

Chapter 1:
Introduction

INTRODUCTION

Diabetes mellitus (DM) is reported to affect more than 100 million people worldwide in 2006 and is projected to affect more than 350 million by 2030 (Kaye *et al.*, 2003; Davies *et al.*, 2006; Pop-Busui *et al.*, 2010). Painful Diabetic Neuropathy (PDN) is one of the most painful complications of diabetes mellitus, involving progressive neuronal damage and dysfunction, and up to 40 % of patients with diabetes mellitus develop diabetic neuropathy (Boulton *et al.*, 2005; Davies *et al.*, 2006; Pluijms *et al.*, 2010). Neuropathic pain has been considered as the most debilitating painful condition as a consequence of primary lesion or dysfunction in the nervous system either the central nervous system (CNS) or the peripheral nervous system (PNS) (Merskey and Bogduk, 1994; Latremoliere and Woolf, 2009). PDN in rodents and humans is characterized by an early thermal/ mechanical hyperalgesia and allodynia (Daulhac *et al.*, 2006; Morgado *et al.*, 2010). Pain caused by diabetes mellitus is debilitating (Hoffman *et al.*, 2009) and often is refractory to classical analgesics, including morphine (Raghavendra *et al.*, 2002; Chen and Pan, 2003b).

The mechanisms involved in genesis of diabetes -induced neuropathy are complex and remain poorly understood (Tesfaye, 2009; Ziegler, 2010). Accumulating line of evidence indicates that one of the major causes of diabetic peripheral neuropathy is oxidative stress (Newsholme *et al.*, 2007; Ozkul *et al.*, 2010), formation of advanced glycation end-product (Cameron *et al.*, 2005; Sugimoto *et al.*, 2008), increased flux through the polyol pathway that leads to accumulation of sorbitol and fructose (Chen *et al.*, 2010), myo-inositol depletion and reduction in Na⁺-K⁺-ATPase activity (Sima *et al.*, 1997; Pop-Busui *et al.*, 2001), deficits in neurotrophism leading to reduced expression and depletion of neurotrophic factors such as nerve growth factor , neurotrophin-3 and insulin-like growth factor (Shimoshige *et al.*, 2010), as well as

alterations in axonal transport (Kuwabara and Misawa, 2008) and PARP over-activation (Negi *et al.*, 2010; Drel *et al.*, 2007; & 2010). In addition to abnormalities of peripheral afferent nerves, altered sensory processing in the spinal cord may contribute to the development of diabetic neuropathic pain (Loseth *et al.*, 2008; Morgado *et al.*, 2010). Other factors, such as up-regulation of spinal excitatory glutamate receptors and increased release of glutamate and substance P, are also implicated in the development of spinal hypersensitivity in diabetes (Anjaneyulu *et al.*, 2008).

Various analgesic agents such as non steroidal anti-inflammatory drugs (NSAID) (Zochodne and Ho, 1992 & 1994), opioids (Gilron *et al.*, 2005), cannabinoids (Ulugol *et al.*, 2004; Bujalska *et al.*, 2008) and neurosteroids (Christine and Ayikoe, 2008) have been used to treat acute and chronic pain. In addition, recently, antidepressant and antiepileptic agents have shown strong antinociceptive effect in various preclinical and clinical studies (Rowbotham *et al.*, 2004; Freeman *et al.*, 2008). NSAIDs are very commonly used over-the-counter analgesics and have been documented to reduce acute and chronic pain via inhibition of cyclo-oxygenase enzymes (Hyllested *et al.*, 2002; Dudhgaonkar *et al.*, 2007). Opioids are centrally acting analgesic agents, which produce analgesia through μ , κ , and δ opioid receptors (Law *et al.*, 2000; Shah *et al.*, 2004). Synthetic cannabinoid receptors agonist such as WIN 55-212-2 is reported to reduce chronic pain in diabetic animals (Ulugol *et al.*, 2004). Furthermore, cannabinoids in combination with opioids (Wilson *et al.*, 2008; Bushlin *et al.*, 2010) or NSAIDs produced synergistic effect (Pinaridi *et al.*, 2005; Ulugol *et al.*, 2006; Hama and Sagen, 2010) and provide adequate pain reliefs in PDN (Toth *et al.*, 2010).

