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Antioxidant and Hepatoprotective Activities of *Carica papaya* (Papaw Leaf) and *Loranthus bengwensis* (Cocoa Mistletoes) against Diclofenac Induced Hepatotoxicity in Rats

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ABSTRACT

In this study, the effect of diclofenac sodium induced liver damage in rats was investigated, biochemically. Damaging effects of reactive oxygen species (ROS) on living systems are well documented, they include the oxidative attack on vital cell constituents. Administration of diclofenac sodium at different concentrations (50 mg/kg, 100 mg/kg, and 150 mg/kg/d) for 14 days produced severe liver injury, as demonstrated by dramatic elevation of plasma hepato specific markers like aspartate amino transaminase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP). In addition, diclofenac sodium administration caused oxidative stress in rats, as evidenced by increased ROS production and malondialdehyde (MDA) concentrations in the liver of rats, along with a remarkable reduction in hepatic catalase (CAT) activity. However, simultaneous treatment with aqueous extract of dry pawpaw leaf (30%) and cocoa mistletoes (30%) significantly attenuated diclofenac sodium induced hepatotoxicity. The results showed that serum marker enzymes and hepatic MDA content as well as ROS production were reduced dramatically and CAT activity content was restored remarkably, when treated with the extracts, as compared to the diclofenac sodium treated rats. It is, therefore, suggested that dry pawpaw leaf and cocoa mistletoes employed in this study can provide a definite protective effect against acute hepatic injury caused by diclofenac sodium in rats, which may mainly be associated with its antioxidative effect.

Key-words: Antioxidant, Drug-induced liver injury, Diclofenac, Hepatotoxicity, Oxidative stress

INTRODUCTION

Liver diseases are considered to be serious health disorders. The liver has one of the highest value of importance for the systemic detoxification and deposition of endogenous and exogenous substances. Liver dysfunction challenges not only health care professionals but also the pharmaceutical industry and drug regulatory agencies. Drug-induced liver injury (DILI) possesses a major clinical problem. DILI has become the leading cause of acute liver failure and transplantation in Western countries.

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Hepatotoxicity associated with most other drugs are idiosyncratic, which implies by definition that DILI develops in only a small proportion of subjects exposed to a drug in therapeutic doses [1]. The risk of acute liver failure associated with idiosyncratic hepatotoxin is usually less than 1 per 10000 exposed patients. However, more than 1000 drugs and herbal products have been associated with idiosyncratic hepatotoxicity.

Diclofenac is Non-steroidal anti-inflammatory drugs (NSAIDs) which are the centerpiece of pharmacotherapy for most rheumatological disorders, and are used in large numbers as analgesics and antipyretics, both as prescription drugs and over the counter purchases. It is the most important cause of the drug induced toxic injury to several organ systems, including well known injury to gastrointestinal tract, liver, heart and kidneys [2]. Diclofenac cardiotoxicity is an archetype of idiosyncratic DIHI (Drug induced heart injury) [2]. About 15% of those patients regularly taking diclofenac develop elevated

levels of heart enzymes, and a threefold rise in transaminase levels has been reported in 5%. Diclofenac is associated with a predominantly hepatocellular and cardiovascular pattern of liver and heart injury.

