PERIPHERAL NEUROPATHY

Peripheral neuropathy is the term for damage to nerves of the peripheral nervous system which may be caused either by diseases or trauma to the nerve or the side-effects of systemic illness (Hughes, 2002). The four cardinal patterns of peripheral neuropathy are polyneuropathy, mononeuropathy, mononeuritis multiplex and autonomic neuropathy. The most common form is (symmetrical) peripheral polyneuropathy, which mainly affects the feet and legs. The form of neuropathy may be further broken down by cause or the size of predominant fiber involvement (i.e., large fiber or small fiber peripheral neuropathy). Neuropathy may be associated with varying combinations of weakness, autonomic changes and sensory changes. Loss of muscle bulk or fasciculations, a particular fine twitching of muscle, may be seen (Gierthmühen et al., 2011). Sensory symptoms encompass loss of sensation and “positive” phenomena including pain. Symptoms depend on the type of nerves affected (motor, sensory, or autonomic) and where the nerves are located in the body (Gijn, 2006). Common symptoms associated with damage to the motor nerve are muscle weakness, cramps and spasms. Loss of balance and coordination may also occur. Damage to the sensory nerve can produce tingling, numbness and pain. Pain associated with this nerve is described in various ways such as the following: sensation of wearing an invisible "glove" or "sock", burning, freezing or electric-like, extreme sensitivity to touch. The autonomic nerve damage causes problems with involuntary functions leading to symptoms such as abnormal blood pressure and heart rate, reduced ability to perspire, constipation, bladder (e.g., incontinence) and sexual dysfunction (Kamenov, 2011; Burakgazi et al., 2012).

Classification of Peripheral Neuropathy

Peripheral neuropathy may be classified according to the number of nerves affected or the type of nerve cell affected (motor, sensory, autonomic) or the process affecting the nerves (e.g. inflammation in neuritis).
**Mononeuropathy** is a type of neuropathy that only affects a single nerve. It is diagnostically useful to distinguish them from polyneuropathies because the limitation in scope makes it more likely that the cause is a localized trauma or infection. The most common cause of mononeuropathy is physical compression of the nerve, known as compression neuropathy (e.g., carpal tunnel syndrome). The "pins-and-needles" sensation of one's "foot falling asleep" (paresthesia) is caused by a compression mononeuropathy which can be resolved merely by moving around and adjusting to a more appropriate position (Ubersicht, 1976). Direct injury to a nerve, interruption of its blood supply (ischemia) or inflammation can also cause mononeuropathy (Kuwabara, 2009; Mauermann *et al.*, 2010).

**Mononeuritis multiplex** is simultaneous or sequential involvement of individual noncontiguous nerve trunks, either partially or completely, evolving over days to years and typically presents with acute or subacute loss of sensory and motor function of individual peripheral nerves. The pattern of involvement is asymmetric, however, as the disease progresses; deficit(s) becomes more confluent and symmetrical, making it difficult to differentiate from polyneuropathy (Pathria *et al.*, 2010). Therefore, attention to the pattern of early symptoms is important. Mononeuritis multiplex may also cause pain, which is characterized as deep, aching pain in the lower back, hip or leg. In people with diabetes mellitus, mononeuritis multiplex is typically encountered as acute, unilateral, severe thigh pain followed by anterior muscle weakness and loss of knee reflex (Diószeghy, 2011).

It is caused by or associated with several medical conditions:

- Diabetes mellitus
- Vasculitides: Polyarteritis nodosa, Wegener granulomatosis and Churg-Strauss syndrome
- Immune-mediated diseases: Rheumatoid arthritis, lupus erythematosus and sarcoidosis
- Infections: Leprosy, lyme disease and HIV
- Amyloidosis
- Cryoglobulinemia
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✓ Chemical agents, including trichloroethylene and dapsone
✓ Sting of certain jellyfish, such as the sea nettle

**Polyneuropathy** is a pattern of nerve damage which is quite different from mononeuropathy. The term "peripheral neuropathy" sometimes used to refer to polyneuropathy. In polyneuropathy many nerve cells in different parts of the body are affected, without regard to the nerve through which they pass. In distal axonopathy, one common pattern, the cell bodies of neurons remain intact, but the axons are affected in proportion to their length. Diabetic neuropathy is the most common cause of this pattern. In demyelinating polyneuropathies, the myelin sheath around axons is damaged, which affects the ability of the axons to conduct electrical impulses. The third and least common pattern affects the cell bodies of neurones directly. This usually picks out either the motor neurones (known as motor neurone disease) or the sensory neurones (known as sensory neuronopathy or dorsal root ganglionopathy). The effect of this is to cause symptoms in more than one part of the body, often on left and right sides symmetrically. As for any neuropathy, the chief symptoms include weakness or clumsiness of movement (motor), unusual or unpleasant sensations such as tingling or burning, reduction in the ability to feel texture, temperature and impaired balance when standing or walking (Latronico and Bolton, 2011). In many polyneuropathies, these symptoms occur first and most severely in the feet. Autonomic symptoms may also occur such as dizziness on standing up, erectile dysfunction and difficulty controlling urination.

Polyneuropathies are usually caused by processes that affect the body as a whole. Diabetes and impaired glucose tolerance are the most common causes (Mondelli et al., 2011; Nowicki et al., 2012). Other causes relate to the particular type of polyneuropathy, and there are many different causes of each type, including inflammatory diseases, vitamin deficiencies, blood disorders and toxins (including alcohol). Most types of polyneuropathy progress fairly slowly, over months or years, but rapidly progressive polyneuropathy also occurs.
Autonomic neuropathy is a form of polyneuropathy which affects the non-voluntary, non-sensory nervous system (i.e., the autonomic nervous system) affecting mostly the internal organs such as the bladder muscles, the cardiovascular system, the digestive tract and the genital organs (Olsovský, 2011; Ejaz et al., 2011). These nerves are not under a person's conscious control and function automatically. Autonomic nerve fibers form large collections in the thorax, abdomen and pelvis outside spinal cord, however they have connections with the spinal cord and ultimately the brain. Most commonly autonomic neuropathy is seen in persons with long-standing diabetes mellitus type 1 and 2. In most but not all cases, autonomic neuropathy occurs alongside other forms of neuropathy, such as sensory neuropathy (Lefrandt et al., 2010; Jawa et al., 2011).

Autonomic neuropathy is one cause of malfunction of the autonomic nervous system, but not the only one; some conditions affecting the brain or spinal cord can also cause autonomic dysfunction, such as multiple system atrophy and therefore cause similar symptoms to autonomic neuropathy.

The signs and symptoms of autonomic neuropathy include the following:

- Urinary bladder conditions: Bladder incontinence or urine retention
- Gastrointestinal tract: Dysphagia, abdominal pain, nausea, vomiting, malabsorption, fecal incontinence, gastroparesis, diarrhea and constipation
- Cardiovascular system: Disturbances of heart rate (tachycardia, bradycardia), orthostatic hypotension and inadequate increase of heart rate on exertion
- Other: Hypoglycemia unawareness and genital impotence

Neuritis is a general term for inflammation of a nerve or the general inflammation of the peripheral nervous system. Symptoms depend on the nerves involved, but may include pain, paresthesia (pins and needles), paresis (weakness), hypoesthesia (numbness), anesthesia, paralysis, wasting and disappearance of the reflexes (Chan, 2011; Pau et al., 2011). The various causes of neuritis include:
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✓ Physical injury: One common cause of neuritis and subsequent inflammation of the nerves to the toes is the wearing of high-heeled shoes or ill-fitting shoes that bind the toes painfully. This can cause temporary numbness and pain in the affected toes for several days.

✓ Infection:
  - Herpes simplex
  - Shingles
  - Leprosy
  - Guillain-Barre syndrome

✓ Chemical injury

✓ Radiation

✓ Underlying conditions causing localized neuritis (affecting a single nerve):
  - Diphtheria
  - Localized injury
  - Diabetes

✓ Underlying conditions causing polyneuritis (affecting multiple nerves):
  - Beriberi
  - Vitamin B12 deficiency
  - Metabolic diseases
  - Diabetes
  - Herpes zoster
  - Hypothyroidism
  - Porphyria
  - Infections, bacterial and/or viral
  - Autoimmune disease, especially Multiple Sclerosis
  - Cancer
  - Alcoholism

Types of neuritis includes (James et al., 2010; Chan, 2011; Mileto and Gaeta, 2011; Pau et al., 2011):

✓ Polyneuritis or Multiple neuritis
✓ Brachial neuritis
✓ Optic neuritis
Vestibular neuritis
Cranial neuritis
Arsenic neuritis

Signs and Symptoms of Peripheral Neuropathy

Those with diseases or dysfunctions in the peripheral nerves may have problems in any of their peripheral nerve functions (Gijn, 2006).

- In terms of sensory function, there are commonly loss of functions (negative symptoms), which include numbness, tremor and gait abnormality.
- Gain of functions (positive symptoms) includes tingling, pain, itching, crawling, and pins and needles. Pain can become intense enough to require use of opioids (i.e. morphine, oxycodone).
- Skin can become so hypersensitive that patients are prohibited from having anything touch certain parts of their body, especially the feet. People with this degree of sensitivity cannot have a bedsheet touch their feet or wear socks or shoes, and eventually become housebound.
- Motor symptoms include loss of function (negative) symptoms of weakness, tiredness, heaviness and gait abnormalities; and gain of function (positive) symptoms of cramps, tremor and muscle twitch (fasciculations).
- There is also pain in the muscles (myalgia) and cramps and there may also be autonomic dysfunction.
- During physical examination, specifically a neurological examination, those with generalized peripheral neuropathies most commonly have distal sensory or motor and sensory loss, though those with a pathology of the peripheral nerves may be perfectly normal, may show proximal weakness, as in some inflammatory neuropathies like Guillain–Barré syndrome or may show focal sensory disturbance or weakness such as in mononeuropathies.
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Causes of Peripheral Neuropathy

The causes of peripheral neuropathy are broadly grouped as follows:

✓ Genetic diseases: Friedreich’s ataxia, Charcot-Marie-Tooth syndrome (Gabriel et al., 1997)
✓ Metabolic/Endocrine: Diabetes mellitus, chronic renal failure, porphyria, amyloidosis, liver failure and hypothyroidism (Kiziltan et al., 2007)
✓ Toxic causes: Drugs (vincristine, phenytoin, nitrofurantoin, isoniazid, ethyl alcohol), organic metals, heavy metals, excess intake of vitamin B₆ (pyridoxine)
✓ Fluoroquinolone toxicity: Irreversible neuropathy is a serious adverse reaction of fluoroquinolone drugs (Cohen, 2001)
✓ Vitamin deficiency states: Vitamin B₁₂ (cyanocobalamin), vitamin A, vitamin E and vitamin B₁ (thiamin)
✓ Physical trauma: Compression, pinching, cutting, projectile injuries (i.e. gunshot wound), strokes including prolonged occlusion of blood flow, electric discharge, including lightning strikes
✓ Others: Shingles, malignant disease, HIV (Gonzalez-Duarte et al., 2007), radiation and chemotherapy (Wilkes, 2007)

NEUROPATHIC PAIN

The International Association for the Study of Pain defines neuropathic pain as "initiated or caused by a primary lesion or dysfunction in the nervous system" and due to disordered peripheral or central nerves (Merskey and Bogduk, 1994; Jensen et al., 2005; Galluzzi, 2007; Veves et al., 2008). This disorder can be caused by compression, transection, infiltration, ischemia or metabolic injury to neuronal cell bodies or in combination. Neuropathic pain may be classified as either peripheral or central in origin (Dworkin, 2002; Pascuzzi, 2009). Examples of the former include diabetic peripheral neuropathy (DPN), alcoholic peripheral neuropathy, postherpetic neuralgia, antineoplastic therapy–induced or HIV-induced sensory neuropathy, tumor
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infiltration neuropathy, phantom limb pain, postmastectomy pain, complex regional pain syndromes (reflex sympathetic dystrophy) and trigeminal neuralgia. Deafferentation syndromes resulting in neuropathic pain include multiple sclerosis, spinal cord injury, central poststroke pain and Parkinson disease. Bennett (1997) provided incidence estimates of common types of neuropathic pain and concluded that if neuropathic low back pain is included in the total, approximately 3.8 million individuals in the United States suffer from this disorder. Such painful conditions are likely to increase as the population grows older and age-related disorders such as herpes zoster, diabetes mellitus, cerebrovascular accidents, Parkinson disease and cancer-diseases of aging-develop.

