Chapter 7

Analgesic and hepatoprotective activity of *M. terminale* Dalz
7.1 Introduction

Pain is an unpleasant feeling often caused by intense or damaging stimuli, such as stubbing of toe, burning of skin, applying phenolic and alcoholic compounds on a cut or cracks in the bone and, internal injuries. According to the international association for the study of pain, the definition is “Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage” (IASP, 1979). Pain alerts the person or animals to withdraw from damaging situations, to protect a damaged body part while it heals, and to avoid similar experiences in the future (Raj, 2007). Most pain resolves promptly once the painful stimulus is removed and the body has healed, but sometimes pain persists despite removal of the stimulus and apparent healing of the body and sometimes pain arises in the absence of any detectable stimulus, damage or disease (Debono et al., 2013). Pain is a major symptom in many medical conditions and can significantly interfere with a person’s quality of life and general functioning (Breivik et al., 2008).

An analgesic or painkillers are the group of drugs used to achieve analgesia-relief from pain. Analgesic drugs act in various ways on the peripheral and central nervous systems. They are distinct from anesthetics, which reversibly eliminate sensation, which include paracetamol, the non-steroidal anti-inflammatory drugs (NSAIDs) such as the salicylates, and opioid drugs such as morphine and opium.

Aspirin suppresses and inhibits the enzymes required for prostaglandins and thromboxanes synthesis. Aspirin is a reversible inhibitor that acts as an acetylating agent where an acetyl group is covalently attached to a serine residue in the active site of the prostaglandin-endoperoxide synthase (PTGS) enzyme. Low-dose, long-term aspirin use irreversibly blocks the formation of thromboxane A2 in platelets, producing an inhibitory effect on platelet aggregation. This antithrombotic property makes aspirin useful for reducing the incidence of heart attacks (Somasundaram, 2000). Aspirin also inhibits the cyclooxygenase enzymes (COX-1 and COX-2) and it is irreversibly inhibits COX-1 and modifies the enzymatic activity of COX-2. The enzyme COX-2 normally produces prostanoids, most of which are proinflammatory. Aspirin- modified PTGS2 produces lipoxins, most of which are anti-inflammatory (Somasundaram, 2000). Endothelial cells lining the microvasculature in the body are proposed to express PTGS2, and by selectively inhibiting PTGS2, prostaglandin production (specifically, PGI2 prostacyclin) is down regulated with respect to

Department of Biochemistry  
Page | 143
thromboxane levels, as PTGS1 in platelets is unaffected. Thus, the protective anticoagulative effect of PGI2 is removed, increasing the risk of thrombus and associated heart attacks and other circulatory problems. Since platelets have no DNA, they are unable to synthesize new PTGS once aspirin has irreversibly inhibited the enzyme, an important difference with reversible inhibitors (Somasundaram, 2000).

Aspiring is shown to have at least three additional modes of action. It uncouples oxidative phosphorylation in cartilaginous mitochondria, by diffusing from the inner membrane space as a proton carrier back into the mitochondrial matrix, where it ionizes once again to release protons (Somasundaram, 2000). Aspirin is readily broken down in the body to salicylic acid, which itself has anti-inflammatory, antipyretic and analgesic effects. The salicylic acid was found to activate AMP-activated protein kinase, and this has been suggested as a possible explanation for some of the effects of both salicylic acid and aspirin (Raffensperger, 2012; Lloyd, 2009). The acetyl portion of the aspirin molecule is not without its own targets. Acetylation of cellular proteins is a well-established phenomenon in the regulation of protein function at the posttranslational level. Recent studies have reported aspirin is able to acetylate several other targets in addition to COX isoenzymes (Lloyd, 2009). These acetylation reactions may explain many previously unexplained effects of aspirin.

The plant and its derived secondary metabolites have, over the years, great contribution to our current understanding of the important mechanisms related to the process of pain transmission and treatment. Furthermore, they have permitted us to characterize receptor types and identify endogenous ligand involved in the mechanism of nociception. Now a day, research has occurred regarding plant-derived substances in the process of development of new analgesic drugs. Plants, such as Papaver, Somniferum, Cannabis sativa and those of the Capsicum and Salix species, have greatly accounted for the development of clinically relevant drugs which are useful for the management of pain disorders. Recent advances in our understanding of the mechanisms of action of plant-derived substances have greatly accelerated attempts to identify promising targets for the discovery of new, safe and efficient analgesic drugs. Despite the great progress which has occurred in the elucidation of pain transmission and despite decades of use, its undesirable side effects are not known. Aspirin continues to be one of the most used drugs in clinical practice for the treatment of
pain disorders. Thus, safer and more efficacious analgesic drugs are urgently needed. A search through the literature reveals that many potentially active anti-nociceptive plant derived compounds have been identified. However, studies aiming to investigate their cellular and molecular mechanisms of action and well-controlled clinical trials to prove their efficacy in humans are still lacking. Nevertheless, natural or synthetic substances that bind to vanilloid or cannabinoid receptors, or even those that are capable of modulating the endogenous ligands which bind to these receptors, are expected to soon appear to assist in the treatment of several pain disorders, including those of neuropathic or neurogenic origin.