Products of higher plant origin have been known to be effective sources of chemotherapeutic agents without any underlying effects. Plants continue to be a major source of medicines, as they have been throughout human history. The use of medicinal plants all over the world predates the introduction of antibiotics and other modern drugs [3,4]. A medicinal plant is any plant with one or more of its organs containing substances that can be used for therapeutic purposes or which are the precursors for the synthesis of useful drugs [5]. They are of great importance to the health of individuals and communities; the medicinal values of certain plants lie in some chemical substances that produce definite physiological action on the human body [6]. The most important of these bioactive constituents of plants are flavonoids, tannins, alkaloids and foods plants sometimes added to foods. Mistletoe is a general term for woody shoot parasites in several plant families, especially in *Loranthaceae* and *Viscaceae* families [6]. The common European mistletoe grows on various trees, usually apples and junipers. It is an evergreen plant with small, greenish flowers and berries. Similar mistletoe, American mistletoe, found in the United States, grows on deciduous trees, particularly red marple elm, from eastern Texas to Florida and northward to Missouri and New Jersey [6]. The leafless flowering dwarf mistletoes depend entirely on the host tree for nourishment. These scrubs are lethal parasites of conifers, such as pine, spruce, fir and hemlock. The plant leaves and berries contain toxic chemicals that can be poisonous and the plant should be kept out of reach of young children who may be tempted to eat the berries [7,8]. *Carica papaya* L., (pawpaw), belongs to the family of *Caricaceae.* Papaya is not a tree but an herbaceous succulent plant that possess self-supporting stems [9]. Papaya is a large perennial herb with a rapid growth rate. The plants are usually short-lived, but can produce fruit for more than 20years. The papaya has a rather complicated means of reproduction. The plants are male, hermaphrodite, or female [9]. The male trees are uncommon, but sometimes occur when homeowners collect their own seeds. Hermaphrodite trees (flowers with male and female parts) are the commercial standard, producing a pear shaped fruit. These plants are self-pollinated. *Carica papaya* plants produce natural compounds (annonaceous acetogenins) in leaf bark and twig tissues that possess both highly anti-tumor and pesticidal properties. It was suggested that a potentially lucrative industry based simply on production of plant biomass could be developed for production of anti-cancer drugs, pending Food and Drug Agency approval, and natural (botanical) pesticides [10]. The high level of natural self-defence compounds in the tree makes it highly resistant to insect and disease infestation. *Carica papaya* L. leaf tea or extract has a reputation as a tumor-destroying agent. The papaya fruit, as well as all other parts of the plant, contain a milky juice in which an active principle known as papain is present. Aside from its value as a remedy in dyspepsia and kindred ailments, it has been utilized for the clarification of beer. The juice has been in use on meat to make it tender. The seed is used for intestinal worms when chewed. The root is chewed and the juice swallowed for cough, bronchitis, and other respiratory diseases. The unripe fruit is used as a remedy for ulcer and impotence [11]. Fresh, green pawpaw leaf is an antiseptic, whilst the brown, dried pawpaw leaf is the best as a tonic and blood purifier. Chewing the seeds of ripe pawpaw fruit also helps to clear nasal congestion, [12]. The green unripe pawpaw has a therapeutic value due to its antiseptic quality. It cleans the intestines from bacteria, more so that (only a healthy intestine is able to absorb vitamin and minerals, especially vitamin B12). The tea, prepared with the green papaya leaf, promotes digestion and aids in the treatment of ailments such as chronic indigestion, overweight and obesity, arteriosclerosis, high blood pressure and weakening of the heart [13]. In view of the above, the present study was planned to determine the antioxidant and hepatoprotective activity of papaw leaf and cocoa mistletoes against diclofenac-induced hepatotoxicity in Rats.

MATERIALS AND METHODS

Collection of Samples- The cocoa mistletoes plants and dry pawpaw leaf were obtained from Ifaki local farm Ekiti State, Nigeria. Some parts of the plants were taken to the Department of Plant Science in Ekiti State University, Ado-Ekiti, Ekiti State, Nigeria for identification/ Authentication.

Experimental Protocol- The study was performed on fourty (40) wistar albino rats (all males) housed in ventilated cages in the Animal House of College of Medicine, Ekiti State University, Ado-Ekiti, Nigeria. They were acclimatized for two weeks before administration of the drugs.

Animals are divided into ten groups of four rats each as followed-

Group 1	Positive Control (Normal) animals
Group 2	Negative control (50 mg/kg Diclofenac treated) animals
Group 3	Negative control (100 mg/kg Diclofenac treated) animals
Group 4	Negative control (150 mg/kg Diclofenac treated) animals
Group 5	50 mg/kg Diclofenac treated animals + mistletoes extract
Group 6	100 mg/kg Diclofenac treated animals + mistletoes extract
Group 7	150 mg/kg Diclofenac treated animals + mistletoes extract
Group 8	50 mg/kg Diclofenac treated animals + dry pawpaw leaf
Group 9	100 mg/kg Diclofenac treated animals + dry pawpaw leaf
Group 10	150 mg/kg Diclofenac treated animals + dry pawpaw leaf

Chemicals/ Reagent kits- All chemicals and drugs were used obtained commercially and of analytical grade. All the diagnostic kits are products of Fortress Chemical Ltd. United Kingdom.

Preparation of Organs homogenate- The animals were quickly dissected, the organs was removed. 10% of each organs homogenate was then prepared in 6.7 mM potassium phosphate buffer, (pH 7.4) using the Teflon homogenizer. The homogenate was centrifuged at 10,000rpm for 10 minutes at 4^0C to obtain a clear supernatant which was stored at 8^0C and used for measurement of biochemical contents.

Biochemical Assay- Standard Fortress kits were used to determine Cholesterol, Total protein, Alkaline Phosphatase (ALP), Aspartate Transaminase (AST) and Alkaline Transaminase.

