 Histological alterations in hippocampus upon GE and BLE supplementation in fluoride intoxicated diabetic mice (400X) (A) Control (B) STZ induced (C) F intoxicated (D) STZ+F exposed (E) STZ+F+GE 150mg/kgbw (F) STZ+F+BLE 150mg/kgbw (G) STZ + F+GE +BLE 150mg/kgbw

N – Hippocampal neurons
Arrows (→) represent degenerated neurons
**Table 6.5** Summary of histopathological alterations observed in the hippocampus of fluoride toxicated diabetic mice and influence of phytoextracts (GE and BLE at 150mg/kgbw dose) in the mitigation of toxicity

<table>
<thead>
<tr>
<th>No</th>
<th>Lesions</th>
<th>Control</th>
<th>STZ</th>
<th>F</th>
<th>STZ + F</th>
<th>STZ+F + GE 150</th>
<th>STZ+F + BLE 150</th>
<th>STZ+F + GE +BLE 150</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Disorganised neuronal layer</td>
<td>--</td>
<td>--</td>
<td>++</td>
<td>+++</td>
<td>+</td>
<td>++</td>
<td>--</td>
</tr>
<tr>
<td>2</td>
<td>Neuronal vacuolisation</td>
<td>--</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>++</td>
<td>--</td>
</tr>
<tr>
<td>3</td>
<td>Celluar necrosis/pyknosis</td>
<td>--</td>
<td>-</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>++</td>
<td>--</td>
</tr>
</tbody>
</table>

n = 6;  Symbols represent; -, nil (0); +, minimal (<12%); ++, mild (< 22%); ++++, moderate (< 45%); and ++++, severe (>45%).
DISCUSSION

Histological changes in liver:

Liver is the major target organ concerned with metabolism of toxic compounds. The normal histoarchitecture of liver constitute hepatic lobules, each consisting of radiating plates and strands of cells forming a network around central vein. Liver strands were alternatively arranged among narrow sinusoids with irregular boundaries having endothelial cells and phagocytic cells called Kupffer cells. Outside the hepatic lobule, there were portal areas of connective tissue with hepatic portal vein, a branch of hepatic artery and bile ductile. Liver, an insulin dependent tissue, plays a pivotal role in glucose and lipid homeostasis which was found to be severely affected during diabetes (Seifter and England, 1982). STZ induction has imposed its toxicity which was evident from liver histology where the major changes observed were cytoplasmic vacuolations and swelling or inflammation in hepatocytes (Al-Rawi et al., 2007). Hepatocytes exposed to fluoride toxicity resulted in parenchymal vacuolar degenerations, necrosis of hepatocytes and/or metabolic enzyme disorders (Anderson, 1985; De Valck et al., 1988; Philips et al., 1987). In addition to the impairments in cytoarchitecture of liver, there were changes in cellular respiration which may interfere with oxido-reduction mechanisms altering the carbohydrate, protein and lipid metabolism (Dabrowska et al., 2006) and consequently leading to cell damage or organelle damage.

In the present study, ballooning degenerations including cell necrosis was seen in the hepatocytes of fluoride exposed mice and the necrotic degeneration was very prominent in fluoride toxicated diabetic mice. Similar alterations were observed in the studies of Bouaziz et al., (2006), thereby this study results corroborate with earlier findings. Several authors have also reported degenerative changes in the liver of animals fed with excessive amounts of fluoride (Chinoy et al., 1993; Kolodziejczyk et al., 2000). Necrotic areas in the liver cells have been also reported by Wang et al., (1993) in rabbits after fluoride exposure. Fluoride exposure to STZ induced diabetic animals has brought additive adverse effects where ballooning and degeneration of hepatocytes were

249
prominent. Irregular sinusoids and alterations apparently observed in fluoride tocalated diabetic mice might have led to liver dysfunction.

**Histological changes in kidney**

Glomerular hypertrophy is one of the prominent alteration(s) indicated in subjects of diabetic nephropathy. The development of diabetic nephropathy is characterized by progressive thickening of the glomerular basement membrane and by expansion of the mesangial matrix which correlates to altered glomerular filtration function (Halim and Misra, 2011). In this study, increased glomerular volume and hypertrophy observed in STZ induced diabetes confirmed the alterations as reported in the earlier studies (Tedong et al., 2006; Teoh et al., 2010). Progressive tubular necrosis with degeneration of brush border was observed in the kidney of STZ induced diabetes. These findings are in consonance with earlier studies made by Kim et al. (2008) and Renno et al. (2008) where authors have showed tubular epithelial changes, enlargement in the lining cells of tubules and accumulation of glycogen in the kidney tubules. The severe pronouncement of changes were evident in fluoride tocalated diabetic mice, leading to decreased glomerular function and cause premature degeneration of the kidneys (Zafar et al., 2009a). The nodular lesions, as reported in diabetic glomerulosclerosis, called K-W nodules were slightly visible in fluoride exposed diabetic mice. It can be inferred from the histological details that the progressive glomerulosclerosis along with decreased renal function, results in end stage renal failure in diabetic nephropathy (Zafar et al., 2009a). Vacuolisation of cellular linings of tubules and widening of its lumen were also observed in the kidney histology of fluoride tocalated mice (Bouaziz et al., 2006). Thereby the results of this study corroborate with the findings of Wadhwani and Ramaswami (1953) and Philips et al. (1934).