**Difference between Nociceptive and Neuropathic Pain**

Response to an acute painful stimulus is an important adaptive mechanism that protects a person from further injury. Pain signals resulting from noxious stimuli (wounds, thermal or inflammatory insults) are converted into electrical impulses within tissue nociceptors whose cell bodies are found in dorsal root ganglions; both nociceptive and neuropathic pain signals utilize the same pain pathways (Galluzzi, 2007). Information regarding intensity, quality and location of pain is conveyed to the sensory cortex from the somatosensory thalamus. The CNS utilizes descending inhibitory pathways via the dorsolateral fasciculus (Lissauer’s tract) of the spinal cord and periaqueductal gray matter to modulate transmission of nociceptive stimuli (Kandel et al., 2000).

Efficient, rapid transmission of acute responses to a painful stimulus is a self protection process. In general, acute pain provides an “alarm” that leads to subsequent protective responses; neuropathic pain, however, signals no imminent danger. The operative difference is that neuropathic pain represents a delayed, ongoing response to damage that is no longer acute but which continues to be expressed as painful sensations (Galluzzi, 2007). Sensory neurons damaged by injury, disease or drugs produce spontaneous discharges leading to sustained levels of excitability. These ectopic discharges begin to “cross talk” with adjacent uninjured nerve fibers, resulting
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in amplification of pain impulses (peripheral sensitization). This hyperexcitability leads to greater transmitter release causing increased response by spinal cord neurons (central sensitization). This process, known as “windup,” accounts for the fact that the level of perceived pain is far greater than what is expected based on what can be observed (Spruce et al., 2003; Ji and Strichartz, 2004; Galluzzi, 2007).

Clinical Symptoms of Neuropathic Pain

Symptoms described by patients with neuropathic pain are myriad representing a variety of possible nerve injuries implicated in causation (Veves et al., 2008; Pascuzzi, 2009). Neuropathic pain sufferers complain of numbness, burning or tingling or a combination, they describe electric shock- like, prickly or pins and needles sensations. Patients completing the McGill Pain Questionnaire described their pain using terms such as “punishing-cruel” and “tiring-exhausting” (Melzack, 1987). Boureau et al. (1990) identified six adjectives used more frequently to describe neuropathic pain; electric shock, burning and tingling were most commonly used (53%, 54% and 48%, respectively), in addition to cold, pricking and itching.

Several types of abnormal sensations, or dysesthesias may occur alone or in addition to other specific complaints in patients with neuropathic pain (Table 1). Unlike usual responses to such discomfort these irritating or painful sensations occur in the absence of an apparent cause. A common example is the severe, aching, “toothache-like” response elicited by a cool draft of air on the cheek of a patient suffering from trigeminal neuralgia. Allodynia is a painful response to an non-noxious stimuli. Taken to the extreme (e.g., inability to remove the stimulus), this response can result in an agonizing neuropathic symptom known as hyperpathia. Another example of this condition is “touch sensitivity” of badly sunburned skin, where even light stroking of an inflamed area causes extreme discomfort like neuropathic pain, this response seems out of proportion to the injury (Galluzzi, 2007; Pascuzzi, 2009).
Table 1. Sensory Symptoms And Signs Associated With Neuropathic Pain

<table>
<thead>
<tr>
<th>Symptoms or Sign</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allodynia</td>
<td>Pain due to non-noxious stimuli (clothing, light touch) when applied to the affected area. May be mechanical (caused by light pressure), dynamic (caused by non-painful movement of a stimulus) or thermal (caused by non-painful warm or cool stimulus)</td>
</tr>
<tr>
<td>Anesthesia</td>
<td>Loss of sensation to the affected region</td>
</tr>
<tr>
<td>Dysesthesia</td>
<td>Spontaneous or evoked unpleasant abnormal sensations</td>
</tr>
<tr>
<td>Hyperalgesia</td>
<td>Exaggerated response to a mildly noxious stimulus applied to the affected region</td>
</tr>
<tr>
<td>Hyperpathia</td>
<td>Delayed and explosive response to a noxious stimulus applied to affected region</td>
</tr>
<tr>
<td>Hypoesthesia</td>
<td>Reduction of normal sensation to affected region</td>
</tr>
<tr>
<td>Paresthesia</td>
<td>Nonpainful spontaneous abnormal sensations</td>
</tr>
<tr>
<td>Phantom Pain</td>
<td>Pain from a specific site that no longer exists (e.g., amputated limb) or where there is no current injury</td>
</tr>
<tr>
<td>Referred Pain</td>
<td>Occurs in a region remote from the source</td>
</tr>
</tbody>
</table>

ALCOHOLIC PERIPHERAL Neuropathy

Alcoholism, the chronic and excessive consumption of alcohol, is a syndrome characterized by severe peripheral as well as CNS toxicity. However, the neurobehavioral deficits induced by alcohol and their impact on quality of life of an individual, are often unrecognized. After ingestion, alcohol distributes throughout body tissues and rapidly crosses the blood-brain barrier (Pan et al., 2008). It is not surprising that ethanol abuse significantly contributes to damage in variety of tissues including liver, the central and peripheral nervous systems, and skeletal and cardiac muscle. The association between alcohol and peripheral neuropathy has been recognized for over 200 years. It was first described by Lettsom in the year 1787 (Tabaraud et al., 1990).
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Definition

Alcoholic peripheral neuropathy is a potentially incapacitating complication of long-term excessive consumption of alcohol characterized by pain and dysesthesias, primarily in the lower extremities and is poorly relieved by available therapies (Ratcliff, 1979; Koike et al., 2001a; 2003; Mellion et al., 2011). Patients with alcoholic neuropathy sustain repeated injury, infection and falls that lead to major head trauma and permanent disablement. The disabilities caused by alcoholic neuropathy compound the already significant health, social and economic consequences of chronic alcoholism (Mellion et al., 2011). Alcohol-related neuropathy is associated with several risk factors such as malnutrition, thiamine deficiency, direct toxicity of alcohol and a family history of alcoholism (Bosch et al., 1979; Monforte et al., 1995; Koike et al., 2003; Koike and Sobue, 2006), but it is not clear which of these plays a primary role in inducing neuropathy (Palliyath and Schwartz, 1993). In the early stages of alcoholic neuropathy, patients complain of pain in the extremities, which may be severe and has been described as burning or ‘like tearing flesh off the bones’ and is characterized by spontaneous burning pain, hyperalgesia and allodynia (Brain and Walton, 1969).

Prevalence of Alcoholic Neuropathy

Using the criteria for alcoholism listed in the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV), studies employing clinical and electrodiagnostic criteria have estimated that in United States neuropathy is present in 25-66% of defined “chronic alcoholics” (Mellion et al., 2011). The factors most directly associated with the development of alcoholic neuropathy include the duration and amount of total lifetime alcohol consumption. Neuropathy is more prevalent in frequent, heavy and continuous drinkers compared to more episodic drinkers (Monforte et al., 1995). Incidence of alcoholic polyneuropathy was found to be higher in women as compared to men (Ammendola et al., 2000). The findings were supported by the results from preclinical studies by Dina et al. (2007) who also found that alcohol induced neuropathy has a rapid onset and more severity in female rats as compared to males.
Clinical Symptoms Associated with Alcoholic Neuropathy

Clinical features of alcoholic neuropathy develop slowly, extending over a period of months and includes abnormalities in sensory, motor, autonomic and gait functions. Painful sensations with or without burning represents the initial and major symptom of alcoholic neuropathy (Koike et al., 2001a; Koike and Sobue, 2006). Sometimes, these symptoms can be very painful and incapacitating. Later on, weakness appears in the extremities, involving mainly the distal parts. Progressively, the sensory and motor symptoms and signs extended proximally into the arms and legs and finally the gait may become impaired (Hawley et al., 1982). Progression of symptoms usually is gradual, continuing over months or years (Koike et al., 2001a; Koike and Sobue, 2006).

Electrophysiologic and pathologic findings mainly indicate axonal neuropathy with reduced nerve fiber densities. Densities of small myelinated fibers and unmyelinated fibers were more severely reduced than the density of large myelinated fibers, except in patients with a long history of neuropathic symptoms and marked axonal sprouting (Koike et al., 2001a). Subperineurial edema is more prominent in thiamine deficient neuropathy whereas segmental de/remyelination resulting from widening of consecutive nodes of Ranvier is more frequent in alcoholic neuropathy (Koike et al., 2003).

Potential Complications Associated with Alcoholic Neuropathy

Complications of untreated alcoholic neuropathy can be serious, even life threatening in some cases. Risk of serious complications can be minimized by following the treatment plan designed by health care professional. Potential complications associated with alcoholic neuropathy are:

- Disability
- Impaired coordination
- Permanent loss of sensation
- Permanent nerve damage
- Permanent or chronic pain
PATHOGENESIS OF ALCOHOLIC NEUROPATHY

The pathogenesis of alcoholic neuropathy is still under debate. It has previously been considered in relationship to nutritional, especially thiamine deficiencies seen in alcoholics. Thiamine deficiency is closely related to chronic alcoholism and can induce neuropathy in alcoholic patients. Ethanol diminishes thiamine absorption in the intestine, reduces hepatic stores of thiamine and affects the phosphorylation of thiamine, which converts it to its active form (Singleton and Martin, 2001). In addition, patients with chronic alcoholism tend to consume smaller amounts of essential nutrients and vitamins and/or exhibit impaired gastrointestinal absorption of these nutrients secondary to the direct effects of alcohol. These relationships make chronic alcoholism a risk factor for thiamine deficiency.

In addition to thiamine deficiency, recent studies indicate a direct neurotoxic effect of ethanol or its metabolites. Axonal degeneration has been documented in rats receiving ethanol while maintaining normal thiamine status (Bosch et al., 1979). Human studies have also suggested a direct toxic effect, since a dose-dependent relationship has been observed between severity of neuropathy and total lifetime dose of ethanol (Monforte et al., 1995; Ammendola et al., 2001). Ethanol inhibits axonal transport and cytoskeletal structure maintenance, which could cause or exacerbate either demyelination or the axonal dying-back process (Malatova and Cizkova, 2002). Acetaldehyde, a major toxic metabolite of ethanol, can also exert direct neurotoxic effects due to increased adduct formation and oxidative stress (Cohen et al., 2007). Other postulated mechanisms of ethanol effect on peripheral nerve include altered lipid peroxidation, activation of atypical protein kinase C (PKC) and disruption of the sympathoadrenal and hypothalamic–pituitary axis (Dina et al., 2008). The role of insulin/IGF resistance vs. oxidative stress as mediators of alcoholic neuropathy is under investigation, but given its role in alcohol-mediated disease in liver and the CNS it makes sense that it would also likely be a factor in alcoholic neuropathy (Mellion et al., 2011).

The exact mechanism behind alcoholic neuropathy is not well understood, but several explanations have been proposed. These include
alcohol-induced impairment in thiamine absorption (Singleton and Martin, 2001), activation of spinal cord microglia after chronic alcohol consumption (Narita et al., 2007), activation of mGlu5 receptors in the spinal cord (Miyoshi et al., 2007), oxidative stress leading to free radical damage to nerves (Cohen et al., 2007), involvement of extracellular signal-regulated kinases (ERKs) or classical MAP kinases (Dina et al., 2007), involvement of opioidergic (Narita et al., 2007) and hypothalamo-pituitary-adrenal system (Gianoulakis et al., 2003; Thayer et al., 2006; Walter et al., 2006). Some other studies have indicated that chronic alcohol intake can decrease the nociceptive threshold with increased oxidative-nitrosative stress and release of pro-inflammatory cytokines coupled with activation of PKC (Dina et al., 2000, 2007). Therefore, alcoholic neuropathy may occur by a combination of direct toxic effects of ethanol or its metabolites and nutritional deficiencies including thiamine deficiency. The precise mechanisms responsible for toxicity on the peripheral nervous system, however, have not yet been clarified and the amount of ethanol which causes clinically evident peripheral neuropathy is still unknown.