Determination of Alanine aminotransferase-

GPT ∝-oxoglutarate + L-alanine→ L-glutamate + pyruvate

Alanine aminotransferase is measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4-dinitrophenylhydrazine.

Determination of AST-

GOT ∝- oxoglutarate + L- aspartate → L-glutamate + oxaloacetate

AST is measured by the monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-dinitrophenylhydrazine.

Determination of ALP- Plasma alkaline phosphatase (ALP) was determined by kinetic method according to Abubakar *et al*. [13].

Determination of Plasma Malondialdehyde (MDA)- Lipid peroxidation was determined by measuring the formation of thiobarbituric acid reactive substances (TBARS). An aliquot of 0.4 mL of the plasma or other organ homogenates was mixed with 1.6 mL of Tris-KCl buffer to which 0.5 mL of 30% trichloroacetic acid (TCA) was added. Then 0.5 mL of 0.75% TBA was added and placed in a water bath for 45 minutes at 80^{o}C. This was then cooled on ice and centrifuged at 3000*g.* The clear supernatant was collected and absorbance measured against a reference blank of distilled water at 532 nm. The MDA level was calculated. Lipid peroxidation in units/mg protein or gram tissue was computed with a molar extinction coefficient of 1.56 x 10^5 M^{-1} cm^{1}.

MDA (units/mg protein)=

$$\frac{\text{Absorbance x volume of mixture}}{E_{532\ nm} \text{ x volume of sample x mg protein}}$$

Determination of Catalase activity of samples- This experiment was carried out using calorimetrically. This method is based on the reduction of dichromate in acetic acid to chromic acetate when heated in the presence of H_2O_2, with the formation of perchromic acid as an unstable intermediate.

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The chromic acetate so produced was measured calorimetrically at 570–610 nm. However, the dichromate has no absorbance at the wavelength and its presence in the assay mixture did not interfere with the determination. The catalase preparation was allowed to split H_2O_2 for different periods of time. The reaction is stopped at a particular time by the addition of dichromate/acetic acid mixture and the remaining H_2O_2 is determined by measuring chromic acetate calorimetrically after heating the reaction mixture.

Calculation- The mononuclear velocity constant, K, for the decomposition of H2O2 by catalase was determined by using the equation for a first-order reaction-

$$K = \frac{1}{t} \log \frac{S0}{S}$$

Where S_0= The initial concentration of H_2O_2 and S= The concentration of the peroxide at t-min. The values of the K= Plotted against time in minutes and the velocity constant of catalase $K_{(0)}$ at 0 min determined by extrapolation.

The catalase contents of the enzyme preparation were expressed in terms of Katalasefeiahigkeit or 'Katf'.

$$Katf = K_{(0)} / \text{mg protein/ ml}$$

Statistical Analysis- All values are presented in Tables and the appropriate comparisons between groups were made using Student's-test. The difference between the groups is taken to be significant at P<0.05.

RESULTS

Fig. 1 represents the effect of pawpaw leaf and cocoa mistletoes on the catalase activity in plasma, heart and liver of diclofenac-induced rat. A significantly lower (P<0.05) plasma and liver levels of Catalase was observed in diclofenac-induced rats compare to normal group but when treated with the pawpaw leaf and mistletoes extracts, the catalase activity was increased towards to normal proportion.

