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COCOA MISTLETOE (*LORANTHUS BENGWENSIS*) AQUEOUS EXTRACT ATTENUATES DICLOFENAC INDUCED ORGANS TOXICITIES IN WISTAR ALBINO RATS

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ABSTRACT

Mistletoe (*Loranthus bengwensis*) has been used as a therapeutic agent and also possesses some beneficial nutrients. This present study therefore investigate the antioxidant, antitoxicity and chemo-protective effects of aqueous extract of cocoa mistletoe on diclofenac induced organsdisorders using male Wistar albino rats. The experiment was performed on twelve Wistar rats weighing between 120g and 150 g which were divided into three groups of four rats each. Group I animals serve as a positive control that fed on water and rat feed while Group II animals were orally administered with 50mg/kg/bodyweight of diclofenac. Similarly Group III rats were administered with 50mg/kg/body weight diclofenac and were also treated at a day interval with 10% aqueous extract of cocoa mistletoe for three weeks. After which the rats were sacrificed; the plasma, liver and heart were obtained to determine the biomarker enzymesAlanine aminotransferase (ALT); Aspartate aminotransferase (AST); Alkaline phosphatase (ALP); Catalase (CAT) and malondialdehyde (MDA). The results of the study showed that diclofenac significantly and progressively lowered the plasma and organs CAT, AST activities while significantly causing elevation in ALT, ALP, MDA concentrations when compared with the control rats group. The 10% aqueous mistletoe

extract significantly lowered the ALP, ALT, MDA and increased CAT and AST activities. However the results of the study showed the cardio-protective, chemoprotective and antioxidant effects of the aqueous extract of Mistletoe (*Loranthus bengwensis*) which may be used in the management of suspected patients with liver and heart problems.

KEYWORD: diclofenac; toxicity; *Mistletoe;* cardio-protection; antioxidants.

INTRODUCTION

Cardiovascular disease (CVD) centers basically on the heart. It is a chronic condition that involves narrowed or blocked blood vessels that lead to heart attack, chest pain (angina) or stroke. Blood flow to the heart, brain or body can be reduced as the result of a blood clot (thrombosis), or by a build-up of fatty deposits inside an artery that cause the artery to harden and narrow (atherosclerosis). Other heart conditions, such as those that affect heart's muscle, valves or rhythm, also are considered forms of heart disease, Coronary heart disease (CHD), Stroke, Aortic disease (Mayo, 2014).

Diclofenac, a nonselective non-steroidal anti-inflammatory drug (nonselective NSAID), classified as COX-2 inhibitor rofecoxib, has been widely used as an anti-inflammatory, analgesic, and antipyretic drug for the treatment of arthritis, back pain, gout, headaches and fever. Medication with diclofenac has many adverse effects on gastrointestinal, renal, hepatic, and the cardiovascular (CV) system (Bort et al. 1998; Kearney et al. 2006). Clinical observations have shown that long-term treatment with diclofenac correlates with the onset or aggravation of the congestive heart failure (CHF), which can cause serious CV thromboembolic events, such as myocardial infarction and stroke (Hudson et al. 2007; Waksman et al. 2007). A recent systemic study claimed that diclofenac has the highest CV risk score of the nonselective NSAIDs (McGettigan and Henry, 2006).

Mistletoe (*Loranthus bengwensis*) an ever green parasitic plant is an excellent medicinal herb that attach itself to the branch of a tree or shrubs, penetrating deep into the tree and thereby absorbing water and nutrient from the host plant. The name mistletoe originally referred to the speices *Viscum album* and letter was classified into different genera and even families such as the Misodendraceae, Loranthaceae. *Loranthus bengwensis* a member of the Loranthesceae family is specific to Africa, and is found mainly in the tropics. It is also known by many natives name in Nigeria such as Ewe Afomo (in Yoruba), Awuruse (in Igbo). The chemical composition of mistletoe varies, depending on the host tree (Luczkiewics et al.

2001; Stein and Berg, 1997). Mistletoe grown on an apple tree has the strongest pharmacologic effects (Hulsen et al. 1986). Mistletoe (*Lanrantus bengwensis*) contains more than 67 nutrients and 35 types of antioxidants. Mistletoe is used to cure about more than hundreds of diseases and almost all the vitamins are found in the vegetable.

