SCOPE AND OBJECTIVES OF THE INVESTIGATION

Neuropathy is a collection of disorders that occurs due to the damage to the nerves. Although more commonly referred to the damage to the peripheral nervous tissue, neuropathy can damage, destruct or cause dysfunction of either the central or peripheral nervous system. The causes may be traumatic injuries, infections, metabolic disorder and chemical insults to the nervous tissue resulting in a crippling complex disease condition. The symptoms of neuropathy are broadly categorized as sensory and motor deficits, comprising numbness, loss of sensation, hypo or hyper sensation and co-ordination and locomotor dysfunctions. Neuropathic pain is mostly burning, shooting, stabbing and cruciate. Neuropathies and the related pain are often classified according to the underlying etiology, eg. diabetic neuropathy, cancer related neuropathy etc.

Current understanding of the pathology encompasses oxidative stress, inflammatory reactions, mitochondrial dysfunction and apoptosis in the nervous tissue. Over production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), a decreased antioxidant defence system or both results in oxidative stress and has been implicated in neuropathy. Although ROS are required for normal cellular functions, accumulation/ enhanced production is deleterious. In the process, inflammatory pathways are triggered, mitochondrial function is affected (membrane damage followed by an imbalance in the ionic homeostasis) which adversely affect energy turn-over and cellular function. The ROS generated are detoxified by antioxidant molecules such as reduced glutathione (GSH) and antioxidant enzymes superoxide dismutase, catalase, glutathione peroxidase and reductases. However, with increased burden of free radicals, significant protein oxidation, lipid peroxidation and RNA/ DNA oxidation is inevitable, thus altering their vital structural and functional role. Dis-regulation of calcium (Ca^{2+}) homeostasis, leading to activation of neuronal nitric oxide synthase (nNOS)/ phospholipase A2, enhanced levels of NO, calpains and initiation of apoptosis. Further, synthesis and expression levels of some of the
neurotrophins or neurotrophic factors are significantly altered affecting the neuronal regenerative process.

Several in vivo and in vitro models are employed to understand the pathophysiology of neuropathy and to develop therapeutic strategies. Rodent models (wild/ transgenic) are often employed as experimental models. In recent times, Drosophila melanogaster (wild/ transgenic) is extensively being utilized as an in vivo model to understand the pathophysiology of various neurodegenerative disorders and neuropathy. The advantages of the fly model include well-known anatomy, short life span, large number of offspring, easy maintenance/ culturing and many genetic tools to modulate/ regulate specific gene expression and knock outs. To obtain mechanistic insights, primary cultures (eg., DRG neurons, CGC cultures) and cell lines (SHSY5Y, Neuro 2a, PC 12) are extensively employed.

Two experimental models have been employed in the present investigation to induce neuropathy in vivo. They are: neurotoxin (Acrylamide, ACR) model and diabetic (STZ) model. ACR, a vinyl monomer, is a human neurotoxin which has wide industrial applications (such as waste water management, ore processing, and dye synthesis), cosmetics and used in gel electrophoresis in biochemistry/ molecular biology laboratories. Carbohydrate foods processed at high temperatures (eg., potato chips, French fries, cookies, biscuits etc.) contributes to the dietary intake of ACR. The neurotoxicity of ACR is characterized by ataxia and distal skeletal muscle weakness among laboratory animals/ humans. Oxidative stress and mitochondrial dysfunctions have been demonstrated to be key mechanisms in ACR-induced cell injury and neuropathy. ACR-induced sensory and motor dysfunctions are also associated with oxidative stress in nervous system (brain, spinal cord and sciatic nerve). A recent study estimated a Tolerable Daily Intake (TDI) 40 µg/ kg bw/ d for ACR induced neurotoxicity. However, ACR exposure in industrial settings is estimated to be much higher. Hence in the first series of investigations this model was employed.
One of the most common complications of diabetes associated microvascular diseases is diabetic neuropathy (DN). The International Diabetic Federation (IDF) estimates that 438 million people world-wide would suffer from diabetes by the year 2030. More than 60% of diabetic people develop/ exhibit some form of neuropathy in terms of sensory deficits, pain and autonomic dysfunction. DNs are a family of nerve disorders caused by persistent hyperglycemia. Diabetes not only damages peripheral neurons, but also impairs their capacity to regenerate. Most of the symptoms such as numbness, tingling and progressive balance difficulties are due to loss of thickly myelinated nerve fibres (large fibre symptoms) and spontaneous shooting/ stabbing pains, hot or cold burning sensations and allodynia are due to dysfunction of the thinly myelinated and unmyelinated nerve fibres (small nerve fibre sensations). Since hyperglycemia can activate many signaling mechanisms in cells, various mechanisms are known to be involved in DN. Excess glucose shunts to the polyol pathway, undergoes auto-oxidation to produce advanced glycation end products (AGEs), activates protein kinase C (PKC) and its down-stream signaling and cause overload and slowing of electron transfer chain (ETC). This further leads to the escape of reactive intermediates. These events result in enhancement of oxidative stress, depletion of cytosolic NADPH and GSH; inflammatory reactions, mitochondrial dysfunction, impaired energy metabolism, discrepancy in Ca\textsuperscript{2+} homeostasis and apoptosis.

In general there exists a need for alternative medicine for management of neuropathy especially in diabetes, chemotherapy induced or toxin induced neuropathy, since usage of various drugs have been found to be effective limited due to their side effects. In the recent past, various herbal actives including spices and phytoconstituents are being extensively employed as complementary therapeutic agents in the management of neuropathy. Humans have been consuming various spices since time immemorial either as a component of various food preparations or medicinal formulations. Spices can be derived from various plant parts - leaf (bay leaf), buds (clove), rhizomes (turmeric), seeds (cumin), bark (cinnamon), and stigma of flower (saffron). The bioactive
compounds are mostly phenolic acids, flavonoids, sterols or terpenes. Currently the role of spice actives in enhancing neuronal health under disease condition or neurotoxin insults is being addressed in various experimental models. Accordingly the present proposal, aims to investigate the propensity of selected spice actives and phyto-constituents to alleviate experimentally induced neuropathy employing various models such as *Drosophila*, cell model and rodent models.

**Hypothesis**

The working hypothesis of this investigation is based on the premise that spice actives owing to their antioxidant and anti-inflammatory properties may possess significant neuromodulatory potential under experimentally induced neuropathic condition.

**Objectives**

- Screening of spice actives/ phytochemicals for the neuroprotective effects in experimentally induced neurotoxicity in *Drosophila* model
- To investigate the modulatory potency of selected spice active principles (eg., eugenol and derivatives) against experimentally induced neuropathy in rat model
- To establish the therapeutic efficacy of spice actives against diabetes-induced neuropathy and examine its correlation with oxidative dysfunctions in brain
- To identify the biochemical targets related to the neuroprotective effects of employing *Drosophila* and cell models.