Activities of Some Antioxidant Enzymes in Some Tissues of Acetaminophen-Induced Hepatotoxic Albino Rats Treated with *Anacardium Occidentale* Stem Bark Aqueous Extract

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ABSTRACT

Liver is a vital organ present in vertebrates, which has a wide range of functions including aiding of digestion, detoxification and protein biosynthesis. But the ability of the liver to perform these functions can be compromised by numerous substances it is daily exposed to, including certain medicinal agents which when taken in over doses. Liver damage, just like many other diseased conditions can lead to oxidative stress, especially when the body's antioxidant system is overwhelmed by the free radicals thus generated. A major component of this antioxidant system are the natural antioxidant enzymes superoxide dismutase and catalase manufactured in the body, which provide an important defense against free radicals usually generated in diseased conditions. Most synthetic anti-hepatotoxicity drugs available present serious side effects and are generally out of reach of the common man. Consequently, the effect of administration of aqueous extract of *Anacardium occidentale* stem bark on the activities of superoxide dismutase and catalase in some tissues of acetaminophen-induced hepatotoxic rats was investigated. There was a significant ($p < 0.05$) reduction in the activities of superoxide dismutase and catalase in the serum, liver, kidney and heart of the hepatotoxic rats. However, treatment of hepatotoxic rats with aqueous extract of *Anacardium occidentale* stem bark led to a significant ($p < 0.05$) increase in the activities of superoxide dismutase and catalase in the serum, liver, kidney and heart of acetaminophen-induced hepatotoxic rats.

Keywords: Liver, Antioxidant, Superoxide dismutase, Catalase, Acetaminophen, Hepatotoxicity.

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I. INTRODUCTION

Liver is an organ in the upper abdomen that aids in digestion and removes waste products and worn-out cells from the blood. It is a vital organ present in vertebrates and some other animals, which has a wide range of functions including detoxification and protein synthesis. It builds complex molecules from simple substances absorbed from the digestive tract, it neutralizes toxins, manufactures bile which aids fat digestion and removes toxins through the bowels [8]. But the ability of the liver to perform these functions is however compromised by numerous substances it is exposed to on a daily basis; these substances include certain medicinal agents which when taken in over doses and sometimes when introduced within therapeutic ranges injures the organ [3].

Liver disease is worldwide problem. Conventional drugs used in the treatment of liver diseases are sometimes

inadequate and can have serious adverse effects. Therefore, the search for alternative drugs for the treatment of liver disease is necessary in order to replace currently used drugs of doubtful efficacy and safety [12].

In the absence of reliable liver-protective drugs and satisfactory remedy for serious liver disease in allopathic medical practices, herbs play a role in the management of various liver disorders because it is believed that most of the herbal drugs speed up the natural healing process of liver, therefore the continued search for effective hepatoprotective drug is inevitable. In the past two decades, there has been a rise of interest in the use of medicinal plants for treating various kinds of ailments. This is because many diseases have defiled or developed a strong resistance to synthetic drugs and also because traditional medicine health system is readily accessible to the rural population in the world today [14]. Drug-induced hepatotoxicity is the most important cause of acute liver failure in many countries of the world

[2]. Almost all drugs are identified as foreign substances by the body, which subject them to various biochemical transformations including reduction of solubility in fat and change of biological activity to make them suitable for elimination. Acetaminophen, or paracetamol, is usually well tolerated in prescribed doses but overdose is the common cause of drug-induced hepatotoxicity worldwide. Damage to the liver is not due to the drug itself but to a toxic metabolite n-acetyl-para-quinone imine (NAPQI) which is produced by cytochrome P₄₅₀ enzymes in the liver [17]. This however has drawn a lot of interest and attention to the curative claims and norms of medicinal plants and other sources all over the world, especially in under developed countries in Africa and some parts of Asia [3]. Plants have a wide variety of medicinal potentials that has remained greatly untapped. This has generated huge and renewed interest in ethnomedicine, ethnobotany and ethnopharmacology. Extracts from these plant parts (especially the roots, stems, leaves and fruits) have been used extensively to treat infectious diseases and inflammatory and oxidative stress related conditions. Traditional medicinal plants have the ability to synthesize a wide variety of chemical compounds that play a major role in primary health care as therapeutic remedies. Additionally, they serve as alternative sources for western medicines that are expensive, synthetic and as consequence, may have adverse side effects [11].

