ANTIOXIDANT AND HEPATOPROTECTIVE EFFECTS OF SELECTED HERBAL PLANTS FROM SABAH, MALAYSIA.

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VERIFICATION

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   (Prof. Dr. Mohd. Harun Abdullah)

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ABSTRACT

In this study, the hepatoprotective effects of *Eleusine indica* and *Thysanolaena latifolia* against CCl₄-induced acute liver damage in albino rats were investigated. Total phenolic content of *E. indica* and *T. latifolia* and 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging were determined *in-vitro*. Rats were pretreated with *E. indica* and *T. latifolia* for fourteen consecutive days prior to the administration of CCl₄ (two doses). The serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), the malondialdehyde (MDA), reduced glutathione (GSH) and some liver enzymatic activities from the post mitochondrial supernatant (PMS), and histopathological changes in rat liver sections were determined. Total phenolic content of *E. indica* and *T. latifolia* were found to be 14.9 ± 0.002 and 20.3 ± 0.007 mg/g total phenolic expressed as gallic acid equivalent per gram of extract. The extracts were able to reduce the stable DPPH in a dose dependent manner. The IC₅₀ value for *E. indica* and *T. latifolia* were found to be 2350 µg/ml and 1525 µg/ml. *E. indica* and *T. latifolia* significantly prevented the increase in serum ALT and AST levels in acute liver injury induced by CCl₄ and produced a marked recovery in the histopathological hepatic injuries. The extent of MDA formation was reduced; the GSH concentration was increased and liver enzymatic activities in PMS were increased in *E. indica* and *T. latifolia* treated groups compared with the CCl₄-intoxicated group. These results indicate that *E. indica* and *T. latifolia* have a significant hepatoprotective effect against oxidative damage induced by CCl₄ in rats, which may be due to their free radical scavenging effect and their ability to increase antioxidant activity. The inhibitory effects of a dietary extract of these plants may be useful as a hepatoprotective agent against chronic chemical-induced hepatic injury *in-vivo*. 
ABSTRAK

# LIST OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>DECLARATION</td>
<td>ii</td>
</tr>
<tr>
<td>VERIFICATION</td>
<td>iii</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>iv</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>v</td>
</tr>
<tr>
<td>ABSTRAK</td>
<td>vi</td>
</tr>
<tr>
<td>LIST OF CONTENTS</td>
<td>vii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xi</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xii</td>
</tr>
<tr>
<td>LIST OF PHOTOS</td>
<td>xiii</td>
</tr>
<tr>
<td>LIST OF SYMBOLS</td>
<td>xiv</td>
</tr>
</tbody>
</table>

## CHAPTER 1: INTRODUCTION

1.1 Background of Free Radicals 1
1.2 Damages and Diseases Caused by Free Radicals 1
1.3 Herbal Plants in Sabah, Malaysia 3
1.4 Objectives of Study 4
CHAPTER 2: LITERATURE REVIEW

2.1 Selected Herbal Plants
   2.1.1 Thysanolaena latifolia (Tiger Grass) 5
   2.1.2 Eleusine indica (Wiregrass) 6

2.2 Carbon Tetrachloride induced Hepatotoxicity 8

2.3 Phenolics 10

2.4 Antioxidant 11

CHAPTER 3: MATERIALS AND METHODS

3.1 Materials
   3.1.1 Chemicals 13
   3.1.2 Plant Material 13
   3.1.3 Animals 14

3.2 Methods
   3.2.1 Preparation of Extracts 14
   3.2.2 Extraction 15
   3.2.3 Determination of Total Phenolic Content 15
   3.2.4 Determination of Antioxidant Activity (DPPH Test) 16
   3.2.5 Experimental Protocol 17
   3.2.6 Preparation of Post-Mitochondrial Supernatant 17
   3.2.7 Determination of Glutathione Reduced 18
   3.2.8 Determination of Lipid Peroxidation 18
   3.2.9 Determination of Glutathione Peroxidase Activity 19
   3.2.10 Determination of Glutathione Reductase Activity 19
3.2.11 Determination of Catalase Activity  
3.2.12 Determination of Glutathione S-Transferase Activity  
3.2.13 Determination of NAD(P)H: Quinone Oxidoreductase Activity  
3.2.14 Determination of Serum Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST)  
3.2.15 Determination of Protein  
3.2.16 Histopathological Assessment  
3.2.17 Statistical Analysis  