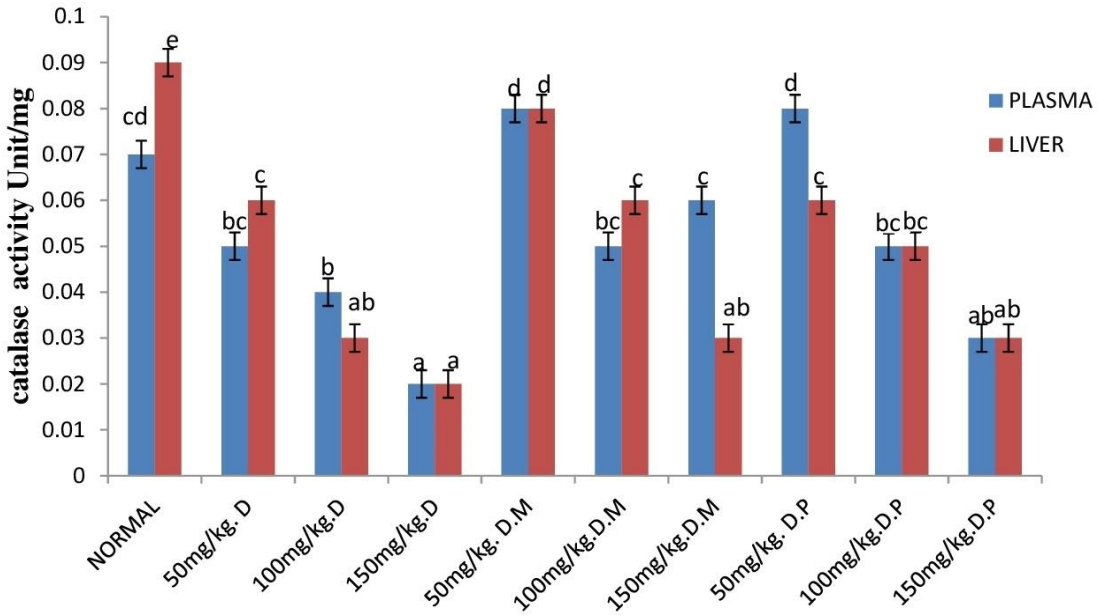

Fig. 1: Effects mistletoes and pawpaw leaf treatment on the catalase activity in plasma, and liver of diclofenac-induced rat

Fig. 2 represents the effect of pawpaw leaf and cocoa mistletoes on the malondialdehyde concentration in plasma and liver of diclofenac-induced rat. A significantly higher (P>0.05) plasma, heart and liver levels of malondialdehyde was observed in diclofenac-induced rats when compare to normal group but when treated with the pawpaw leaf and mistletoes extracts, the malondialdehyde concentration was decreased towards to normal level.

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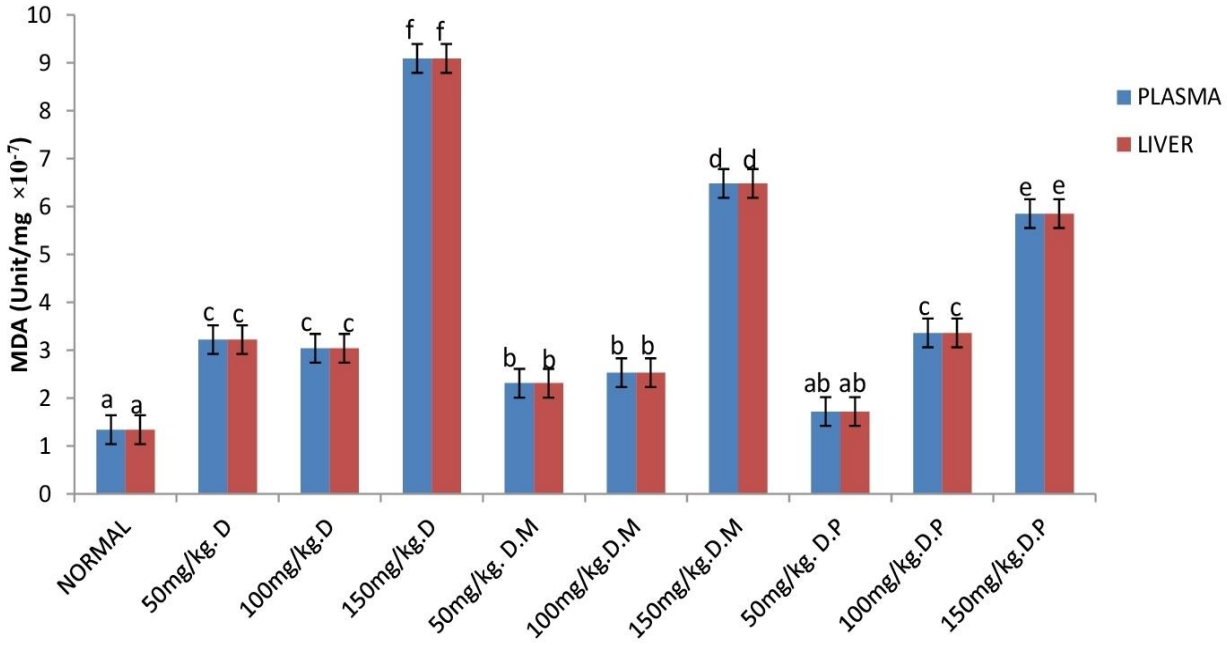

Fig. 2: Effects mistletoes and pawpaw leaf treatment on the Malonaldehyde concentration in plasm and liver of diclofenac-induced rat

Administration of diclofenac produced significant adverse effects on the functionalities of liver and plasma, which is evidenced by a significant elevation in the actions of ALT, AST, and ALP enzymes in (negative group) diclofenac-induced rats group compared to (Normal control) normal. Treatment of cardiotoxic and hepatotoxic rats with pawpaw leaf and mistletoes exhibited improvement in the actions of ALT, AST and ALP enzymes compared to (negative control) rats (Fig. 3 to Fig. 5).