Anacardium occidentale is a medium-sized evergreen tree, spreading, much branched and can grow up to a height of 12 m. When grown on lateritic, gravelly, coastal sandy areas, it rarely exceeds 6 m and develops a spreading habit and globose shape with crown diameter to 12 m. When grown in land on loams, it reaches 15 m and is much branched, with a smaller (4-6 m) crown diameter. The root system of a mature *Anacardium occidentale*, when grown from the seed, consists of a very prominent taproot and a well-developed and extensive network of lateral and sinker roots [9].

Natural antioxidant enzymes manufactured in the body provide an important defense against free radicals usually generated in diseased condition such as liver damage. Glutathione peroxidase, glutathione reductase, catalase and superoxide dismutase, are among the most important antioxidant enzymes. The antioxidant enzymes catalase and glutathione peroxidase removes H₂O₂, while superoxide dismutase catalyzes the dismutation of O₂⁻ to form H₂O₂ and O₂. Glutathione peroxidase is generally thought to be more important than catalase as a H₂O₂ removing system in humans. Catalase is located in peroxisomes, whereas glutathione peroxidase is localized in the mitochondria and cytosol, a similar distribution to that of SOD [7].

The available therapies currently used in managing liver toxicity are not only limited in their mechanisms of action, but also seen to bring about some adverse effects [5]. This limitation has therefore geared up the search for natural products with hepatoprotective compounds for the management of hepatotoxicity [5]. This study was therefore designed to investigate the hepatoprotective effect of the aqueous bark extract of *Anacardium occidentale* on the acetaminophen-induced liver damage with respect to the

activities of some antioxidant enzymes in some tissues of albino rats.

II. MATERIALS AND METHODS

A. Collection of plant material

Fresh stem bark of *nacardium. occidentale* was obtained from the premises of The Federal Polytechnic, Ado Ekiti, and identified at the Department of Science Technology of the same institution. This was air-dried in the laboratory for about sixty-one days, pulverized and then stored in an airtight container.

B. Extraction of the air-dried plant material

The air-dried samples were ground to fine powder using a blender. 500 g each of the powdered was soaked in 3000 ml of distilled water for 72 hours with frequent stirring. It was then filtered using filter paper and freeze-dried to obtain the dried extract. The extract was kept in a closed container and kept inside the fridge at 40 °C for further studies

C. Reagents and Chemicals

All reagents and chemicals used were all of analytical grade from BDH, Sigma and Aldrich Chemicals, UK.

D. Animals' protocol

Forty-two (42) albino rats (male and female) weighing 150 kg – 170 kg were obtained from the Animal House at The Federal Polytechnic, Ado Ekiti, Ekiti State, Nigeria. They were acclimatized for 2 weeks and allowed to have free access to food (commercial pelletized diet from Vital Feed Mill) and drinking water *ad libitum* daily.

E. Experimental Design

Randomized Complete Block Design (RCBD) was used. Forty-two (42) male and female albino rats were randomly divided into six groups (1-6) of seven animals in each group. All the rats in group 1 were left without being treated with acetaminophen and then given distilled water for fourteen days while the rats in groups 2-6 were administered 3500 mg/kg bw acetaminophen single dose orally, and then treated as shown in the table below daily for fourteen days.

Group 1: Non-hepatotoxic rats

Group 2: Hepatotoxic control

Group 3: Hepatotoxic rats + Silymarin (200 mg/kg bw)

Group 4: Hepatotoxic rats + Extract (50 mg/kg bw)

Group 5: Hepatotoxic rats + Extract (100 mg/kg bw)

Group 6: Hepatotoxic rats + Extract (200 mg/kg bw)

F. Dissection of Rats

On the fourteenth day, the rats were anaesthetized with diethyl ether, dissected and blood was collected in heparinised sample bottles and allowed to stand for 1 hour. Serum was prepared by centrifugation at 3000 rpm for 15 min at 25 °C. The clear supernatant was collected and used for the estimation of serum enzymes' activities.