Mistletoe leaves contain choline and acetylcholine. Though these compounds act directly on the autonomic nervous system, the berries contain alkaloids and toxic substances and should not be ingested (Ogunmefun et al. 2010). However, some medicinal plants have sufficient pre-clinical and more clinical data, indicating their botanical dietary supplement and their constituents that have a possible use in strategies' to reduce the prevalence's and mortality of cardiovascular diseases, cancer, diabetes and many more diseases, either in general population or subsets of individual at high risk. These plants prevent lipid peroxidation that initiates some of these diseases because of its antioxidant properties (Mahady, 2005). In view of the above considerations the present study was designed to investigate the antitoxic, antioxidant, chemo-protective effects of Mistletoe (*Loranthus bengwensis*) aqueous leaf extract on heart and liver diclofenac induced toxicity using male Wistar albino rats.

MATERIAL AND METHOD

Sample collection and preparation

Mistletoe (*Loranthus bengwensis*) leaves were collected from a branch of a cocoa tree plantation at Ifaki-Ekiti and were authenticated at the department of Plant Science, Ekiti State University, Ado-Ekiti.

Extract preparation

The fresh mistletoe leaves collected were washed without squeezing to remove debris and dust particles. The leaves were then air dried at room temperature for two weeks, blended to powdering form with the aid of a laboratory blender. 10g of the powdered mistletoe was extracted with 100ml of distilled water, shaken overnight on mechanical shaker which was filtered using muslin cloth to obtain 10% aqueous extract.

Experimental protocol

The study was performed on twelve male Wistar albino rats, 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 were divided into

three groups of four rats each. The drug administrations and extract treatment lasted for three weeks.

Group 1: Control animals fed with feed and water only.

Group 2: Negative control induced with 50mg/kg body weight of diclofenac.

Group 3: Treated animals induced with 50mg/kg body weight of diclofenac in addition with 10% aqueous extract of cocoa mistletoe.

Preparation of Organ homogenate

The heart and the liver were harvested from the rats, each being weighed on a chemical balances and placed in a separate tubes. 10% of each organ homogenate was prepared in 6.7mmole of potassium phosphate buffer, (pH 7.4) at the concentration ratio of 1:10 weight per volume using hand held electrically driven homogenizer. The homogenate were centrifuged using refrigerated centrifuge at 6,000 rpm for 10 minutes at 8^{o}C in order to obtain a clear supernatant which was stored at 4^{0}C and used for measurement of biochemical assays.

Biochemical Analyses

Fortress standard diagnostic kits were used in the determination of Aspartate aminotransferase (AST), Alkaline phosphate (ALP), Alanine aminotransferase (ALT) while Catalase and malondialdehyde were analyzed using standard methods.

Alanine-Aminotransferase (ALT)

Principle

Alanine-aminotransferase or ALT is a transaminase enzyme also known as serum glutamic-pyruvate transaminase (SGPT). ALT is found at highest concentration in the liver, but also found in the red blood cells, heart cells, muscle tissue and other organs.

ALT catalyzes the transmination of L-alanine to α-ketogluterate forming Pyruvate and L-glutamate. The pyruvate formed is reduced to lactate by lactate dehydrogenase (LDH) with simultaneous oxidation of nicotinamide-adenine dinucleotide (NADH).

$$\propto -oxoglutarate + L-alanine \longrightarrow L-glutamate + pyruvate$$

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

Aspartate-Aminotransferase (AST)

Principle

Aspartate-aminotransferase (AST) also known as serum glutamic oxaloacetic transaminase (GOT). It's an enzyme that is found at higher concentration in the liver and less concentration found in other organs. The enzyme catalyzes the transamination of L- aspartate to α-ketoglutarate.

L-Aspartate + 2-Oxaloacetate + L-Glutamate ⟶ Oxaloacetate + L-Glutamate

Determination of Malondialdehyde (MDA)

Lipid peroxidation was determined by measuring the formation of thiobarbituric acid reactive substances (TBARS) according to the method of (Varshney and Kale, 1990). An aliquot of 0.4mL of the plasma or other organ homogenates was mixed with 1.6mL of Tris-KCl buffer to which 0.5mL of 30% trichloroacetic acid (TCA) was added. Then 0.5mL 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 532nm. The MDA level was calculated according to the method of (Adam-Vizi and Seregi, 1982). Lipid peroxidation in units/mg protein or gram tissue was computed with a molar extinction coefficient of 1.56 x 105 $M^{-1}Cm^{-1}$.