Chronic diabetes or persistent hyperglycemia has been reported to reduce: i) threshold of pain (Raz *et al.*, 1988; Kamei *et al.*, 1994 & 1998), ii) reduce opioids and

cannabinoids agonist responsiveness, which may be due to receptor desensitization or down-regulation (Chen *et al.*, 2002; Chen, 2003; Tanya *et al.*, 2008), iii) modulate several enzymes, neurotransmitter and endogenous mediator involved in regulation of pain sensitivity, including PKC (Gerald and King, 2010) and NMDA (Sang *et al.*, 2002; Chen *et al.*, 2009; Guo *et al.*, 2009), iv) induce generation of ROS (Ozkul *et al.*, 2010), v) advanced glycation end products (AGEs) (Sugimoto *et al.*, 2008; Friederich *et al.*, 2009), vi) modulate the release and production of pro-inflammatory cytokines (Carter *et al.*, 1998; Doupis *et al.*, 2009), vii) activation of astrocyte and micro-glial cells in spinal cord (McMahon *et al.*, 2005; Revsin *et al.*, 2005; Surcheva *et al.*, 2009) that induce increase in bioactive endogenous mediators, including pro-inflammatory cytokines and NO (Flodstrom *et al.*, 1996; Inoue, 2006; Ledebor *et al.*, 2007), viii) activate COX- pathway and increase the generation of prostaglandins (PGs) (Du *et al.*, 2004; Bujalska *et al.*, 2009), ix) increase superoxide generation that activate NF- κ B (Bierhaus *et al.*, 2001) and, x) increase the cytokines formation (Doupis *et al.*, 2009; Goldberg *et al.*, 2009) which enhance expression of iNOS and the generation of NO (Flodstrom *et al.*, 1996; Grover *et al.*, 2000; Rashid *et al.*, 2004; Joharchi and Jorjani, 2007).

Superoxide anion avidly combines with NO, forming the powerful cytotoxic oxidant; peroxynitrite (ONOO⁻) (Obrosova *et al.*, 2007; Zheng *et al.*, 2009) and the generated cytotoxin ONOO attacks various biomolecules in the vascular endothelium, vascular smooth muscle and myocardium, neuronal cells and leading to cardiovascular disorders and PDN *via* multiple mechanisms (Obrosova *et al.*, 2005 & 2007; Negi *et al.*, 2010). These studies indicate that hyperglycaemia -induced activation of multiple mechanisms and enhanced oxido-nitrosative stress that are involved in the development of PDN.

The multiple, low dose, administration of streptozotocin (STZ) (MLDS) in non-obese diabetic (NOD) mouse, provides a relevant experimental model for insulin-dependent diabetes mellitus in man (IDDM) (Kolb and Kroncke, 1993). The role of autoimmune mediated destruction of islet of β -cells is indicated by islet infiltration by mononuclear cells (Kono and Matsuzawa, 1993), mainly T-lymphocytes and macrophages (Bradley *et al.*, 1999; Beyhum *et al.*, 1997), induction of co-stimulatory molecules and cytotoxic enzymes i.e iNOS and COX (Eizirik *et al.* 1996; Beyhum *et al.*, 1997). It has been shown that prevention of IDDM is possible with neonatal thymectomy (Boitard *et al.*, 1991) or splenectomy, and by in vivo/vitro treatment with cytokines inhibitor such as cyclosporine /thalidomide (Hernan *et al.*, 1989) or cytokines antibodies, anti-CD4⁺, or anti-I-A m Abs (Chatenoud *et al.*, 1997; Pickersgill and Mandrup-Poulsen, 2009; Taliyan *et al.*, 2010). Moreover, it has been demonstrated that spleen or spleen derived factor (s) modulate pain threshold and was involved in hyperglycemia-induced hyperalgesia (Kamie *et al.*, 1992; Khan *et al.*, 2009). Spleen derived factor(s) is also reported to be involved in diabetes induced morphine analgesic tolerance (Kamei *et al.*, 1992; 1994 & 1994; Taliyan *et al.*, 2010a & 2010b). Splenectomy or splenectomised beije-j mice regain the antinociceptive effect of analgesic and transfer of their mononuclear cells to their normal littermate's decreased antinociceptive effect of morphine (Kamie *et al.*, 1992: 1994: 1995 a & 1995b; Taliyan *et al.*, 2010 a & 2010b). Furthermore, splenectomy in diabetic animals restored the decrease analgesic effect of morphine (Kamei *et al.*, 1992; Grover *et al.*, 2000) and DAMGO, a μ -opioid agonist (Ohsawa and Kamei., 1997; Tasatargil and Sadan, 2004). These observations strongly indicate that some factor (s) derived from spleen mononuclear cells may be involved in decreasing analgesic effect of opioids analgesic in diabetes mellitus. Therefore, it is possible that spleen or spleen derived factor (s) is also involved in

modulation of antinociceptive effect to various analgesics, including cannabinoids, NSAIDs and neurosteroids in diabetic animals.

Thus, even though, diabetic neuropathy is one of the most common etiologies of chronic pain in patients, the underlying mechanism (s) involved in the development of analgesics resistance to various class of analgesic drugs in diabetic patients, remains, poorly understood. The present study was, therefore, designed to investigate the possible mechanism of diabetes - induced decrease in antinociceptive effect of various analgesics.