CHAPTER IV

Efficacy of spice actives/ phytoconstituents to alleviate experimentally induced diabetes associated neuropathy
1.0 INTRODUCTION

Diabetes is one of the major health concerns world-wide since it is a leading cause of morbidity, mortality and disability across the world (Singh et al., 2012). One of the most common complications of diabetes mellitus is DN, a microvascular complication leading to the damage of the nervous system. The International Diabetic Federation (IDF) estimates that 438 million people worldwide would suffer from diabetes by the year 2030 (Singh et al., 2012) and 50-60% diabetics will exhibit some form of neurological disorder. A series of neurochemical, neurophysiological and structural abnormalities found in CNS is referred to as diabetic encephalopathy and those found in PNS is diabetic peripheral neuropathy (Kamboj and Sandhir, 2011; Urban et al., 2012). Involvement of oxidative stress, inflammatory response and energy depletion in the development of DN has been well appreciated. Commonly associated symptoms with DN include numbness, tingling, allodynia and hyperalgesia. It is often said that DN has no definite pharmacological solution due to its multifactorial etiology (Yorek, 2011).

Although glucose supplies energy essential for the maintenance and neuronal activities of the nervous system, it also controls the mitochondrial death cascade. All the pathological pathways are related to the metabolic and/or redox state of the cell (Obrosova, 2009). These pathways include glucose flux through the polyol pathway, hexosamine pathway, inappropriate activation of protein kinase C isoforms, accumulation of advanced glycation end products and activation of nuclear enzyme poly (ADP-ribose) polymerase and decreased antioxidant defense (Vincent et al., 2004; Brownlee, 2005; Edwards et al., 2008; Obrosova, 2009). These collectively cause an imbalance in the mitochondrial redox state of the cell and lead to excessive formation of ROS and RNS. Increased ROS/ RNS result in inflammatory reactions followed by neuronal damage. In the process, neurons are not only lost but their ability to regenerate is also impaired; worst affected are the nerve fibres- small and large. While the small and unmyelinated nerve fibres (C fibres) are mediators of pain transmission, the large and myelinated nerve fibres (Aβ fibres) mediate touch and pressure sensations (Vincent et al., 2004; Tatiraju et al., 2013).
For mechanistic understanding of the hyperglycemic effects, several *in vitro* models have been employed. Both primary cultures and established cell lines have proved to be excellent systems to decipher the underlying mechanisms (Hattangady and Rajadhyaksha, 2009). One of the most extensively employed cell line for the study of hyperglycemic effects is SHSY5Y, a cloned sub-line of the neuroblastoma SK-N-SH cell line established from metastatic bone tumor. Several other cell lines has been widely used for mechanistic studies to understand the effects of neurotoxins, modeling of PD and AD (Li et al., 2003; Wapen et al., 2004; Du et al., 2012b). Several other cell lines have also exploited to obtain basic insights on the molecular implications of hyperglycemia (Li et al., 2003; Kuhla et al., 2004; Russo et al., 2012). Interestingly, a recent study demonstrated that fluctuating glucose levels had a greater adverse effect on energy turn-over than either persistent high or persistent low glucose levels (Russo et al., 2012). Previously, protective effects of green tea polyphenols have been studied in SHSY5Y cells against 6-OHDA induced ROS/ NO (Guo et al., 2005).

Many herbal actives *per se* or in various formulations are in use in traditional medicines of Indian (Ayurveda) and Chinese origin. Phyto-constituents such as curcumin, cinnamaldehyde, berberine, withanoloides, bacopasides have been shown to possess preventive or curative properties in experimental diabetes, irrespective of the degree of secondary complication such as DN (Parihar et al., 2004; Sharma et al., 2006; Cao et al., 2010). These are presumed to be less toxic than oral hypoglycemic agents (eg., sulfonylureas, metformin) which are demonstrated to possess side effects. Due to the involvement of complex and integrated mechanisms in DN pathology, search for new therapeutic interventions remains critical for its treatment and/ or to off-set its secondary complications such as DN.

Geraniol (GE), an acyclic monoterpene is a constituent of various spices and aromatic herbs. While it is mainly used in food/ beverages for its aromatic properties it has large application in a variety of other products like perfumes, cosmetics, cleansing products etc., for its fragrance (Lapczynski et al., 2008). It is widely recognized for its pharmacological properties *viz.*, antioxidant, anti-
inflammatory and anticancer potential, besides being insecticidal and antimicrobial (Tiwari and Kakkar, 2009; Chen and Viljoen, 2010; Katsukawa et al., 2011; Ahmad et al., 2011). EU, a major component of clove is known to possess anti-oxidant and anti-inflammatory effects (Hidalgo and Rosa, 2009; Chen et al., 2009). The depressant activity on CNS and the general/local anesthetic effect of EU has been well documented (Dallmeier and Carlini, 1981; Guenette et al., 2006; Reiner et al., 2013). Further EU prevented depletion of DA in rodent model emphasizing its role in dopaminergic neurotransmission (Kabuto et al., 2007). The neuromodulatory effects of EU in ACR model of Drosophila and ACR model of neurotoxicity in rats has been demonstrated (Chapters II and III).

Based on the efficacy of spice actives such as geraniol and eugenol in the ACR model of neuropathy in rat model, it was hypothesized that these spice actives are likely to alleviate diabetes-associated neuropathic conditions. The hypothesis was tested both in cell model and in vivo diabetic model. Initially hyperglycemic condition was induced in SHSY5Y cells and the ameliorative effects of selected spice actives were studied in terms of cell survivability, oxidative stress and associated physiological perturbations. Further, in the STZ rat model of diabetes, the modulatory effect of spice active was investigated with a focus on (i) behavioral dysfunction and (ii) biochemical perturbations in SN and brain regions. The results have been presented under three sections A, B and C. Section A describes in vitro studies carried out in SHSY5Y neuronal cells. Section B describes the studies related to the standardization of STZ rat model and the modulatory effects of GE-co-exposure paradigm and the curative effects of EU-an intervention model. Section C describes studies pertaining to the vulnerability of streptozotocin induced diabetic rats to the neurotoxin ACR.
2.0 OBJECTIVE

The primary focus of this series of investigations was to obtain insights on the modulatory role of spice actives/phytoconstituents against hyperglycemia associated biochemical perturbations in cell model (SHSY5Y). Further the protective efficacy of spice actives/phytoconstituents validated in the DN model employing two different paradigms. Additionally, in an interactive model the susceptibility of diabetic rats to the neurotoxin ACR was also studied.

3.0 EXPERIMENTAL DESIGN

SECTION – A

3.1 In vitro model of hyperglycemia in SHSY5Y cells

3.1.1 Induction of hyperglycemia

SHSY5Y, a human neuroblastoma cell line, was maintained in DMEM media supplemented with 10% FBS and penstrep. The growth conditions include humid atmosphere of 5% CO₂ and 95% O₂ at 37°C. Cells were plated in 96 well plates at a density of 5 x 10⁵ cells/well. Cells were exposed to different concentrations of glucose (25 to 300 mM) for 24 h. Cell survival was determined by MTT assay. IC₅₀ value was calculated using statistical analysis.

3.1.2 Effect of spice actives on cell survivability

A 10 mM stock solution of EU and GE were prepared in 95% ethanol and CU in 0.5 M NaOH solution. All the working standards were prepared in the culture media. Initially cells were plated in 96 well plates at a density of 5 x 10⁵ cells/well containing different concentrations (1 to 100 µM) of each spice active - CU, EU and GE for 24 h. Cell survival was determined by MTT assay. The non-toxic concentrations of each of these spice actives were ascertained. These concentrations were employed to assess the potential to modulate glucose (Glc) induced cellular aberrations and cell death.
3.1.3 Neuroprotective effects of spice actives

The non-toxic concentrations of each spice active was co-exposed with IC$_{50}$ concentration of Glc for 24 h. Cell survival and protection rendered by the actives against Glc induced cell death was determined by MTT assay.

Biochemical alterations

Experiments were carried out only with the concentrations of the spice actives at which they rendered protection against Glc induced cell death and various markers for oxidative stress (ROS, HP, GSH/ GSSG) among the untreated cells, spice active (per se) treated cells, Glc (100 mM) exposed cells with or without spice actives at specific concentrations were determined.

Further, the modulatory effect of spice actives on the levels of 3-nitrotyrosine, HSP70 and neurofilaments was determined using slot blot analysis in cells exposed to Glc.

SECTION – B

DIABETIC NEUROPATHY (DN): STREPTOZOTOCIN RAT MODEL AND AMELIORATIVE EFFECTS OF SPICE ACTIVES

3.2 STZ model of neuropathy: Onset and progression

Adult male rats were rendered diabetic by a single i.p., of streptozotocin (STZ) at 55 mg/ kg bw in freshly prepared ice-cold citrate buffer (0.1 M, pH 4.5). Age matched rats which received an equal volume of the vehicle served as controls. STZ injected rats were provided with glucose water (5% w/v) for 48 h to prevent hypoglycemic shock. Induction of hyperglycemia was ascertained by measuring the blood glucose levels by Accuchek comfort sensor glucometer; the animals with glucose levels ≥ 350 mg/ dL after 72 h of STZ injection were included in the study. By the end of one week about 30% mortality occurred among STZ rats. The following determinations were made in both control and diabetic rats.
**Feed intake, growth and blood glucose levels**

All rats were monitored daily for feed intake and weekly for body weights throughout the experimental period of 12 weeks. Further, both control and diabetic rats were monitored for blood glucose levels biweekly (as described in Chapter I).

**Behavioral assessments**

STZ treated rats were assessed for the development of sensory and motor dysfunctions by tail immersion tests (hyperalgesia and allodynia) and narrow beam tests respectively (as described in Chapter I).

**Biochemical alterations**

Terminally rats were sacrificed under anesthesia, SN and brain were excised washed several times in saline and blotted dry before sample preparation. The following biochemical markers were determined in SN and different brain regions (as described in Chapter I).

*Induction of oxidative damage and GSH levels:* The induction of oxidative damage assayed by measuring the levels of ROS, MDA and PC levels in the cytosolic fractions of SN and brain regions. As a marker of redox status, GSH levels were also determined.

*Induction of inflammatory response and calcium levels:* The measurement of HP and NO levels in the cytosolic fractions of SN and brain regions were indicative of the induction of inflammatory reaction in response to hyperglycemia. The calcium levels were also determined in the cytosolic fractions of SN and brain regions since it contributes significantly to the oxidative/inflammatory burden and is in part a trigger to pre-apoptotic cascade.

*Determination of neurotoxicity markers:* The activity of AChE and DA levels were determined in the cytosolic fractions of SN and brain regions.
3.3 Amelioration of DN: Behavioral and biochemical evidences

3.3.1 Suppressive effects of oral supplements of geraniol

The experimental procedure adopted in this study was similar in terms of induction of diabetes in adult rats. One week post STZ administration induction of hyperglycemia was ascertained and both non-diabetic and diabetic rats were grouped as follows:

- **Group I**: Control (received equi-volume of edible oil);
- **Group II**: geraniol control (GE, 100 mg/ kg bw/ d, po, 8 weeks);
- **Group III**: Diabetic rats untreated (received equi-volume of edible oil);
- **Group IV**: Diabetic rats supplemented with geraniol (Diabetic + GE; 100 mg/ kg bw/ d, po, 8 weeks).

Rats from all the groups were housed individually and fed with commercial powdered diet and water ad libitum.

**Feed intake, growth and blood glucose levels**

Feed intake of all rats was monitored daily, while body weights were monitored weekly throughout the experimental period of 8 weeks. Blood glucose levels were monitored once in two weeks (as described in Chapter I).

**Behavioral assessments**

Rats of various groups were subjected to behavioral assessments to determine the development of sensory (hyperalgesia and allodynia) and motor dysfunctions (as described in Chapter I).

**Biochemical alterations**

Terminally samples of SN and brain regions were analyzed biochemically. Markers of oxidative stress, inflammatory response, mitochondrial function and neurochemical markers were determined in SN and different brain regions (as described in Section 3.2).

Further the protective effect of GE on mitochondrial oxidative stress and dysfunctions were determined by estimating the activities of certain functional
enzymes (complex I – III, SDH and CS) along with quantification of MDA, HP, NO and PC levels.

3.3.2 Efficacy of eugenol to abrogate DN: intervention model
Hyperglycemia was induced as described previously among adult male rats. After 6 weeks, the control and diabetic rats were sub-divided as follows:

Group I: Control (received equi-volume of DMSO);
Group II: eugenol (EU, 10 mg/ kg bw, ip., in DMSO, alternate days, 6 wks);
Group III: Diabetic rats untreated (received equi-volume of DMSO);
Group IV: Diabetic rats supplemented with eugenol (EU, 10 mg/ kg bw, ip., in DMSO, alternate days, 6 wks).

