PDF - EFFECT OF ETHANOL EXTRACT OF DennettiatripetalaON LIVER AND KIDNEY ANTIOXIDANT ENZYME ACTIVITY AND MALONDIALDEHYDE CONCENTRATION OF ALBINO WISTAR RATS EXPOSED TO CCl4. - researchcub.info**ABSTRACT**

The effect of ethanol extract of *Dennettiatripetala* on rats exposed to carbon tetrachloride was investigated. Ethanol extract of the plant was prepared using standard procedure. Sets of 30 female wistar albino rats were divided into 6 groups containing five animals each and were treated orally with increasing doses of ethanol extract of *Dennettiatripetala* for two weeks. CCl_4 was diluted with olive oil in a 1:1 ratio and administered once by oral route at the end of the extract administration. Results from the study showed non-significant decreases in the levels of catalase and SOD activities ($P>0.05$) in the CCl_4 group compared to the control. The extract treatment however produced a higher activity for the antioxidant enzymes compared to the CCl_4treated groups. The results also showed increased levels of MDA concentration ($P<0.05$) in the CCl_4 group whereas extract treated rats showed lower concentrations of MDA. The overall results suggests that the ethanolic extract of *Dennettiatripetala* may have moderate hepatoprotective effect in the CCl_4 induced rats.

CHAPTER ONE

1.0 INTRODUCTION

Natural plant products and their derivatives represent more than 50% of all the drugs in clinical use in the world (Ben-Eric, 2002). *Dennettiatripetala*also known as pepper fruit tree is a well-known Nigerian spicy medicinal plant. It is found in the tropical rainforest region of Nigeria and sometimes in Savanna areas (Okwu *et al.*, 2005). It is locally called “Nkarika” by the Efiks of Calabar. The young leaves and fruits have distinctive spicy taste. The mature fruits constitute the main edible portions. Some communities in parts of Southern Nigeria also utilize the leaves and roots, in addition to the fruits for medicinal purpose. *Dennettiatripetala*has been found tocontain lots of minerals, vitamins, alkaloids and trace elements which are of medicinal importance. It was also indicated that the rich presence of essential oil (oleoresins) determines the aromatic flavoring, coloring and pungent properties of pepper fruits. (Nwaogu*et al*., 2007) investigated phytochemical content of *Dennettiatripetala* and detected the presence of saponins, flavonoids, tannins and cyanogenic glycosides. The intake of flavonoids in any fruit and vegetable tends to decrease cancer risk (Neuhouser, 2004; Graf*et al.,* 2005). Flavonoid contributes to the color of plants, their fruits and flowers. The use of medicinal plants in traditional medicine is not intended in any way to replace modern medical science but rather an aid in conventional therapy (Ben-Eric, 2002).

Carbon tetrachloride (CCl_4) is an industrial chemical that does not occur naturally. Most of the carbon tetrachloride produced is used in the production of chlorofluorocarbons (CFCs) and other chlorinated hydrocarbons. It was once used widely as a solvent, cleaner and degreaser, both for industrial and home use. Today, the scientific database on the effects of haloalkanes is so vast that it is no longer employed for such purposes although it is used as a model of experimental liver injury (Weber *et al.,* 2003).

CCl_4 is a well-known hepato- and nephrotoxicant (Thrall *et al.,* 2000; Ogeturk*et al.,* 2005), and proves highly useful as an experimental model for the study of certain hepatotoxic effects (Muriel *et al.,* 2003; Moreno and Muriel, 2006). CCl_4-induced toxicity, depending on dose and duration of exposure, covers a variety of effects. At low doses, transient effects prevail, such as loss of Ca^{2+} homeostasis, lipid peroxidation, release of noxious or beneficial cytokines (Kyung-Hyun *et al.,* 2006; Muriel, 2007) and apoptotic events followed by regeneration. Other effects, with higher doses or longer exposure, are more

serious and develop over a long period of time, such as fatty degeneration, fibrosis, cirrhosis and even cancer (Weber *et al.,* 2003). In addition, acute intoxication with CCl_4 at high doses, when the hepatocellular necrosis exceeds the regenerative capacity of the liver, fatal liver failure will ensue. Extreme doses of CCl_4 result in nonspecific solvent toxicity, including central nervous system depression and respiratory failure and death.

This study aims at investigating the effect of ethanol extract of *Dennettiatripetala*on liver and kidney antioxidant enzyme activity and malondialdehyde concentration of rats exposed to CCl_4.