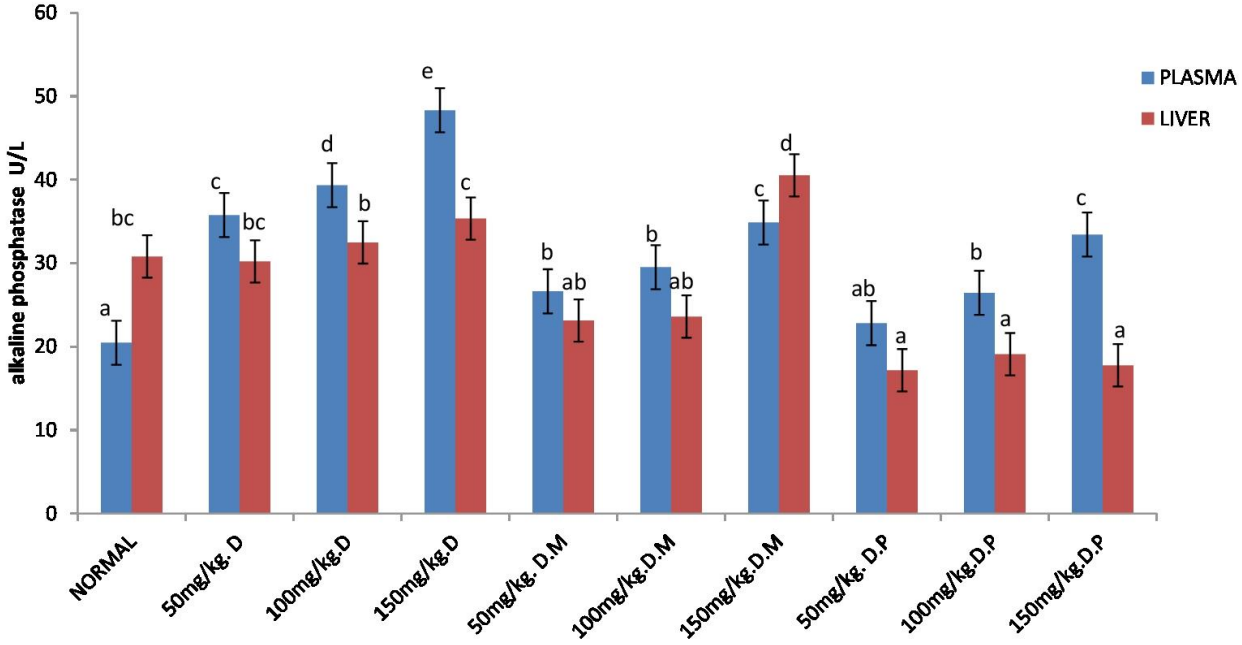

Fig. 3: Effects of mistletoes and pawpaw leaf on treatment on the alkaline phosphatase activity in plasma and liver of diclofenac-induced rat