CHAPTER 4: RESULTS  
4.1 Data from In-vitro Studies  
4.1.1 DPPH Test Results  
4.1.2 Total Phenolic Content  
4.2 Data from In-Vivo Studies  
4.2.1 Blood Serum Analysis (ALT and AST)  
4.2.2 Malondialdehyde (MDA) Formation from Lipid Peroxidation (LPO)  
4.2.3 Glutathione Reduced (GSH) Levels in PMS  
4.2.4 Antioxidant Enzymes Level in PMS  
4.3 Results of Histopathology  

CHAPTER 5: DISCUSSION  
5.1 Antioxidant Activity in Selected Herbal Plants  
5.2 Hepatoprotective Effects of Selected Herbal Plants
LIST OF TABLES

<table>
<thead>
<tr>
<th>Table No.</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1</td>
<td>The percentage of DPPH free radical scavenging in different concentrations of plant extracts.</td>
<td>24</td>
</tr>
<tr>
<td>4.2</td>
<td>The effect of <em>E. indica</em> and <em>T. latifolia</em> on activities of hepatic antioxidant enzymes in rats</td>
<td>30</td>
</tr>
<tr>
<td>4.3</td>
<td>The effect of <em>E. indica</em> and <em>T. latifolia</em> on activities of hepatic phase-II metabolizing enzymes in rats</td>
<td>31</td>
</tr>
</tbody>
</table>
### LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure No.</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1</td>
<td>Effects of <em>E. indica</em> and <em>T. latifolia</em> on serum ALT and AST levels after CCl₄ treatment in rats.</td>
<td>26</td>
</tr>
<tr>
<td>4.2</td>
<td>Effects of <em>E. indica</em> and <em>T. latifolia</em> on MDA levels after CCl₄ treatment in rats.</td>
<td>27</td>
</tr>
<tr>
<td>4.3</td>
<td>Effects of <em>E. indica</em> and <em>T. latifolia</em> on GSH levels after CCl₄ treatment in rats.</td>
<td>28</td>
</tr>
<tr>
<td>4.4</td>
<td>Histopathological changes in rat livers (H &amp; E).</td>
<td>33</td>
</tr>
</tbody>
</table>
LIST OF PHOTOS

<table>
<thead>
<tr>
<th>Photo No.</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td><em>Thysanolaena latifolia</em> (Togiung)</td>
<td>6</td>
</tr>
<tr>
<td>2.2</td>
<td><em>Eleusine indica</em> (Sohinatad)</td>
<td>8</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
<td></td>
</tr>
<tr>
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</tr>
</tbody>
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CHAPTER 1

INTRODUCTION

1.1 Background of Free Radicals

Oxidative stress is defined as elevated levels of free radicals or other reactive oxygen species (ROS) which can elicit either direct or indirect damage to the body (Chin et al., 2009). The ROS metabolites are generated either by transfer of radiant energy to oxygen molecules or by its stepwise reduction. In the former case, the molecular oxygen absorbing energy is raised to an excited state to produce singlet oxygen, whereas its stepwise reduction leads to the generation of series of oxidant molecules, such as superoxide anions, hydrogen peroxide and hydroxyl radicals (Athar et al., 1992). Usually free radicals are generated by exposure of tissue to ionizing radiation and ultraviolet light (Nakayama et al., 1983). The metabolism of certain pesticides, drugs, cigarette smoke and various other pollutants generate a number of ROS and free radicals in biological systems (Nakayama et al., 1983). Many enzymes such as xanthine oxidase, NAD(P)H oxidase, aldehyde oxidase and flavin dehydrogenase produce superoxide anions (Athar et al., 1992).