1.1 LITERATURE REVIEW

1.1.0 THE LIVER

The liver is the largest organ of the human body weighing approximately 1500 g, and is located in the upper right corner of the abdomen on top of the stomach, right kidney and intestines and beneath the diaphragm. The liver performs more than 500 vital metabolic functions (Naruse*et al.,* 2007). It is involved in the synthesis of products like glucose derived from glycogenesis, plasma proteins, clotting factors and urea that are released into the bloodstream. It regulates blood levels of amino acids.

Liver parenchyma serves as a storage organ for several products like glycogen, fat and fat soluble vitamins. It is also involved in the production of a substance called bile that is excreted to the intestinal tract. Bile aids in the removal of toxic substances and serves as a filter that separates out harmful substances from the bloodstream and excretes them (Saukonen*et al.,* 2006). An excess of chemicals hinders the production of bile thus leading to the body's inability to flush out the chemicals through waste.

Smooth endoplasmic reticulum of the liver is the principal 'metabolic clearing house' for both endogenous chemicals like cholesterol, steroid hormones, fatty acids and proteins, and exogenous substances like drugs and alcohol. The central role played by liver in the clearance and transformation of chemicals exposes it to toxic injury (Saukonen*et al.,* 2006).

1.1.0.1 FUNCTIONS OF THE LIVER

The liver has three main functions: storage, metabolism, and biosynthesis. Glucose is converted to glycogen and stored; when needed for energy, it is converted back to glucose. Cholesterol uptake also occurs in the liver. Fat, fat-soluble vitamins and other nutrients are also stored in the liver. Fatty acids are metabolized and converted to lipids, which are then conjugated with proteins synthesized in the liver and released into blood stream as lipoproteins. Numerous functional proteins such as, enzymes and blood-coagulating factors are also synthesized by the liver. In addition, the liver, which contains numerous xenobiotic metabolizing enzymes, is the main site of xenobiotic metabolism (Hogson and Levi, 2004).

1.1.0.2BIOTRANSFORMATION OF HEPATOTOXICANTS

Liver plays a central role in biotransformation and disposal of xenobiotics.

The close association of liver with the small intestine and the systemic circulation enables it to maximize the processing of absorbed nutrients and minimize exposure of the body to toxins and foreign chemicals. The liver may be exposed to large concentrations of exogenous substances and their metabolites. Metabolism of exogenous compounds can modulate the properties of hepatotoxicant by either increasing its toxicity (toxication or metabolic activation) or decreasing its toxicity (detoxification).

Most of the foreign substances are lipophilic thus enabling them to cross the membranes of intestinal cells. They are rendered more hydrophilic by biochemical processes in the hepatocyte, yielding water-soluble products that are exported into plasma or bile by transport proteins located on the hepatocyte membrane

and subsequently excreted by the kidney or gastrointestinal tract (Totsmann*et al.,* 2008).

The hepatic biotransformation involves Phase I and Phase II reactions. Phase I involves oxidative, reductive, hydroxylation and demethylation pathways, primarily by way of the cytochrome P-450 enzyme system located in the endoplasmic reticulum, which is the most important family of metabolizing enzymes in the liver. The endoplasmic reticulum also contains a NADPH-dependent mixed function oxidase system, the flavin-containing monooxygenases, which oxidizes amines and sulphur compounds.

Phase I reactions often produce toxic intermediates which are rendered non-toxic by phase II reactions. Phase II reactions involve the conjugation of chemicals with hydrophilic moieties such as glucuronide, sulfate or amino acids and lead to the formation of more water-soluble metabolite which can be excreted easily. Another Phase II reaction involves glutathione which can covalently bind to toxic intermediates by glutathione-S- transferase. As a result, these reactions are usually considered detoxification pathways. However, this phase can also lead to the formation of unstable precursors to reactive species that can cause hepatotoxicity.

The activities of enzymes are influenced by various endogenous factors and exogenous drugs or chemicals (Lee and Boyer, 2000). Many substances can influence the cytochrome P450 enzyme mechanism. Such substances can serve either as inhibitors or inducers. Enzyme inhibitors act immediately by blocking the metabolic activity of one or several cytochrome P450 enzymes. Enzyme inducers act slowly and increase cytochrome P450 activity by increasing its synthesis (Lynch and Price, 2007).

1.2 KIDNEY

The kidneys are bean-shaped organs that serve several essential regulatory roles in vertebrates. They remove excess organic molecules from the blood and their best known function is the removal of waste products of metabolism. They serve homeostatic functions such as the regulation of electrolytes, maintenance of acid-base balance, and regulation of blood pressure (via maintaining the salt and water balance). In producing urine, the kidneys excrete wastes such as urea and ammonium. They are responsible for the reabsorption of water, glucose, and amino acids. They also produce hormones like calcitriol and erythropoietin.