Rats from all the groups were housed individually and fed with commercial powdered diet and water ad libitum.

Feed intake, growth and blood glucose levels
All rats were monitored daily for feed intake and weekly for body weights throughout the experimental period. Rats were tested for blood glucose levels once in two weeks.

Behavioral assessments and biochemical alterations
Rats from all the groups were assessed for sensory and motor deficits in a battery of behavioral tests as mentioned in Section 3.3.1. Terminally, the samples of SN and brain regions were assessed for alterations in biochemical markers of oxidative stress, inflammatory response, mitochondrial function and neurochemical markers as described in Section 3.3.1. In addition, histopathological examination of SN (LS) from control and diabetic (EU treated and untreated) was conducted following standard staining technique.
SECTION – C

VULNERABILITY OF DIABETIC RATS TO ACRYLAMIDE: INTERACTIVE EFFECTS

3.4 Neurotoxic response of STZ diabetic rats to ACR exposure

Hyperglycemia was induced as described previously among adult male rats. After 1 wk, the control and diabetic rats were sub-divided as follows:

- **Group I:** Control (received equi-volume of saline);
- **Group II:** ACR control (25 mg/ kg bw, ip, 3x/ wk, for 5 wks);
- **Group III** – Diabetic control (received equi-volume of saline);
- **Group IV** – Diabetic administered with ACR (Diabetic + ACR; 25 mg/ kg bw, ip, 3x/ wk, for 5 wks).

Rats from all the groups were housed individually and fed with commercial powdered diet and water ad libitum. ACR dosage was selected based on the dose standardization studies described in Chapter III. At a dosage of 25 mg/ kg bw, i.p., 3x/ wk, for 5 wks, ACR did not cause death or induce robust sensory dysfunction or motor deficits among adult male rats.

**Feed intake, growth and blood glucose levels**
All rats were monitored daily for feed intake and weekly for body weights and blood glucose levels throughout the experimental period.

**Behavioral assessments and biochemical alterations**
Rats from all the groups were assessed for sensory and motor deficits in a battery of behavioral tests as mentioned in Section 3.3.1. Further, terminally the samples of SN and brain regions were assessed for alterations in biochemical markers of oxidative stress, inflammatory response and neurochemical markers as described in Section 3.3.1.
4.0 RESULTS

SECTION – A

4.1 In vitro model of hyperglycemia in SHSY5Y cells

4.1.1 Induction of hyperglycemia
Exposure of SHSY5Y cells to graded concentrations of Glc resulted in a concentration dependent cell death as evidenced by MTT reduction assay (Fig. 4.1 A and B). While at 50 mM concentration 23% cell death was evident, 77% cell death ensued at 300 mM. However, non-linear regression analysis revealed IC\textsubscript{50} value as 100.8 mM. Hence for studying modulatory potency of spice actives 100 mM concentration of Glc was used.

4.1.2 Effect of spice actives on cell survivability
Exposure of cells to different concentrations of spice actives did not cause cell death in the range 5 - 50 µM. All the actives at concentrations higher than 50 µM caused significant reduction in cell survival (Data not shown). Hence the concentrations used for studying the modulatory potency of spice actives against hyperglycemia were 5 - 50 µM.

4.1.3 Protective effects of spice actives

4.1.3.1 Cell survival
Spice actives in the concentration range of 5 - 20 µM modulated Glc induced cell death (Fig. 4.2). Maximum protection (31%) was rendered by CU at 20 µM concentration (p ≤ 0.001). EU rendered consistent protection (22-26%) only at 5 and 10 µM concentrations (p ≤ 0.001) while GE offered marginal protection at 10 µM. The representative photo micrographs of cells are presented in Fig. 4.3.

4.1.3.2 Modulatory effects of spice actives on Glc induced oxidative stress
Glc exposure caused significant induction of oxidative stress as evident by elevation in the ROS (20%) and HP (50%) levels as well as depletion in GSH levels (35%) with concomitant increase (30%) in GSSG levels.

CU reduced the endogenous levels of ROS and HP significantly (48% and 23% respectively) and under co-exposure with Glc (Fig. 4.4A and B). CU caused no significant alteration in the endogenous levels of GSH, further no
effect was apparent when co-exposed with Glc, however GSSG levels were enhanced (Table 4.1).

With only EU exposure, the endogenous levels of ROS and HP were markedly diminished (Fig 4.5A and B). When co-exposed with Glc, the ROS levels were reduced by 35% at both the concentrations and HP levels displayed robust reduction (5 µM: 62%; 10 µM: 76%) (Fig 4.5A and B). Although no significant alterations in the levels of GSH/ GSSG was apparent among EU treated cells, the depleted levels of GSH and enhanced levels of GSSG were significantly altered with EU in the co-exposure paradigm. Interestingly EU treatment caused robust enhancement in the GSH levels (5 µM: 2.39 fold; 10 µM: 1.87fold) with normalization of GSSG levels at 5 µM concentration and insignificant change at 10 µM concentration (Table 4.2).

GE exposed cells exhibited a significant reduction in the endogenous levels of ROS (37%) and HP (27%). While ROS levels were reduced marginally by GE when co-exposed with Glc, the HP levels were normalized (Fig 4.6A and B). Further, the depleted GSH levels among Glc exposed cells were enhanced by 2 folds (Table 4.3).

4.1.3.3 Modulatory effects of spice actives on 3-NT and HSP70 levels
The endogenous levels of 3-NT were reduced by the spice active treatment (Fig. 4.7). While Glc exposure resulted in an increase (46%) in 3-NT levels, a marked diminution occurred with spice active treatments (EU 10 µM: 74%) and (GE 10 µM: 64%). Further a marked elevation in the HSP70 expression was observed among cells exposed to Glc (91%) and EU (45%). While GE had no effect, CU caused a marginal increase (17%) (Fig. 4.8). Interestingly, when co-exposed with spice actives, Glc induced elevation of HSP70 expression levels were markedly diminished, albeit differentially (CU: 44%; EU: 60%; GE: 32%).

4.1.3.4 Effect of spice actives on Glc induced alterations in NF levels
Glc resulted in elevation of NF levels (NF-L: 80%; NF-H: 18%) in SHSY5Y cells at 24 h exposure (Fig. 4.9). The endogenous levels of NF-L were significantly elevated with spice active treatment (CU- 30%; EU- 20%; GE- 34%), while NF-H levels were affected differentially. Co-exposure of cells with Glc and spice
actives resulted in a reduction (15%) only with EU and GE. Marked reduction (31%) in the NF-H levels was evident only with EU.

SECTION – B

DIABETIC NEUROPATHY (DN): STREPTOZOTOCIN RAT MODEL AND AMELIORATIVE EFFECTS OF SPICE ACTIVES

4.2 STZ model of neuropathy: Onset and progression

4.2.1 Feed intake and growth
Although the feed intake was relatively higher among the diabetic rats, the body weight gain was markedly lower compared to control rats (Data not shown). The terminal body weights (g) were: Control: 345 ± 6; Diabetic: 168 ± 15. In general nearly 30% deaths occurred among STZ administered rats within one wk and nearly 20% death was evident during the experimental period of 12 weeks.

4.2.2 Blood glucose levels
Induction of diabetes was ascertained by measuring the blood glucose levels 3 days post STZ injection. While the glucose level among control rats was 99 ± 7 mg/ dL, the rats injected with STZ the mean glucose level of 509 ± 31 mg/ dL. The terminal mean blood glucose levels among the survivors of the diabetic group were 506 ± 58 mg/ dL.

4.2.3 Sensory and motor function
While the diabetic rats developed sensitivity to hot stimuli as early as two weeks, they displayed reduction in latency period to the cold stimuli only during week 4 (Fig. 4.10A and B). The reduction in latency period for hot hyperalgesia was as follows- 4 wks: 37%; 8 wks: 46%; 12 wks: 55%. The reduction in latency period for cold allodynia was- 4 wks: 28%; 8 wks: 60%; 12 wks: 70%. However diabetic rats exhibited a marginal motor dysfunction only by wk 6 and all diabetic rats developed motor dysfunction by the end of week 12. The narrow beam test score were- Control: 1 ± 0.2; Diabetic: 2.8 ± 0.2 (Fig. 4.10C).
4.2.4 Biochemical markers in SN and brain regions

Alterations in oxidative stress markers

While significant elevation in the levels of oxidative markers viz., ROS, HP and PC was evident at all sampling time points, the levels of NO and MDA were enhanced in SN only at 8 and 12 weeks (Table 4.2). Brain regions also showed differential elevation in the levels of ROS and HP. However, the PC levels among diabetic rats showed progressive elevation (20 - 52%) over the experimental period. Likewise, the elevation in the MDA levels was also temporal among diabetic rats (40% and 97% at 8 and 12 wks) (Table 4.2). The NO levels among diabetic rats were robust at 8 wks (80%) and showed decreasing trend at 12 wks (36%). Concomitantly, a significant reduction (35%) in the GSH levels was also evident at 12 weeks (Fig. 4.11A).

Alterations in calcium levels and neurochemical markers

Diabetic rats exhibited a marked increase in the calcium levels only at 8 (44%) and 12 (72%) weeks (Fig. 4.11B). Further, a consistent depletion (~ 50%) in DA levels was evident at 8 and 12 weeks (Fig. 4.11C). However, an increase in the activity of AChE was apparent at all sampling time points (Fig. 4.11D).

4.3 Amelioration of DN: Behavioral and biochemical evidences

4.3.1 Suppressive effects of oral supplements of geraniol (GE)

Effect of GE on body weight and blood glucose levels

GE supplementation had no effect on feed intake or body weight gain among non-diabetic (control) rats throughout the experimental period. A general decrease in body weight gain was evident among diabetic rats irrespective of GE supplements. Terminally, the body weights of diabetic rats were significantly lower (50%) compared to control rats (Table 4.3). The increase (4-5 fold) in blood glucose levels among the diabetic rats was consistent throughout the experimental period (Fig. 4.12).
GE improves sensory and motor function
Tail immersion tests for noxious (52°C) and non-noxious (10°C) stimuli revealed a lowered latency period among diabetic rats irrespective of GE supplements (Fig. 4.13 A and B). While the decreased tail flick to thermal stimuli (hot hyperalgesia) was evident as early as week 2 among diabetic rats, significant decrease in response to cold stimuli (cold allodynia) was apparent from week 4 onwards. Although GE supplemented diabetic rats showed progressive improvement in hot hyperalgesia test, from week 4 onwards, they showed increased resistance in cold allodynia test (evidenced by delayed response at each time point). Terminally diabetic rats exhibited significant motor deficits, while GE supplemented diabetic rats did not develop locomotor phenotype and their locomotor functions was comparable to those of control rats (Fig. 4.13 C).

GE ameliorates status of oxidative stress
No significant alterations occurred in the endogenous levels of oxidative markers in SN among GE supplemented non-diabetic rats. While, diabetic rats exhibited robust elevation in the levels of oxidative markers in SN (Fig. 4.14), GE supplements markedly reduced the levels. Further, GE supplements differentially attenuated the levels of oxidative markers in brain regions among diabetic rats (Fig. 4.15 and Fig. 4.16). While ROS levels were reduced uniformly, HP levels were reduced only in Ct and Hc. Further, MDA levels were reduced in all regions (except striatum) among GE supplemented diabetic rats (Fig. 4.16A). While diabetic rats showed elevated protein oxidation levels, GE supplements reduced the levels in all brain regions (Fig. 4.16B).

GE modulates cytosolic calcium and NO levels
A significant elevation in the cytosolic calcium levels was evident among untreated diabetic rats in SN (44%) and brain regions (Fig. 4.17). GE supplementation significantly ameliorated the calcium levels in SN and brain regions (except Hc) (Fig. 4.17A). Similarly, a robust increase in the NO levels was evident in both SN (81%) and brain regions among untreated diabetic rats. Interestingly, GE supplementation markedly lowered the NO levels in SN and brain regions (except Hc) (Fig. 4.17B).
**Effects of GE on GSH and activities of antioxidant/ detoxifying enzymes**

In general, depletion in GSH levels was accompanied with reduced activities of antioxidant enzymes such as GR and TRR among untreated diabetic rats in both SN and brain regions (Table 4.4). In general, GE supplementation unaltered the endogenous levels of GSH and activities of GR and TRR, however, among diabetic rats showed a significant increase (Table 4.4). Although, no significant alteration in the activity of SOD was evident in SN or the brain regions, a reduction in the CAT activity and a differential effect on GST activity was apparent among untreated diabetic rats (Table 4.5). However, with GE supplementation an increase in the activities of SOD, CAT and GST enzymes was evident.