G. Preparation of Homogenates

The liver, heart and kidney were excised using scissors and forceps. They were trimmed of fatty tissue, washed in distilled water, blotted with filter paper and weighed. They

were then chopped into bits and homogenized in ten volumes of the homogenizing phosphate buffer (pH 7.4) using a Teflon homogenizer. The resulting homogenates were centrifuged at 3000 rpm at 4 °C for 30 minutes. The supernatant obtained was collected and stored under 40 °C and then used for biochemical analyses.

H. Assay of Antioxidant Enzymes

1. Assay of Superoxide Dismutase (SOD) Activity

This was determined by the method of Misra and Fridovich [10]. 1.0 ml of sample (serum, and liver, heart, and kidney homogenates' supernatant) was diluted in 9 ml of distilled water to make a 1 in 10 dilution. An aliquot of the diluted sample was added to 2.5 ml of 0.05 M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer. The reaction was initiated by the addition of 0.3 ml of freshly prepared 0.3 mM adrenaline to the mixture which was quickly mixed by inversion. The reference cuvette contained 2.5 ml buffer, 0.3 ml of substrate (adrenaline) and 0.2 ml of water. The increase in absorbance at 480 nm was monitored every 30 seconds for 150 seconds.

2. Assay of Catalase Activity

This was carried out using the method described by Sinha [15]. 0.2 ml of sample (serum, and liver, heart, and kidney homogenates' supernatant) was mixed with 0.8 ml distilled H₂O to give 1 in 5 dilution of the sample. The assay mixture contained 2 ml of solution (800 µmol) and 2.5 ml of phosphate buffer in a 10 ml flat bottom flask. Properly diluted enzyme preparation (0.5 ml) was rapidly mixed with the reaction mixture by a gentle swirling motion. The reaction was run at room temperature. A 1 ml portion of the reaction mixture was withdrawn and blown into 1 ml dichromate/acetic acid reagent at 60 seconds intervals. The hydrogen peroxide content of the withdrawn sample was determined.

I. Statistical Analysis

All values were expressed as mean of six determination $\pm$ SEM. Statistical evaluation was done using One Way Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) by using SPSS 20.0 for windows (Anthony and Richard, 2006). The significance level was set at $p < 0.05$.

III. RESULTS

The activities of superoxide dismutase and catalase in the serum, of acetaminophen-induced hepatotoxic rats were found to be increased significantly ($p < 0.05$) to normal following the administration of *Anacardium occidentale* stem bark aqueous extract at concentrations of 50, 100 and 200 mg per kg body weight of the rats. This restoration in the groups treated with 100 and 200 mg/kg body weight was also found to compare well with those of hepatotoxic rats treated with standard anti-hepatotoxic drug, Silymarin. However, the activities of superoxide dismutase and catalase reduced significantly ($p < 0.05$) in the serum of the rats in the untreated hepatotoxic control group (Fig. 1).

Fig. 2 showed that the activities of superoxide dismutase and catalase in the liver of acetaminophen-induced

hepatotoxic rats were increased significantly ($p < 0.05$) following the administration of *Anacardium occidentale* stem bark aqueous extract at concentrations of 50, 100 and 200 mg per kg body weight of the rats. This restoration in the groups treated with 100 and 200 mg per kg body weight compare favourably well with those of the non-hepatotoxic rats. However, the activities of superoxide dismutase and catalase reduced significantly ($p < 0.05$) in the liver of the rats in the untreated hepatotoxic control group.

Fig. 1. Activities of superoxide dismutase and catalase in the Serum of Acetaminophen-induced hepatotoxic rats following administration of *Anacardium occidentale* stem bark aqueous extract.

Values are means of six determinations $\pm$ S.E.M. Values with different superscript in a cluster differ significantly ($p < 0.05$).

Fig. 2. Activities of superoxide dismutase and catalase in the Liver of Acetaminophen-induced hepatotoxic rats following administration of *Anacardium occidentale* stem bark aqueous extract.

Values are means of six determinations $\pm$ S.E.M. Values with different superscript in a cluster differ significantly ($p < 0.05$).