1.2.1 FUNCTIONS OF THE KIDNEY

Many of the kidney's functions are accomplished by relatively simple mechanisms of filtration, reabsorption, and secretion, which take place in the nephron. Filtration, which takes place at the renal corpuscle, is the process by which cells and large proteins are filtered from the blood to make an ultrafiltrate that eventually becomes urine. The kidney generates 180 litres of filtrate a day, while reabsorbing a large percentage allowing for the generation of only approximately 2 litres of urine. Reabsorption is the transport of molecules from this ultrafiltrate into the blood. Secretion is the reverse process, in which molecules are transported in the opposite direction, from blood to the urine.(Bard *et al.,* 2003).

1.2.1.0 Excretion of wastes

The kidneys excrete a variety of waste products produced by metabolism into the urine. These include the nitrogenous wastes urea, from protein catabolism, and uric acid, from nucleic acid metabolism. The ability of mammals and some birds to concentrate wastes into a volume of urine much smaller than the volume of blood from which the wastes were extracted is dependent on an elaborate countercurrent multiplication mechanism. This requires several independent nephron characteristics to operate: a tight hairpin configuration of the tubules, water and ion permeability in the descending limb of the loop, water

impermeability in the ascending loop, and active ion transport out of most of the ascending limb. In addition, passive countercurrent exchange by the vessels carrying the blood supply to the nephron is essential for enabling this function.

1.2.1.1 Reabsorption of the vital nutrients

Glucose at normal plasma levels is completely reabsorbed in the proximal tubule. The mechanism for this is the Na^+/glucose cotransporter. A plasma level of 350mg/dL will fully saturate the transporters and glucose will be lost in the urine. A plasma glucose level of approximately 160 is sufficient to allow glucosuria, which is an important clinical clue to diabetes mellitus.

Amino acids are reabsorbed by sodium dependent transporters in the proximal tubule. Hartnup disease is a deficiency of the tryptophan amino acid transporter which results in pellagra (Le Tao, 2013)

1.2.1.2 Acid-base homeostasis

Two organ systems, the kidneys and lungs, maintain acid base homeostasis, which is the maintenance of pH around a relatively stable value. The lungs contribute to acid-base homeostasis by regulating carbon dioxide (CO_2) concentration. The kidneys have two very important roles in maintaining the acid-base balance: to reabsorb and regenerate bicarbonate from urine, and to excrete hydrogen ions and fixed acids (anions of acids) into urine (Seldin*et al.,* 1989).

1.2.1.3 Osmolality regulation

Any significant rise in plasma osmolality is detected by the hypothalamus, which communicates directly with the posterior pituitary gland. An increase in osmolality causes the gland to secrete antidiuretic hormone (ADH), resulting in water reabsorption by the kidney and an increase in urine concentration. The two factors work together to return the plasma osmolality to its normal levels.

ADH binds to principal cells in the collecting duct that translocate aquaporins to the membrane, allowing water to leave the normally impermeable membrane and be reabsorbed into the body by the vasa recta, thus increasing the plasma volume of the body.

There are two systems that create a hyperosmotic medulla and thus increase the body plasma volume: urea recycling and the ‘single effect’.

Urea is usually excreted as a waste product from the kidneys. However, when plasma blood volume is low and ADH is released the aquaporinsthat are opened are also permeable to urea. This allows urea to leave the collecting duct into the medulla creating a hyperosmotic solution that attracts water. Urea can then re-enter the nephron and be excreted or recycled again depending on whether ADH is still present or not. The ‘single effect’ describes the fact that the ascending thick limb of the loop of henle is not permeable to water but is permeable to NaCl. This allows for a countercurrent exchange system whereby the medulla becomes increasingly concentrated, but at the same time setting up an osmotic gradient for water to follow should the aquaporins of the collecting duct be opened by ADH (Vander, 1985).

1.2.1.4 Blood pressure regulation

Although the kidney cannot directly sense blood, long term regulation of blood pressure predominantly depends upon the kidney. This primarily occurs through maintenance of the extracellular fluid compartment, the size of which depends on the plasma sodium concentration. Renin is the first in the series of important chemical messengers that make up the renin-angiotensin system. Changes in rennin ultimately alter the output of this system, principally the hormones angotensin II and aldostrone. Each hormone acts via multiple mechanisms, but both increase the kidney’s absorption of sodium chloride, thereby expanding the

extracellular fluid compartment, and an increase in blood pressure. Conversely, when rennin levels are low, angiotensin II and aldosterone levels decrease, contracting the extracellular fluid compartment, and an increase in blood pressure. Conversely, when rennin levels are low, angiotensin II and aldosterone levels decrease, contracting the extracellular fluid compartment, and decreasing blood pressure.