GE modulates mitochondrial functions

**Oxidative stress markers and MTT reduction:** Diabetic rats showed an increase in oxidative markers with concomitant decrease in MTT reduction in the mitochondrial fractions (except in Hc region) (Table 4). While, GE supplementation induced alterations in the endogenous levels of oxidative markers were insignificant, their levels were lowered among diabetic rats. Further elevation in the levels of MTT reduction was apparent with GE supplementation among diabetic rats (Table 4.6).

**Activities of complex I – III, SDH and CS:** Diabetic rats in general exhibited reduced activities of complex I – III, SDH and CS (Fig. 4.18A, B and C). Although GE supplementation did not alter the endogenous levels of these enzyme activities, a significant increase was ensued among diabetic rats (Ct, Cb and St). Interestingly, in Hc region of diabetic rats only the activities of complex I – III and SDH were reduced, however, with GE supplementation a significant increase was evident in the activities of SDH and CS.

**Modulatory effects of GE on DA levels and AChE activity**

Among untreated diabetic rats, a significant depletion in DA levels was evident only in SN and St (Fig. 4.19). Although no alteration was apparent in the endogenous levels of DA among GE supplemented rats, a significant elevation was evidenced among diabetic rats (Fig. 4.19A). The activity of AChE enzyme...
was elevated significantly in SN and brain regions (except Hc) among the untreated diabetic rats. Interestingly, the elevated activity of AChE was reduced significantly among the GE treated diabetic rats in SN and brain regions (except Hc, Data not shown). The alteration in the activity of AChE in SN and St is presented in Fig. 4.19B.

### 4.3.2 Efficacy of EU to abrogate DN: Intervention model

**Effect of EU on body weight and blood glucose levels**

EU supplementation to non-diabetic rats did not affect the feed intake or body weight gain throughout the experimental period. A general decrease in body weight gain was evident among both EU treated and untreated diabetic rats. Terminally, the mean body weight of diabetic rats was ~50% lower than the controls (Fig. 4.20A). Interestingly a significant reduction in the mean blood glucose level among EU treated diabetic rats was evident at the end of the experimental period (Fig. 4.20B).

**EU improves sensory and motor function**

A similar response to hot (hyperalgesia) and cold (allodynia) stimuli was evident among all the diabetic rats at the end of six weeks (Fig. 4.21A and B). However EU supplementation resulted in a marked improvement in both responses (hyperalgesia: 53%; allodynia: 71%). Interestingly, the tail withdrawal latency was normalized by the end of six weeks of EU treatment. Similarly, complete abrogation of motor impairments was evident with EU treatment at the end of the experimental period (Fig. 4.21C).

**Cytosolic markers in SN and brain regions**

#### Modulatory effects EU on oxidative stress markers

With EU treatment the endogenous levels of HP and NO were significantly reduced only in SN and St regions (Fig. 4.22A and B) and the ROS levels were diminished only in St and Hc (Fig. 4.22C). The elevated levels of HP (~ 50%) in SN and among all the regions of brain (except a robust increase in Cb) of untreated diabetic rats were normalized with EU treatment. While, an increase (30-50%) in the NO and ROS levels was observed in SN and brain regions (except Cb region) of untreated diabetic rats, marked reduction was evident with
EU treatment. The protein oxidation levels (PC) was significantly elevated among untreated diabetic rats was apparently normalized with EU treatment (except in Hc) (Fig. 4.23A). A robust increase (about 100%) in the MDA levels among the diabetic rats in SN and brain regions (Ct, Hc) was observed. In Cb and Ct, the increase in MDA levels was about 30% (Fig. 4.23B). However among the EU treated diabetic rats a marked reduction was evident in SN/ Hc, while normalizing the same in Cb and St (Fig. 4.23B).

*EU modulates cytosolic levels of calcium*

While a marked elevation in the cytosolic calcium levels was evident among untreated diabetic rats in SN (44%), the increase (2-3 fold) was robust in the brain regions. EU supplementation normalized the calcium levels in SN/ Cb and significantly ameliorated the same in other brain regions (Fig. 4.23C).

*Effect of EU on redox status*

While a marked depletion (30%) in the levels of GSH occurred in both SN and brain regions of diabetic rats, TSH levels were depleted only in SN (50%) and St (Fig. 4.24). While the GSH levels were restored in SN and St, it was further elevated marginally in other brain regions by EU treatment (Fig. 4.24A). A marked elevation in the TSH levels was evident in Ct (38%) and Hc (56%) and a marginal increase ensued in SN and St of EU treated diabetic rats (Fig. 4.24B).

*Effect of EU on the activities of antioxidant/ detoxifying enzymes*

EU treatment did not affect the endogenous activity levels of various antioxidant/detoxifying enzymes in SN (Table 4.7). In general among diabetic rats, the activities of antioxidant enzymes were decreased and EU treatment restored the levels. However, the activity levels of the enzymes in brain regions were differentially affected (Table 4.8 and 4.9). The SOD levels in Ct and St were diminished and EU treatment normalized the levels. Further, a similar trend was also evident in the activity levels of GR and TRR in all the brain regions. The activity of phase II enzyme, GST which was enhanced in St and Hc remained unaffected with EU treatment.
Modulatory effect of EU on mitochondrial functions

Oxidative stress markers and MTT reduction: Among untreated diabetic rats, the mitochondrial fractions of all the brain regions showed an increase in oxidative markers with a concomitant decrease in MTT reduction (Fig. 4.25 and Fig. 4.26). EU supplementation among diabetic rats caused a marked reduction in the levels of ROS and PC (Fig. 4.25A and Fig. 4.26C). Further a similar effect was also observed in the levels of HP, NO (Fig. 4.25B and C) and MDA (Fig. 4.26B). Among diabetic rats MTT reduction was enhanced significantly with EU treatment (Fig. 4.26A).

Activities of complex I – III, SDH and CS: In general the activities of complex I – III and SDH (Fig. 4.27A and B) were reduced among the diabetic rats. EU supplementation significantly enhanced the activities of these enzymes among diabetic rats in all brain regions examined. Further, a similar trend of results was apparent in the activity of CS in all regions (except Hc).

Modulatory effect of EU on AChE activity

The endogenous activity level of AChE was diminished in Ct, Cb and Hc with EU treatment (Fig. 4.28). Diabetes caused a marked increase (40%) in the activity of AChE in SN and St, while the increase was less marked in other regions. Interestingly EU treatment resulted in varying degree of reduction in its activity in brain regions.

Effects of EU on histopathology in SN

The pathological alterations in SN of diabetic rats (untreated and EU treated) have been presented in Fig. 4.29. In general histological sections of SN of untreated diabetic rats exhibited prominent gaps between the nerve bundles with intermittent grouping of the bundles. Further, varying degree of vacuolization was also evident. In contrast, sections of SN of EU treated diabetic rats, showed typical parallel striations of nerve bundles which were largely comparable to those observed among SN of control rats. Interestingly, vacuolization or gaps was completely absent with EU treated rats.
SECTION – C

VULNERABILITY OF DIABETIC RATS TO ACRYLAMIDE: INTERACTIVE EFFECTS

4.4 Response of STZ diabetic rats to ACR exposure

*Feed intake and growth*

Significant reduction in body weights by the end of first week was evident among diabetic rats (with or without ACR administration). While the feed intake was higher (20-50%) among diabetic rats from second week onwards, marginal reduction was evident among Diabetic + ACR rats (Data not shown). While no reduction in body weight gain was evident among control and ACR rats, significant reduction occurred among diabetic and diabetic + ACR rats. Terminal body weights (g) of various groups were as follows: Control: 278 ± 10; ACR: 265 ± 20; Diabetic: 184 ± 20; Diabetic + ACR: 145 ± 7. At the end of 5 weeks 10% mortality ensued among Diabetic + ACR group.

*BLOOD GLUCOSE LEVELS*

The blood glucose levels among both control and ACR rats were highly comparable. ACR at the administered dosage did not affect the blood glucose levels in the diabetic rats (Data not shown). Terminally, the mean blood glucose levels (mg/ dL) among various groups were as follows: Control: 98 ± 3; ACR: 95 ± 6; Diabetic: 507 ± 40; Diabetic + ACR: 511 ± 34.

*Effect on sensory and motor function*

*Hot hyperalgesia:* Among diabetic rats, a marginal decrease in the latency period was evident. However diabetic rats administered with ACR displayed a marked reduction (36%) in the latency period. At the end of five weeks, the percent reduction in the latency period was: Diabetic: 50%; ACR: 34%; Diabetic + ACR: 64% (Fig. 4.30A).

*Narrow beam test:* Diabetic rats did not develop motor deficits throughout the experimental period (Fig. 4.30B). ACR administered rats developed marginal motor deficits by the end of four weeks. However, diabetic rats administered
with ACR displayed the robust signs of motor dysfunction as early as three weeks and was progressive (Fig. 4.30B).

**Biochemical markers in SN and brain regions**

Effect on oxidative stress markers and GSH levels
While the ROS levels were enhanced only in SN (28%) and Ct of diabetic rats, no alteration was evident among ACR administered rats (Fig. 4.31A). However ACR administered diabetic rats showed significantly enhanced ROS levels in SN (46%) and brain regions. A similar trend was evident in HP levels in SN (Fig. 4.31B). A marked increase in MDA levels was apparent in SN (38%) and brain regions (Ct: 52%; Cb: 80%) of Diabetic + ACR group (Fig. 4.32A). While a significant increase in PC levels was evident only in SN of diabetic rats (Fig. 4.32B), a marked increase in SN (56%) and a moderate elevation in brain regions occurred among Diabetic + ACR group (Fig. 4.32B).

Effects of ACR on the activities of antioxidant/ detoxifying enzymes
Perturbations in the activities of GST, SOD and CAT in SN and brain regions of various treatment groups are presented in Table 4.10. SN of diabetic rats showed a significant increase only in the activity of SOD. However, the activities of the antioxidant enzymes were higher among the ACR administered diabetic rats. Further, the activity of GST was enhanced significantly in both Ct and Cb of ACR administered rats. But among diabetic rats administered with ACR, the activity of GST was markedly diminished only in Ct and Cb. Among diabetic rats, the activities of SOD and CAT was enhanced in SN (SOD: 53%; CAT: 48%) and Ct (SOD: 74%; CAT: 77%). In general, among ACR administered diabetic rats the SOD activity was increased. The CAT activity was further elevated in SN and Ct (Table 4.10).

Modulatory effect of EU on cytosolic levels of calcium
Among diabetic rats, a robust elevation in the cytosolic calcium levels was evident only in SN (44%) and Ct (75%) (Fig. 4.33A). However ACR administration to diabetic rats resulted in robust enhancement in its levels in SN (74%) and the brain regions (Ct: 78%; Cb:47%).
Effect of ACR on neurochemical markers

**AChE activity:** The activity level of AChE was significantly elevated among diabetic rats in SN and Ct (Fig. 4.33B). Although the activity of AChE was enhanced in SN, a robust increase (62%) occurred among Diabetic + ACR group. A similar potentiated effect was also evident in Ct and Cb among ACR administered diabetic rats.

**DA levels:** While diabetic rats did not exhibit depletion in DA levels either in SN or brain regions, a significant depletion (about 20%) ensued among ACR administered rats only in SN and Cb (Fig. 4.33C). However, among diabetic rats administered with ACR showed significant depletion in DA levels in SN (28%) and in Ct and Cb (Fig. 4.33C).
Fig. 4.1

Representative photographs of SHSY5Y cells exposed to varying concentrations of glucose for 24 h: (A) Typical morphology (B) Cell survivability

A

Control
Glucose 50 mM
Glucose 100 mM
Glucose 300 mM

B

Optical density

Glucose (mM)

0 50 100 200 300

0.0 1.0 2.0 3.0 4.0

Control Glucose 50 mM Glucose 100 mM Glucose 300 mM
Fig. 4.2

Photomicrographs of SHSY5Y cells exposed to glucose and spice bio-actives in a co-exposure paradigm (24 h)

Glucose: Glc 100 mM; Curcumin: CU 20 µM; Eugenol: EU 10 µM; Geraniol: GE 10 µM
Fig. 4.3

Modulatory effect of selected phyto-constituents on the survivability of SHSY5Y cells under hyperglycemia

![Graph showing optical density of different treatments]

Glc 100: Glucose 100 mM; CU 5, 10, 20: Curcumin 5, 10, 20 µM; EU 5, 10, 20: Eugenol 5, 10, 20 µM; GE 5, 10, 20: Geraniol 5, 10, 20 µM.