Calculation

$$\text{MDA (units/mg protein)} = \frac{\text{Absorbance x volume of mixture}}{\text{E532nm x volume of sample x mg protein}}$$

Determination of Catalase activity

This experiment was carried out using the method described by (Sinha, 1972). 0.2mL of sample was mixed with 0.8mL distilled H_2O to give 1 in 5 dilution of the sample. The assay mixture contained 2mL of solution (800µmol) and 2.5mL of phosphate buffer in a 10mL flat bottom flask. 0.5mL of properly diluted enzyme preparation was rapidly mixed with the reaction mixture by a gentle swirling motion. The reaction was run at room temperature. A 1.0mL portion of the reaction mixture was withdrawn and blown into 1.0mL dichromate/acetic acid reagent at 60 seconds intervals. The hydrogen peroxide content of the withdrawn sample was determined by the method described above. The mononuclear velocity constant, K, for the decomposition of H_2O_2 by catalase was determined by using the equation for a first-order reaction: K = 1/t log S0/S, where S0 is the initial concentration of H_2O_2 and S

is the concentration of the peroxide at t min. The values of the K are 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 Katalase feiahigkeit or 'Kat f' according to (von Euler and Josephson, 1927).

$$\text{Kat. f} = \frac{\text{K (0)}}{\text{mg protein/mL}}$$

A unit of Catalase activity was defined as an absorbance change of 0.01 as units/min.

Statistical Analysis

Data were evaluated using the statistical test of variance one-way ANOVA, and $p< 0.05$ was considered significant, the results were expressed as mean ± SD (standard deviation) and the probability of significance donated by superscript (a, b, c), which was used to compare the changes occurring due to the effect of the extract drug on the plasma, liver and heart to prove the chemo-protectiveness effect of Mistletoe (*Loranthus bengwensis*) on diclofenac induce cardiovascular disordered in Wistar albino rats.

RESULTS AND DISCUSSION

The values are given as Mean±SD for all the parameters of all the groups. Superscript a, b, c within a column signifies that means with different superscript differs significantly at $p<0.05$, while mean's with the same superscript do not differ significantly at $p<0.05$ (using one way ANOVA with Duncan multiple range test).

Table 1.0: Effect of Aqueous Extract of Mistletoe (*Loranthus bergwensis*) leaf on Alanine aminotransferase (ALT) level in diclofenac induced toxicity using Wistar Albino rats.

ALT (U/L)			
	Plasma	**Liver**	**Heart**
Group 1	3.79 ± 0.89^{a}	5.16 ± 1.76^{c}	5.10 ± 1.44^{a}
Group 2	4.28 ± 2.23^{b}	3.25 ± 0.03^{a}	7.39 ± 1.25^{c}
Group 3	3.69 ± 0.35^{a}	3.57 ± 0.10^{b}	6.41 ± 0.81^{b}

The same letter shows they are significantly different from normal control group ($p<0.05$).
Different letter shows significantly different from normal and controls ($p<0.05$).

Table 1 shows the result of Alanine aminotransferase analysis in which there was a significant increase ($p < 0.05$) in the plasma of diclofenac treated animals as seen in Group 2

which also witnessed a significant decrease and restoration to normal in the plasma of Group 3 animals in the aqueous extract of mistletoe treatment. Conversely, a comparative significant decrease ($p < 0.05$) in the ALT activity was observed in the liver in Group 2 animals when compared to Group 1normal control animals as a result of diclofenac treatment. However, treatment with the aqueous extract of mistletoe in Group 3 resulted in slightly significant increase to restore to the control value. There was a significant increase in the heart ALT activity in the diclofenac treated animals in Group 2 while treatment with the plant extract resulted in a significant decrease that tend to restore to the control value. The overall result of the ALT activity showed that diclofenac caused significant depletion of the enzyme in the liver but aggravated in the heart, though treatment with the extract produced a reversal in both organs in Group 3. Alanine aminotransferase is a known biomarker enzyme used to predict possible toxicity in organs (Rahman et al. 2001). The marker enzyme is mostly found higher in the liver than any other body tissue, and used as a clinical and toxicology measurement to indicate heart damage just as observed in the liver damage by toxicants/oxidants or in diseased conditions (Singh et al. 2010). It can be deduced from Table 1 that the elevation in the plasma alanine aminotransferase and reduction of the enzyme in the liver indicates a hepatocellular injury leading to liver damage, also the elevation in the ALT level in the heart compared to the normal control in group 1 may indicate a disease condition of the heart which may lead to cardiac disorder which may also suggest a leakage of the enzyme from other organs like the liver, thereby causing an increase in the heart which is as a result of the inducement with diclofenac drug. Furthermore in this study, mistletoe orally administered to the animals in Group 2 resulted to elevating the concentration of this enzyme which was also restored to normal in both the plasma and the heart in Group 3. However, aqueous extract of mistletoe showed chemoprevention and organ protection against diclofenac induced toxicity, which was similar to what was observed by Oseni et al. (2015) in effects of aqueous extract of nutmeg in potassium bromate induced organ toxicity.