**Nutritional Factors Responsible for Alcoholic Neuropathy (Indirect Toxicity)**

**Contribution of Metabolic Pathways**

The primary axonal damage and secondary demyelination of motor and sensory fibers (especially small diameter fibers) are considered to constitute the morphologic basis of alcoholic damage to nerve tissue (Ludin and Tackmann, 1984). The demyelination is explained as the result of a slowing-down (deceleration) of axoplasmic flow and a degradation of the quality of biological properties of axonal enzymes and proteins. This type of degeneration so called "dying-back" resembles the Wallerian degeneration. Ethanol and its toxic metabolites affect neuronal metabolism including the metabolic pathways of nucleus, lysosomes, peroxisomes, endoplasmatic reticule and cytoplasm (Kucera et al., 2002). Alcohol enters the blood as early as 5 minutes after ingestion and its absorption peaks after 30-90 minutes. The key role in the degradation of ethanol is played by alcohol dehydrogenase and acetaldehyde dehydrogenase-two step enzymatic systems by which ethanol is converted to acetate which is further metabolised in humans. The
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Acetaldehyde dehydrogenase is a mitochondrial enzyme that underwent a single amino acid substitution (mutation) in about 50% of the Asian population in a way similar to the genetic changes in sickle cell anaemia (Kucera et al., 2002). Thus, in alcoholics with mutated dehydrogenase enzyme, the acetaldehyde levels may reach values about 20 times higher than in individuals without the mutation. A certain amount of acetaldehyde is not metabolized in usual pathways (Fig. 2) and binds irreversibly to proteins which results in the creation of cytotoxic proteins which adversely affect the function of nervous system cells. These abnormal proteins influence other cell populations especially the hepatocytes where the damage to hepatic mitochondria results in hepatic cirrhosis with reduction of energetic substrates in the liver. The action of these abnormal proteins is explained by the competition with normal proteins causing the damage to function and metabolism of the cell (Achord, 1995).

One of the other important issues in alcoholic individuals is the source of their calorie intake. These individuals draw the majority of calories from the calorie rich alcoholic beverages with low nutritive value. Chronic abuse of alcohol depletes the pool of liver proteins which are consumed for energy production and the insufficient intake of proteins only worsens this imbalance. Resulting disturbances in protein and lipid metabolism leads to undernourishment which adversely influences other metabolic pathways, including those influencing the function of the nervous system. While the CNS has its own barrier systems (blood-brain barrier), which may defy the metabolic and toxic influences and their effect on brain functions for a significant period of time, the peripheral nervous system lacks this protective barrier which can contribute to the fact that peripheral nervous system disorders are present in 12-30 % of alcohol abusers (Scheid, 1980).

Relationship between Alcoholic Neuropathy and Thiamine Deficient Neuropathy

There is both clinical and experimental evidence of a direct neurotoxic effect of ethanol, while some have argued that it results from a nutritional deficiency, especially thiamine deficiency. The relationships between alcoholic
neuropathy and commonly associated nutritional deficiencies, especially thiamine deficiency have been discussed in terms of the apparent clinical and pathologic presentations (Victor and Adams, 1961; Novak and Victor, 1974).

Figure 2. Multiple pathways involved in metabolism of ethanol

Koike et al. (2004) compared the clinicopathologic features of thiamine-deficiency neuropathy caused by a dietary imbalance with those caused by gastrectomy, including strict biochemical determination of thiamine status. Although clinical manifestations varied widely between patients with either type of thiamine-deficiency neuropathy, overall clinicopathologic features including the spectrum of clinical variability did not differ significantly by cause. Thus, clinicopathologic features of postgastrectomy polyneuropathy with thiamine deficiency are identical to those of beriberi neuropathy, and the results further confirmed that thiamine deficiency can be a major cause of postgastrectomy polyneuropathy (Koike et al., 2001b). In another clinical study by Koike et al. (2008), the cause of the thiamine deficiency was found to be associated with gastrectomy to treat cancer in a 46-year-old man and with dietary imbalance in a 33-year-old man. In both patients, the upper and lower extremities showed a rapidly progressive weakness over the course of 1
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Month. Muscle weakness in the first patient progressed even after admission to the hospital, urinary retention, Wernicke's encephalopathy, lactic acidosis, paralytic ileus and heart failure appeared subsequently. Clinical symptoms in both patients showed improvement after initiation of thiamine administration, although some residual deficit remained. Clinically, sensory disturbance and weakness, especially in the distal part of the lower extremities are common features of both alcoholic and thiamine deficient neuropathies (Victor and Adams, 1961; Ohnishi et al., 1980). Electrophysiologic and histopathologic findings of axonal neuropathy have also been considered as common features (Bosch et al. 1979; Ohnishi et al., 1980; Koike et al., 2001a). These similarities have led to a belief that these two neuropathies are identical and that polynuropathy associated with chronic alcoholism most likely is caused by thiamine deficiency (Victor and Adams, 1961; Novak and Victor, 1974). Thus, the concept of alcoholic neuropathy encompasses both direct neurotoxicity of ethanol or its metabolites and the concomitant effects of nutritional status especially thiamine deficiency (Fig. 3).

In one clinical study, aimed at studying distinct clinicopathologic features of alcoholic neuropathy, 64 patients were assessed out of which in 47 patients, sural nerve biopsy was performed, with discrimination in terms of their thiamine status (Koike et al., 2003). The ethanol consumption of these patients was more than 100 g per day for more than 10 years. These patients were divided into two groups based on thiamine status. The subgroup without thiamine deficiency consisted of 36 patients, while the subgroup with thiamine deficiency consisted of 28 patients. In addition, 32 patients with nonalcoholic thiamine-deficiency neuropathy were also evaluated for comparison. The subgroup without thiamine deficiency, considered to be a pure form of alcoholic neuropathy, uniformly showed slowly progressive, sensory-dominant symptoms. Superficial sensation, especially nociception was predominantly impaired and painful symptoms were the primary complaint in most patients in this group. In contrast, the neuropathic symptoms of nonalcoholic thiamine-deficiency neuropathy, considered to be identical to beriberi neuropathy, were variable, but typically were motor dominant and acutely progressive, affecting both superficial and deep sensation. The histologic features of sural nerve
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biopsy specimens demonstrated small-fiber-predominant axonal loss as characteristic of the pure form of alcoholic neuropathy (Koike et al., 2004).

Figure 3. A Schematic Flowchart Depicting Role of Thiamine and other Nutrients in Alcoholic Neuropathy

Role of nutritional status other than thiamine deficiency

Deficiency of vitamins other than thiamine may also contribute to clinical features of alcoholic neuropathy. Chronic alcoholism can alter the intake, absorption, and utilization of various nutrients (nicotinic acid, vitamin B2, vitamin B6, vitamin B12, folate or vitamin E). Deficiencies of B vitamins other than thiamine also may contribute to variation in clinical features, but characteristic symptoms of multiple-vitamin deficiency were not seen in patients with thiamine-deficiency neuropathies due to gastrectomy and dietary imbalance (Koike et al., 2004). These clinical features include anorexia, diarrhea, erythematous and hyperkeratotic dermatitis, and mental changes in pellagra (nicotinic acid deficiency), cheilosis, glossitis, keratoconjunctivitis and dermatitis in vitamin B2 deficiency, and myelopathy in vitamin B12 and folate
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deficiencies. Thus, these vitamin deficiencies were not considered to be major
causal factors of neuropathy (Koike et al., 2004).

Behse and Buchthal (1977) compared 37 Danish patients with
alcoholic neuropathy with six patients with nonalcoholic postgastrectomy
polyneuropathy. The authors noted that Danish beer at the time of the study
contained thiamine and vitamin B6. Thus, deficiency of these vitamins was felt
to be unlikely in Danish beer drinkers at that time and, indeed, measured
vitamin concentrations were mostly normal. Clinical features of neuropathies
in the alcoholic and postgastrectomy patients were similar. These two groups,
however, were distinct from the standpoint that nerve conduction velocities
were slower and sural nerve biopsy specimens revealed more segmental
demyelination in the postgastrectomy group. The authors concluded that
malnutrition, including low blood concentrations of B vitamins, is not a
prerequisite for the development of alcoholic neuropathy, and ethanol per se
plays a role in the pathogenesis of alcoholic neuropathy. Another study by
Zambelis et al. (2005) also suggested the participation of the direct toxic effect
of ethanol on the peripheral nervous system in the pathogenesis of alcoholic
neuropathy, although long-standing hyperglycemia and impaired vitamin B12
utilization were also suggested to be involved.

Direct Toxic Effects of Ethanol or its Metabolites (Direct Toxicity)
Role of Acetaldehyde in Alcoholic Neuropathy

Ethanol can exert its harmful effects through its metabolism. One
possible mediator of the direct neurotoxic effect of ethanol is acetaldehyde, a
highly toxic metabolite of ethanol with extraordinary reactivity. The
mechanisms of the toxicity for liver include production of acetaldehyde-protein
adduct formation, depletion of glutathione, microtubular impairment, inhibition
of DNA repair, impairment of mitochondrial electron transport chain and
stimulation of immunologic reactivity. There is evidence that acetaldehyde-
protein adducts are present even in organs that do not seem to produce
acetaldehyde efficiently themselves, due to lack of ADH expression (Masaki
et al., 2004). In such cases, acetaldehyde may be formed by induction of the
microsomal ethanol oxidizing system (Lieber, 1998). Alternatively,
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Acetaldehyde may reach those organs by blood flow. Given these possibilities, the mechanisms by which acetaldehyde has toxic effects on peripheral nerves may be similar to those in the liver and other organs. Dose-dependent increases in neuronal cell death was demonstrated by incubation of neuronal cell cultures with acetaldehyde-derived advanced glycation end-products (AA-AGE), and the neurotoxicity of AA-AGE is attenuated by the addition of an anti-AAAG-specific antibody (Takeuchi and Saito, 2005). These results suggest that the neurotoxicity due to accumulation of acetaldehyde may be associated with the pathogenesis of alcoholic neuropathy (Fig. 4).

Oxidative-Nitrosative Stress and Alcoholic Neuropathy

Oxidative stress is known to play a very important role in experimental animal models of neuropathic pain. Lee et al., (2007) suggested that reactive oxygen species are importantly involved in the development and maintenance of capsaicin-induced pain, particularly in the process of central sensitization in the spinal cord in rats. Padi and Kulkarni (2008), demonstrated that chronic administration of minocycline when started early before peripheral nerve injury could attenuate the development of neuropathic pain by inhibiting pro-inflammatory cytokines release and oxidative and nitrosative stress in mononeuropathic rats. Naik et al. (2006) suggested the involvement of oxidative stress in experimentally induced chronic constriction injury of sciatic nerve model in rats. Endoneural oxidative stress leads to nerve dysfunction in rats with chronic constriction injury (Kim et al., 2004). A significant decrease in the activity of anti-oxidant enzymes (superoxide dismutase and catalase) and an increase in lipid peroxidation were observed in sciatic nerves of diabetic rats with established neuropathic pain (Sharma et al., 2006). ROS triggers second messengers involved in central sensitization of dorsal horn cells (Ali and Salter, 2001) or they activate spinal glial cells which in turn plays an important role in chronic pain (Raghavendra et al., 2003). Reduced glutathione is a major low molecular weight scavenger of free radicals in cytoplasm. Depletion of glutathione increases the susceptibility of neurones to oxidative stress and hyperalgesia (Cooper and Kristal, 1997; Wullner et al., 1999).
Nitric oxide is also implicated in neuropathic pain (Levy and Zochodne, 2004; Sung et al., 2004). It sensitizes spinal neurones and contributes to sensitization of central neurones by disinhibition (Lin et al., 1999). Moreover, unfettered production of nitric oxide coupled with deficient superoxide dismutase leads to production of peroxynitrite, which is several times multiple of its parents in terms of tissue toxicity.

Ethanol is oxidized to acetaldehyde by cytochrome P450 which increases reactive oxygen species, with concomitant changes in redox balance (Mantle and Preedy, 1999; Zima et al., 2001). Rats given chronic ethanol show enhanced production of oxidative markers, such as thiobarbituric acid-reactive substances, hydrogen peroxide, and OH' like species (Dicker and Cederbaum, 1992). Studies have suggested that chronic ethanol increases oxidative damage to proteins, lipids, and DNA (Mansouri et al., 2001; McDonough, 2003). Bosch-Morell et al. (1998) demonstrated that chronic ethanol feeding promotes oxidative stress in rat peripheral nerve. Malondialdehyde, a lipid peroxidation product, content increases in sciatic nerves of rats fed an ethanol-containing diet, when compared with pair-fed animals. Moreover, glutathione content and glutathione peroxidase activity in this same tissue decrease in ethanol-fed vs pair-fed rats suggesting the probable involvement of alcohol induced oxidative stress in pain like state associated with chronic alcohol intake.