1.2 Damages and Diseases Caused by Free Radicals

The generation and subsequent involvement of free radicals in large number of diseases such as myocardial ischemia, carcinogenesis, liver damage, inflammatory diseases,
cataract formation and Alzheimer’s disease have been recognized (Floyd, 1990; Fridovich, 1988; Nakayama et al., 1983). Under normal circumstances, ROS are efficiently kept in check by body’s complex antioxidant defense and there is equilibrium between ROS formation and dissipation. However, an overproduction of ROS and/or inadequate antioxidant defense hinders this equilibrium favoring ROS upsurge that ends in oxidative stress.

Oxidative stress, through a series of events, disregulates cellular physiology and its sustained presence may lead to pathogenesis of several chronic ailments (Floyd, 1990; Fridovich, 1988; Nakayama et al., 1983). Oxidative stress plays a vital role in pathophysiology of cancer, influencing all stages of carcinogenesis right from initial DNA damage to final metastasis (Cook et al., 2004). It has been observed that lipids, carbohydrates, proteins and DNA are the major target of oxidative injury (Athar et al., 1987; Ames, 1989; Agarwal et al., 1990). ROS can directly attack DNA and mutate it or afflict other cellular components such as proteins and lipids giving rise more oxidants that can couple to DNA bases mutating the DNA. ROS also modulate cell signaling pathways implicated in cellular proliferation and thus influence tumor promotion. A number of transcription factor pathways such as MAP-kinase or AP-1 and NF-KB are activated by ROS and have direct effect on cell proliferation and apoptosis. Of these, DNA damage may be of particular importance as its role has been recognized in large number of genetic disorders such as Alzheimer’s disease (Jenner, 1991), Parkinson’s disease, Hodgkin’s disease, Bloom’s (Halliwell and Gutteridge, 1981; Imlay and Linn, 1988) including cancer (Emerit and Cerutti, 1981). Therefore, inhibition of oxidative DNA damage may be one of the strategies in the chemopreventive of large number of clinical disorders.

Several clinical disorders, implicate a deficient natural antioxidant defense as their etiological or pathological factor. The onset or progression of these disorders can be held or delayed by supplementing with the antioxidant defense. Therefore, a lot of scientific research is focused on exploring safe and effective antioxidant compounds.
Plant extract and plant derived antioxidant compounds potentiate body’s antioxidant defense or act as antioxidant and are antioxidants of choice because of their safety over synthetic. The World Health Organization (WHO) has estimated more than 75 percent of the world’s total population to be dependent on herbal drugs for their primary health care needs. Thus, a huge body of research is based on discovering plants that protect against various kinds of injuries or diseases including cancer with antioxidant potential that may be used for human consumption (Rajbir et al., 2008).

1.3 Herbal Plants in Sabah, Malaysia

Malaysia is one of the 12 mega diversity nations in world, which is rich with natural resources especially plants. There is a distribution of over 12,000 species of flowering plants of which about 1,300 are said to be medicinal (Burkill, 1935), and only about a hundred have been investigated fully for their potential. Meanwhile, Sabah has the most outstanding flora and this feature is the highlight of this state. According to YB Datuk K.Y. Mustafa from Sabah State Government, Sabah is found to have more than 10,000 species of wild plant species and about more than 4,000 types have already been indentified (Daily Express, 2004). Medicinal plants that are used in traditional healing by the various ethnic groups in Malaysia have not yet been scientifically proven and many of these plants have received little or no attention from natural product chemists. But this situation is healing nowadays as more herbal plants from the interior are being brought out to light by researchers by scientific proofs and publications. Medicinal plants have been used widely to cure diseases among the local people from time to time (Kulip, 1997).