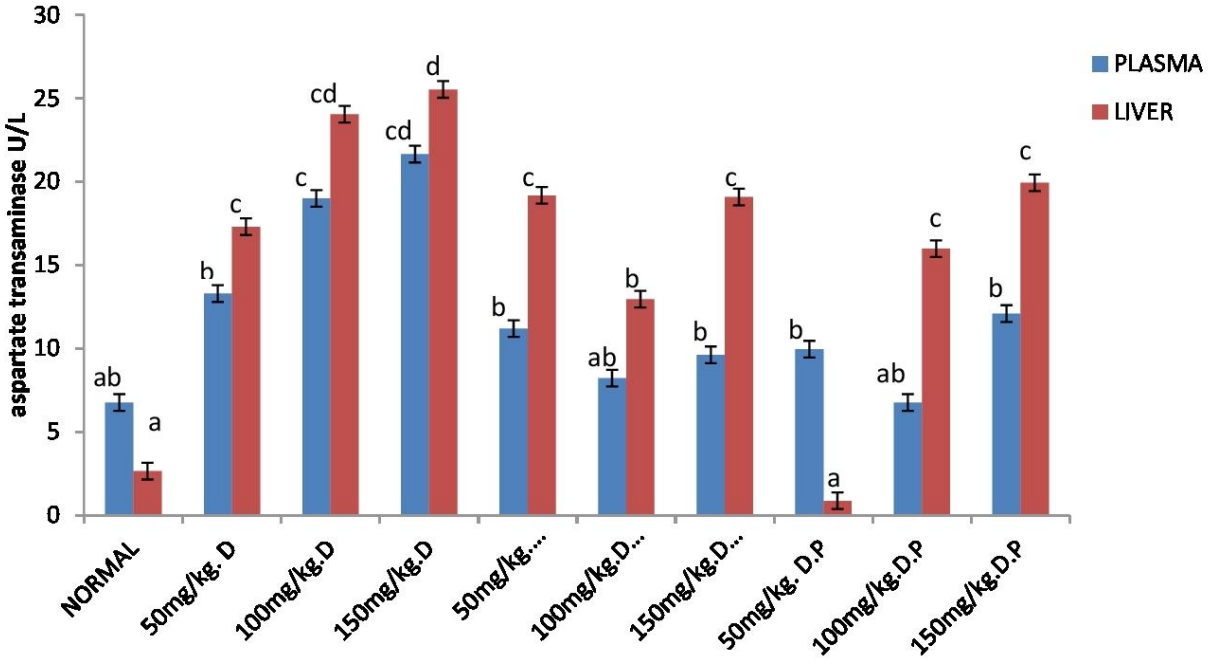

Fig. 4: Effects of mistletoes and pawpaw leaf on treatments on the alkaline phosphatase activity in plasma and liver of diclofenac-induced rat

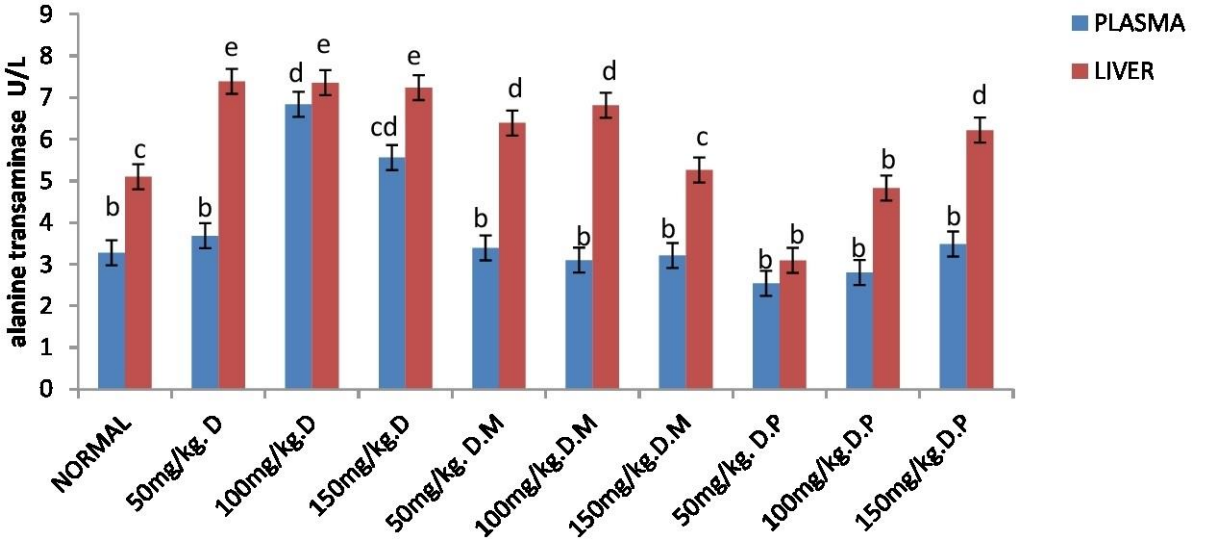

Fig. 5: Effects of mistletoes and pawpaw leaf on treatments on the alanine transaminase activity in plasma of diclofenac-induced rat

DISCUSSION

However, Fig. 1 above showed the effects of aqueous extract of mistletoes and pawpaw leaf treatment on the catalase activity in plasma and liver of diclofenac-induced rat. Free radical scavenging enzyme,such as CAT, are the first line of defense against oxidative injury. The H_2O_2 formed by SOD and other processes is scavenged by catalase that catalyzed the dismutation of H_2O_2 into water and molecular oxygen. Thus, the antioxidant enzyme catalase is responsible for detoxification of H_2O_2. The catalase enzyme may also be released into the extracelular environment in which it has the potential to function as a potent antioxidant and thereby regulated cell survival [14]. In light of these considerations, it seems plausible that extracellular catalase might function as an important autocrine antioxidant and survival factor. A large number of the metabolites produced by NSAIDs are found to generate superoxide anion and other free