Recently, we also observed marked hyperalgesia and allodynia coupled with significantly enhanced lipid peroxide levels and marked decrease in reduced glutathione, super oxide dismutase and catalase activity in sciatic nerve of rats chronically administered ethanol (10 g/kg, oral gavage) for 10 weeks (Tiwari et al., 2009a, 2011). Thus, following ethanol intoxication, the balance between prooxidants and antioxidants is disturbed to such an extent that it results in the oxidative damage of biomolecules such as fats, proteins or DNA and finally leading to cell injury and thus alcoholic neuropathy (Fig. 4).
Role of Protein Kinases in Alcoholic Neuropathy

Protein kinase C is a family of protein kinases consisting of 10 isozymes. Recurring reports suggest that PKC is involved in receptor desensitization, modulating membrane structure events, regulating transcription, mediating immune responses, regulating cell growth and learning and memory. These functions are achieved by PKC mediated phosphorylation of other proteins (Dina et al., 2000). Apart from above function, over-activation of epsilon form of PKC are known to be involved in mediating neuropathic pain such as pain induced by cancer chemotherapy (Aley and Levine, 2002) and diabetes (Ahlgren and Levine, 1994). PKC and PKA are both known to be important in nociceptor function (Taiwo et al., 1989; Ahlgren and Levine, 1994; Khasar et al., 1999).

There are several evidences suggesting the involvement of protein kinases in alcoholic neuropathy. Dina et al. (2000) maintained rats on a diet to simulate chronic alcohol consumption in humans, found mechanical hyperalgesia by the fourth week which was maximal at 10 weeks. Thermal hyperalgesia and mechanical allodynia were also present with decreased mechanical threshold of C-fibers. The hyperalgesia was acutely attenuated by intradermal injection of nonselective PKC or selective PKC\(\varepsilon\) inhibitors injected at the site of nociceptive testing. Western immunoblot analysis indicated a higher level of PKC\(\varepsilon\) in dorsal root ganglia from alcohol-fed rats, supporting a role for enhanced PKC\(\varepsilon\) second messenger signaling in nociceptors contributing to alcohol-induced hyperalgesia (Dina et al. 2000). Miyoshi et al. (2007) found that significant decrease in the mechanical nociceptive threshold was observed after 5 weeks of chronic ethanol consumption in rats. This hyperalgesia was significantly attenuated by repeated intraperitoneal injection of (S)-2,6-diamino-N-[[1-(oxotridecyl)-2-piperidinyl]methyl] hexanamide dihydrochloride (NPC15437), a selective PKC inhibitor, once a day for a week after 4 weeks of ethanol treatment. Moreover, phosphorylated-PKC was significantly increased in the spinal cord following chronic ethanol consumption. These findings constitute direct evidence that spinal PKC plays substantial roles in the development and maintenance of an ethanol-induced neuropathic pain-like state in rats (Fig. 4).
PKA and PKC epsilon signaling is also known to play highly sexually dimorphic role in alcoholic neuropathy (Dina et al., 2007). In gonad-intact female rats both PKCε inhibitor as well as a PKA inhibitor; Walsh inhibitor peptide (WIPTIDE), injected intradermally at the site of nociceptive testing after establishing alcohol induced hyperalgesia significantly inhibited hyperalgesia. Following ovariectomy, alcohol failed to induce hyperalgesia in female rats while estrogen replacement reinstated alcoholic neuropathy in the female rats. PKA inhibitor (WIPTIDE) also attenuated alcohol-induced hyperalgesia in estrogen-replaced female rats. In addition, the magnitude of analgesia induced by PKCε inhibitor was greater in female rats as compared to males. However, in male rats, the PKCε inhibitor, but not PKA inhibitor, attenuated alcohol-induced hyperalgesia (Dina et al., 2007). The mechanism underlying the sexually dimorphic contribution of PKA and PKCε to pain associated with alcohol-induced neuropathy remains to be determined.
A Connection between MEK/ERK Signaling and Alcoholic Neuropathy

Extracellular signal-regulated kinases (ERKs) or classical MAP kinases are widely expressed protein kinase intracellular signalling molecules which are involved in functions including the regulation of meiosis, mitosis and postmitotic functions in differentiated cells. Many different stimuli, including growth factors, cytokines, virus infection, ligands for heterotrimeric G protein-coupled receptors, transforming agents and carcinogens, activate the ERK pathway. There are many studies suggesting the role of MEK/ERK signaling in inflammatory pain in male (Aley et al., 2001, Dina et al., 2003, 2005, Zhuang et al., 2005) and female rats (Dina et al., 2007). Dina et al. (2007) for the first time, evaluated the contribution of MEK/ERK signaling in alcohol-induced peripheral neuropathy and found that intradermal injection of PD98059 (1 μg/μl), a selective inhibitor of mitogen and extracellular regulated kinase and U0126 (1 μg/μl), a specific inhibitor of ERK1/2 attenuated ethanol-induced hyperalgesia similarly in male and female rats. Results from the above study confirmed the role for MEK/ERK signaling in chronic alcohol-induced hyperalgesia in rats of both sexes (Fig. 4).

Role of Spinal cord microglia

Spinal cord glial cells are implicated in exaggerated pain state created by diverse manipulations such as subcutaneous inflammation, neuropathy and spinal immune activation (Ledeboer et al., 2005; Watkins et al., 2001). It has been recognized that spinal cord glial cells, astrocytes and microglia, are activated by neuropathic pain or peripheral inflammation (Raghavendra et al., 2003). Furthermore, astrocytes and microglia are activated by such pain-relevant substances as substance P, calcitonin-gene related peptide (CGRP), ATP and excitatory amino acid from primary afferent terminals, in addition to virus and bacteria (Norenberg, 1994; Julius and Basbaum, 2001). In another study by Narita et al. (2007) five weeks of ethanol treatment resulted in significantly decreased mechanical nociceptive threshold along with microglia activation in the spinal cord of rats implicating role of proliferated and activated microglia in the expression of neuropathic pain-like state following chronic ethanol consumption.
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Role of Caspases in Alcoholic Neuropathy

Caspases, or cysteine-aspartic acid proteases, are a family of cysteine proteases, which play essential role in apoptosis (programmed cell death), necrosis and inflammation. Translocation of NFκβ to the nucleus has been reported to result in activation of the endogenous proteolytic enzyme system caspases (Robbins et al., 2003). Consequently, the cascade events promote further apoptosis (Krebs et al., 1999). Joseph and Levine (2004) suggested that activity in signalling pathways that ultimately lead to apoptosis plays a critical role in the generation of neuropathic pain before death of sensory neurones becomes apparent. Activator and effector caspases, defining components of programmed cell death (apoptosis) signalling pathways, also contribute to pain-related behaviour in animals with small-fibre peripheral neuropathies and that the death receptor ligand, tumour necrosis factor-α and its downstream second messenger, ceramide, also produce pain-related behaviour via this mechanism. In two models of painful peripheral neuropathy, HIV/AIDS therapy (induced by the nucleoside reverse transcriptase inhibitor, dideoxycytidine), and cancer chemotherapy-induced peripheral neuropathy and for pain-related behaviour induced by tumour necrosis factor-α and its second messenger, ceramide, inhibition of both activator (1, 2, 8 and 9) and effector (3) caspases attenuates neuropathic pain-related behaviour. This suggests that these pathways are potential targets for novel pharmacological agents for the treatment of inflammatory as well as neuropathic pain (Joseph and Levine, 2004).

Chronic exposure to ethanol results in increased amounts of oxidative damage, translocation of PKC, activation of PKC and NF-kβ which results in DNA fragmentation and ultimately increased neuronal death through apoptosis or other mechanisms that are responsible for the behavioral deficits (Jung et al., 2005). Izumi et al. (2005) also demonstrated that a single day of ethanol exposure in rats on postnatal day 7 results in significant apoptotic neuronal damage throughout the forebrain after twenty-four hours of ethanol administration. Thus, it is quite possible that chronic alcohol consumption is responsible for inducing neuropathy by activation of caspase cascade and may be an important target for the treatment of alcoholic neuropathy (Fig. 4).
Involvement of Glutamate receptors

Accumulating evidence suggests a pivotal role for mGluRs in nociceptive processing, inflammatory pain and hyperalgesia (Meller et al. 1996; Young et al. 1997). Several mGluR subtypes have been identified in the superficial dorsal horn of the spinal cord (Jia et al., 1999; Valerio et. al., 1997), and on primary afferent fibres (Hudson et al., 2002). Glutamate levels are elevated in the superficial dorsal horn of rats after chronic ligature of the sciatic nerve (al-Ghoul et al. 1993). Miyoshi et al. (2006) found that five weeks after ethanol treatment, the mechanical nociceptive threshold was significantly decreased which gets further reduced upto 10th week. As supported by immunostaining, the membrane fraction showed that spinal mGluR5 levels in ethanol-treated rats were significantly increased compared to those in the control-diet group. These findings support the idea that the increased number of membrane-bound mGluR5 following chronic ethanol consumption may lead to a long-lasting activation of neuronal PKC in the dorsal horn of the spinal cord. This phenomenon may be responsible for the induction of the neuropathic pain like behavior following chronic ethanol consumption. Not only metabotropic glutamate (mGlu) receptor but ionotropic glutamate (NMDA) receptors are also involved in alcoholic-induced neuropathic pain. Narita et al. (2007) found that p- Ser1303-NR2B subunit protein (subunit of NMDA receptor) in the spinal cord of rats was significantly increased following chronic ethanol treatment suggesting that PKC-dependent NR2BRs in the spinal cord may be activated following chronic ethanol consumption and may be involved in the induction of the ethanol-induced neuropathic pain-like state.

Involvement of Opioidergic system

Narita et al. (2007) found that chronic alcohol consumption was associated with long lasting hyperalgesia during and even after ethanol withdrawal along with opioid receptor dysfunctioning specific for μ opioid receptors (MOR), but not delta and kappa opioid receptors. These findings suggest that chronic ethanol treatment causes the specific dysfunction of MOR. Thus, up-regulation of cPKC activity may, at least in part, be involved in MOR dysfunction (may be an increase in MOR phosphorylation) following
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chronic ethanol treatment. This phenomenon may explain the reduced sensitivity to morphine-induced antinociception under the ethanol-dependent neuropathic pain-like state.

Involvement of Sympathoadrenal and Hypothalamo-pituitary-adrenal (HPA) axis in Alcoholic Peripheral Neuropathy

Alcohol consumption potently activates the two major neuroendocrine stress axes, leading to the sustained release of glucocorticoids and catecholamines (Gianoulakis et al., 2003; Thayer et al., 2006; Walter et al., 2006). Increased activity in the sympathetic nervous system has been implicated in some forms of neuropathic pain (Tracey et al., 1995; Singh et al., 2003) and glucocorticoids have been reported to exacerbate pain in some animal models of peripheral neuropathy (Wang et al., 2004).

Dina et al. (2008) demonstrated the involvement of the sympathoadrenal stress axis and its final common mediator, epinephrine, in painful alcoholic neuropathy by showing that adrenal medullectomy prevented and reversed the pro-nociceptive effects of alcohol consumption. Moreover, they found reconstitution of hyperalgesic phenotype in rats that had undergone adrenal medullectomy by administering stress levels of epinephrine. The critical contribution of stress hormones to the pain associated with alcohol-induced peripheral neuropathy, combined with the demonstration of stress-induced hyperalgesia, dependent on neuroendocrine stress axes (Quintero et al., 2003; Khasar et al., 2005), suggest that the mechanisms described in the study of Dina et al. (2008) may have implications for other types of pain in which patients experience repeated exposure to stress.

Thus, stress hormones, catecholamines and glucocorticoids, from the sympathoadrenal and HPA neuroendocrine stress axes, respectively plays a very important role in initiation and maintenance of alcoholic neuropathy. The combined actions of catecholamines and glucocorticoids, via their receptors on sensory neurones, demonstrate a novel mechanism by which painful alcoholic neuropathy is induced and maintained.
**Effects on axonal transport and cytoskeletal properties**

Axonal transport and cytoskeletal properties are impaired by ethanol exposure (Koike and Sobue, 2006). Since alcoholic neuropathy manifests with length-dependent axonal degeneration, the axonal transport system, which supplies essential proteins and other cellular components, may be the primary site exhibiting vulnerability to the toxicity of ethanol. Yerdelen et al. (2008) suggests that alcoholic neuropathy is a primary axonal neuropathy characterized by wallerian degeneration of the axons and a reduction in the myelination of neural fibers. An *in-vitro* study of axonal transport using dorsal root ganglion–sciatic nerve preparations from the rat showed that transport was reduced following long-term ethanol feeding (McLane, 1987). *In-vivo* studies using rats have demonstrated impairment of retrograde axonal transport (Hellweg et al. 1996; Malatova and Cizkova, 2002).