Liver is the largest organ of the body and play important roles in regulating various physiochemical functions of our body, including bile, protein, and cholesterol synthesis, regulation of amino acids, synthesis of glycogen and metabolism of toxic chemicals. Damage in hepatic parenchyma cells of liver cause deleterious effect to these physiochemical functions (Wolf, 1999). Many factors are responsible for liver damage, including infectious agents and hepatotoxic chemicals. Carbon tetrachloride (CCL₄) is one of the most commonly used hepatotoxins for inducing liver damage in experimental animal studies (Johnston and Kroening, 1998). Paracetamol is also one of the causative agents of liver damage and it is a synthetic compound widely used as antipyretic (fever reducer) and analgesic (pain reliever) drug. This drug is primarily metabolized in the liver into non-toxic compounds and then gradually excreted through kidney but over dosage of paracetamol can damage multiple organs in humans. The N-acetyl-p-benzoquinoneimine (NAPQI) is one of the byproducts of paracetamol and is the main reason for toxicity of drug in the body. Paracetamol hepatotoxicity is most common cause of acute liver failure (Larson et al., 2005). Apart from these, there are various factors responsible for liver damage and dysfunction.

The liver dysfunction or damage include both physical signs and a variety of symptoms related to digestive problems, coagulopathies, blood sugar problems, immune disorders, abnormal absorption of fats and metabolism problems. The malabsorption of fats may lead to symptoms that include indigestion, reflux, deficit of fat soluble vitamins, hemorrhoids, gallstones, intolerance to fatty foods, intolerance to alcohol, nausea and vomiting attacks, abdominal bloating, and constipation. The liver damage also effect nervous system including depression, mood changes, anger,
irritability, poor concentration and foggy brain, overheating of the body, especially the face and torso and recurrent headaches associated with nausea. Hypoglycemia is also one of the problems during the liver damage conditions. The LDL level may vary; the HDL level decreases and triglyceride level may vary in different extent. The high blood pressure is caused by the clogged arteries, heart attacks and strokes, buildup of fat in other body organs, lumps of fat in the skin, excessive weight gain, inability to lose weight even while dieting, sluggish metabolism, protuberant abdomen, cellulite fatty liver and a roll of fat around the upper abdomen.

7.1.1 Different types of liver damage

People around the world face more than hundred kinds of liver disease. The most important ones are as follows:

7.1.1.1 Hepatitis

Hepatitis is a condition defined by the inflammation of liver and characterized by the presence of inflammatory cells in tissue of the organ. The hepatitis name was originated from Greek word hepatitis meaning "liver" and suffix-itis, meaning "inflammation" (WHO, 2013). The condition normally healed on its own or can progress to fibrosis and cirrhosis. The important characteristic of hepatitis is that it may occur with limited or no symptoms, but often or finally leads to jaundice, anorexia and malaise. Hepatitis is acute when it lasts less than six months and chronic when it persists longer. Hepatitis is acute if it lasts less than six months and chronic if it persists longer. The hepatitis viruses are the most common cause of the cirrhosis conditions, but hepatitis can be caused by other infection, toxic substances (alcohol, certain medication, some industrial organic solvents, fungal toxins, plants), and autoimmune diseases.

Viral hepatitis is the most common cause of hepatitis worldwide. Most common causes of viral hepatitis are hepatotrophic viruses; hepatitis A, hepatitis B, hepatitis C, hepatitis D and hepatitis E. Other common causes of non-viral hepatitis include toxic and drug-induced, alcoholic, autoimmune, fatty liver, and metabolic disorders (Longo, 2012). In certain complication of pregnancy and decreased blood flow to the liver can induce hepatitis (Baq, 2011). The Cholestasis due to hepatocellular dysfunction, biliary tract obstruction, or biliary atresia can result in liver damage and hepatitis (Santos et al., 2010, Geller, 2010).
7.1.1.2 Acute hepatitis

The initial feature of acute hepatitis are nonspecific and it show flu-like symptoms, and also include malaise, muscle and joint aches, fever, nausea or vomiting, diarrhea and headache. More specific symptoms, which can be present in acute hepatitis from any cause, are profound loss of appetite, aversion to smoking among smokers, dark urine, yellowing of the eyes and skin and abdominal discomfort. The physical findings are usually minimal, apart from jaundice, tender hepatomegaly, lymphadenopathy and splenomegaly. Few people with acute hepatitis progress to acute liver failure, in which the liver is unable to remove harmful substances from the blood and produce blood proteins. This may become life-threatening and occasionally requires a liver transplant.