Various parts of the plants are involved in the treatment such as leaves, roots, rhizomes and bark of the plants. This is due to the availability of them throughout the year compared to the flowers, fruits or seeds which can be found in certain time in the year. Medicinal plants are used internally or externally. For internal uses, plants are used
to treat illness such as asthma, ulcers, fever, inflammation, diabetes, hypertension, kidney, liver and bladder problems by boiling and drinking as tea. While for external uses, plants are crushed and mixed with oil or water and made into paste and directly applied to wounds or affected areas for example, for hair growth, stomach pain, skin diseases and so on. Keeping those in view, we have selected two plants viz. *Thysanolaena latifolia* and *Eleusine indica* to evaluate their hepatoprotective effects against liver damage caused by carbon tetrachloride. The selection of these plants is based on documents reporting on the medicinal importance of these plants (Kulip, 1996; Pasok, 2003).

### 1.4 Objectives of Study

The objectives of this study are:

(I) To prepare crude extract of selected herbal plants viz. *Thysanolaena latifolia* and *Eleusine indica*.

(II) To evaluate the total phenolic contents and antioxidant properties (*in-vitro*) of selected herbal plants viz. *Thysanolaena latifolia* and *Eleusine indica*.

(III) To evaluate the hepatoprotective effects of crude extract of herbal plants viz. *Thysanolaena latifolia* and *Eleusine Indica* on hepatotoxicity induced by carbon tetrachloride.
CHAPTER 2

LITERATURE REVIEW

2.1 Selected Herbal Plants

2.1.1 *Thysanolaena latifolia* (Tiger Grass / Togiung)

*Thysanolaena latifolia*, or Tiger grass, better known as Togiung by locals in Sabah, is a strongly tufted, very robust perennial grass with erect or slightly spreading solid bamboo-like culms up to 3.5 m tall. It is a member of the Poaceae family. In light shade, seedlings grow slowly at first, but are then able to compete with other low-growing plants. Tiger grass can be propagated by rhizomes, rooted culms or seeds. It is cultivated for broom making, but not so much for forage. Weeding is required at the early stage of establishment. Leaf-sheath of Tiger grass is hairy along outer margin and is usually 1-2 mm long. Young leaves and stem tips are used to feed cattle and buffaloes. Its large inflorescences are used in making brooms. The grass is occasionally planted for ornamental purposes and as a hedge. Studies have been carried out on the capability of *T. latifolia* to be daily food supplement for highland cattle and it was found that the *in vitro* digestibility of leaves ranged from 40% to 60% (Falvey *et al.*, 1981). Tiger grass occurs from India to Indo-China and China and throughout Southeast Asia. It is also occasionally cultivated outside this region. *Thysanolaena latifolia* is considered as one of the important herbal plant that has medicinal values to treat febrifuges and cough by the locals of Sabah, and is being used traditionally for treatment of ailment for the past many years. The leaves of the plant are boiled in water and the decoctions are
consumed as medication for febrifuges (Kulip, 1997). It is also known that *Thysanolaena latifolia* is being used in traditional medication in places other than Sabah, such as Philippines, Indonesia, India and China.

![Photo 2.1 Thysanolaena latifolia (Togiung)](image)

2.1.2 *Eleusine indica* (Wiregrass / Sohinatad)

*Eleusine indica*, or Wiregrass, also known as Sohinatad by the local people in the area of Tambunan, Sabah is an invasive species in the grass family Poaceae. It is a small annual weed distributed throughout the warmer areas of the world to about 50 degrees latitude. *Eleusine indica* is an important weed in cultivated crops, lawns, and golf courses. It thrives in disturbed areas with compacted soils in full sun. Both tillage and herbicides are used in its control. This low-growing grass is capable of setting seed even
when closely mowed. *Eleusine indica* performs C4 photosynthesis and therefore can grow in hot climates and in the hotter months of the temperate zone. Its seeds germinate later in spring than most temperate zone grasses that are weeds in the same sites, such as crab grasses. Though usually considered an annual, it may survive for more than a year in climates not subject to frost.