Ethanol exposure reduces neurofilament protein levels in primary cultured hippocampal neurones (Saunders et al., 1997). Studies using the rat spinal cord indicate that chronic ethanol exposure causes a reduction in neurofilament-associated phosphatase activity and an increase in phosphate content of neurofilament proteins (Guru et al., 1991). An *in-vitro* study using rat brain has demonstrated that phosphorylation of microtubule-associated proteins, which modulate the functional properties of microtubules is altered by ethanol exposure (Ahluwalia et al., 2000). A study using hepatoma-derived cells has shown altered integrity of proteins associated with microtubules following ethanol exposure (Kannarkat et al., 2006). Altered expression of neuronal protein 22 which interacts with microfilament and microtubule matrices may also be involved in the pathogenesis of alcoholic neuropathy (Depaz et al., 2005). Thus, defects in axonal transport and cytoskeletal properties of axons may be one of the important pathways involved in alcohol induced peripheral neuropathy.

Thus, it is clear that all the above pathways are potential targets for novel pharmacological agents for the treatment of alcoholic neuropathy.
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THERAPEUTIC STRATEGIES FOR ALCOHOLIC NEUROPATHY

Alcoholic peripheral neuropathy presents with considerable morbidity and can result in significant decrease in quality of life. While conventional medicine can offer some relief, the potential side effects or addictive nature of many of the medications render long term use undesirable. Such treatments, furthermore, merely mask the symptoms and do not address the underlying pathologies. Alternative therapies, on the other hand, are typically without side effects and address nutrient deficiencies, oxidative stress and other etiological factors associated with the development of peripheral neuropathy.

Current treatment of alcohol-related neuropathy is largely aimed at relieving pain, which is the most disabling and distressing symptom. A specific vitamin B complex preparation significantly improved the symptoms of alcohol-related polyneuropathy over a 12-week treatment period (Peters et al., 2006). However, in the setting of ongoing ethanol use, vitamin supplementation alone has not been convincingly shown to be sufficient for improvement in most patients. Painful dysesthesias associated with alcoholic neuropathy can be treated using gabapentin or amitriptyline with other OTC pain medications, such as aspirin or acetaminophen. But these drugs are being used only for the management of acute pain and are ineffective in targeting the basic pathological pathways involved in alcoholic neuropathy. Cochrane reviews of carbamazepine and gabapentin for acute and chronic pain (Wiffen et al., 2005a, 2005b) and of antidepressant drugs for neuropathic pain (Saarto and Wiffen, 2007) did not specifically address any studies on alcohol-related neuropathy. Other pain modifying therapies that have been tried in alcohol-related neuropathy include morphine (Narita et al. 2007), mexiletine (Nishiyama and Sakuta, 1995) and berlition (Kovrazhkina et al., 2004) but none of them were found to provide adequate relief without side effects.

Vitamin supplementation treatments for alcoholic neuropathy

Vitamin supplementation and medications to reduce pain and discomfort are the mainstays of treatment for alcoholic neuropathy. In alcoholic
neuropathy, supplementation with the following vitamins may improve symptoms:
- Biotin
- Folic acid
- Vitamin A
- Vitamin B₁ (thiamine)
- Vitamin B₃ (niacin)
- Vitamin B₅ (pantothenic acid)
- Vitamin B₆ (pyridoxine)
- Vitamin B₁₂ (cobalamin)
- Vitamin E (tocopherol)

Benfotiamine for the Treatment of Alcohol Related Peripheral Neuropathy

A deficiency of vitamin B₁ in chronic alcoholics can be due to inadequate dietary intake, reduced capacity for hepatic storage, inhibition of intestinal transport and absorption or decreased formation of the active coenzyme form. In an animal study, it has been found that chronic alcohol consumption in rats resulted in a significant depletion in thiamine diphosphate (TDP)—the active coenzyme form of thiamine. Supplementation with benfotiamine, a synthetic S-acyl derivative of thiamine (vitamin B₁), significantly increased levels of TDP and total thiamine compared to supplementation with thiamine hydrochloride (Netzel et al., 2000). An eight-week, randomized, multicenter, placebo-controlled, double-blind study compared the effect of benfotiamine alone to a benfotiamine complex (Milgamma-N) or placebo in 84 alcoholic patients. Benfotiamine was given in a daily oral dose of 320 mg (two 40-mg tablets four times daily) during weeks 1-4, followed by 40 mg three times daily (120 mg total daily dose) during weeks 5-8. A second group received Milgamma-N (providing a total daily dose of 320 mg benfotiamine, 720 mg pyridoxine, and 2,000 mcg cyanocobalamin) during weeks 1-4 and a total daily dose of 120 mg benfotiamine, 270 mg pyridoxine and 750 μg cyanocobalamin during weeks 5-8; a third group received placebo (Woelk et al., 1998). Parameters measured included vibration perception in the great toe, ankle, and tibia; neural pain intensity;
motor function and paralysis; sensory function and overall neuropathy score and clinical assessment. Although benfotiamine therapy was superior to Milgamma-N or placebo for all parameters, results reached statistical significance only for motor function, paralysis and overall neuropathy score. The reason for better results with benfotiamine-alone group than the Milgamma-N group, despite the fact that the benfotiamine dosage was equivalent is not completely understood. The authors hypothesized that vitamins B₆ and B₁₂ might have competed with the effects of vitamin B₁ in the Milgamma-N group. On the other hand, in the case of diabetic neuropathy, the positive effects of the combination may be due to the fact that deficiencies of vitamins B₁, B₆, and B₁₂ are all implicated in its possible pathogenesis whereas alcoholic neuropathy is associated with only vitamin B₁ deficiency (Woelk et al., 1998).

In another small Russian study also, benfotiamine was found beneficial in patients with alcoholic neuropathy. Fourteen chronic alcoholic men with polyneuropathy were given 450 mg benfotiamine daily for two weeks, followed by 300 mg daily for an additional four weeks demonstrated regression of neuropathy symptoms (Anisimova and Danilov, 2001).

**Symptomatic Treatment of Alcoholic Peripheral Neuropathy**

The lack of understanding of the pathogenesis of this disorder precludes the development of mechanism-specific therapies (Feldman et al., 2002). Therefore, currently accepted medical approaches are only partially successful and are often ineffective (Dworkin et al., 2007). Antiseizure medications that are effective in the treatment of nerve pain resulting from alcoholic neuropathy include:

- Carbamazepine
- Gabapentin
- Phenytoin
- Pregabalin
- Topiramate

Medications containing opioids, such as codeine, that are physically addicting and may lead to dependence will be prescribed only when other
options are not successful in relieving severe pain. Additional options to treat nerve pain associated with alcoholic neuropathy include antidepressants, such as amitriptyline, nortriptyline and duloxetine.

As there is no effective therapeutic intervention available for relieving the neuropathic pain due to chronic alcohol consumption, thus there is a need to understand the basic pathophysiological mechanisms involved in alcohol induced neuropathic pain so that new newer therapeutic modalities targeting at disrupted molecular events can be developed for prevention as well as clinical management of alcoholic neuropathy. The use of well-researched nutrients and the possible addition of new cutting-edge treatments should decrease the morbidity associated with alcoholic peripheral neuropathy and the side effects associated with the commonly prescribed conventional pain-relieving treatments.

**ALCOHOL-INDUCED COGNITIVE DEFICITS: Alcoholic Encephalopathy**

Alcoholic encephalopathy is a serious complication of chronic alcohol consumption which involves loss of specific brain functions including cognitive functioning (Liu et al. 2010). Up to 50–75% of long-term alcoholics may show permanent cognitive impairment, making chronic alcoholism the second leading cause of dementia behind Alzheimer’s disease (Eckardt and Martin, 1986). Alcoholics consistently show deficits in executive functioning, declarative memory, short-term memory and frequent impairments in spatial learning and memory, effects which indicate hippocampal dysfunctioning (Parsons, 1998; Sullivan et al., 2000).

Parallel to the behavioral and cognitive impairments are observations of “brain shrinkage” or neurodegeneration in alcoholics (Harper, 1998; Sullivan and Pfefferbaum, 2005; Zahr et al., 2011). Human imaging studies, animal models and postmortem analysis of brain structure suggest that chronic alcoholism is closely associated with brain damage or neurodegeneration. Alcoholics show significant volume loss in cortical and subcortical brain structures that includes both gray and white matter shrinkage. Both postmortem and in vivo imaging studies of brain morphology reveal abnormally reduced brain volumes of gray and white matter across
multiple regions. The frontal lobes are the most insulted region in the alcoholic brain with the superior frontal cortex showing significant neuronal loss (Kubota et al., 2001; Sullivan and Pfefferbaum, 2005). The frontal lobes regulate complex cognitive skills such as working memory, temporal ordering, discrimination and reversal learning that underlie judgement, attention, risk taking and motivation. Disorders in these behaviors are central if not causal to the consumption of dangerous amounts of alcohol despite the knowledge of negative consequences. Accordingly, chronic alcoholics demonstrate impaired judgment, blunted affect, poor insight, social withdrawal, reduced motivation, distractibility, attention and impulse-control deficits (Parsons, 1987; Sullivan et al., 2000; Sullivan and Pfefferbaum, 2005). Both clinical observations (Parsons, 1993; Sullivan et al. 2000) and animal studies have shown a direct relationship between chronic alcohol and learning and memory deficits (Bond and Di Giusto, 1976; Arendt et al., 1989; Lukoyanov, 1999; Matthews and Morrow, 2000).

Alcohol consumption during pregnancy is a significant public health problem and results in a wide range of adverse outcomes for the child. Heavy prenatal alcohol exposure has been associated with widespread neuropsychological deficits across several domains including general intelligence, memory, language, attention, learning, visuospatial abilities, executive functioning, motor skills, and social and adaptive functioning (Mattson and Riley, 1998). Children prenatally exposed to alcohol also have decreased academic achievement and higher rates of learning disabilities than non-exposed children, which may relate to impairments in verbal and non-verbal learning and memory (Roebuck-Spencer and Mattson, 2004; Greenbaum et al., 2011).

In the United States, England and Canada, 20-32% of pregnant women drink and in some European countries the rate is even higher exceeding 50% (May et al., 2005). In India, alcohol use is more prevalent in tribal women, tea plantation workers, women of lower socioeconomic status, commercial sex workers and to a limited upper crust of the rich and is not favored by women from the middle or upper socioeconomic classes (Mohan et al., 2001). The clinical description of Fetal Alcohol Syndrome (FAS) was first published in
1973 by Jones and Smith. Since then, FAS has come to be accepted as the leading identifiable cause of mental retardation and neurologic deficit in the western world (Abel and Sokol, 1986). O’Leary (2004) summarized the epidemiological research on fetal alcohol syndrome (FAS) concluding that its estimated worldwide prevalence is around 1/100 making it the most common cause of learning difficulties. The cost of caring for children with FAS has been estimated at approximately US$ 74.6 million per year, with three quarters of this cost associated with the care of FASD cases with mental retardation (Abel and Sokol, 1991).

Therefore, understanding how chronic alcohol consumption produces behavioral and cognitive deficits in adults as well as in neonates with prenatal alcohol exposure is of great medical and economic importance.

ETIOPATHOGENESIS OF ALCOHOL-INDUCED COGNITIVE DEFICITS

The cellular, biochemical and molecular mechanisms behind alcohol-induced neuronal damage and cognitive deficit are not fully understood but several explanations have been proposed including severe acute deficiency of thiamine, oxidative-nitroductive stress leading to free radical damage (Cohen-Kerem and Koren, 2003; Crews et al., 2004; Haorah et al., 2008a), alcohol-induced neuroinflammation (Alfonso-Loeches et al., 2010; Alikunju et al., 2011), activation of NFκB (Crews et al., 2006) and toll like receptor-4 (TLR-4) signaling (Alfonso-Loeches et al., 2010), neuronal apoptosis (Jung et al., 2005), NMDA receptor supersensitivity (Prendergast et al., 2004), suppression of growth factors (Breese and Sonntag, 1995), disruption of the hypothalamus–pituitary–thyroid axis (Scott et al., 1998) and inhibition of neurogenesis (Nixon and Crews, 2002).