7.1.1.3 Chronic hepatitis

Chronic hepatitis cause nonspecific symptoms such as malaise, tiredness and weakness and often never showed any physical symptoms. The jaundice is the first indication of the liver damage and liver enlargement is also seen in this hepatitis. The chronic hepatitis cause extensive damage to liver and leads to weight loss, easy bruising and bleeding, peripheral edema and accumulation of ascites. Eventually, cirrhosis may lead to various complications like esophageal varices (enlarged in the wall of the esophagus that can cause life-threatening bleeding), hepatic encephalopathy and hepatorenal syndrome.

7.1.1.4 Alcoholic hepatitis

The over consumption of alcohol significantly cause the hepatitis and liver damage (cirrhosis). Alcoholic hepatitis usually caused by the long exposure to alcohol. If the men and women take 80 grams and 40 grams of alcohol, respectively, a day then it may be associated with development of alcoholic hepatitis. Alcoholic hepatitis can vary from mild asymptomatic disease to severe liver inflammation and finally leads to liver failure. The symptoms of alcoholic hepatitis are very similar to other hepatitis. The liver enzyme like aspartate transaminase (AST), alanine transaminase (ALT) and alanine phosphatase (ALP) levels vary according to the extent of liver damage (Papadaikis and Maxine, 2014).
7.1.1.5 Toxic and drug-induced hepatitis

The drugs used to cure fever, pain reliever, anticancer drug, antimalarial drug etc., in excess dosage and other toxic chemicals can cause hepatitis. In the United States, acetaminophen, antibiotics and central nervous system medications are among the most common causes of drug-induced hepatitis. The herbal medicine and dietary supplements can also cause hepatitis and these are the most common causes of drug-induced hepatitis in Korea (Suk et al, 2012). The extent of drug-induced hepatitis include; increasing age, female sex and previous drug-induced hepatitis (Ghabril, 2010; Navarro, 2006). Toxic compounds and drug can cause liver damage through a different mechanisms, including direct cell damage, disrupting cell metabolism and structural architecture of liver cells (Lee and William, 2003). The drug, like acetaminophen, cause predictable dose-related liver damage, whereas others cause idiosyncratic reactions that vary among individuals (Navarro, 2006).

7.1.1.6 Autoimmune hepatitis

It is a chronic disease caused by an abnormal immune response against liver cells. The disease is thought to have a genetic predisposition as it is associated with certain human leukocyte antigens (Teufel and Andreas, 2009). The symptoms of autoimmune hepatitis are similar to other hepatitis and may have a fluctuating course form mild to very severe. The females who are suffering from autoimmune hepatitis may have abnormal menstruation or become amenorrheic. The people who are suffering from the autoimmune hepatitis also have other autoimmune diseases (Krawitt and Edward, 2008). The autoimmune hepatitis may happen in all age groups, commonly in young women.

7.1.1.7 Non-alcoholic fatty liver hepatitis

Non-alcoholic fatty liver hepatitis (NAFLD) is the occurrence of excess of fat content in liver or fatty liver in people who have little or no history of alcohol use. As other hepatitis the NAFLD also unable to show any symptoms, as the disease progresses chronic hepatitis may develop. NAFLD is associated with metabolic syndrome, obesity, diabetes and hyperlipidemia (Cohen and Anania, 2012). Severe NAFLD leads to inflammation, fibrosis and cirrhosis, a state referred to as non-alcoholic steatohepatitis (NASH). The liver biopsy can demonstrate inflammation and
fibrosis characteristic or NASH (Masuoka and Chalasani, 2013). The NASH is recognized as the third most common cause of liver disease in the United States.

The search for an effective hepatoprotective drug is still going on as currently such drugs are lacking for the treatment of variety of liver diseases. In the past, the traditional healers around the world were relying upon various medicinal plant extracts for many centuries to cure several diseases. In recent years, the interest in the use of natural products becoming extremely popular because of their reduced side effects as compared to synthetic drugs. In parallel, many folk remedies from plant origin have also been in use for the treatment of liver diseases (Luper, 1999). Several studies have shown that these plant based medicines could be very effective to treat various liver diseases. Some of the herbs that are reported to possess hepatoprotective activity include *Tridax procumbens* (Ravinkumar, 2006), *Silybum marianum* (Flora et al, 1998), *Strychnos potatorum* (Sanmugapriya and venkataraman, 2006), *Picrorhiza kurroa* (Saraswat et al, 1999), *Aquilegia vulgaris* (Liebert et al., 2005) and *Andrographis paniculata* (Pramyothin et al., 1994).

Other than the above mentioned liver diseases, followings problems are also causes liver damage in human beings:

- The fatty liver disease (hepatic steatosis) is one of the major disease caused western world. It is a reversible condition where large vacuoles of triglyceride fat accumulate in liver cells. The non-alcoholic fatty liver diseases are a spectrum of diseases associated with obesity and metabolic syndrome, among other causes.

- Cirrhosis is causes of chronic liver failure, it is caused by the formation of fibrous tissue (fibrosis) in the place of liver cells that have died due to a variety of causes, including viral hepatitis, alcohol overconsumption and other forms of liver toxicity.