Fig 4.4

Protective effect of curcumin on hyperglycemia-associated elevation of Reactive Oxygen Species (A) and Hydroperoxides (B) in SHSY5Y cells

![Graph showing DCF and Hydroperoxides levels]

Glc: Glucose 100 mM; CU 20: Curcumin 20 µM

(Values are mean ± SE (n = 12). Data analyzed by one way analysis of variance (ANOVA) followed by Tukey’s test for comparison of means. *** significant at p ≤ 0.001; * significant p ≤ 0.05.)
**Fig. 4.5**

Modulatory effect of eugenol on hyperglycemia-induced elevation of Reactive Oxygen Species (A) and Hydroperoxides (B) in SHSY5Y cells

Glc: Glucose 100 mM; EU 5, 10: Eugenol 5, 10 µM

**Fig. 4.6**

Modulatory effect of geraniol on hyperglycemia-induced elevation of Reactive Oxygen Species (A) and Hydroperoxides (B) in SHSY5Y cells

Glc: Glucose 100 mM; GE 20: Geraniol 20 µM

(Values are mean ± SE (n=6). Data analyzed by one way analysis of variance (ANOVA) followed by Tukey's test for comparison of means. * significant against control, # against hyperglycemic condition, p ≤ 0.001)
Table 4.1

Modulatory effect of curcumin, eugenol and geraniol on glutathione levels (reduced - GSH; oxidized - GSSG) in SHSY5Y cell model

<table>
<thead>
<tr>
<th>Group</th>
<th>GSH (nmol/ mg protein)</th>
<th>GSSG (nmol/ mg protein)</th>
<th>GSSG/ GSH ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>22.28 ± 0.7</td>
<td>8.03 ± 0.1</td>
<td>0.360</td>
</tr>
<tr>
<td>CU, 20 µM</td>
<td>23.37 ± 0.1</td>
<td>7.60 ± 0.1</td>
<td>0.325</td>
</tr>
<tr>
<td>EU, 5 µM</td>
<td>23.99 ± 0.8</td>
<td>7.97 ± 0.1</td>
<td>0.332</td>
</tr>
<tr>
<td>EU, 10 µM</td>
<td>22.78 ± 0.7</td>
<td>9.02 ± 0.1*</td>
<td>0.396</td>
</tr>
<tr>
<td>GE, 10 µM</td>
<td>22.52 ± 1.2</td>
<td>8.60 ± 0.1</td>
<td>0.382</td>
</tr>
<tr>
<td>Glc, 100 mM</td>
<td>14.92 ± 0.8*</td>
<td>10.33 ± 0.3</td>
<td>0.692</td>
</tr>
<tr>
<td>Glc + CU, 20 µM</td>
<td>15.40 ± 0.3*</td>
<td>12.44 ± 0.3*#</td>
<td>0.808</td>
</tr>
<tr>
<td>Glc + EU, 5 µM</td>
<td>35.56 ± 0.2*#</td>
<td>8.27 ± 0.1#</td>
<td>0.233</td>
</tr>
<tr>
<td>Glc + EU, 10 µM</td>
<td>27.80 ± 1.1#</td>
<td>10.96 ± 0.1*</td>
<td>0.394</td>
</tr>
<tr>
<td>Glc + GE, 10 µM</td>
<td>29.16 ± 0.3#</td>
<td>8.53 ± 0.2#</td>
<td>0.293</td>
</tr>
</tbody>
</table>

Values are mean ± SE (n=6).

Glc, 100 mM: Glucose 100 mM; CU, 20: Curcumin 20 µM; EU, 5 µM: Eugenol 5 µM; EU, 10 µM: Eugenol 10 µM; GE 10 µM: Geraniol 10 µM

(Data analyzed by one way analysis of variance (ANOVA) followed by Tukey’s test for comparison of means. * significant against control, # against hyperglycemic state, p ≤ 0.001)
Fig. 4.7

Modulatory effect of spice bio-actives on 3 nitrotyrosine (3-NT) levels in SHSY5Y cell model: Levels of 3-NT (A) and Ratio of 3 NT to β actin (B)

Glc – Glucose 100 mM; CU – Curcumin 20 µM; EU – Eugenol 10 µM; GE: Geraniol 10 µM
Fig. 4.8

Modulatory effect of spice bio-actives on HSP 70 levels in SHSY5Y cell model: Levels of HSP 70 (A) and Ratio of HSP 70 to β actin (B)

Glc – Glucose 100 mM; CU – Curcumin 20 µM; EU – Eugenol 10 µM; GE: Geraniol 10 µM
Fig. 4.9

Modulatory effect of spice bio-actives on the levels neurofilaments (NF) in SHSY5Y cell model: Levels of NF (A) and Ratio of NF to β actin (B)

NF – L: Low Mol wt; NF – H: High Mol wt

Glc – Glucose 100 mM; CU – Curcumin 20 µM; EU – Eugenol 10 µM; GE: Geraniol 10 µM
Fig. 4.10

Progression of neuropathic signs with respect to sensory (A - Hot hyperalgesia; B - Cold allodynia) and motor functions (C) among control and diabetic rats.

(Values are mean ± SE (n = 126). Data analysed by one way analysis of variance (ANOVA) followed by Tukey’s test for comparison of means. * - significant; p ≤ 0.05)
Table 4.2

Status of oxidative stress markers at different sampling times in sciatic nerve of adult rats rendered diabetic with an acute dose of streptozotocin

<table>
<thead>
<tr>
<th></th>
<th>ROS (pmol/ min/mg protein)</th>
<th>HP (nmol/mg protein)</th>
<th>NO (nmol/mg protein)</th>
<th>MDA (nmol/mg protein)</th>
<th>PC (nmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>4 weeks</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>98.2 ± 3</td>
<td>10.5 ± 0.4</td>
<td>6.91 ± 0.2</td>
<td>1.52 ± 0.05</td>
<td>16.6 ± 0.6</td>
</tr>
<tr>
<td>Diabetic</td>
<td>118.1 ± 6*</td>
<td>12.1 ± 0.8*</td>
<td>7.05 ± 1.0</td>
<td>1.61 ± 0.04</td>
<td>19.8 ± 1.0*</td>
</tr>
<tr>
<td><strong>8 weeks</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>93.5 ± 8</td>
<td>11.2 ± 0.4</td>
<td>7.06 ± 0.2</td>
<td>1.42 ± 0.04</td>
<td>17.7 ± 0.8</td>
</tr>
<tr>
<td>Diabetic</td>
<td>198.4 ± 2*</td>
<td>18.9 ± 1.6*</td>
<td>12.8 ± 0.6*</td>
<td>1.97 ± 0.06*</td>
<td>23.7 ± 0.3*</td>
</tr>
<tr>
<td><strong>12 weeks</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>86.1 ± 6</td>
<td>10.9 ± 0.3</td>
<td>6.81 ± 0.2</td>
<td>1.39 ± 0.08</td>
<td>16.1 ± 0.4</td>
</tr>
<tr>
<td>Diabetic</td>
<td>130.2 ± 3*</td>
<td>15.9 ± 0.5*</td>
<td>9.25 ± 0.3*</td>
<td>2.74 ± 0.05*</td>
<td>24.3 ± 1.8*</td>
</tr>
</tbody>
</table>

Values are mean ± SE (n=6)

A single dose of streptozotocin was administered (ip.) to rats at a dosage of 55 mg/ kg bw

ROS – Reactive Oxygen species
HP – Hydroperoxides
NO – Nitric oxide
MDA – Malondialdehyde
PC – Protein carbonyls

(Data analyzed by one way analysis of variance (ANOVA) followed by Tukey’s test for comparison of means. *significant; p ≤ 0.05)
Fig. 4.11

Levels of reduced glutathione (A), cytosolic calcium (B), dopamine (C) and activity of acetylcholinesterase (D) in the sciatic nerve of diabetic rats

(Values are mean ± SE (n = 12). Data analysed by one way analysis of variance (ANOVA) followed by Tukey's test for comparison of means. * - significant p ≤ 0.05)
Table 4.3

Effect of geraniol supplements on body weight gain among diabetic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weights (g)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Terminal</td>
</tr>
<tr>
<td>Control</td>
<td>176 ± 3</td>
<td>332 ± 9.2</td>
</tr>
<tr>
<td>GE</td>
<td>175 ± 5</td>
<td>335 ± 9.5</td>
</tr>
<tr>
<td>Diabetic</td>
<td>176 ± 4</td>
<td>170 ± 7.1*</td>
</tr>
<tr>
<td>Diabetic + GE</td>
<td>176 ± 3</td>
<td>182 ± 20.5*</td>
</tr>
</tbody>
</table>

Values are mean ± SE (n=6)

GE: Geraniol, 100 mg/ kg bw, po., for 8 weeks

(Data analyzed by one way analysis of variance (ANOVA) followed by Tukey’s test for comparison of means. *significant; p ≤ 0.05)

Fig. 4.12

Effect of geraniol supplements on blood glucose levels among diabetic rats

GE: Geraniol, 100 mg/ kg bw, po., for 8 weeks

(Values are mean ± SE (n=6). Data analyzed by one way analysis of variance (ANOVA) followed by Tukey’s test for comparison of means. *significant against control and # against diabetic at p ≤ 0.05)
Fig. 4.13

Effect of oral supplements of geraniol on tail immersion tests- hot hyperalgesia (A), cold allodynia (B) and Narrow beam test (C) among control and diabetic rats.

GE: Geraniol, 100 mg/kg bw, po., for 8 weeks

(Values are mean ± SE (n=6). Data analyzed by one way analysis of variance (ANOVA) followed by Tukey’s test for comparison of means. *significant against control and # against diabetic at p ≤ 0.05)
Fig. 4.14

Effect of oral supplements of geraniol on oxidative markers in sciatic nerve of control and diabetic rats

Geraniol: GE, 100 mg/ kg bw, po., for 8 weeks

A – Reactive Oxygen species
B – Hydroperoxides
C – Malondialdehyde
D – Protein carbonyls

(Values are mean ± SE (n=6). Data analyzed by one way analysis of variance (ANOVA) followed by Tukey’s test for comparison of means. *significant against control and # against diabetic at p ≤ 0.05)
Fig. 4.15

Effect of oral supplements of geraniol on oxidative markers in brain regions of control and diabetic rats

GE: Geraniol, 100 mg/ kg bw, po., for 8 weeks

A – Reactive Oxygen species
B – Hydroperoxides

(Values are mean ± SE (n=6). Data analyzed by one way analysis of variance (ANOVA) followed by Tukey's test for comparison of means. *significant against control and # against diabetic at p ≤ 0.05)
Fig. 4.16

Effect of oral supplements of geraniol on oxidative markers in brain regions of control and diabetic rats

GE: Geraniol, 100 mg/ kg bw, po., for 8 weeks

A – Malondialdehyde
B – Protein carbonyls

(Values are mean ± SE (n=6). Data analyzed by one way analysis of variance (ANOVA) followed by Tukey’s test for comparison of means. *significant against control and # against diabetic at p ≤ 0.05)
Fig. 4.17

Effect of oral supplements of geraniol on cytosolic levels of calcium (A) and NO (B) in sciatic nerve and brain regions of control and diabetic rats

GE: Geraniol, 100 mg/kg bw, po., for 8 weeks

SN: Sciatic nerve; Ct: Cortex; Cb: Cerebellum; St: Striatum; Hc: Hippocampus

(Values are mean ± SE (n=6). Data analyzed by one way analysis of variance (ANOVA) followed by Tukey’s test for comparison of means. *significant against control and # against diabetic at p ≤ 0.05)
Table 4.4