Thus, although the occurrence of alcoholic dementia and neurodegeneration are well supported by multiple studies, the mechanisms of neurotoxicity are still poorly understood. Multiple pathways involved in alcohol-induced cognitive deficits are summarized here.
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Role of Alcohol-induced Neuronal Oxidative-Nitrodative Stress

Oxidative and nitrodative stress has been implicated in a variety of neurodegenerative disorders, including multiple sclerosis, Parkinson’s disease and Alzheimer’s disease and may also play an important role in the behavioral deficits (such as dementia) produced by ethanol (Butterfield et al., 2002; Jung et al., 2005). Oxidative stress results from an imbalance between the endogenous antioxidant defense system and free radical generation. Excessive oxidative challenges impair the brain antioxidant defense systems and can activate secondary events leading to apoptosis by affecting DNA integrity, protein function and membrane lipids (Behl and Moosmann, 2002) and ultimately producing neuronal death (Butterfield et al., 2002). Ethanol enhances oxidative stress directly through generation ofoxyfree radicals and lipid peroxidation (Nordmann et al., 1990) and depletion of endogenous antioxidants such as α-tocopherol, glutathione, ascorbate and vitamin E. Ethanol is converted into acetaldehyde via intracellular oxidation, eventually generating ROS such as superoxide anion, hydrogen peroxide and hydroxyl radical (Sagara et al., 1998). Neurons are highly dependent on glucose for ATP generation necessary for many biochemical processes and produce ROS as by-products of the oxidative phosphorylation within the mitochondria. The CNS is particularly susceptible to ROS-induced damage because (i) it has a high consumption of oxygen; (ii) it contains high levels of membrane polyunsaturated fatty acids susceptible to free radical attack; (iii) it is relatively deficient in oxidative defenses (poor catalase activity and moderate superoxide dismutase and glutathione peroxidase activities); and (iv) a high content in iron and ascorbate can be found in some regions of the CNS, enabling the generation of more ROS through the Fenton/Haber Weiss reaction (Halliwell, 1992). In addition, ethanol suppresses antioxidant enzymes such as glutathione peroxidase/glutathione reductase (Siler-Marsiglio et al., 2004). Certain regions of the CNS, such as the hippocampus and cerebellum, may be particularly sensitive to oxidative stress because of their low endogenous levels of Vitamin E, an important biochemical antioxidant, relative to other brain regions (Abel and Hannigan, 1995). Such a depressed defense system may be adequate under normal circumstances.
However, in pro-oxidative conditions, such as during alcohol exposure, these low antioxidant defenses can predispose the brain to oxidative damage. High dose or chronic exposure to alcohol (even at low dose) induces iNOS in the CNS and an excess amount of nitric oxide (NO) suppresses various physiological functions. The relevance of these data is supported by the findings that NOS induction was detected in cerebellar cortical neurons of alcoholics (Konovko et al., 2004). Peroxynitrite, a harmful oxidant formed by reaction between superoxide and NO, reacts with protein and non-protein-thiols, unsaturated fatty acids and DNA, thus affecting energy conservation mechanisms and oxidative post-translation modification of protein, and ultimately causing neuronal cell death (Fig. 5).

![Figure 5. The formation and degradation of reactive oxygen species (Adapted from Murray et al. 2003)](image)

Highly reactive oxygen molecules can be generated during normal cellular respiration and following toxic injury. Oxygen in the presence of electrons can form the free radical superoxide (O$_2^-$). Superoxide can be rapidly converted by superoxide dismutase (SOD) to hydrogen peroxide. Superoxide can also combine with nitric oxide to form hydroxyl and nitrogen dioxide radicals (NO$_2^-$) via peroxynitrite anion (OONO$^-$). Catalase and glutathione (GSH) remove ROS via enzymatic mechanisms that convert hydrogen peroxide to water and O$_2$. Under normal conditions, cells use free-radical scavengers and antioxidants to neutralize reactive-oxygen species to prevent damage.

**Oxidative Stress Mediated Pro-inflammatory Signaling in Brain**

Many findings suggest that ethanol-induced brain damage is related to oxidative stress from pro-inflammatory enzymes activated during ethanol intoxication. During the presence of ethanol, there are changes in protein transcription with increased DNA binding of NF-kB and reduced DNA binding of CREB. CREB family transcription factors are activated by phosphorylation and promote neuronal survival, protecting neurons from excitotoxicity and
apoptosis through regulating the transcription of pro-survival factors (Lon and Ginty, 2002). Conversely, NF-κB is a transcriptional factor that is widely known for its ubiquitous roles in inflammatory and immune responses (O’N and Kaltschmidt, 1997). The balance in expression and activation of the transcription factors, and thus the balance of prosurvival versus proinflammatory states, suggests a mechanism by which alcohol induces brain damage in alcoholic neuropathology (Fig. 6).

![Figure 6. A Schematic Presentation of Multiple Pathways Involved in Alcohol-induced Cognitive Deficits](image)

Activation of NF-κB transcription is associated with increases in proinflammatory cytokines such as tumor necrosis factor-α (TNF-α). A role for cytokines in alcoholic neuropathology is suggested by several studies (Crew et al., 2006). Acute ethanol increases cytokine induction by TLR-2 and TLR ligands (Oak et al., 2006). Both in vivo and in vitro evidence support the involvement of a proinflammatory cascade including increased NF-κB-driven induction of oxidative stress enzymes as a key factor in alcohol-induced brain damage. TNF-α can directly potentiate glutamate neurotoxicity by inhibiting glutamate uptake through NF-κB mechanisms (Zou and Crews, 2005). Human astroglial cells, which normally regulate extracellular glutam...
concentrations, ethanol enhances NF-κβ-DNA binding and the induction of iNOS (Davis and Syapin, 2004). Similarly, ethanol-induces COX-2, iNOS and NADPH oxidase and increases reactive oxygen species producing enzymes that are downstream of NF-κβ (Knapp and Crews, 1999). NADPH oxidase is a multimeric enzyme composed of multiple subunits that in the active form catalyze the transfer of one electron from NADPH to oxygen, giving rise to superoxide. Ethanol significantly increases the brain expression of NADPH oxidase subunits, gp91phox and p67phox, that persists for at least 8 days of abstinence (Qin et al., 2008). Thus, ethanol promotes a proinflammatory and anti-survival environment through the activation of proinflammatory transcription factors and the inhibition of prosurvival transcription factors (Fig. 6).

**Activation of NFκB Signaling Pathway**

Reactive oxygen species producing enzymes including NOS, COX-2 and NADPH oxidase are all induced by NF-κβ activation suggesting that ethanol-induced ROS in brain may be related to NF-κβ activation (Crews et al., 2006). There is indirect connection between ethanol and NF-κβ, as large acute doses or chronic administration of ethanol alter the fluidity of mitochondrial membranes and produce acetaldehyde, which generates oxidative species (Kukielka et al., 1994), including free radicals, hydrogen peroxide and hydroxyl radicals, which are all known to rapidly and significantly activate NF-κβ (Kono et al., 2000). Crews et al. (2006) suggested that alcohol-induced neurodegeneration involves NF-κβ activation, microglial activation and increased COX-2 immunoreactivity, all of which are indicative of an enhanced neuro-inflammatory response (Fig. 6). Valles et al. (2004) also found that 5 months of ethanol liquid diet induces inflammatory mediators IL-1β, COX-2, and iNOS in brain via NF-κβ induction. Izumi et al. (2005) also demonstrated that a single day of ethanol exposure in rats on postnatal day 7 resulted in significant apoptotic neuronal damage throughout the forebrain after twenty-four hours of ethanol administration.

Jung et al. (2005) suggested a cascade of events in which oxidative insults induced by chronic ethanol leads to activation of PKC, which
subsequently phosphorylates IkB (the NF-kB inhibitor) of NF-kB-IkB complex. On phosphorylation, a cell death signal NF-kB is released to its active form and translocates to the nucleus. The NF-kB then binds to DNA, induces the expression of target genes and results in DNA fragmentation and apoptosis through activation of caspases (Basheer et al., 2001). Numerous factors can induce apoptosis of CNS cells, including insufficient blood supply to the brain, dysfunction of the cell's energy-generating organelles (mitochondria), disruption of the normal calcium levels in the cells and oxidative stress. Alcohol can also induce apoptosis and this has been demonstrated both in animal models of alcohol exposure (Cartwright et al., 1998) and in isolated CNS cells grown in culture, including cells from the hypothalamus (De et al., 1994). Heavy, binge-like alcohol exposure during the period of brain development that is comparable to that of the human third trimester has been shown to produce death of postmitotic neurons in the hypothalamus (De et al., 1994), cerebral cortex (Ikonomidou et al., 2000), cerebellum (Light et al., 2002) and associated brain-stem structures (Napper and West, 1995). It has been reported that administration of ethanol to immature mice during the synaptogenesis period induces widespread apoptotic cell death in the developing brain (Ikonomidou et al., 2000), and caspase-3 activation is believed to be responsible for generating the cytological changes that characterize neuronal apoptosis (Fig. 6).

**Toll Like Receptor-4-induced Neuroinflammation and Brain Damage**

TLRs are a family of pattern-recognition receptors that enable the recognition of conserved structural motifs in a wide array of pathogens. Activation of TLRs triggers the downstream stimulation of nuclear factor-kB (NF-kB) and the induction of genes that encode inflammation-associated molecules and cytokines (O'Neill, 2003; Akira and Takeda, 2004). Recent evidence demonstrates that these receptors respond to pathogens and host tissue injury (Owens et al., 2005; Trendelenburg, 2008) and they not only play a role in the innate immunity in response to infections but also participate in CNS neurodegeneration and neural injury (Jin et al., 2008; Okun et al., 2009; Alfonso-Loeches et al., 2010). Activation of the TLR response significantly contributes to neuroinflammation (Chen et al., 2007) and TLR4-deficient mice
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are protected against ischemic brain damage and injury (Caso et al., 2007; Tang et al., 2007). The role of TLR-4 in brain injury has been indicated in a number of recent studies demonstrating that elimination of TLR-4 protects against oxidative stress in Alzheimer’s disease (Tang et al., 2008), focal cerebral ischemia (Kilic et al., 2008), human immunodeficiency virus-associated neurodegeneration (Salaria et al., 2007) and ischemic brain injury (Tang et al., 2007).

Chronic ethanol consumption increases cytokines and inflammatory mediators in the rat brain, activating signaling pathways associated with neuroinflammation and triggering cell damage (Valles et al., 2004). It was also found that ethanol activates TLR-4 signaling in astrocytes (Blanco et al., 2005), microglia and macrophages (Fernandez-Lizarbe et al., 2009), suggesting that activation of the TLR-4 response by ethanol could be an important mechanism of ethanol-induced neuroinflammation (Fig. 6). Although chronic ethanol treatment increased the expression of iNOS and COX-2 in the cerebral cortices of the ethanol-treated wild type mice, the induction of these proteins did not take place in the cortices of the TLR4-knock-out mice. Previous findings demonstrate that ethanol at low/moderate concentrations activates the TLR-4 receptors in astrocytes, triggers NFκβ activation and leads to the induction of an inflammatory response (Blanco et al., 2005) suggesting that TLR-4 activation in glial cells is a critical event in the ethanol-induced inflammatory processes. In vivo findings also support the pivotal role of the TLR-4 receptors in the activation of both microglia and astroglia induced by ethanol, since the deficiency of TLR-4 function markedly reduces astroglia hypertrophy and completely abolishes microglia activation. A deficient TLR-4 function prevents both glial activation and the inflammatory reaction, thus supporting the role played by the TLR-4 function in these processes. Elimination of the TLR-4 receptor function prevents ethanol-induced NF-κβ activation and cytokine upregulation suggesting the critical role of TLR-4/NF-κβ in the ethanol-induced inflammatory process in the brain (Fig. 6).
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**NMDA Receptor Supersensitivity**

Neuronal death can also be induced by excess activity of certain neurotransmitters, including glutamate. Early studies, mostly in vitro culture models, suggested that acute ethanol exposure inhibited glutamatergic N-methyl-D-aspartate (NMDA) receptors but long term exposure to ethanol resulted in NMDA receptor supersensitivity (Chandler et al., 1993; Roh et al., 2011). Under certain conditions, when glutamate interacts with the NMDA receptor, it causes calcium to flow into the signal-receiving neuron. Calcium influx is a powerful regulator of the activity and function of a neuron. Excessive activation of the NMDA glutamate receptor, however, can lead to dangerously high calcium accumulation inside the neuron (Choi, 1995). If sufficiently severe or prolonged, the rise in intracellular calcium can lead to cell death by either apoptosis or necrosis (Choi, 1995; Kroemer et al., 1997).

Conditions of excitotoxicity can also occur during withdrawal from high levels of alcohol and may thereby contribute to alcohol-induced damage to the fetal brain, particularly when the mother binge drinks (Thomas and Riley, 1998). In these cases, the fetus experiences periods of heavy alcohol exposure, followed by withdrawal episodes. High levels of alcohol acutely inhibit NMDA receptor function. During withdrawal after a binge-drinking episode, however, glutamate stimulation of NMDA receptor activity increases temporarily and may lead to excitotoxicity (Thomas et al., 1997). Although some experimental support exists for the potential contribution of withdrawal-related events to alcohol-induced fetal brain damage (Thomas et al., 1997), including the potential role of excitotoxicity, this hypothesis requires more research.