- The liver cancer is most commonly manifests as hepatocellular carcinoma and cholangiocarcinoma, rarer forms include angiosarcoma and hemangiosarcoma of the liver.

- The primary sclerosing cholangitis is a serious chronic inflammatory disease of the bile duct, which is believed to be autoimmune in origin.
- Budd-chiari syndrome is the clinical picture caused by occlusion of the hepatic vein, which in some causes may lead to cirrhosis.

- Gilbert’s syndrome is genetic disorder of bilirubin metabolism found in about 5% of the population, can cause mild jaundice.

- The transthyretin-related hereditary amyloidosis, the liver produces a mutated transthyretin protein which has severe neurodegenerative and cardiopathic effects.

- There are also many pediatric liver diseases including: biliary atresia, alpha-1 antitrypsin deficiency, alagelle syndrome and progressive familial intrahepatic cholestasis.

*Memecylon* species, one of the important medicinal plants, is being widely used in southern part of Karnataka state in India for treating many diseases. It is an important folk medicine and found to be effective in treating dysentery, fever, diabetes, diarrhea, piles and haemoptysis. Keeping in view of the need for a potent analgesic and hepatoprotective drug, in this study, an attempt has been made to validate the plant extract for its hepatoprotective activity using experimental animals against most widely used hepatotoxin, CCl₄.
7.2 Materials and Method

7.2.1 Chemicals and Reagents

Silymarine was obtained from Sigma Chemical Co. (St. Louis, USA). All other chemicals and reagents were of analytical grade procured from Himedia labs, Mumbai. The solvents used were distilled prior to use.

7.2.2 Animals

Wistar rats (weighing 140±20 g) and Swiss albino mice (weighing 25±2 g) of either sex were procured from the S.S.I medical college, Davangere, Karnataka, India. The animals were housed under controlled conventional condition (temperature 22±2°C, relative humidity 50±10%, 12 h light-dark cycle) and fed with the standard pellet and drinking water ad libitum throughout the experiment. The animals were kept under starvation for 24 h before starting the experiment. All the studies were performed in accordance with the guidelines for the care and use of laboratory animals, as adopted and promulgated by the Institutional Animal Care Committee, CPCSEA, India (Reg.no.No-628/02/c/CPCSEA).

7.2.3 Analgesic activity

The central analgesic activity of the test drug was studied against thermal stimuli using the hot plate test (Eddy and Leimbach, 1953). In this test, the initial reaction times of all the animals of control and test groups were recorded by placing on the hot plate which is maintained at 55±0.5°C. Licking of paw or jumping was taken as the index of reaction of heat. The albino mice were divided into five groups, each groups consisting of 6 male mice. Plant extracts (100 mg/kg bw) were administered orally. The first groups served as control and received vehicle only (10% Tween 80 in distilled water). The post-treatment reaction time of each animal was recorded at 30, 60 and 90 min.

The peripheral analgesic activity of the test drug was evaluated using the acetic acid-induced writhing test (Koster et al., 1959). In this test, the albino mice were divided into five groups. Plant extracts (100 mg/kg bw) or aspirin (100 mg/kg bw) were administered orally one hour prior to intra-peritoneal injection of 0.6% v/v acetic acid. Five minutes after the injection of acetic acid, the number of writhing
during the following 20 min was counted. The control mice received only the vehicle (10% Tween-80 in distilled water).

7.2.4 Hepatoprotective activity

The hepatoprotective activity of different extracts of the plant was carried out according to the procedure of Naveen et al (2005), by using CCl₄ with suitable modifications. The plant extracts and standard drug silymarin were prepared in 1% sodium carboxymethyl cellulose (CMC). The rats were divided into twelve groups of six animals each. Group 1 was normal control and received only the vehicle (1 ml/kg/day, orally). Group 2 received CCl₄ (0.5 ml/kg, W/V) through i.p., daily once for seven days. Group 3 received CCl₄ through i.p., and silymarin (200 mg/kg, orally) for seven days. Groups 4-6 received CCl₄ through i.p., and petroleum ether extract (100, 250 and 500 mg/kg, orally) for seven days, respectively. Group 7-9 were administered with CCl₄ through i.p., and chloroform extract (100, 250 and 500 mg/kg, orally) for seven days, respectively. Group 10-12 received CCl₄ through i.p., and methanolic extract (100, 250 and 500 mg/kg, orally) for seven days, respectively.

The experimental animals were sacrificed 24 h after the last treatment. The whole blood was collected by cardiac puncture with and without the anticoagulant (EDTA) and the liver was dissected for investigations on histopathological alterations. The organs were immediately rinsed with phosphate buffer, cleaned and fixed in 10% formalin solution. A portion of the whole blood collected without anticoagulant was centrifuged after coagulation and serum was separated. The serum sample was stored frozen at -80°C until further use.