Effect of geraniol supplements on reduced glutathione (GSH) and activities of Glutathione reductase (GR) and Thioredoxin reductase (TRR) in sciatic nerve and brain regions of control and diabetic rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>GE</th>
<th>Diabetic</th>
<th>Diabetic + GE</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH&lt;sup&gt;1&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sciatic nerve</td>
<td>4.73 ± 0.12</td>
<td>4.80 ± 0.10</td>
<td>4.18 ± 0.04*</td>
<td>4.32 ± 0.09*</td>
</tr>
<tr>
<td>Brain</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortex</td>
<td>5.13 ± 0.03</td>
<td>5.01 ± 0.10</td>
<td>3.68 ± 0.25*</td>
<td>5.92 ± 0.09*#</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>5.19 ± 0.09</td>
<td>5.98 ± 0.20*</td>
<td>4.77 ± 0.32*</td>
<td>5.81 ± 0.23*#</td>
</tr>
<tr>
<td>Striatum</td>
<td>5.19 ± 0.06</td>
<td>5.20 ± 0.05</td>
<td>4.03 ± 0.40*</td>
<td>5.74 ± 0.10*#</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>5.07 ± 0.14</td>
<td>5.11 ± 0.10</td>
<td>4.04 ± 0.09*</td>
<td>5.04 ± 0.22#</td>
</tr>
<tr>
<td>GR&lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sciatic nerve</td>
<td>4.19 ± 0.21</td>
<td>4.22 ± 0.09</td>
<td>2.17 ± 0.06*</td>
<td>2.80 ± 0.05*#</td>
</tr>
<tr>
<td>Brain</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortex</td>
<td>30.0 ± 0.5</td>
<td>35.0 ± 0.5</td>
<td>15.0 ± 0.5*</td>
<td>21.8 ± 0.8*#</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>29.3 ± 0.8</td>
<td>27.9 ± 0.5</td>
<td>26.4 ± 2.8*</td>
<td>34.3 ± 2.2#</td>
</tr>
<tr>
<td>Striatum</td>
<td>13.5 ± 0.9</td>
<td>14.1 ± 1.0</td>
<td>14.3 ± 2.5</td>
<td>24.8 ± 0.7*#</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>14.3 ± 1.0</td>
<td>14.8 ± 1.0</td>
<td>26.7 ± 1.2*</td>
<td>21.8 ± 1.0*#</td>
</tr>
<tr>
<td>TRR&lt;sup&gt;3&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sciatic nerve</td>
<td>3.15 ± 0.02</td>
<td>3.25 ± 0.05</td>
<td>2.18 ± 0.06*</td>
<td>3.09 ± 0.21#</td>
</tr>
<tr>
<td>Brain</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortex</td>
<td>5.92 ± 0.14</td>
<td>5.82 ± 0.20</td>
<td>5.07 ± 0.36</td>
<td>6.97 ± 0.50#</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>5.58 ± 0.08</td>
<td>6.30 ± 0.20*</td>
<td>4.82 ± 0.41*</td>
<td>6.25 ± 0.25*#</td>
</tr>
<tr>
<td>Striatum</td>
<td>5.12 ± 0.16</td>
<td>5.05 ± 0.10</td>
<td>4.32 ± 0.51*</td>
<td>6.82 ± 0.25*#</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>5.23 ± 0.07</td>
<td>5.20 ± 0.10</td>
<td>3.91 ± 0.40</td>
<td>4.52 ± 1.04</td>
</tr>
</tbody>
</table>

Values are mean ± SE (n=6).

GE: Geraniol, 100 mg/ kg bw, po., for 8 weeks

1 - µg/ mg protein
2 - nmol NADPH oxidized/ min/ mg protein
3 - nmol substrate/ min/ mg protein

(Data analyzed by one way analysis of variance (ANOVA) followed by Tukey’s test for comparison of means. *significant against control and # against diabetic at p ≤ 0.05)
Table 4.5

Effect of geraniol supplements on the activity levels of antioxidant/detoxifying enzymes among control and diabetic rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>GE</th>
<th>Diabetic</th>
<th>Diabetic + GE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SOD</strong>&lt;sup&gt;1&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sciatic nerve</td>
<td>2.91 ± 0.38</td>
<td>2.85 ± 0.15</td>
<td>2.34 ± 0.23</td>
<td>3.72 ± 0.17*</td>
</tr>
<tr>
<td>Brain</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortex</td>
<td>68.9 ± 1.2</td>
<td>75.0 ± 2.0</td>
<td>59.1 ± 7.8</td>
<td>71.6 ± 1.2</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>72.6 ± 6.1</td>
<td>70.5 ± 2.5</td>
<td>67.0 ± 11</td>
<td>109 ± 3.7**#</td>
</tr>
<tr>
<td>Striatum</td>
<td>21.7 ± 1.0</td>
<td>22.2 ± 1.0</td>
<td>27.4 ± 1.4</td>
<td>24.9 ± 2.2</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>29.9 ± 0.8</td>
<td>28.5 ± 1.5</td>
<td>26.1 ± 5.0</td>
<td>56.4 ± 5.7**#</td>
</tr>
<tr>
<td><strong>CAT</strong>&lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sciatic nerve</td>
<td>3.60 ± 0.12</td>
<td>3.75 ± 0.15</td>
<td>2.98 ± 0.07</td>
<td>6.85 ± 0.27**#</td>
</tr>
<tr>
<td>Brain</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortex</td>
<td>6.32 ± 0.03</td>
<td>9.50 ± 0.05*</td>
<td>5.52 ± 0.13*</td>
<td>4.77 ± 0.12**#</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>6.14 ± 0.23</td>
<td>9.05 ± 0.05*</td>
<td>4.95 ± 0.50*</td>
<td>5.00 ± 0.21</td>
</tr>
<tr>
<td>Striatum</td>
<td>4.88 ± 0.11</td>
<td>4.75 ± 0.10</td>
<td>3.41 ± 0.47*</td>
<td>4.81 ± 0.18**#</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>4.39 ± 0.07</td>
<td>4.50 ± 0.10</td>
<td>3.43 ± 0.09*</td>
<td>4.07 ± 0.17**#</td>
</tr>
<tr>
<td><strong>GST</strong>&lt;sup&gt;3&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sciatic nerve</td>
<td>19.9 ± 0.4</td>
<td>18.9 ± 1.0</td>
<td>15.1 ± 0.3</td>
<td>19.2 ± 1.2</td>
</tr>
<tr>
<td>Brain</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortex</td>
<td>42.8 ± 0.8</td>
<td>40.5 ± 1.5</td>
<td>29.3 ± 2.3*</td>
<td>45.8 ± 1.8**#</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>46.6 ± 0.6</td>
<td>45.5 ± 1.0</td>
<td>43.0 ± 1.9*</td>
<td>50.0 ± 1.4**#</td>
</tr>
<tr>
<td>Striatum</td>
<td>49.6 ± 1.1</td>
<td>48.6 ± 1.5</td>
<td>82.5 ± 3.3*</td>
<td>97.5 ± 5.0**#</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>40.9 ± 1.6</td>
<td>41.0 ± 1.0</td>
<td>53.8 ± 3.2*</td>
<td>61.8 ± 1.9**#</td>
</tr>
</tbody>
</table>

Values are mean ± SE (n=6).

Geraniol: GE, 100 mg/kg bw, po., for 8 weeks

1 – Superoxide dismutase, Units/mg protein
2 – Catalase, nmol H₂O₂ hydrolyzed/min/mg protein
3 – Glutathione S transferase, µmol Conjugate/min/mg protein

(Data analyzed by one way analysis of variance (ANOVA) followed by Tukey’s test for comparison of means. *significant against control and # against diabetic at p ≤ 0.05)
Table 4.6

Effect of geraniol supplements on mitochondrial oxidative markers and MTT reduction in control and diabetic rats

<table>
<thead>
<tr>
<th>Brain regions</th>
<th>Control</th>
<th>GE</th>
<th>Diabetic</th>
<th>Diabetic + GE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MDA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortex</td>
<td>0.98 ± 0.05</td>
<td>1.01 ± 0.05</td>
<td>1.41 ± 0.07*</td>
<td>1.00 ± 0.06*#</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>1.14 ± 0.02</td>
<td>1.01 ± 0.10</td>
<td>1.63 ± 0.11*</td>
<td>1.07 ± 0.11*#</td>
</tr>
<tr>
<td>Striatum</td>
<td>1.08 ± 0.03</td>
<td>1.11 ± 0.05</td>
<td>1.50 ± 0.09*</td>
<td>1.10 ± 0.04*#</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>0.92 ± 0.04</td>
<td>0.95 ± 0.05</td>
<td>0.98 ± 0.04</td>
<td>0.87 ± 0.05</td>
</tr>
<tr>
<td><strong>HP</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortex</td>
<td>14.4 ± 0.7</td>
<td>14.0 ± 1.1</td>
<td>22.3 ± 2.6*</td>
<td>13.2 ± 1.3*#</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>12.7 ± 0.4</td>
<td>13.1 ± 1.0</td>
<td>16.6 ± 0.6*</td>
<td>13.1 ± 1.1*#</td>
</tr>
<tr>
<td>Striatum</td>
<td>13.9 ± 0.5</td>
<td>13.5 ± 0.9</td>
<td>17.7 ± 0.3*</td>
<td>18.0 ± 0.1*#</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>12.5 ± 0.7</td>
<td>11.8 ± 1.0</td>
<td>12.2 ± 0.3</td>
<td>11.0 ± 0.3</td>
</tr>
<tr>
<td><strong>NO</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortex</td>
<td>22.6 ± 0.9</td>
<td>21.7 ± 0.7</td>
<td>31.7 ± 0.7*</td>
<td>22.4 ± 2.3*#</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>23.3 ± 1.0</td>
<td>22.5 ± 0.5</td>
<td>50.6 ± 7.3*</td>
<td>22.1 ± 2.2*#</td>
</tr>
<tr>
<td>Striatum</td>
<td>21.9 ± 0.5</td>
<td>21.5 ± 1.0</td>
<td>27.8 ± 2.4*</td>
<td>17.7 ± 0.6*#</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>24.2 ± 0.5</td>
<td>23.5 ± 1.0</td>
<td>51.3 ± 6.0*</td>
<td>41.1 ± 5.0*</td>
</tr>
<tr>
<td><strong>PC</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortex</td>
<td>15.4 ± 0.5</td>
<td>14.6 ± 1.0</td>
<td>20.3 ± 2.9*</td>
<td>13.1 ± 0.8*#</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>11.9 ± 0.8</td>
<td>11.0 ± 1.0</td>
<td>17.4 ± 1.5*</td>
<td>12.9 ± 0.9*#</td>
</tr>
<tr>
<td>Striatum</td>
<td>12.9 ± 0.7</td>
<td>12.1 ± 0.9</td>
<td>17.3 ± 0.9*</td>
<td>12.2 ± 0.3*#</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>11.6 ± 0.4</td>
<td>10.5 ± 1.0</td>
<td>16.3 ± 3.0</td>
<td>8.40 ± 0.6*</td>
</tr>
<tr>
<td><strong>MTT reduction</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortex</td>
<td>11.4 ± 0.1</td>
<td>12.1 ± 0.8</td>
<td>8.19 ± 0.1*</td>
<td>11.9 ± 1.2*#</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>10.3 ± 0.2</td>
<td>10.5 ± 0.5</td>
<td>7.62 ± 0.1*</td>
<td>8.57 ± 0.2*#</td>
</tr>
<tr>
<td>Striatum</td>
<td>6.97 ± 0.2</td>
<td>7.01 ± 0.5</td>
<td>4.36 ± 0.5*</td>
<td>6.87 ± 0.1*#</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>7.37 ± 0.1</td>
<td>7.25 ± 0.5</td>
<td>6.30 ± 0.4</td>
<td>7.39 ± 0.6</td>
</tr>
</tbody>
</table>

Values are mean ± SE (n=6).

Geraniol: GE, 100 mg/ kg bw, po., for 8 weeks

1 – Malondyaldehyde, nmol/ mg protein; 2 – Hydroperoxides, nmol/ mg protein;
3 – Nitrites, nmol/ mg protein; 4 – Protein carbonyls, nmol/ mg protein;
5 – Optical density/ mg protein

(Data analyzed by one way analysis of variance (ANOVA) followed by Tukey’s test for comparison of means. *significant against control and # against diabetic at p ≤ 0.05.)
Fig. 4.18

Effect of geraniol supplements on mitochondrial markers: Complex I - III (A), succinate dehydrogenase (B) and citrate synthase (C) in brain regions of control and diabetic rats

Geraniol: GE, 100 mg/kg bw, po, for 8 weeks

(Values are mean ± SE (n=6). Data analyzed by one way analysis of variance (ANOVA) followed by Tukey’s test for comparison of means. *significant against control and # against diabetic at p ≤ 0.05)
Fig. 4.19

Effect of geraniol supplements on dopamine levels (A) and activity of acetylcholinesterase (B) among control and diabetic rats

Geraniol: GE, 100 mg/ kg bw, po., for 8 weeks

(Values are mean ± SE (n=6). Data analyzed by one way analysis of variance (ANOVA) followed by Tukey’s test for comparison of means. *significant against control and # against diabetic at p ≤ 0.05).
Fig. 4.20

Modulatory effect of eugenol supplements on body weights (A) and blood glucose levels (B) among diabetic rats in an intervention model

Eugenol: EU, 10 mg/kg bw/ ip., alternate days 6 weeks, post 6 weeks of diabetes induction

(Values are mean ± SE (n=10). Data analyzed by one way analysis of variance (ANOVA) followed by Tukey’s test for comparison of means. *significant against control and # against diabetic at p ≤ 0.05)
Fig. 4.21

Effect of eugenol supplements on tail immersion tests- hot hyperalgesia (A), cold allodynia (B) and narrow beam test (C) among diabetic rats in an intervention model

Eugenol: EU, 10 mg/kg bw/ ip., alternate days 6 weeks, post 6 weeks of diabetes induction