radicals in the biological systems [15]. However, at a higher dose of diclofenac (150 mg/kg) and consistent using of diclofenac, intermediate metabolites accumulate and cause liver damage. The reduction in catalase activity after induction with diclofenac was another significant finding in this study. The decreased concentration of plasma catalase is attributable in part to the reduced synthesis of this antioxidant enzyme (which functions in the detoxification of hydrogen perioxide) whose concentrations would have fallen with the diclofenac that was injected into the animals. Though some studies have reported that no alterations in the activity of red cell catalase. However, this study is in agreement with earlier reports by Redmond and Redmond [16], who reported a decreased in red blood cell catalase activity cardiovascular and hepato-cellular disorder. While treatment with Pawpaw leaf and mistletoes extracts caused significant increase in catalase activity in their respective groups due to bioactive compounds presented in the plants, which aid the activities of the antioxidant enzymes, which is in agreement with the previous study and can also explain the increased detoxification of the reactive metabolites generated from the diclofenac metabolism in the liver of plant extracts treated animals. In present studies, the Pawpaw leaf and mistletoes co-administered rats showed significantly decreased levels of these lipids peroxidation markers as compared with diclofenac-induced rats. This present study is accordance with the previous study of Sarker *et al.* [17], which they found that pawpaw leaf and mistletoes respectively contain substances that delay the rate of oxidation by directing the breakdown of peroxides into stable substances that do not promote further oxidation or by sweeping free radicals away. Damaged membranes were recovered by the treatment with plans extracts by enhancing antioxidants' status and decreasing lipid peroxidation [18]. The inhibition of lipid peroxidation by Pawpaw leaf and mistletoes extracts, therefore, may be one of the mechanisms by which the plants exert their protection against diclofenac sodium mediated tissue injury.

Estimating the activities of serum marker enzymes, like AST, ALT, and ALP, can make assessment of liver function. When liver cell plasma membrane is damaged, a variety of enzymes normally located in the cytosol are released in to the blood stream. Their estimation in the serum is a useful quantitative marker of the extent and type of hepatocellular damage [19,20]. The enhanced activities of these serum marker enzymes observed in diclofenac sodium treated rats in present study correspond to the extensive liver damage induced by diclofenac sodium. The tendency of these enzymes to return towards a near normal level in group I (Normal) rats is a clear manifestation of anti-hepatotoxic effect of mistletoes and dry pawpaw leaf. Consistent and high dosage administration of diclofenac in this study produced significant adverse effects on the organs functions of the rats such as liver, heart and kidney, which is evidenced by a significant increase in the actions of ALT, AST, and ALP enzymes in (negative groups) diclofenac-induced rats group compared to (control) normal. Similar result has also been recorded that stated that when enzymes leak from the liver cytosol into the blood stream, it lead to higher level of ALP in serum is an indicative of hepatotoxic of diclofenac-induced rats [20]. High levels of ALP in serum indicate liver damage which is similar to this present study where, the increase in ALP activity in diclofenac-induced rats shows the liver damage, as a result of metabolic changes such as administration of toxin, liver cirrhosis, hepatitis, and cancer of the liver [20]. Thus, it can be used involved as markers to estimate the extent of liver damage. Also Aminotransferases (ALT and AST) occupy a central position in the metabolism of amino acids as they help in retaining amino groups (to form new ones) during degradation of amino acids. They are also involved in the biochemical regulation of amino acid pool and in providing necessary intermediate to predict possible toxicity in some organs such as kidney cytolysis and the heart and liver of animals [21], which similar result occurred this present study. The measurement of the activities of these enzymes is of clinical and toxicological significance. Since the enzymes were only being released into cellular flow when organs are damaged, the reduction in the levels of these enzymes in the plasma of the animals maintained on the dry pawpaw leaf and mistletoes extracts treated groups.

CONCLUSIONS

The oxidative stress has been imposed due to the imbalance of biochemical processes that involved in the generation of reactive oxygen and nitrogen species (ROS and RNS) and their neutralization by the inherent antioxidant (enzymatic or non-enzymatic) defense

system of the cells. Mistletoes and pawpaw leaf are one of the most abundant natural antioxidants with variety of applications. The present study shows that the Hepatotoxicity in rats treated with diclofenac was markedly reduced by simultaneous administration of mistletoes and pawpaw leaf extract. The results of this study show protective effect of mistletoes and pawpaw leaf on plasma and liver function which might be due to the presence of some bioactive compound in the extract. Further investigation should be conducted to identify this bioactive compounds present in mistletoes.