7.2.5 Measurement of serum ALP, AST, ALT and Bilirubin

The level of enzymes, Alkaline phosphatase (ALP), Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) was used as a biochemical marker for liver injury. The enzyme and bilirubin level in serum supernatants was determined by using a commercial kit from Lab care Diagnostics (India) Pvt. Ltd.

7.2.6 Histopathological study of the liver

The left lobe of liver dissected from all the animals in each group was used for the histopathological studies. The slices of liver were fixed in 10% formalin for 24 h,
embedded in paraffin and cut into 3-4 μm thick sections using a microtome. The sections were then stained with hematoxylin and eosin (H&E) and examined using a light microscope (Olympus, Tokyo, Japan) for necrosis, apoptosis and inflammatory changes of liver.

7.2.7 Statistical analysis

The data obtained were analyzed by one way analysis of variance (ANOVA) followed by Turkey’s multiple comparison test using IBM SPSS (version 20). All statement of significance were based on a probability of p<0.05.

7.3 Results

The central analgesic activity of different extracts of *M. terminale Dalz* was evaluated using acetic acid induced paw writhing test. Mice were treated with different extracts of plant (50-200 mg/kg bw of mice, orally) 30 minutes before i.p injection of acetic acid 0.6% in vehicle (saline or DMSO 2% diluted in saline). It was observed that plant extracts inhibited, in a dose dependent manner, the pain caused by the acetic acid treatment. In the central analgesic activity, petroleum ether extract unable to show pain curing activity. On the other hand, chloroform extract showed a comparatively less activity in lower concentrations (50 and 100 mg/kg) and moderately at higher concentrations (150 and 200 mg/kg). The methanolic extract (50 and 100 mg/kg) did not much alter the response, but there was a significant reduction in the flinching and licking responses. On the other hand, higher concentration of the extracts (150 and 200 mg/kg) showed a significant reduction in the number of flinches and licking time.

The anti-nociceptive potential of *M. terminale Dalz* was also evaluated using classical pharmacological models such as Eddy’s hot plate method. The contemporaneous results revealed that plant extracts possesses anti-nociceptive activity in the models of pain test. Inflammation is the primary responses are characterized by pain, heat, redness, edema and loss of function. Among these effects edema and pain are fundamental and essential outcomes to be considered when evaluating potential anti-nociceptive compounds. The pretreatment with standard or different extracts of plant did not produce any significant a change of the licking time was observed in mice treated with methanolic extract and standard drug. The very
promising activity was observed with methanolic extract (100 mg/kg bw, orally) at 90 min time interval, which is comparable to the standard drug Aspirin. On the other hand chloroform extract is impotent to show good and comparable activity that of standard and methanolic extract. The petroleum ether extract fails to show analgesic activity.

The liver protective potency of different extracts of *M. terminale Dalz* was studied by estimating the serum marker enzymes and total bilirubin in CCl₄ administered experimental rats (Table 7.3). Hepatic injury induced by CCl₄ in the experimental rats significantly increased the concentration of marker enzymes such as AST (500.6±5.1U/L), ALT (477.6±2.5 U/L) and ALP (185.3±5.0 U/L) and total bilirubin (0.23±0.04 mg/L) as compared to control group (Table 7.3). On the other hand, the CCl₄ induced diabetic rats showed significant improvement in the level of serum enzymes and bilirubin when treated with standard hepatoprotective drug, silymarine (200 mg/kg bw) as well as different extracts of *M. terminale Dalz* (Table 7.3). Among different extracts, the methanolic extract (500 mg/kg bw) showed comparable liver protective activity as that of standard drug. The concentration of AST (258.2±2.4 U/L), ALT (155.3±3.1 U/L), ALP (128.8±3.4 U/L) and bilirubin (0.75±0.06 mg/L) were comparable to that of control group. On the other hand, the petroleum ether and chloroform extracts were unable to show significant liver protection against CCl₄ induced liver toxicity.

As shown in Fig. 7.2, the liver cells of CCl₄ treated rats showed high degree of damage when compared to normal animals and the extent of damage was characterized by the degenerated nuclei, cell vacuolization, pyknosis and damage to the walls of bile capillaries. Intra lobular vein is damaged and their wall is broken at many places and endothelium is disrupted at few places. Cell lysis is visible around the intralobular vein and wide spaces are formed at some sinusoids. The hepatic cells adjoining to intralobular vein showed atrophy (Fig. 7.1).

The petroleum ether extract (500 mg/kg) treatment did not exhibit significant hepatoprotective activity and showed a nodular transformation of liver architecture with loss of structure of hepatic lobules. In the liver cells of rats treated with chloroform extract (500 mg/kg) and intoxicated with CCl₄, the nuclei are not very clear as in normal hepatocytes, but there appears to be little protection against damage induced by CCl₄. In these liver sections, pyknotic nucleus and vacuolation in cytoplasm are observed and the intra lobular vein is almost normal in structure but
showed little damage in the wall showing space formation. On the other hand, the liver sections of rats treated with methanolic extract (500 mg/kg) showed more or less normal lobular pattern with tiny and short septa of connective tissue and a mild degree of fatty change, necrosis and lymphocyte infiltration which was comparable to the control and silymarin treated groups. The vacuolation although present to a very small extent, it is similar to that of normal. The hepatic cells of methanolic extract treated rats are mostly normal with few vacuoles and some damaged cells, but no pyknosis in the nucleus were observed.