1.2.1.5 Hormone secretion

The kidneys secrete a variety of hormones, including erythropoietin, and the enzyme rennin. Erythropoietin is released in response to hypoxia (low levels of oxygen at tissue level) in the renal circulation. It stimulates erythropoiesis (production of red blood cells) in the bone marrow. Calcitriol, the activated form of vitamin D, promotes intestinal absorption of calcium and the renal reabsorption of phosphate. Part of the renin-angiotensin-aldosterone system, renin is an enzyme involved in the regulation of aldosterone levels (Valtin, 1983).

1.3 HEPATOTOXICITY

Hepatotoxicity refers to liver dysfunction or liver damage that is associated with an overload of drugs or xenobiotics(Navaro*et al.,* 2006). The chemicals that cause liver injury are called hepatotoxins or hepatotoxicants. Hepatotoxicants are exogenous compounds of clinical relevance and may include overdoses of certain medicinal drugs, industrial chemicals, natural chemicals like microcystins, herbal remedies and dietary supplements (Willett *et al.,* 2004).

Certain drugs may cause liver injury when introduced even within the therapeutic ranges. Hepatotoxicity may result not only from direct toxicity of the primary compound but also from a reactive metabolite or from an immunologically-mediated response affecting hepatocytes, biliary epithelial cells and/or liver vasculature (Saukkonen*et al.,* 2006).

The hepatotoxic response elicited by a chemical agent depends on the concentration of the toxicant which may be either parent compound or toxic metabolite, differential expression of enzymes and concentration gradient of cofactors in blood across the acinus. Hepatotoxic response is expressed in the form of characteristic patterns of cytolethality in specific zones of the acinus.

1.3.1 SYMPTOMS OF HEPATOTOXICITY

Hepatotoxicity related symptoms may include a jaundice or icterus appearance causing yellowing of the skin, eyes and mucous membranes due to high level of bilirubin in the extracellular fluid, pruritus, severe abdominal pain, nausea or vomiting, weakness, severe fatigue, continuous bleeding, skin rashes, generalized itching, swelling of the feet and/or legs, abnormal and rapid weight gain in a short period of time, dark urine and light colored stool (Bleibel*et al.,* 2007; Chang and Shaino, 2007).

The symptoms of hepatotoxicity can be subdivided into clinical and drug-induced pathological symptoms.

1.3.1.0 CLINICAL MANIFESTATION

The manifestation of drug induced hepatotoxicity is highly variable, ranging from a symptomatic evaluation of liver enzymes to fulminant hepatic failure. The injury may suggest a hepatocellular injury with evaluation of aminotransferases levels as the predominant symptom or a cholestatic injury, with elevated alkaline phosphatase levels with or without hyperbiliruminemia being the main feature.

In addition, drugs that cause mild amino transferase elevation with subsequent adaptation are differentiated from those that result in true toxicity that require discontinuation.

Hepatotoxicity can be induced in the laboratory by exposing laboratory animals to toxic chemicals such as

carbon tetrachloride.

1.3.1.1 PATHOLOGICAL MANIFESTATION

Acute hepatocellular damage

Chronic hepatocellular damage

Chronic cholestasis

Vascular lesions / venocclusive disease

Angiosarcoma (Bleibel*et al.,* 2007; Chang and Shaino, 2007).

1.4 CARBON TETRACHLORIDE

Carbon tetrachloride (CCl_4) is an industrial chemical that does not occur naturally. Most of the carbon tetrachloride produced is used in the production of chlorofluorocarbons (CFCs) and other chlorinated hydrocarbons. It was once used widely as a solvent, cleaner and degreaser, both for industrial and home use. Today, the scientific database on the effects of haloalkanes is so vast that it is no longer employed for such purposes although it is used as a model of experimental liver injury (Weber *et al.,* 2003).

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CCl_4 metabolism begins with the formation of the trichloromethyl free radical, CCl_3 through the action of the mixed function cytochrome P-450 oxygenase system of the endoplasmic reticulum. This process involves reductive cleavage of a carbon-chlorine bond. Free radical activation of CCl_4 in mitochondria has also been observed and may contribute significantly to its toxicity. The major cytochrome iso-enzyme to execute biotransformation of CCl_4 is cytochrome P-450 iso-enzyme 2E1 (CYP2E1). This is evidenced by the absence of toxicity in CYP2E1 knockout mice.

In humans, CYP2E1 dominates CCl_4 metabolism at environmentally relevant concentrations, but at higher concentrations other cytochromes, particularly CYP3A, also contribute importantly (Zanger*et al.,* 2000). The CCl_3 radical reacts with several important biological substances, like fatty acids, proteins, lipids, nucleic acids and amino acids (Weber *et al.,* 2003). CCl_3 also acts by abstracting hydrogen from unsaturated fatty acids to form chloroform

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