**Glia and Alcoholic Neurodegeneration**

Normal brain development and function require not only neurons, but also non-neuronal cells, called glia, that support the growth and development of the neurons. Glia may also contribute to alcoholic neurodegeneration. Alcohol exposure can reduce the overall number of astrocytes in the cortex and interfere with response to specific growth factors. Alcohol also causes astroglia to degenerate, leaving a void in trophic and metabolic support and
then neurons degenerate (Kimelberg and Aschner, 1994). The loss of astroglia results in reduced ability to take up excess glutamate, buffer K+ (ion homeostasis) and eliminate free radicals (Dringen, 2000). Such alcohol-induced changes in astrocyte development and function could have serious consequences on neuronal migration and survival and on the correct formation of connections among neurons (Miguel-Hidalgo et al., 2002). Careful studies in postmortem human hippocampus found a statistically significant loss of 37% of the glial cells in alcoholic hippocampus that included a reduction of astrocytes and oligodendrocytes but no loss of neurons (Korbo, 1999). Long-term alcohol exposure decreases an intermediate neurofilament, glial fibrillary acidic protein (GFAP), that is a characteristic of astrocytes, in the cerebellum of male and females rats (Rintala et al., 2001). The loss of GFAP expression suggests a loss of astrocytes (Rintala et al., 2001) consistent with the finding that the number of astrocytes identified by giemsa staining in human hippocampus is reduced in alcoholics (Korbo, 1999; Miguel-Hidalgo, 2005).

**Involvement of Hypothalamic Pituitary axis (HPA) axis**

Ethanol-exposed males and female rats show increased corticosterone, adrenocorticotropic hormone (ACTH) and/or corticotropin releasing hormone responses to stressors such as repeated restraint, foot shock and lipopolysaccharide (LPS) challenges or to morphine administration (Lee et al., 2000). The mechanisms that underlie HPA-axis hyper-responsiveness in ethanol-exposed offspring are not well understood. However, several reports suggest an abnormal production and/or release of CRH after a stress challenge may be one of the causes for the altered stress regulation process in the ethanol-exposed offspring (Sarkar et al., 2007; Przybycien-Szymanska et al., 2011).

**Disruption of Growth-Factor Signaling**

Alcohol can also interfere with the activity of growth factors that regulate cell proliferation and survival. Numerous growth factors are needed for cell division to proceed normally, including two factors called insulin like growth factors (IGF) I and II. Alcohol can interfere with the activity of the IGF-I
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receptor. As a result, IGF-I still binds to its receptor but the receptors signaling function is blocked and IGF-I-mediated cell division cannot proceed (Resnicoff et al., 1993). Thus, alcohol can prevent the normal production of CNS cells by interfering with the growth factors that regulate cell division (Fig. 6). Alcohol may also induce cell death by inhibiting several growth factors that support cells which have attained their final function (i.e., that are differentiated) and no longer divide (Zhang et al., 1998). Cohen et al. (2007) found that ethanol-induced neurodegeneration in adult rats is mediated by insulin/IGF resistance, persistent oxidative stress and impaired acetylcholine biosynthesis.

Alcohol Intoxication Inhibits Neurogenesis

Neurogenesis is the net result of four components: cell proliferation, cell differentiation, cell migration and cell survival. Alcohol could potentially affect neurogenesis at any of these stages of cell development. Indeed, over 30 years of research on the effects of alcohol on fetal neurogenesis has shown that alcohol affects each of these components in the developing brain (Goodlett et al., 2005). Longer alcohol exposure durations, specifically a four-day binge, affects both cell proliferation and newborn cell survival. Reduced cell survival in this binge exposure model is consistent with both evidence of cell death in the dorsol root ganglion (DG) following binge alcohol exposure (Obernier et al., 2002) and also the seminal finding of DG granule cell loss following chronic alcohol exposure (Walker et al., 1980). Thus, inhibition of adult neurogenesis should be considered as a new mechanism underlying alcohol-induced neurodegeneration. Inhibiting neurogenesis has shown detrimental effects on hippocampus-based learning (Shors et al., 2001). These findings imply that events that inhibit neurogenesis would have downstream effects on learning and memory. The learning and memory performance was examined at 3 weeks following binge exposure and deficits in hippocampus dependent task were observed at the same time point where neurogenesis was inhibited (Nixon and Crews, 2002; Hamilton et al., 2011). Several groups have consistently shown that progenitor cell survival is also reduced, which suggests another mechanism by which alcohol reduces neurogenesis in rats (Nixon and Crews, 2002; Herrera et al., 2003; He et al., 2005). Further, ethanol treatment during adult neurogenesis blunted the
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growth of progenitor’s dendritic arbor (He et al., 2005). Taken together, these studies indicate that ethanol reduces neurogenesis during intoxication contribute to neurodegeneration through loss of cell generation. Intriguingly, inflammatory processes may inhibit neurogenesis (Monje et al., 2003). Thus, ethanol activation of proinflammatory cytokine-induced oxido-nitrosative stress cascades likely inhibits neurogenesis as well as mediates the other necrotic degenerative processes (Fig. 6).

Disruption in Glucose Transport and Uptake

Some of the harmful effects of prenatal alcohol exposure may be associated with alcohol-induced disruption of the brain’s utilization of the glucose (Abdul Muneer et al., 2011). Glucose has several crucial functions in the body, including the brain. First, it serves as an energy source in all cells. Second, it is used in the production of various important types of molecules, including DNA and RNA building blocks (i.e., nucleic acids), fat molecules (i.e., lipids), certain hormones (i.e., steroids) and certain neurotransmitters. To enter cells from the blood and fulfill its functions, glucose must cross the cell membrane. To this end, most mammalian cells contain specific glucose transporter proteins designated GLUT-1 through GLUT-7. The principal glucose transporter proteins of the brain are GLUT-1 and GLUT-3. In cultured rat neurons and astrocytes, short-term alcohol exposure reduced cellular glucose uptake as well as the levels of glucose transporter proteins (Hu et al., 1995). Similarly, prolonged prenatal exposure of rats to alcohol reduced both glucose uptake and GLUT-1 gene expression (Singh et al., 1992). Because of the central role that glucose plays in the body, alcohol-induced changes in glucose transport have broad implications and must be considered as an important potential contributor to both growth deficiency and CNS damage associated with prenatal alcohol exposure.

Effects on Cell Adhesion

Yet another mechanism through which alcohol may interfere with normal brain development is by reducing cell adhesion (Fitzgerald et al., 2011). Neurons must establish cell-to-cell contact during growth and development in order to survive, migrate to their final destination and develop
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appropriate connections with neighboring cells. Numerous cell adhesion molecules assist in various aspects of this process. Defects in one particular cell adhesion molecules called L1 can lead to abnormal brain development in humans, characterized by mental retardation, complete absence of the corpus callosum and abnormal development of the cerebellum. These brain abnormalities are similar to those found in patients with FAS suggesting that prenatal alcohol exposure also may affect the L1 molecule and thereby contribute to several aspects of the FAS phenotype. This hypothesis is supported by findings that when cultured brain cells are exposed to low levels of alcohol (less than 0.05 percent) the L1-mediated clumping of the cells is inhibited (Ramanathan et al., 1996). Researchers found that only certain alcohol molecules such as ethanol interfere with L1-mediated cell adhesion. Conversely, types of alcohol molecules such as a octanol, actually block ethanol's effect on cell adhesion in tissue cultures (Wil kemeyer et al., 2000). Octanol even prevented the harmful effects of ethanol on mouse fetuses grown in culture (Chen et al., 2001a), suggesting that ethanol's effect on cell adhesion is an important contributer to the harmful consequences of prenatal alcohol exposure.

Altered Developmental Regulation of Gene Expression

Another candidate mechanism through which prenatal alcohol exposure could damage the CNS and lead to such devastating consequences as FAS is through interfering with the normal regulation of the genes that control brain development. Researchers have not yet been able to elucidate these processes, leaving a major gap in their understanding of candidate mechanisms underlying FAS. Although investigators have identified genes whose expression is altered by alcohol in vitro, studies of alcohol effects on gene expression as it relates to the development of various body structures and the CNS are still in their infancy. Detailed studies of alcohol-induced changes in gene expression during critical periods of development constitute one of the highest priorities for new research. Cutting-edge technologies, such as the gene microchip array and proteomic technologies may provide the means to make rapid advances on this frontier in the near future (Goodlett and Horn, 2001).
THERAPEUTIC STRATEGIES FOR ALCOHOL-INDUCED COGNITIVE DEFICITS

Treatment of alcoholic dementia requires vitamin replacement, correction of medical problems, and management of behavior problems. The brain damage produced by alcohol may be arrested by cessation of drinking. Prolonged periods of sobriety for patients with alcohol-induced dementia may result in slow small improvements of intellectual function. Memantine can be used to improve memory and cognitive functioning (Preuss et al., 2001). Psychiatric problems produced by alcohol consumption are treated with appropriate psychotropic medications. Antidepressants or antipsychotics are more effective for alcohol-induced psychiatric problems. Apathy rarely responds to antidepressants or other psychotropic medications. Impulsive or hostile behavior can be managed with anti-convulsants, anti-psychotics or Lithium. Benzodiazepines can sometimes be use to manage irritability or anxiety (Martin and Nimmerrichter, 1993).

It is unlikely that health service providers can deliver therapeutic interventions to women who are heavily abusing alcohol during pregnancy in time to be effective, thus it is essential that treatments for children with prenatal alcohol-induced brain damage should be developed. One promising set of pharmacotherapeutic candidates comprises the neuroprotective peptides, which may prevent neuroteratogenic effects of alcohol exposure by antagonizing ethanol-induced inhibition of L1 cell adhesion, by reducing oxidative stress or by mechanisms not yet identified (Spong et al., 2001). Treatments with antioxidant supplements may also prove feasible and effective for at least some pathogenic effects of fetal alcohol exposure based on animal model (Heaton et al., 2000; Peng et al., 2005) and in vitro studies (Mitchell et al., 1999; Siler-Marsiglio et al., 2004).

Though considerable progress has been made in understanding the pathophysiology and pathways involved in alcohol-induced cognitive deficits in both adults and children exposed to alcohol, strategies regarding therapeutic management of the disease needs to be investigated so that novel pharmacotherapeutic agents can be developed for targeting multiple disrupted pathways involved in alcohol-induced cognitive deficits.
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PHARMACOLOGICAL INTERVENTIONS EMPLOYED IN THE STUDY

TOCOTRIENOL

Tocotrienols are fat-soluble vitamins belonging to the family of tocopherols i.e. tocochromanols. Tocochromanols are group of amphipathic, lipid-soluble organic molecules composed of a polar moiety derived from tyrosine and a hydrophobic polypropyl side chain originating from the isoprenoid pathway. Tocochromanols with a saturated phytol-derived side chain are termed tocopherols whereas those with unsaturated geranylated side chain are termed tocotrienols. Structurally, tocopherols and tocotrienols share some resemblance consisting of a common chromanol head and a side chain at the C-2 position. Tocopherols and tocotrienols are further separated into individual compounds assigned by the Greek letter prefixes (α, β, γ and δ) depending on the number and position of methyl substitution on the chromanol ring. The alpha form has three methyl groups, the beta & gamma forms have two methyl groups and the delta form has only one methyl group (Theriault et al. 1999). Each of these forms of vitamin E has a different biopotency.