7.4 Discussion

The present study showed the anti-nociceptive effect of different extracts of *M. terminale Dalz* plant in different anti-nociceptive responses generated by a chemical or thermal noxious stimulus. The anti-nociceptive effects of the extracts of *M. terminale Dalz* plant occurred at doses that evoked no modification in the overall behavior of the animals. In this study, standard drug prevented the anti-nociception induced by acetic acid, corroborating with the hypothesis that μ-opioid receptors must be involved. The acetic acid-induced writhing is a sensitive test for assessment of analgesic drug. However, it can be seen as a general non-selective model for anti-nociceptive studies, since acetic acid indirectly induces the release of endogenous mediators, stimulating the peripheral nociceptor and sensitive neurons that were sensitive to the inflammatory mediator such as cytokines and prostanoids (Couture et al., 2001; Deraedt et al., 1980; Koster et al., Ribeiro et al., 2000).

The results presented here showed that methanolic extract of *M. terminale Dalz* plant significantly inhibited acetic acid induced writhing responses. Therefore, one possible mechanism of anti-nociceptive activity of methanolic extract could be due to the blockade of the effect or the release of endogenous substances that sensitize and activate peripheral nociceptors. The result of this test, however, does not ascertain whether the anti-nociceptive effect was mediated by central or peripheral process. There is an initial acute period (phase 1) and, after a short period of remission, phase 2 begins and consists of a longer period of sustained activity. The phase 1 corresponds to acute nociceptive neurogenic pain, and phase 2 corresponds to acute nociceptive neurogenic pain, and is sensitive to analgesic drugs that interact with opioid system. The phase 2 corresponds to an inflammatory pain, dependent of several nociceptive behavior in this phase is very sensitive to non-steroid anti-inflammatory drugs as the
cyclooxygenase inhibitors. Drugs that act primarily as central analgesics inhibit both phases while peripherally acting drugs inhibit only the second phase (Abram and Olson, 1994; Manning, 1998; Rosland et al., 1990; Yamamoto et al., 2002; Yamamoto and Nozaki-Taguchi, 2002). Both first and second phase behavioral hypernociception were affected by the methanolic extract of *M. terminale Dalz* plant. It can be suggested that not only anti-inflammatory action (Muko and Ohiri, 1999) but also direct analgesic function can be present here.

Therefore, the anti-nociceptive activity of plant extracts in acetic acid induced test strongly attributed to peripherally acting as well as centrally acting pain mediators. This would not be the first time that an anti-inflammatory agent had parallel and independent direct analgesic effect. In fact, the analgesic effect of both methanolic extract involves not only their anti-inflammatory potential, but a peripheral anti-nociceptive effect is associated with ATP-sensitive K⁺ channel (Alves and Duarte, 2002; Alves et al., 2004). The involvement of this channel may explain both spinal and peripheral analgesia, and the anticonvulsive use of the plant. To confirm the participation of central analgesic system in the anti-nociceptive activity of plant extract of *M. terminale Dalz*, hot plate test were employed. Hot plate test is predominantly a spinal reflex or behavioral reaction and used to test supra-spinal analgesia in plant extracts. The plate heated to a constant temperature produces two behavioral components that can be measured in terms of their reaction times, paw licking and jumping. Both are considered to be supraspinaly integrated responses, it is therefore, selective for centrally acting analgesic drugs, like morphine, while peripheral anti-inflammatory anti-nociceptive agents are found to be inactive on thermal stimulus. These tests also revealed that the anti-nociceptive effect of *M. terminale Dalz* extracts on mice remained present for at least up to 90 min after administration of the extracts. The plant extracts was found to have anti-nociceptive activity in the hot plate test, which is a specific central anti-nociceptive test. The anti-nociceptive effects of plant extract involve supraspinal as well as spinal components, as demonstrated by the use of the hot plate test (Yaksh and Rudy, 1976; Yaksh and Rudy, 1977; Yeung et al., 1977).

One of the main strategies in nociception studies has been the search for opioid analgesics acting at opioid receptors outside the central nervous system (CNS), with the prospect of avoiding centrally mediated side effects as tolerance and dependence (Benyhe, 1994; Vanegas and Tortorici, 2002). For the assessment of
CHAPTER 7

opoid system involvement in the analgesic activity, the mice were pretreated with an opioid antagonist, naloxone. In this study, naloxone prevented the anti-nociceptive effect on both phases of the formalin test, as well in writhing, tail-flick and hot plate tests. Those results suggest that, at least part of the anti-hyperalgesic effect observed for the fractions is due to involvement of this system (μ-opioid) since naloxone reverted the antinociceptive activity.