**Histological changes in discrete brain regions**

Fluoride intoxication resulted to bring alterations, not only on biochemical aspects, but also in the histoarchitecture of discrete brain regions. Cerebral cortex, the major brain region, which participates in the memory, attention, perceptual awareness and consciousness, is shown to be vulnerable to
fluoride toxicity. In this study, normal histoarchitecture of cerebral cortex showed distinctive layers consisting of molecular, granular and pyramidal layers. Neurodegenerative changes including severe vacuolisation and neuronal necrosis were observed in the CC of fluoride toxicated diabetic mice. The present study results were in consonance with studies of several authors (Uzar et al., 2012; Sonneville et al., 2012). Where the results depict disorganised tissue layers, vacuolar spaces around the pyramidal cells and neurons with pyknotic nuclei as a consequence of high fluoride exposure (Basha et al., 2011a; Shivarajashankara et al., 2002). Severe degenerative changes with more vacuolisations in neuronal cells were evident in positive control group indicating the vulnerability of the fluoride toxicated diabetic animals. As cerebral cortex is responsible for cognitive functions, any alterations in this region attribute to impairments in learning, memory and coordination (Basha et al., 2011).

Cerebellum has characteristic axonal networks of purkinje's cells which are vital for the expression of behaviour and other motor functions (Goodlett et al., 1992). Ample evidences highlight the functions of cerebellum viz., cognition, behaviour and emotion (Schmahmann and Caplan, 2006). In the present study, the histology of cerebellum showed irregular/degenerative neurons and disorganised purkinje cell layer in diabetic mice. These histological alterations attribute to cerebellar dysfunction in hypoglycemic and hyperglycemic conditions (Antony et al., 2010). Exposure to fluoride has led to the disorganisation of purkinje cell layer, as seen in multiple layer rather than changes in a single layer. Severity in the loss of purkinje cell layer was observed in fluoride toxicated group (Basha et al., 2011a). Interference of fluoride ions in mitosis of granular layer cells was attributed to the adverse changes in granular cells which would interrupt the motor functioning as well as behaviour (Trabelsi et al., 2001; Kaur et al., 2009). A severity with disorganised purkinje cells was observed in the positive control group of the present study. As the degenerated purkinje cells failed to establish contact with the granule cells, this will lead to lack of normal synchronism leading to depriviation of motor functions (Trabelsi et al., 2001).
Hippocampus is characterised by well organised/aranged hippocampal neurons. In diabetic and fluoride intoxicated mice, neurons displayed darkly stained small nuclei and distorted in shape. Thereby results of this study are in agreement with previously reported findings of Basha et al. (2011a). These changes in CA1 and CA3 subregions may form the neural basis for impaired learning and memory, abnormal behaviour patterns, and disturbed overall body physiology (Shivarajashankara et al., 2002) and severity of alterations observed in fluoride toxicated STZ induced diabetic mice confer the neuropathy and its vulnerability in hampering the neuronal functions.

**Ameliorative role of phytoextract supplementation**

There are various herbal antidiabetic remedies used in various traditional systems of medicine prevailing around the world, although only some of them have been scientifically assessed for their efficacy. Ginsenosides have the object for countless researches as they are believed to be key principle behind the efficacy and potential effects of ginseng. Similarly corosolic acid from banaba has shown to be potential for diabetic complications. The histological assessments made in this study upon alone and co-supplementations of GE and BLE at dose 150mg/kgbw to fluoride toxicated diabetic mice showed significant protection to liver, kidney and brain tissues. Supplementation of GE and BLE at dose 150mg/kgbw show almost normal hepatocytes and kupffer cells thereby reducing hepatic alterations. In renal tissues, normal glomerular size, GBM and mesangial matrix and further absence of KW nodules and tubular dilations were noticed upon supplementation of GE and BLE at a dose of 150mg/kgbw. In GE and BLE supplemented group appreciable improvement was observed in the organization of cerebral layers and reducing the vacuolar spaces among the neuronal cells. GE and BLE supplementation proved advantageous as the molecular, granular and purkinje cell layers were found to be intact and significant improvement was observed in distribution and structural abnormality of hippocampal neurons.
This resurgence found in GE and BLE (150mg/kg bw) supplemented groups may attributed to the role of these antioxidants in hepatic, renal and neuroprotection. The pharmacological actions of ginseng extract are attributed to ginsenosides, which are tetracyclic triterpenoid saponin glycosides of the saponins family of steroids and are known to possess the ability to scavenge free radicals (Kim et al., 1996; Chu and Chen, 1990). Similarly, the pentacyclic triterpenoids of corosolic acid of banaba extract is shown to be unique in its antioxidant and free radical scavenging activity (Van Kampen et al., 2003). Consistent with earlier reports on the antioxidative abilities of these phytoextracts, the present study has also found that the supplementation of GE and BLE alone and together showed a reduction in alleviating the oxidative stress in diabetes as well as fluorosis. It can be postulated from the results that the synergy among the extracts of GE and BLE enhances the triterpenoid bioavailability or efficacy by forming a chelate complex which could be responsible for the synergistic actions, thereby removes free toxic radicals from the cells and in turn inhibits oxidative damage. Although the primary function of corosolic acid and ginsenosides appears to be related to their free radical scavenging activity, some ginsenoside fractions have been shown to induce antioxidant enzyme superoxide dismutase (Cu–Zn–MnSOD) via enhanced nuclear protein binding to its promoter (Kim et al. 1996; Chang et al. 1999). The co-supplementation of GE and BLE, therefore, could simultaneously improve the overall ability of scavenging free radicals from the cellular components, thereby offer a synergistic action to protect biological tissues from toxic burden.

In brief, GE and BLE co-supplementation at dose of 150mg/kg bw led to enhance the mitochondrial oxidative phosphorylation machinery as well as TCA enzymes which prevented the ROS toxicity induced oxidative damage and attenuated the histological alterations in liver, kidney and brain of fluoride exposed diabetic mice.