Fig. 3 revealed that the activities of superoxide dismutase and catalase in the kidney of acetaminophen-induced hepatotoxic rats were increased significantly ($p < 0.05$) following the administration of *Anacardium occidentale* stem bark aqueous extract at concentrations of 100 and 200 mg per kg body weight of the rats. This restoration in the group treated with 200 mg per kg body weight was also found to compare favourably well with those of the non-hepatotoxic rats. However, the activities of superoxide dismutase and catalase reduced significantly ($p < 0.05$) in the kidney of the rats in the untreated hepatotoxic control group.

The activities of superoxide dismutase and catalase in the heart tissues of acetaminophen-induced hepatotoxic rats were found to be increased significantly ($p < 0.05$) following the administration of *Anacardium occidentale* stem bark aqueous extract at concentrations of 50, 100 and 200 mg per kg body weight of the rats. This restoration in the group treated with 200 mg/kg body weight was also found to compare well with those of non-hepatotoxic rats and

hepatotoxic rats treated with standard anti-hepatotoxic drug, Silymarin. However, the activities of superoxide dismutase and catalase reduced significantly ($p < 0.05$) in the heart of the rats in the untreated hepatotoxic control group (Fig. 4).

Fig 3. Activities of superoxide dismutase and catalase in the Kidney of Acetaminophen-induced hepatotoxic rats following administration of *Anacardium occidentale* stem bark aqueous extract. Values are means of six determinations + S.E.M. Values with different superscript in a cluster differ significantly ($p < 0.05$).

Fig. 4. Activities of superoxide dismutase and catalase in the Heart of Acetaminophen-induced hepatotoxic rats following administration of *Anacardium occidentale* stem bark aqueous extract. Values are means of six determinations + S.E.M. Values with different superscript in a cluster differ significantly ($p < 0.05$).

IV. DISCUSSION

The ability of the liver to perform its functions is often compromised by numerous substances it is exposed to on a daily basis; these substances include certain medicinal agents which when taken in over doses and sometimes even when introduced within therapeutic ranges injures the organ [3].

Recent development in medicinal field reports a number of disease associated with free radicals. The risk of diseases due to oxidative stress is compounded by ostentatious lifestyle and indiscriminate exposure to chemicals, pollution, cigarette smoking, drugs, illness, stress etc. Many of the recent landmarks in scientific research have shown that in human beings, oxidative stress has been implicated in the progression of major health problems by inactivating the metabolic antioxidant enzymes and damaging important cellular components leading to cardiovascular diseases, joint disorders, neurological diseases, cancer, aging etc. [4].

The production of oxidants such as reactive oxygen

species like superoxide anions, hydrogen peroxide and hydroxyl radicals by activated Kupffer cells has been identified as central to hepatic injuries [18]. Kupffer cells, also known as hepatic macrophages, are one type of non-parenchyma cells that help maintain the integrity of liver cells. However, these phagocytic cells are also susceptible to the effects of oxidative stress produced by the surrounding cells and its own immune reactions [13]. Antioxidants are the substances which can scavenge free radicals and help to decrease the incidence of oxidative stress-induced tissue damage. The body's first line of defense against oxidative stress are the endogenous antioxidant enzyme system including Superoxide dismutase (SOD) and Catalase (CAT) [6].

The significantly ($p < 0.05$) decreased activities of superoxide dismutase and catalase in the various tissues of the hepatotoxic rats may be due to inactivation caused by reactive oxygen species generated during hepatic injury such as superoxide anion, hydrogen peroxide and hydroxyl radicals. This reduction may also be due to the channeling of these antioxidant enzymes towards the removal of these reactive oxygen species [16]. Also, impairment of antioxidant machinery may be described by both the damage of antioxidant enzymes caused by protein glycation and consumption by excess demand. However, in the treatment groups the increased catalase activity could be as a result of higher production of H_2O_2 . It is also possible that catalase activity, which in turn would protect superoxide dismutase inactivation by H_2O_2 causes an increase in superoxide dismutase activity [1].

V. CONCLUSION

The activities of antioxidant enzymes superoxide dismutase and catalase decreased in the serum, liver, kidney and heart of rats with induced liver damage. However, there was a dose-related increase in the activities of these enzymes in the studied tissues of the hepatotoxic rats following the administration of aqueous extract of *Anacardium occidentale* stem bark. Consequently, *Anacardium occidentale* stem bark aqueous extract could be a drug lead in anti-hepatotoxicity.

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