The hepatoprotective results presented in this study have shown that the administration of extracts of *M. terminale Dalz* prevents the liver damage induced by CCl₄ in a dose-dependent manner. Among these, the methanolic extract clearly reduced the levels of bilirubin and serum enzymes (AST, ALT and ALP) which were elevated by the action of CCl₄. Liver fibrosis is one of the major damage mainly due to environmental toxicity, alcohol and viral infection, which is also responsible for liver cirrhosis and hepatocellular carcinomas (Bataller and Brenner, 2005). The alteration in the architecture of liver produced by the toxic effect of CCl₄ are very similar to that of acute viral hepatitis and thus CCl₄ treated rats are widely used as an experimental model for studying hepatoprotective activity (Recknagel *et al*, 1989; James and Pickering, 1976). The hepatotoxicity induced by CCl₄ is due to its metabolite action on polyunsaturated fatty acids, in the presence of oxygen, to produce lipid peroxides, leading to liver damage (Bishayee *et al*, 1995). The toxic metabolite, CCl₃ radical first produced is further converted to trichloromethyl peroxy radical by cytochrome P450 2E1 enzyme which is further transformed to trichloromethyl radicals (Recknagel *et al*, 1989). This radical binds covalently to the macromolecules and causes peroxidative degradation of cellular membrane leading to the necrosis of hepatocytes (Bataller and Brenner, 2005). Hepatocellular necrosis and oxidative stress causes liver damage, consequently releasing liver enzymes into blood that lead to elevation of the serum marker enzymes (Ashok *et al*, 2001). Effective control of AST, ALT, ALP and bilirubin levels in methanolic extract treated group pointing towards an early improvement in the secretary mechanism of hepatocytes. These biochemical findings were further substantiated by histopathological studies. The ability of a hepatoprotective drug to reduce the injurious effects or to preserve the normal hepatic physiological conditions, which have been disturbed by a hepatotoxin, is the index of its protective effects (Boll, 2001). In addition, methanolic extract displays a protective efficiency similar to that of silymarin, indicating that methanolic extract possesses potential protective effect against liver toxicity. Further, reduced
lipid deposition and liver fibrosis were also observed in the animals treated with methanolic extract. These data clearly indicates that the total methanolic extract of *M. terminale Dalz* is a potential source of drug molecules that can be used against liver damage.

The data obtained in the present study indicates that the methanolic extract of *M. terminale Dalz* possessed strong analgesic and hepatoprotective activity against acetic acid induced analgesic activity, hot plate test and CCl₄-induced liver damage as observed in mice and rat model. Although the exact mechanism is unclear, the analgesic and hepatoprotective activity of methanolic extract of *M. terminale Dalz* may be due to its antioxidant activity resulting from the presence of flavonoids and phenolic compounds.
7.5 Tables and figures

**Table 7.1:** Effect of *Memecylon terminale Dalz* plant extracts on acetic acid-induced writhing in test mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% of inhibition</th>
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<tbody>
<tr>
<td></td>
<td>50</td>
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<tr>
<td>Aspirin (100 mg/kg body weight)</td>
<td>44.2±0.2</td>
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<tr>
<td>Petroleum ether extract</td>
<td>9.3±0.4</td>
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<tr>
<td>Chloroform extract</td>
<td>21.4±0.4</td>
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<tr>
<td>Methanol extract</td>
<td>35.1±0.2</td>
</tr>
</tbody>
</table>

Values are mean± SD., n=6, p<0.05, Significant as compared to control.

**Table 7.2:** Effect of different plant extracts of *Memecylon terminale Dalz* on latency to hot plate test in mice.

<table>
<thead>
<tr>
<th></th>
<th>Mean latency (s) before and after drug administration(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>Control</td>
<td>2.30±0.4</td>
</tr>
<tr>
<td>Petroleum ether extract</td>
<td>2.48±0.3</td>
</tr>
<tr>
<td>Chloroform extract</td>
<td>2.52±0.8</td>
</tr>
<tr>
<td>Methanol extract</td>
<td>2.41±0.5</td>
</tr>
<tr>
<td>Pentazocine</td>
<td>2.70±0.6</td>
</tr>
</tbody>
</table>

**Fig. 7.1:** Analgesic effect of petroleum ether, chloroform and methanol extract of *Memecylon terminale Dalz* on heat stimulation response in the hot plate test. The values are mean ± SEM (n = 6), *P < 0.05, **P < 0.01 compared to control group control (vehicle) – 10 % Tween 80 in distilled water; standard drug – pentazocine.
### Table 7.3: Hepatoprotective activity of different extracts of *M. terminale Dalz*