(Values are mean ± SE (n=10). Data analyzed by one way analysis of variance (ANOVA) followed by Tukey’s test for comparison of means. *significant against control and # against diabetic at p ≤ 0.05)
Fig. 4.22

Modulatory effect of eugenol supplements on oxidative markers in sciatic nerve (SN) and brain regions of diabetic rats in an intervention model

A – Hydroperoxides; B – Nitric oxide; C – Reactive oxygen species
Eugenol: EU, 10 mg/kg bw/ ip., alternate days 6 weeks, post 6 weeks of diabetes induction

(Values are mean ± SE (n=10). Data analyzed by one way analysis of variance (ANOVA) followed by Tukey’s test for comparison of means. *significant against control and # against diabetic at p ≤ 0.05)
Fig. 4.23

Effect of eugenol supplements on PC, MDA and Ca$^{2+}$ levels in sciatic nerve (SN) and brain regions of diabetic rats in an intervention model

A – Protein carbonyls (PC); B – Malondialdehyde (MDA); C – Cytosolic calcium (Ca$^{2+}$)
Eugenol: EU, 10 mg/kg bw/ ip., alternate days 6 weeks, post 6 weeks of diabetes induction

(Values are mean ± SE (n=10). Data analyzed by one way analysis of variance (ANOVA) followed by Tukey’s test for comparison of means. *significant against control and # against diabetic at p ≤ 0.05)
Fig. 4.24

Modulatory effect of eugenol supplements on reduced glutathione (A) and total thiols (B) in sciatic nerve (SN) and brain regions in diabetic rats

Eugenol: EU, 10 mg/kg bw/ ip., alternate days 6 weeks, post 6 weeks of diabetes induction

(Values are mean ± SE (n=10). Data analyzed by one way analysis of variance (ANOVA) followed by Tukey’s test for comparison of means. *significant against control and # against diabetic at p ≤ 0.05)
Table 4.7

Modulatory effect of eugenol supplements on the activities of selected enzymes in sciatic nerve among diabetic rats in an intervention model

<table>
<thead>
<tr>
<th>Enzymes / Group</th>
<th>Control</th>
<th>EU</th>
<th>Diabetic</th>
<th>Diabetic + EU</th>
</tr>
</thead>
<tbody>
<tr>
<td>GST¹</td>
<td>19.2 ± 0.8</td>
<td>22.7 ± 2.6</td>
<td>10.8 ± 1.4*</td>
<td>17.1 ± 0.6*#</td>
</tr>
<tr>
<td>SOD²</td>
<td>5.36 ± 0.03</td>
<td>5.27 ± 0.12</td>
<td>4.68 ± 0.14*</td>
<td>6.55 ± 0.29*#</td>
</tr>
<tr>
<td>CAT³</td>
<td>2.53 ± 0.09</td>
<td>2.58 ± 0.02</td>
<td>1.68 ± 0.23*</td>
<td>2.82 ± 0.15#</td>
</tr>
<tr>
<td>GR⁴</td>
<td>4.24 ± 0.12</td>
<td>4.27 ± 0.24</td>
<td>1.51 ± 0.15*</td>
<td>2.60 ± 0.14#</td>
</tr>
<tr>
<td>TRR⁵</td>
<td>4.82 ± 0.14</td>
<td>4.90 ± 0.25</td>
<td>1.18 ± 0.07*</td>
<td>3.17 ± 0.17#</td>
</tr>
</tbody>
</table>

Values are mean ± SE (n=6)

Eugenol: EU, 10 mg/kg bw/ ip., alternate days 6 weeks, post 6 weeks of diabetes induction

¹ – Glutathione-S-transferase, ηmol conjugate formed/ min/ mg protein;
² – Superoxide dismutase, U/ mg protein;
³ – Catalase, nmol hydrogen peroxide decomposed/ min/ mg protein;
⁴ – Glutathione Reductase, nmol NADPH oxidized/ min/ mg protein;
⁵ – Thioredoxin Reductase, nmol substrate/ min/ mg protein

(Data analyzed by one way analysis of variance (ANOVA) followed by Tukey’s test for comparison of means. * significant against control; # against diabetic at p ≤ 0.05.)
Table 4.8

Effect of eugenol supplements on the activity levels of selected enzymes in cortex and cerebellum of diabetic rats in an intervention model

<table>
<thead>
<tr>
<th>Enzymes/ Group</th>
<th>Control</th>
<th>EU</th>
<th>Diabetic</th>
<th>Diabetic + EU</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cortex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GST (^1)</td>
<td>43.2 ± 1.5</td>
<td>40.5 ± 0.5</td>
<td>29.1 ± 1.6(^*)</td>
<td>47.7 ± 1.0 (^#)</td>
</tr>
<tr>
<td>SOD (^2)</td>
<td>63.1 ± 0.9</td>
<td>67.0 ± 1.2</td>
<td>55.2 ± 1.4(^*)</td>
<td>66.4 ± 1.9 (^#)</td>
</tr>
<tr>
<td>CAT (^3)</td>
<td>6.00 ± 0.08</td>
<td>7.65 ± 0.65(^*)</td>
<td>7.54 ± 0.32(^*)</td>
<td>5.77 ± 0.18 (^#)</td>
</tr>
<tr>
<td>GPx (^4)</td>
<td>29.7 ± 1.0</td>
<td>34.1 ± 2.7</td>
<td>21.5 ± 0.7(^*)</td>
<td>37.1 ± 2.9 (^#)</td>
</tr>
<tr>
<td>GR (^5)</td>
<td>31.6 ± 1.0</td>
<td>47.3 ± 1.2 (^*)</td>
<td>23.4 ± 1.2(^*)</td>
<td>47.2 ± 2.8 (^#)</td>
</tr>
<tr>
<td>TRR (^6)</td>
<td>6.01 ± 0.08</td>
<td>8.85 ± 0.28(^*)</td>
<td>5.20 ± 0.11(^*)</td>
<td>6.18 ± 0.39</td>
</tr>
</tbody>
</table>

| Cerebellum     |         |    |          |               |
| GST \(^1\)     | 41.7 ± 0.7 | 42.8 ± 1.5 | 48.7 ± 2.9 | 49.1 ± 1.5 |
| SOD \(^2\)     | 67.5 ± 1.5 | 62.7 ± 1.3 | 72.9 ± 3.8 | 62.2 ± 7.0 |
| CAT \(^3\)     | 6.05 ± 0.06 | 7.19 ± 0.35 | 7.30 ± 0.4 | 7.02 ± 0.28 |
| GPx \(^4\)     | 26.2 ± 1.1 | 27.6 ± 2.1 | 37.2 ± 1.3\(^*\) | 35.8 ± 1.4\(^*\) |
| GR \(^5\)      | 31.6 ± 0.5 | 29.5 ± 1.0 | 22.1 ± 1.5\(^*\) | 27.4 ± 0.6\(^#\) |
| TRR \(^6\)     | 7.90 ± 0.08 | 10.8 ± 0.38 | 5.91 ± 0.40\(^*\) | 13.1 ± 0.17\(^#\) |

Values are mean ± SE (n=6)

Eugenol: EU, 10 mg/kg bw/ip., alternate days 6 weeks, post 6 weeks of diabetes induction

\(^1\) Glutathione-S-transferase, nmol conjugate formed/ min/ mg protein;
\(^2\) Superoxide dismutase, Unit/ mg protein;
\(^3\) Catalase, nmol hydrogen peroxide decomposed/ min/ mg protein;
\(^4\) Glutathione peroxidase, nmol NADPH oxidized/ min/ mg protein;
\(^5\) Glutathione Reductase, nmol NADPH oxidized/ min/ mg protein;
\(^6\) Thioredoxin Reductase, nmol substrate/ min/ mg protein

(Data analyzed by one way analysis of variance (ANOVA) followed by Tukey’s test for comparison of means. * significant against control; # against diabetic at p ≤ 0.05)
Table 4.9

Modulatory effect of eugenol supplements on the activities of selected enzymes in striatum and hippocampus among diabetic rats in an intervention model

<table>
<thead>
<tr>
<th>Enzymes/Group</th>
<th>Control</th>
<th>EU</th>
<th>Diabetic</th>
<th>Diabetic + EU</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Striatum</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GST&lt;sup&gt;1&lt;/sup&gt;</td>
<td>44.9 ± 1.3</td>
<td>48 ± 1.0</td>
<td>57.1 ± 0.7*</td>
<td>56.9 ± 3.2*</td>
</tr>
<tr>
<td>SOD&lt;sup&gt;2&lt;/sup&gt;</td>
<td>28.8 ± 1.1</td>
<td>26.3 ± 1.0</td>
<td>9.17 ± 0.8*</td>
<td>26.9 ± 0.6 #</td>
</tr>
<tr>
<td>CAT&lt;sup&gt;3&lt;/sup&gt;</td>
<td>3.86 ± 0.04</td>
<td>3.18 ± 0.21*</td>
<td>1.96 ± 0.12*</td>
<td>2.61 ± 0.04*#</td>
</tr>
<tr>
<td>GPx&lt;sup&gt;4&lt;/sup&gt;</td>
<td>17.9 ± 0.3</td>
<td>25.2 ± 0.9*</td>
<td>31.1 ± 1.2*</td>
<td>29.9 ± 2.0*</td>
</tr>
<tr>
<td>GR&lt;sup&gt;5&lt;/sup&gt;</td>
<td>11.5 ± 0.99</td>
<td>11.4 ± 0.34</td>
<td>4.80 ± 0.31*</td>
<td>8.82 ± 1.01 #</td>
</tr>
<tr>
<td>TRR&lt;sup&gt;6&lt;/sup&gt;</td>
<td>5.35 ± 0.10</td>
<td>5.52 ± 0.06</td>
<td>1.43 ± 0.17*</td>
<td>4.81 ± 0.59 #</td>
</tr>
<tr>
<td><strong>Hippocampus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GST&lt;sup&gt;1&lt;/sup&gt;</td>
<td>40.0 ± 0.7</td>
<td>46.1 ± 2.3</td>
<td>58.9 ± 3.5*</td>
<td>58.4 ± 2.5*</td>
</tr>
<tr>
<td>SOD&lt;sup&gt;2&lt;/sup&gt;</td>
<td>30.8 ± 0.7</td>
<td>30.1 ± 0.8</td>
<td>43.8 ± 6.6*</td>
<td>29.3 ± 1.8 #</td>
</tr>
<tr>
<td>CAT&lt;sup&gt;3&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>GPx&lt;sup&gt;4&lt;/sup&gt;</td>
<td>31.7 ± 1.9</td>
<td>34.3 ± 1.1</td>
<td>26.1 ± 0.8*</td>
<td>34.7 ± 1.3 #</td>
</tr>
<tr>
<td>GR&lt;sup&gt;5&lt;/sup&gt;</td>
<td>11.0 ± 0.4</td>
<td>10.7 ± 1.26</td>
<td>4.91 ± 0.70*</td>
<td>13.5 ± 0.91 #</td>
</tr>
<tr>
<td>TRR&lt;sup&gt;6&lt;/sup&gt;</td>
<td>4.95 ± 0.11</td>
<td>5.34 ± 0.28</td>
<td>3.21 ± 0.33*</td>
<td>4.10 ± 0.28</td>
</tr>
</tbody>
</table>

Values are mean ± SE (n=6); ND – Not determined

Eugenol: EU, 10 mg/kg bw/ ip., alternate days 6 weeks, post 6 weeks of diabetes induction

1 – Glutathione-S-transferase, nmol conjugate formed/ min/ mg protein;
2 – Superoxide dismutase, U/ mg protein;
3 – Catalase, nmol hydrogen peroxide decomposed/ min/ mg protein;
4 – Glutathione peroxidase, nmol NADPH oxidized/ min/ mg protein;
5 – Thioredoxin Reductase, nmol NADPH oxidized/ min/ mg protein;
6 – Glutathione Reductase, nmol substrate/ min/ mg protein

(Data analyzed by one way analysis of variance (ANOVA) followed by Tukey’s test for comparison of means. * significant against control; # against diabetic at p ≤ 0.05.)
Fig. 4.25

Effect of eugenol supplements on ROS (A), HP (B) and NO (C) levels in mitochondria of brain regions of diabetic rats in an intervention model

Eugenol: EU, 10 mg/kg bw/ ip., alternate days 6 weeks, post 6 weeks of diabetes induction

(Values are mean ± SE (n=6). Data analyzed by one way analysis of variance (ANOVA) followed by Tukey’s test for comparison of means. *significant against control; # against diabetic at p ≤ 0.05)
Fig. 4.26

Effect of eugenol supplements on MTT reduction (A), malondialdehyde (B) and protein carbonyls (C) levels in mitochondria of brain regions of diabetic rats in an intervention model