Tocopherol

\[ \text{Tocopherol} \]

\[ \text{Tocotrienol} \]
Sources of Tocotrienols

While tocopherols are generally present in nuts (almonds) and common vegetable oils (wheat germ, sunflower), tocotrienols are minor constituents especially abundant in palm oil, cereal grains and rice (McLaughlin and Weihrauch, 1979; Abidi, 2003; Ko et al., 2003). Crude oil extracted from the fruits of oil palm (*Elaeis guineensis*) particularly contains a high amount of tocotrienols (up to 800 mg/kg), mainly consisting of γ-tocotrienol and alpha-tocotrienol. Tocotrienols are also found in oil derived from rice bran, barley, wheat germ and rye.
Structurally, tocotrienols differ from tocopherols by the presence of three trans double-bonds in the hydrocarbon tail. Because of the unsaturations in the isoprenoid side-chain, tocotrienols are thought to assume a unique conformation (Atkinson, 2006). α-Tocotrienol possesses number...
functions that are not shared by α-tocopherol (Sen et al., 2006). Tocotrienols possess powerful cardioprotective (Das et al. 2008), neuroprotective (Khanna et al., 2006; Shichiri et al. 2007), radioprotective (Ghosh et al., 2009), anti-angiogenic (Nakagawa et al., 2004; Shibata et al., 2009), potent natural antioxidant (Schroeder et al., 2006; Maniam et al., 2008), anti-cancer (Nesaretnam et al., 2004; Wada et al., 2005), anti-inflammatory (Wu et al., 2008), cyclooxygenase-2 inhibitory (Yam et al., 2009), anti-nociceptive (Kuhad and Chopra, 2009; Tiwari et al., 2009a), insulin sensitizing, hypoglycemic (Chen and Cheng, 2006; Budin et al., 2009) and cholesterol lowering (Chou et al., 2009) properties that often differ from the properties of tocopherols (Serbinova et al., 1991; Serbinova and Packer, 1994; Sen et al., 2007; Budin et al., 2009; Tiwari et al., 2009a; Kuhad and Chopra, 2009). The unsaturated side chain of tocotrienol allows for more efficient penetration into tissues that have saturated fatty layers such as the brain and liver (Suzuki et al., 1993; Atkinson et al., 2006). Experimental research examining the antioxidant effects of tocopherol and tocotrienols has revealed that tocotrienols appear superior due to (a) a more uniform distribution in the membrane lipid bilayer, (b) a more efficient interaction of the chromanol ring with lipid radicals, and (c) a higher recycling efficiency from chromanoxyl radicals (Serbinova et al., 1991; Suzuki et al., 1993; Kawakami et al., 2007; Tsuzuki et al., 2007; Maniam et al., 2008).

**EPIGALLOCATECHIN GALLATE (EGCG)**

Since past decade, tea polyphenols have been picked up by scientific community for its diverse biological activities. Phenolic compounds are widely present in plants and they have recently received considerable attention due to their antioxidant property (Rajamurugan et al., 2011; Zimmer et al., 2012). There are four primary polyphenols present in green tea (*Camellia sinensis*), the most important polyphenols, called catechins (sometimes referred to as tea flavonoids). The family of catechins includes: Galallocatechin (GC), Epigallocatechin (EGC), Epicatechin (EC) and Epigallocatechin Gallate (EGCG). EGC is the most abundant catechin (30-35%) present in tea and is considered as the main active ingredient in green tea (Reto et al., 2007).
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Green Tea

Catechin gallates are gallic acid esters of the catechins. Catechins were initially discovered in the 1970s, when medical researchers were looking for the reason: why Japanese in Shizuoka district, a major tea growing area, had much lower rates of cancer than other Japanese, even though they were very heavy smokers (Jankun et al., 1997). Numerous studies found EGCG to be effective in preventing and inhibiting cancer growth. EGCG converts carcinogens into benign substances (Hsu and Liou, 2011; Vu et al., 2010). Epigallocatechin gallate possesses the most potent antioxidant activity of catechins. EGCG is 100 times more powerful than vitamin C and 25 times more effective than vitamin E (Murray, 2000).

\[
\text{[(2R,3R)-5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)chroman-3-yl] 3,4,5-trihydroxybenzoate (EGCG)}
\]

Dietary Source of Catechins

Catechins constitute about 25% of the dry weight of fresh tea leaves, although total catechin content varies widely depending on clonal variation, growing location, seasonal/light variation and altitude (Balentine et al., 1999). They are present in nearly all teas made from *Camellia sinensis*, including white tea, green tea, black tea and oolong tea. Catechins are also present...
the human diet in chocolate (Hammerstone et al., 2000), and in fruits and vegetables (Ruidavets et al., 2000).

EGCG, the principal active constituent of green tea, is known to have anti-inflammatory, anticarcinogenic and free radical-scavenging properties (Sueoka et al., 2001; Hsu et al., 2005). It also inhibits COX-2 without affecting COX-1 expression (Hussain et al., 2005). EGCG possesses neuroprotective effects against a variety of toxic insults and inflammatory neuronal injury (Mandel et al., 2004; Antonio and Druse, 2008). EGCG also blocks induction of nitric oxide synthase by down-regulating lipopolysaccharide induced activity (Lin and Lin, 1997).

RESVERATROL

Resveratrol (trans-3,4',5-trihydroxystilbene), a natural polyphenolic non-flavonoid antioxidant, is a phytoalexin found in many plants including grapes, nuts and berries (Das and Das, 2007).
Plants produce resveratrol in response to stress, injury, fungal infection or ultraviolet (UV) radiation (Aggarwal et al., 2004). Resveratrol is a fat-soluble compound that occurs in a trans and a cis configuration. Scientists became interested in exploring potential health benefits of resveratrol in 1992 when its presence was first reported in red wine (Siemann and Creasey, 1992). More recently, reports on the potential for resveratrol to inhibit the development of cancer (Jang et al., 1997) and extend lifespan (Howitz et al., 2003) in cell culture and animal models have continued to generate scientific interest.

**Dietary Sources of Resveratrol**

Resveratrol is found in grapes, wine, grape juice, peanuts and berries of *Vaccinium* species, including blueberries, bilberries and cranberries (Sanders and McMichael, 2000; Burns et al., 2002; Rimando et al., 2004). In grapes, resveratrol is found only in the skins (Creasey and Coffee, 1988). The amount of resveratrol in grape skins varies with the grape cultivar, its geographic origin and exposure to fungal infection (Fremont, 2000). The amount of fermentation time a wine spends in contact with grape skins is an important determinant of its resveratrol content. Consequently, white and rosé wines generally contain less resveratrol than red wines (Siemann and Creasey, 1992). Red or purple grape juices may also be good sources of resveratrol (Romero-Perez et al., 1999). The predominant form of resveratrol in grapes and grape juice is trans-resveratrol glucoside but wines also contain significant amounts of resveratrol aglycones, thought to be the result of sugar cleavage during fermentation (Burns et al., 2002). Red wine is a relatively rich source of resveratrol, but other polyphenols are present in red wine at considerably higher concentrations than resveratrol (Burns et al., 2001). The total resveratrol content of some beverages and foods are listed in the tables.

Resveratrol possesses diverse biochemical and physiological actions, which includes antiplatelet (Goçmen et al., 2011), cardioprotective (Petrovski et al., 2011; Sebai et al., 2011; Wu and Hsieh, 2011), neuroprotective (Sharma et al., 2007; Kanthasamy et al., 2011), renoprotective (Chander and Chopra, 2006), cancer preventive (Baur and Sinclair, 2006) and anti-
Review of inflammatory properties (Bertelli et al., 1996; Zang et al., 2011; Kanel 2011). Resveratrol has been found to protect the heart, kidney and brain ischemic-reperfusion injury (Das et al., 1999; Bastianetto et al; Giovannini et al., 2001). Resveratrol also prevents memory deficits, increase in acetylcholinesterase activity in streptozotocin-induced diabetes (Schmatz et al., 2009). In kidney cells, resveratrol was found to have a protective action through upregulation of NO (Giovannini et al., 2001).

<table>
<thead>
<tr>
<th>Food</th>
<th>Serving</th>
<th>Total resveratrol (ng)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peanuts (raw)</td>
<td>1 cup (146 g)</td>
<td>0.01-0.26</td>
</tr>
<tr>
<td>Peanuts (boiled)</td>
<td>1 cup (180 g)</td>
<td>0.32-1.28</td>
</tr>
<tr>
<td>Peanut butter</td>
<td>1 cup (258 g)</td>
<td>0.04-0.13</td>
</tr>
<tr>
<td>Red grapes</td>
<td>1 cup (160 g)</td>
<td>0.24-1.25</td>
</tr>
</tbody>
</table>

**Total Resveratrol Content of Selected Foods (Sobolev and Col; Sanders and McMichael, 2000; Burns et al., 2002)**

<table>
<thead>
<tr>
<th>Beverage</th>
<th>Total resveratrol (mg/liter)</th>
<th>Total resveratrol (5-oz glass)</th>
</tr>
</thead>
<tbody>
<tr>
<td>White wines (Spanish)</td>
<td>0.05-1.80</td>
<td>0.01-0.2</td>
</tr>
<tr>
<td>Rosé wines (Spanish)</td>
<td>0.43-3.52</td>
<td>0.06-0.5</td>
</tr>
<tr>
<td>Red wines (Spanish)</td>
<td>1.92-12.59</td>
<td>0.29-1.8</td>
</tr>
<tr>
<td>Red wines (Global)</td>
<td>1.98-7.13</td>
<td>0.30-1.0</td>
</tr>
<tr>
<td>Red grape juice (Spanish)</td>
<td>1.14-8.69</td>
<td>0.17-1.3</td>
</tr>
</tbody>
</table>

Resveratrol is not known to be toxic or cause adverse effects in humans, but there have been only a few controlled clinical trials. A recent trial that evaluated the safety of oral resveratrol in ten subjects, single dose up to 5 grams resulted in no serious adverse effects (Boal et al., 2007). In rats, daily oral administration of trans-resveratrol at doses of 300 mg/kg of body weight for four weeks resulted in no apparent effects (Juan et al., 2002; Crowell et al., 2004).
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CURCUMIN

Curcumin is the principal curcuminoid of the popular Indian spice turmeric which is obtained from rhizome of Curcuma longa Linn. (Family-Zingiberaceae). The other two curcuminoids are desmethoxycurcumin and bis-desmethoxycurcumin. The curcuminoids are natural phenols and are responsible for the yellow color of turmeric. In the crude extract of rhizomes of C. longa about 70–76% curcumin is present along with about 16% demethoxycurcumin and 8% bisdemethoxycurcumin (Huang et al., 1995).

\[
\text{(1E,6E)-1,7-bis (4-hydroxy- 3-methoxyphenyl) -1,6- heptadiene-3,5-dione (Curcumin)}
\]

Turmeric has been used historically as a component of Indian Ayurvedic medicine since 1900 BC to treat a wide variety of ailments (Aggarwal et al., 2007). Research in the latter half of the 20th century has identified curcumin as responsible for most of the biological activity of turmeric (Aggarwal et al., 2007). Curcumin acts as a free radical scavenger and antioxidant, inhibiting lipid peroxidation (Molina-Jijón et al., 2011) and oxidative DNA damage. In vitro and animal studies have suggested curcumin...
may have antitumor (Ströfer et al., 2011) antioxidant (Molina-Jijón et al., 2011), antiarthritic (Moon et al., 2010), antiamyloid (Wang et al., 2010), anti-ischemic (Shukla et al., 2008) and anti-inflammatory properties (Basnet and Skalko-Basnet, 2011; Buhrmann et al., 2011). In addition it may be effective in treating malaria, prevention of cervical cancer, and may interfere with the replication of the human immunodeficiency virus (HIV) (Reddy et al., 2005; Gandapu et al., 2011; Singh and Singh, 2011).

The Siegel Life Project funded an initial study on curcumin for Alzheimer’s in 1997-1998 through the UCLA Center on Aging. They found that curcumin was particularly effective in reducing neurodegeneration, oxidative damage, diffuse plaque deposition, aberrant inflammation and impaired inflammatory clearance following beta-amyloid infusion (Frautschy et al., 2001). This led to testing in a transgenic animal model where it was shown to dramatically diminish plaque burden and overall inflammation, but also increase plaque associated inflammatory cells suggesting clearance (Lim et al., 2001). Numerous studies have demonstrated curcumin, amongst only a few other things, such as high impact exercise, learning, bright light, and antidepressant usage, has a positive effect on neurogenesis in the hippocampus and concentrations of brain-derived neurotrophic factor (BDNF), reductions in both of which are associated with stress, depression, and anxiety (Bala et al., 2006; Wu et al., 2006; Xu et al., 2007). Curcumin was found to be pharmacologically safe in human clinical trials with doses up to 10g/day. A phase 1 human trial with 25 subjects using up to 8000 mg of curcumin per day for three months found no toxicity from curcumin (Chainani, 2003).

Although many preclinical studies suggest curcumin may be useful for the prevention and treatment of several diseases, the effectiveness of curcumin has not yet been demonstrated in randomized, placebo-controlled, double-blind clinical trials (Mancuso and Barone, 2009). Numerous clinical trials in humans were underway, studying the effect of curcumin on various diseases, including multiple myeloma, pancreatic cancer, myelodysplastic
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syndromes, colon cancer, psoriasis and Alzheimer's disease (Hatcher et al., 2008; Singh and Sankhla, 2010).