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Bilirubin (mg/l)</th>
<th>AST (U/L)</th>
<th>ALT in U/L</th>
<th>ALP in U/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>0.64±0.02</td>
<td>234.3±4.5</td>
<td>102.6±7.5</td>
<td>150.1±7.2</td>
</tr>
<tr>
<td>CCl₄ control</td>
<td>0.23±0.04</td>
<td>500.6±5.1</td>
<td>477.6±2.5</td>
<td>185.3±5.0</td>
</tr>
<tr>
<td>STD (silymarin 200 mg/kg bw)</td>
<td>0.87±0.03**</td>
<td>233.4±2.6**</td>
<td>147.3±2.5</td>
<td>186.0±4.2</td>
</tr>
<tr>
<td>Petroleum ether extract</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>0.21±0.02*</td>
<td>455.3±5.0**</td>
<td>448.0±2.6*</td>
<td>188.6±3.2**</td>
</tr>
<tr>
<td>250 mg/kg</td>
<td>0.20±0.01**</td>
<td>434.4±4.0*</td>
<td>429.3±3.0**</td>
<td>184.7±5.2**</td>
</tr>
<tr>
<td>500 mg/kg</td>
<td>0.61±0.03*</td>
<td>398.3±7.6*</td>
<td>365.0±5.2*</td>
<td>181.2±3.2*</td>
</tr>
<tr>
<td>Chloroform extract</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>0.68±0.06**</td>
<td>452.4±5.3**</td>
<td>289.5±6.4**</td>
<td>166.0±2.7***</td>
</tr>
<tr>
<td>250 mg/kg</td>
<td>0.68±0.12***</td>
<td>420.2±2.8*</td>
<td>325.3±3.9**</td>
<td>144.6±4.7**</td>
</tr>
<tr>
<td>500 mg/kg</td>
<td>0.92±0.04**</td>
<td>275.6±4.5**</td>
<td>209.1±8.4**</td>
<td>198.0±8.5**</td>
</tr>
<tr>
<td>Methanol extract</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>0.95±0.06***</td>
<td>286.1±5.3**</td>
<td>350.1±4.3*</td>
<td>211.3±2.6*</td>
</tr>
<tr>
<td>250 mg/kg</td>
<td>0.65±0.13**</td>
<td>286.5±6.2*</td>
<td>350.7±5.3***</td>
<td>211.1±5.7*</td>
</tr>
<tr>
<td>500 mg/kg</td>
<td>0.75±0.06***</td>
<td>258.2±2.4**</td>
<td>155.3±3.1**</td>
<td>128.8±3.4**</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M, ***P<0.001 - Highly significant when compared with CCl₄ control, **P<0.005 - Significant when compared with CCl₄ control. *P<0.05 - Not significant when compared with CCl₄ control.
Table 7.4: Pathological changes in the liver morphology of rats treated CCl₄, standard drug, ilymarin and various extracts of *M. terminale Dalz.*

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mononuclear cell infiltrations</th>
<th>Vacuolization, enlarged dimensions of cells</th>
<th>Increased density of chromatin, compact nuclear structure</th>
<th>Increased number of Kupffer cells</th>
<th>Blurred trabecular structure of the lobes</th>
<th>Pycnotic nuclei, strongly acidophilic cytoplasm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CCl₄ Control</td>
<td>+++²</td>
<td>++³</td>
<td>+++³</td>
<td>++²</td>
<td>+++¹</td>
<td>+++³</td>
</tr>
<tr>
<td>STD (silymarin 200 mg/kg bw)</td>
<td>+¹</td>
<td>-</td>
<td>+¹</td>
<td>-</td>
<td>++¹</td>
<td>++¹</td>
</tr>
<tr>
<td>Petroleum ether extract</td>
<td>+++²</td>
<td>+++¹</td>
<td>+++³</td>
<td>++²</td>
<td>+++¹</td>
<td>+++³</td>
</tr>
<tr>
<td>Chloroform extract</td>
<td>++²</td>
<td>+³</td>
<td>++¹</td>
<td>+¹</td>
<td>+¹</td>
<td>+²</td>
</tr>
<tr>
<td>Methanol extract</td>
<td>+¹</td>
<td>+++¹</td>
<td>+¹</td>
<td>+¹</td>
<td>+²</td>
<td>+¹</td>
</tr>
</tbody>
</table>

+++², a change was found in most of the lobes in all the animals of a group; ++, a change was found in some lobes in all the animals of a group; +, a change was found in some lobes in a majority of animals of a group; ±, a change was sporadic in a group; −, a change was absent in all animals of a group.

1 A change was present in single cell in a group.
2 A change was found in some cells in lobes.
3 A change occurred in a majority of the cells in lobes.
Fig. 7.2. The photomicrographs of control and CCl₄ treated liver sections stained with haematoxylin and eosin. (A) Normal Control rat liver. (B) CCl₄ treated rats. (C) Silymarin 200 mg/kg bw. (D) Petroleum ether extract (500 mg/kg bw). (E) Chloroform extract (500 mg/kg bw). (F) Methanolic extract (500 mg/kg bw).