Eugenol: EU, 10 mg/kg bw ip, alternate days 6 weeks, post 6 weeks of diabetes induction

(Values are mean ± SE (n=6). Data analyzed by one way analysis of variance (ANOVA) followed by Tukey's test for comparison of means. * significant against control; # against diabetic at p ≤ 0.05)
Fig. 4.27

Modulatory effect of eugenol supplements on the activities of complex I – III (A) Succinate dehydrogenase (B) and Citrate synthase (C) in mitochondria of brain regions of diabetic rats in an intervention model

Eugenol: EU, 10 mg/kg bw/ ip., alternate days 6 weeks, post 6 weeks of diabetes induction

(Values are mean ± SE (n=6). Data analyzed by one way analysis of variance (ANOVA) followed by Tukey’s test for comparison of means. * significant against control; # against diabetic at p ≤ 0.05)
Fig. 4.28

Modulatory effect of eugenol supplements on the activity of acetylcholinesterase in sciatic nerve (SN) and brain regions of diabetic rats in an intervention model of diabetes

Eugenol: EU, 10 mg/kg bw ip, alternate days 6 weeks, post 6 weeks of diabetes induction

(Values are mean ± SE (n=6). Data analyzed by one way analysis of variance (ANOVA) followed by Tukey's test for comparison of means. * significant against control; # against diabetic at p ≤ 0.05)
Fig. 4.29

Histological section (LS, H&E) of sciatic nerve showing the effect of eugenol treatment among diabetic rats in an intervention model

SN were sampled after 12 wks of diabetes; eugenol (EU) was administered at a dosage of 10 mg/ kg bw, ip., alternate days after 6 weeks of diabetes induction

H&E: Hematoxylin and eosin; light microscopy (20X). Arrows highlight the differences in the histoarchitecture of the nerve bundles in SN of the control, diabetic and EU treated diabetic rats
Effect of acrylamide intoxication on sensory function—hot hyperalgesia (A) and motor function—narrow beam test (B) among diabetic rats

ACR, 25 mg/kg bw, ip., 3 times/wk, 5 weeks

(Values are mean ± SE (n=6). Data analyzed by one way analysis of variance (ANOVA) followed by Tukey's test for comparison of means. * significant against control; # against diabetic, at p ≤ 0.05)
Fig. 4.31

Effect of acrylamide intoxication on the levels of ROS (A) and HP (B) in sciatic nerve and brain regions of diabetic rats

ACR, 25 mg/kg bw, ip., 3 times/wk, 5 weeks

(Values are mean ± SE (n=6). Data analyzed by one way analysis of variance (ANOVA) followed by Tukey’s test for comparison of means. * significant against control; # against diabetic, at p ≤ 0.05)
Fig. 4.32

Effect of acrylamide intoxication on the levels of MDA (A) and PC (B) in sciatic nerve and brain regions of diabetic rats

ACR, 25 mg/ kg bw, i.p., 3 times/ wk, 5 weeks

(Values are mean ± SE (n=6). Data analyzed by one way analysis of variance (ANOVA) followed by Tukey’s test for comparison of means. *significant against control; # against diabetic, at p ≤ 0.05)
### Table 4.10

Effect of acrylamide intoxication on the activities of antioxidant enzymes in sciatic nerve and brain regions of diabetic rats

<table>
<thead>
<tr>
<th>Parameters/Group</th>
<th>Control</th>
<th>Diabetic</th>
<th>ACR</th>
<th>Diabetic + ACR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sciatic nerve</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GST(^1)</td>
<td>19.5 ± 1.4</td>
<td>19.2 ± 2.4</td>
<td>22.9 ± 1.4</td>
<td>21.8 ± 2.5</td>
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<td>SOD(^2)</td>
<td>4.50 ± 0.2</td>
<td>5.87 ± 0.4*</td>
<td>4.80 ± 0.2</td>
<td>6.90 ± 0.2 *#</td>
</tr>
<tr>
<td>CAT(^3)</td>
<td>2.72 ± 0.2</td>
<td>3.25 ± 0.3</td>
<td>2.65 ± 0.4</td>
<td>4.05 ± 0.4 *#</td>
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<tr>
<td><strong>Cortex</strong></td>
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</tr>
<tr>
<td>GST(^1)</td>
<td>40.6 ± 1.6</td>
<td>35.4 ± 1.8</td>
<td>47.0 ± 1.7*</td>
<td>20.6 ± 3.1*#</td>
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<td>SOD(^2)</td>
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<td>89.8 ± 6.5</td>
<td>66.0 ± 3.6</td>
<td>126.0 ± 4.0*#</td>
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<td>CAT(^3)</td>
<td>5.91 ± 0.3</td>
<td>8.51 ± 0.2*</td>
<td>7.08 ± 0.2</td>
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<tr>
<td>GST(^1)</td>
<td>38.9 ± 2.1</td>
<td>39.2 ± 4.1</td>
<td>43.5 ± 1.7*</td>
<td>24.3 ± 1.5 *#</td>
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<tr>
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<td>71.1 ± 2.9</td>
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<td>CAT(^3)</td>
<td>6.11 ± 0.3</td>
<td>4.48 ± 0.3*</td>
<td>6.88 ± 0.3</td>
<td>4.33 ± 0.5 *</td>
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</table>

Values are mean ± SE (n=6)

ACR, 25 mg/ kg bw, i.p., 3 times/ wk, 5 weeks

\(^1\)-Glutathione-S-transferase, µmol/ min / mg protein
\(^2\)-Superoxide dismutase, U/ mg protein
\(^3\)-Catalase, nmol of hydrogen peroxide decomposed/ min/ mg protein

(Data analyzed by one way analysis of variance (ANOVA) followed by Tukey's test for comparison of means. * Significant against control; # significant against diabetic, p ≤ 0.05)
Fig. 4.33

Effect of acrylamide intoxication on the levels of calcium (A), acetyl cholinesterase activity (B) and dopamine levels (C) in sciatic nerve and brain regions of diabetic rats

ACR, 25 mg/ kg bw, ip., 3 times/ wk, 5 weeks

(Values are mean ± SE (n=6). Data analyzed by one way analysis of variance (ANOVA) followed by Tukey's test for comparison of means. *significant against control; # against diabetic, at p ≤ 0.05)
5.0 DISCUSSION

Under diabetic condition, neuronal injury occurs due to hyperglycemia. It is well accepted that persistent high glucose levels triggers polyol pathway, hexosamine pathway, activation of protein kinase C isoforms, accumulation of advanced glycation end products and activation of nuclear enzyme poly (ADP-ribose) polymerase, imbalance in calcium homeostasis and decreased antioxidant defense (Obrosova, 2009). These pathways cause oxidative stress/inflammatory response, mitochondrial dysfunction and trigger apoptotic pathways in the nervous system. Oxidative stress, a key mediator in DN, provokes deleterious downstream cellular events. These affects phosphorylation and de-phosphorylation of key enzymes, activation and deactivation transcription factors, regulation of protein turnover, mitochondrial function, nucleic acid metabolism, regulation of cell cycle and apoptosis. Since in vivo models provide data on the behavioral and physiological impact associated with hyperglycemia, cell models are often employed to understand the molecular mechanisms.

In the present investigation undifferentiated SHSY5Y cells were employed to understand the implications of hyperglycemia and subsequently the modulatory effects of selected spice actives were determined. Consistent with previous reports, the IC$_{50}$ value of glucose was 100 mM in SHSY5Y cell line (Li et al., 2003). At sub-lethal concentration, glucose induced significant oxidative stress as evidenced by elevated levels of ROS and HP with concomitant reduction in GSH levels. SHSY5Y cells co-exposed with glucose and spice actives- EU, CU and GE exhibited lower levels of oxidative markers- ROS, HP and 3-NT, a marker of protein oxidation.

Further in the cell model, the effect of hyperglycemia on neurofilaments was studied. The integrity of the cytosol is mainly contributed by the neurofilaments (NFs) along with the actin and tubulins. NFs provide strength and caliber to the neurons especially the axons. They are involved in a dynamic network comprising neuronal differentiation, axon outgrowth and regeneration (Perrot and Eyer, 2013). These proteins facilitate the movement of vesicles from the interior to the cell membrane and vice-versa. This event is more crucial at the synapse, as the release and up-take of neurotransmitters governs the extent of
neurotransmission and the degree of action potential. But under DN, neurofilaments accumulate in the sensory neurons affecting the neuronal transport and function (Sayers et al., 2003). Consistent with the notion in the present study, SHSY5Y cells under hyperglycemic condition exhibited accumulation of NFs (mainly the NF-L). When cells were co-exposed with spice actives and glucose, EU significantly lowered the levels of both NF-L and NF-H. Although specific reason for this effect cannot be deduced from this study, it is speculated that the antioxidant property EU may prevent the accumulation/aggregation of oxidatively altered cytoskeletal proteins such as NFs.

Previously few studies have shown that that the expression of HSPs both in diabetic rats and in humans is reduced. Under diabetic condition, NFκB is activated via protein kinase C resulting in an inflammatory reaction by enhancing the cytokine gene expression. HSP 70 prevents this pathway by blocking NFκB activation. Higher levels of HSPs are required to prevent apoptosis in vitro (Yan et al., 2013). However, in the cell model- SHSY5Y, at sub-lethal concentration of glucose, an elevation of HSP70 levels was evident, this may be in response to the stress induced by hyperglycemia, as an adaptive measure by the cells (Hooper and Hooper, 2005). It has been reported earlier that the levels of HSP70 is enhanced in the dorsal root ganglion of diabetic rats and has been termed as a survival element (Kamiya et al., 2005). However, cells co-exposed with spice actives and glucose exhibited diminished levels of HSP70. This effect may be indicative of lowered trigger for enhanced HSP70 expression due to the antioxidative properties of actives. In general HSP family, an endogenous defence system, renders protection against tissue damage by correcting the misfolding of the denatured proteins and maintaining structural integrity by acting as molecular chaperons (Al-Khatib, 2013).

**In vivo neuropathy model**

Initially employing the diabetogen STZ, the development/progression of neuropathy was assessed in a rat model. Further, hyperglycemia associated biochemical perturbations were also investigated in SN and brain regions (Ct, Cb, St, Hc). During the course of development of DN, the small and large nerve fibres
are known to be affected giving rise to phenotypes such as spontaneous shooting pain, allodynia and hyperalgesia. In the present study, at varying sampling time points, a STZ diabetic model was used to mimic these neuropathic features (allodynia and hyperalgesia) since long term diabetic condition in humans are known to be associated with DN (Gul et al., 2000; Kamei et al., 2001). In diabetes, sensory neurons are damaged before significant motor deficits develop (Zochodne et al., 2008). Consistent with this, sensory function – hyperalgesia/ allodynia developed significantly within week four of diabetic induction, while significant motor deficits were evident only by seventh week. Further, among diabetic rats, marked induction of oxidative stress was discernable at different sampling times in SN (peripheral nerve tissue) and brain regions (Kamboj et al., 2010; Andersen, 2012). The biochemical assessments in SN clearly showed pronounced aberrations in the levels of oxidative markers, GSH and calcium at weeks 8 and 12 and onset of behavioral manifestations. The effect was more pronounced with longer duration of diabetic condition. However the alterations in the brain regions were differential; the extent of oxidative stress and perturbations in the activity levels of various enzymes did not exhibit a time dependent pattern. These data clearly suggest that although alterations in both CNS and PNS are involved in the behavioral deficits, the effect in SN is a delayed outcome under hyperglycemic condition.

Modulatory effects of spice actives

It is well accepted that, other than strict control of blood glucose levels there are no therapies that can explicitly off-set progressive DN. Drugs (anticonvulsants and antidepressants), which are currently in use to treat neuropathic pain are known to fail among nearly 50% of the patients, while in the others total alleviation is not achieved normally (Yasmina et al., 2011). Despite the evidence about the pivotal role of oxidative stress in the development of DN, classical antioxidants such as vitamin E, have failed to show anticipated beneficial effects in clinical trials (Shelton et al., 2005; Ceriello 2006). Therefore, there exists a greater need to identify antioxidants that would be effective in ameliorating/ preventing nerve damage under hyperglycemic condition. In this regard the potential of herbal actives to attenuate the endogenous redox status in
vivo has been considered as an effective tool to achieve neuro-protection (Rio et al., 2009; Dumont and Beal, 2011). Accordingly, in the present study, the potential of GE/ EU to modulate endogenous redox markers in brain regions was examined. Under diabetic condition, owing to their antioxidant (free radical quenching ability) and anti-inflammatory property, GE/ EU significantly improved the nociceptive thresholds in thermal hyperalgesia and cold allodynia.