*Eleusine indica* is a glabrous plant which can grow up to 1 meter in height. Its leaves are 10-30 cm long, 3-7 mm wide, flaccid, with flattened sheaths. Its spikes are in a terminal whorl and the spikelets are numerous, crowded and about 3-4 mm long. The roots and seeds of this plant are edible; roots can be eaten raw and young seedlings are usually cooked or sometimes eaten raw, but yields are low. In addition, the ability of weedy *E. indica* to live in trampled, contaminated soils increases the risk that its seeds may contain undesirable chemicals. Stems of the plant are used for making mats, hats and are also suitable for paper making. It is found that *E. indica* has developed herbicide resistance and has become a threat to crops all around it (Zeng and Baird, 1999). The first report of a biotype of *E. indica* resistant to glyphosate in Malaysia was made in 1997 (Lim and Ngim, 2000). Many researches were carried out in order to overcome the herbicide resistance in *E. indica* but many researchers were not aware of the medicinal values of this plant. *Eleusine indica* is being used as traditional medicine in treating ailments such as diuretic, anti-helminthic, diaphoretic, febrifuges and cough by the locals in Sabah and also in other places Philippines, Indonesia, India, Venezuela and Colombia. The whole plant, from root to flower is used in treatment. The decoctions of boiled plant are consumed as treatment for anti-helminthic and febrifuges (Kulip, 1997). Studies also found that *Eleusine Indica* has anti inflammatory effects on LPS induced lung airway inflammation on mice. The infusion of aerial parts (EI) of *Eleusine indica* Gaertn (Poaceae) is used in Brazil against airway inflammatory processes like influenza and pneumonia (DeMelo *et al.*, 2005).
Liver is considered to be one of the most vital organs that functions as a centre of metabolism of nutrients such as carbohydrates, proteins and lipids and excretion of waste metabolites. Additionally, it is also handling the metabolism and excretion of drugs and other xenobiotics from the body thereby providing protection against foreign substances by detoxifying and eliminating them. The bile secreted by the liver has, among other things, plays an important role in digestion. Liver cell injury caused by various toxicants such as certain chemotherapeutic agents, carbon tetrachloride, thioacetamide, chronic alcohol consumption and microbes is well-studied. Enhanced lipid peroxidation during metabolism of ethanol may result in development of hepatitis leading to cirrhosis (Saleem et al., 2010).
It is emphasized that hepatotoxins which cause acute hepatitis should have close resemblance with viral hepatitis, clinically, biochemically and histologically. Certain drugs are responsible for chronic hepatic disease. Chemically-induced hepatic injury for experimental studies should be severe enough to cause cell death or to modify hepatic functions. The mechanism of acute hepatic injury depends upon the chemical compounds used to induce toxicity. Carbon tetrachloride (CCl₄) is one of the most powerful hepatotoxins in terms of severity of injury. It causes toxic necrosis leading to biochemical changes, having clinical features similar to those of acute viral hepatitis (Pradeep et al., 2009). It has been suggested that hepatic necrosis caused by CCl₄ involves bioactivation by the microsomal cytochrome P450-dependent monooxygenase system, resulting in the formation of trichloromethyl free radicals and ROS that initiate lipid peroxidation and protein oxidation (Hsiao et al., 2003).

CCl₄ intoxication in the rat is an experimental model widely used to study necrosis and steatosis of the liver. The toxic manifestations of CCl₄ in isolated rat hepatocytes have been described by several researchers who find a suitable correlation with the induced cell injury in vivo (Adzet et al., 1983). Liver injury induced by CCl₄ is the most intensively studied system for xenobiotic induced oxidative hepatotoxicity. It is well known that alanine aminotransferase (ALT) and aspartate aminotransferase (AST) serum enzyme activities served as parameters to demonstrate the extent of hepatotoxicity in the rats. Furthermore, the increase in the level of lipid peroxidation, the decrease in superoxide dismutase (SOD) activity, and the breakdown of the glutathione (GSH)-dependent antioxidant defense system are obviously seen along with the liver damage induced by CCl₄ (Yang et al., 2010). Antioxidant property is claimed to be one of the mechanisms of hepatoprotective effect of Indigenous substances against the hepatotoxicity caused by CCl₄ (Shenoy et al., 2001).
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