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CONTRIBUTION OF AUTHORS

Research concept- Oseni OA
Research design- Odesanmi EO
Supervision- Oloyede OI
Materials- Odesanmi EO
Data collection - Oseni OA
Data analysis and interpretation- Odesanmi EO
Literature search- Odesanmi EO
Writing article- Odesanmi EO
Critical review- Oloyede OI
Article editing- Ogundare MAB
Final approval- Oseni OA
Research support- Adebayo OD

REFERENCES

[1] Biour M, Poupon R, Grange JD, Chazouilleres O. Druginduced hepatotoxicity, 13th ed. of the bibliographic database of drug-related liver injuries and responsible drugs. Gastroenterol. Clin. Biol., 2000; 24(11): 1052-91.

[2] Dahlin DC, Miwa GT, Lu AYH, Nelson SD. N-acetyl-pbenzoquinoneimine: acytochrome p-450 mediated oxidation product of acetaminophen. Proceeding of the Nat. Acad. Sci. USA, 1984; 81: 1327–31.

[3] Dick G. Papaya: A tantalizing taste of the Tropics Maricopa County Master Gardener Volunteer information, University of Arizona Cooperative Extension, 2003. www.papayaMaricopa-hort@ag.arizo.edu.

[4] Dutta AC. Botany for Degree Students. 6th Edition. Oxford University Press, 2005; pp. 592.

[5] Fahey JW, Zhang Y, Talalay P. Proc. Nat. Acad. Sci. USA, 1997; 94: 10367-72.

[6] Gill LS, Onyibe HI. Mistletoes on rubber trees in Nigeria. Haustorium, 23: 1-2. Am. Eurasian 2007. J. Sustainable Agric., 1990; 5: 360–61.

[7] Kearney P, Baigent C, Godwin J, Halls H, Emberson J, et al. Do selective cyclo-oxygenase-2 inhibitors and traditional non-steroidal anti-inflammatory drugs increase the risk of atherothrombosis? Meta-analysis of randomised trials. BMJ, 2006; 332(7553): 1302-08.

[8] Khammash A. "Holly Parasite or Christmas Mistletoe" Jordan Times Weekender. Copyright © 2005, http: www.jordanflora.com/holly.htm., 2005.

[9] Kingsbury JM, Redmond WA. "Poisonous Plants." Microsoft ® Student [DVD]. Microsoft Corporation, 2007.

[10] Kokowaro J O. *Asparagus africanus*. Medicinal plants of East Africa, 1976; pp. 239.

[11] Mitchell JR, Jollow DJ, Potter WZ, Gillette JR, Brodie BB. Acetaminophen inducedhepatic necrosis. Protective role of glutathione. Pharmacology and Experimental Therapeutics 1973; 187: 211–15.

[12] National Cancer Institute. Mistletoe Extract (PDG) National Cancer Institute. Website. Accessed on July 3, 2007.

[13] Abubakar MS, Musa AM, Ahmed A, Hussaini IM. The Perception and Practice of Traditional Medicine in the Treatment of Cancers and Inflammations by the Hausa and Fulani Tribe of Northern Nigeria. J. Ethnopharmacol., 2007; 3(3): 625–29.

[14] Orecnius S, Moldeus P. The multiple roles of glutathione in drug metabolism. TIPS, 1984; 5: 432–35.

[15] Prochaska HJ, Santamaria AB, Talalay P. Proc. Natl. Acad. Sci. USA, 1992; 89: 2394-98.

[16] Redmond WA. Redmond WA. "Mistletoe" Microsoft ® Student 2008 [DVD]. Redmond, W.A.: Microsoft Corporation, 2007.

[17] Sarker MSK, Kim GM, Hossain ME, Sharmin MF, Huque KS, et al. Effect of mixed probiotics treated with *Alisma canaliculatum, Laminaria japonica* and *Cornus officinalis* on oxidation, cholesterol and fatty acids profile in broiler meat. In: Proceedings of 9th Asia Pacific Poultry Conference. Taipei, Taiwan, 2011.

[18] Shin DD, Brandimarte F, De Luca L, Sabbah HN, Fonarow GC, et al. Review of current and

DOI:10.21276/ijlssr.2018.4.5.3

investigational pharmacologic agents for acute heart failure syndromes. Am. J. Cardiol., 2007; 99: 4A-23A.

[19]Tang W. The metabolism of diclofenac enzymology and toxicology perspectives. Curr. Drug Metab., 2003; 4(4): 319-29.

[20]Trichopoulos D, Willett WC, eds. Cancer Causes Control**,** 1996; 1: 103-80.

[21]Udoh AE, Ntu I, Essien O, Ndon M. Red cell catalase activity in diabetics. J. Nutr., 2007; 6: 511-15.