Following the standardization of STZ model, modulatory effects of selected spice actives were investigated employing two paradigms: (i) Co-exposure (with GE supplementation) and (ii) Intervention model (with EU supplementation). While a time dependent progression in neuropathic signs was evident among diabetic rats, GE supplemented diabetic rats exhibited significant improvement against both noxious (hyperalgesia) and non-noxious (allodynia) stimuli from week 4 onwards. In the intervention study, EU treatment was able to significantly restore the sensory function. The protective effect of EU in terms of alleviating neuropathic pain is probably related to its specific action in the dorsal horn of spinal cord (Lionnet et al., 2010). Interestingly, GE supplemented diabetic rats did not develop significant motor deficits during the entire experimental period. In the curative approach, treatment with EU offset DN induced motor deficits. This action of EU may be related to its ability to modify the excitability of SN and superior cervical ganglion neurons (Moreira-Lobo et al., 2010).

Both the bioactives, GE and EU did not affect the body weight gain, blood glucose levels and behavioral tests in comparison to the control rats. However, both caused a significant reduction in the endogenous levels of oxidative markers with elevated activities of SOD, CAT and GR in brain regions clearly suggesting their antioxidant property. Previously, under diabetic condition, elevated levels of ROS/ RNS and compromised antioxidant defense, leading to lipid peroxidation, protein oxidation and DNA damage have been demonstrated (Obrosova, 2009; Negi et al., 2011). Although, no significant change was evident with respect to body weights among the treated and untreated diabetic rats, biochemical alterations in SN and brain regions were more pronounced with these actives treatment. Although no reduction in blood glucose levels was evident among GE
treated rats, interestingly EU treated diabetic rats showed significant reduction in a time dependent manner.

In the present model diabetic rats exhibited a marked increase in oxidative markers in SN and brain regions, while GE/ EU supplementation restored their levels. This effect is consistent with previous studies that have shown the ability of GE/ EU to scavenge different types of ROS (Prasad et al., 2004; Khatun et al., 2006; Tiwari and Kakkar, 2009; Ahmad et al., 2011). In rat model of renal cancer, depletion in GSH levels were restored to normalcy with concomitant increase in the activities of various antioxidant enzymes (CAT, GST and GPx) in the renal tissue of GE treated rats (Ahmad et al., 2011). Similarly in SN and brain regions an elevation in the levels of GSH and total thiols (Data not shown) along with the enhancement in the activities of related enzymes were observed in GE supplemented diabetic rats. These data corroborates with the findings in D. melanogaster where GE/ EU enriched diet caused reduction in the levels of ROS and enhanced the activities of antioxidant enzymes as well as offered marked protection against ACR-induced lethality and locomotor dysfunction (Prasad and Muralidhara, 2012, 2013b). Further in the rat model, GE/ EU conferred protection against acrylamide induced neuropathy and altered biochemical markers of oxidative stress in SN and brain regions (Prasad and Muralidhara, 2013b, c).

Although speculative, in the present model, elevated levels of free radicals among diabetic rats was probably caused by disruption of cellular Ca\textsuperscript{2+} homeostasis via mitochondrial dysfunction and activation of transient receptor potential (TRP) channels (de Arriba et al., 2006; Naziroglu, 2011). The accumulating evidence implicating Ca\textsuperscript{2+} dysregulation and over production of oxidative stress products in DN, along with recent advances in understanding cation channels such as TRP channels, suggests modulation of neuronal Ca\textsuperscript{2+} handling an increasingly attractive approach for therapeutic interventions against the painful and degenerative aspects of DN (Latham et al., 2009; Naziroglu et al., 2012). Interestingly, GE/ EU treatment lowered the levels of Ca\textsuperscript{2+} in SN and brain regions. Further, elevation in the Ca\textsuperscript{2+} levels activates NOS resulting in elevation of NO levels. However GE/ EU treatment could lower NO levels in SN and brain regions, probably by lowering the cellular Ca\textsuperscript{2+} levels.
further emphasizing the anti-inflammatory property of GE/ EU \textit{in vivo} (Katsukawa et al., 2011).

Mitochondrial oxidative stress has been proposed as a major mediator of neurodegeneration in diabetes (Fernyhough et al., 2010; Kamboj and Sandhir, 2011). Accordingly, the elevation in the oxidative markers in mitochondrial fraction was associated with significant reduction in the activities of the marker enzymes among the diabetic rats. It was earlier postulated that, with impairment of mitochondrial physiology (such as mitochondrial swelling and decreased transmembrane potential), a significant reduction occurs in electron transport chain function (Vincent et al., 2005; Venditti et al., 2007; Fernyhough et al., 2010). This abnormal activity may predispose mitochondria to generate more ROS, further hampering the bioenergetic status of neurons. Interestingly, GE/ EU supplemented rats, exhibited diminished levels of oxidative markers and enhanced activities of Complex I – III, SDH and CS. It would be logical to assume that the above effects of GE/ EU may be largely responsible for the observed protective effects.

In a recent study, GE was shown to activate peroxisome proliferator activated receptor (PPAR α and γ), a nuclear regulatory protein involved in the transcription of genes regulating glucose and fat metabolism (Katsukawa et al., 2011). The ethanol extract of clove (rich in EU) was also shown to possess the ability to activate PPAR- γ (Kuroda et al., 2012). PPARs act on peroxisome proliferator responsive elements (PPRE), which influences insulin sensitive genes. The increase in the production of mRNAs of insulin dependent enzymes results in better use of glucose by the cells. Although speculative, the protective effects of GE/ EU may be related to the activation of PPAR. In contrast, enhanced lipid peroxidation and protein oxidation observed among untreated diabetic rats are likely to result in loss of cellular membranes integrity, ion channels, receptors of various ligands and neurotransmission.

Significant alteration in the neurotransmission markers such as AChE activity and DA levels has been previously reported under diabetic condition (Peeyush et al., 2010). AChE being an important membrane component, contributes to its integrity and alters permeability occurring during synaptic
transmission and conduction (Schmatz et al., 2009). Previous findings have shown that enhanced activation of AChE causes a reduction of cholinergic neurotransmission due to decreased levels of acetylcholine, thereby affecting related functions such as cognition and cell proliferation (Jin et al., 2004; Schmatz et al., 2009; Peeyush et al., 2010). In our study in the Drosophila model of ACR neurotoxicity, both spice actives attenuated significantly the activity levels of AChE in the head and body regions. A similar effect of GE and EU were also evident in the diabetic model clearly suggesting a specific effect on cholinergic function (Prasad and Muralidhara, 2012; 2013b). Earlier few workers have demonstrated the potency of these actives to inhibit AChE activity in other models (Howes et al., 2003; Perry et al., 2003; Dohi et al., 2009; Kumar et al., 2009). Further GE supplementation also restored the depleted DA levels in SN and brain regions among diabetic rats. In all probability the restoration of DA levels emphasizes the anti-hypersensitivity effect of GE (Dang et al., 2010). This finding of GE is consistent with the earlier effects observed in Drosophila model.

Local anesthesia has a soothing effect against pain, hence such properties of EU correlate well with its analgesic activity (Moreira-Lobo et al., 2010). The electrophysiological effect of EU on TRPV1 channels has been described and is suggested to be more specifically related to nociception. Previously, EU was reported to reduce acetic acid induced abdominal contortions and such effect was antagonized by capsazepine induced inward current in the neurons of the dorsal root ganglia which was partially sensitive to capsazepine (Ohkubo and Shibata, 1997; Ohkubo and Kitamura, 1997). EU alleviates neuropathic pain (allodynia and hyperalgesia) probably by acting centrally at the level of the dorsal horn of the spinal cord where vannilloid receptors are found (Lionnet et al., 2010).

In conclusion, it is proposed that GE/ EU have the potential to modulate the markers of oxidative stress, mitochondrial dysfunction and aberrations in neurotransmission under diabetic condition. Based on behavioral assessments and biochemical evidence, it is proposed that GE/ EU are promising therapeutic adjuvants to treat/ manage diabetes associated neuropathy. Although the protective action of GE/ EU is largely attributed to but not limiting to their
antioxidant and anti-inflammatory properties, further studies are warranted to decipher the underlying molecular mechanism(s).

*Interactive model: Diabetic and ACR*

The primary objective of the interactive model was to understand whether diabetic condition predisposes to exogenous neurotoxin insult. Data obtained in this model clearly suggests that diabetic rats were indeed predisposed to the neurotoxic effects of ACR (low dose). Interestingly, development of neuropathic signs was advanced among the diabetic rats administered with ACR. The intensity of deficits in both sensory and motor functions was higher among ACR administered diabetic rats. The degree of oxidative impairments among diabetic rats administered with ACR was evident in SN and brain regions; the effects being more pronounced in SN. The elevated activity of AChE among diabetic rats was further enhanced with ACR administration in SN suggesting the probable potentiating effect of ACR on cholinergic function. Among diabetic rats intoxicated with ACR, higher degree of depletion in DA levels indicates that the diabetic rats are more susceptible to ACR induced effects on dopaminergic function. These factors probably contribute to the development of neuropathic signs earlier than in diabetic rats or in ACR administered control rats.

6.0 SUMMARY

1. The IC$_{50}$ value for glucose in SHSY5Y cells was 100 mM. Spice actives *viz*., EU (5 - 10 µM), CU (20 µM) and GE (10 µM) significantly attenuated the impact of hyperglycemic response in a co-exposure paradigm (24 h) as evidenced by diminution in the levels of ROS/HP and enhanced levels of GSH.

2. Treatment of cells with EU/GE significantly attenuated glucose induced elevation in the levels of 3-nitro tyrosine and HSP70 levels.

3. While glucose (100 mM) enhanced the accumulation of neurofilaments (NF-L and NF-H), the levels of NF-H were reduced only among EU treated cells.
4. Adult rats rendered diabetic developed characteristic signs of neuropathy in a temporal manner and the onset of sensory deficits preceded (at 2 weeks) the development of motor dysfunction (at 6 weeks).

5. Among diabetic rats, biochemical analysis revealed enhancement in the levels of oxidative markers in brain regions at different sampling points (4, 8 and 12 weeks). Pronounced alterations in SN were evident at 8 and 12 weeks.

6. GE (100 mg/ kg bw/d, 8 wks) supplements among diabetic rats resulted in a marginal reduction in the blood glucose levels (20% of rats displayed normal levels) and increased the resistance to hot and cold stimuli (tail immersion tests).

7. While untreated diabetic rats developed motor deficits as early as 6 wks, only 20% of GE treated rats displayed a marginal induction of motor deficit terminally.

8. Among diabetic rats, GE ameliorated the elevated levels of oxidative markers in SN and brain regions with concomitant elevation in GSH levels, activities of GR/ TRR and lowered the calcium levels in SN and brain regions.

9. GE supplements attenuated diabetes associated enhanced levels of mitochondrial oxidative markers and activities of CS (except Hc) and SDH.

10. GE supplements significantly restored the DA levels in SN/ St and also reduced the activity of AChE (SN, Cb and St) among diabetic rats.

11. In the intervention model, EU treatment to diabetic rats significantly ameliorated both sensory (hyperalgesia and allodynia) and motor deficits. Significant reduction in the blood glucose levels without improvement in the body weight gain was apparent among EU treated diabetic rats.

12. Diabetic rats exhibited robust increase in the levels of oxidative markers with depletion in GSH levels in both SN and brain regions (Ct, Cb, St, Hc) and EU treatment markedly abrogated the levels of oxidative markers and calcium levels.
13. In this model, activities of GR and TRR, were enhanced among EU treated rats in both SN and brain regions, while the activities of SOD and CAT were enhanced only in SN.


15. Robust increase in the levels of oxidative markers in the mitochondrial fraction of diabetic rats was significantly reduced with EU treatment. Marked decrease in the activities of mitochondrial enzymes (SDH, CS, complex I – III and MTT reduction) among diabetic rats was enhanced by EU treatment.

16. Further EU treatment significantly improved the anatomical aberrations in the SN of diabetic rats as evidenced by histological studies.

17. Taken together, these data suggest the spice bio-actives tested (EU, GE) were able to significantly attenuate hyperglycemia induced oxidative impairments in the SN and brain regions in a STZ diabetic rat model.

18. In the interactive model, diabetic rats exhibited relatively higher susceptibility to the neurotoxin ACR, as evidenced by advancement in the development of sensory and motor dysfunctions.

19. The degree of oxidative impairments among diabetic rats administered with ACR were more pronounced in both SN and brain regions.

20. The elevated activity of AChE among diabetic rats was further enhanced with ACR administration in SN. Dopaminergic function appeared more vulnerable as evidenced by higher depletion in the levels of DA in SN and brain regions of ACR administered diabetic rats.