Table 2.0: Effect of Aqueous Extract of Mistletoe (*Loranthus bergwensis*) on Aspartate aminotransferase (AST) level in diclofenac induced toxicity using Wistar Albino rats.

AST (U/L)			
	Plasma	**Liver**	**Heart**
Group 1	6.76 ± 0.00^{a}	25.27 ± 0.28^{c}	19.18 ± 0.92^{c}
Group 2	13.29 ± 0.77^{c}	15.75 ± 0.55^{a}	12.64 ± 0.17^{a}
Group 3	11.20 ± 2.59^{b}	18.26 ± 2.80^{b}	17.30 ± 0.65^{b}

The same letter shows they are significantly different from normal control group ($p<0.05$).

Different letter shows significantly different from normal and controls ($p<0.05$).

In Table 2, there was a significant increase in the plasma AST activity ($p < 0.05$) in Group 2 diclofenac induced rat, while significant reduction was noted in both the heart and liver when compared to Group 1 normal control animals. Treatment with aqueous extract of mistletoe leaf however showed statistically significant decreases in the AST plasma activity, while the enzyme was elevated in both the liver and heart of the aqueous extract of mistletoe treated animals in Group 3.

The elevation in the level of Aspartate aminotransferase (AST) in the plasma of Group 2 is an indication of leakage of the enzyme from damaged organs. The results obtained in this study corroborate those of Obi, et al. (1998) and Reinke, et al. (1988) who also reported the elevation in the plasma content of the AST which could be an indication of heart damage. AST is located in the cell cytoplasm and is emptied into the circulation once the cellular membrane is damaged. Treatment of the animals with the plant aqueous extract significantly reduced the plasma AST, thereby increasing its concentrations in liver and the heart when compared with values in Group 2.

Table 3: Effect of Aqueous Extract of Mistletoe (*Loranthus bergwensis*) leaf on Alkaline Phosphatase (ALP) level in diclofenac induced toxicity using Wistar Albino rats.

ALP (U/L)			
	Plasma	**Liver**	**Heart**
Group 1	20.47 ± 4.67^{a}	22.50 ± 0.71^{ab}	33.81 ± 4.88^{b}
Group 2	35.77 ± 2.66^{c}	19.45 ± 2.51^{a}	60.21 ± 5.28^{c}
Group 3	26.63 ± 1.04^{b}	23.52 ± 2.04^{b}	23.12 ± 1.49^{a}

The same letter shows they are significantly different from normal control group ($p<0.05$).
Different letter shows significantly different from normal and controls ($p<0.05$).

In Table 3, the result of alkaline phosphatase (ALP) analysis in the experimental rats shows a significant increase ($p < 0.05$) in both the plasma and heart after the inducement with diclofenac in comparison to the normal control Group 1. But thereafter a significant decrease ($p < 0.05$) in both heart and plasma with a significant increase in the liver was noted in the rat treated with mistletoe extract (Group 3). The significant increase ($p < 0.05$) in ALP concentration observed in the heart of Group 2 (diclofenac treated) compared with the control may be attributable to loss of membrane components due to a possible reaction between diclofenac sodium atoms and the heart tissues. Therefore, enzymes from diseased organs may become manifested in the plasma resulting in increased concentration of ALP, since it must have leaked from the diseased liver. The increased concentration of the plasma enzyme is

often accompanied by a corresponding decrease in enzyme activity of the liver. While the pronounced reduction in ALP activity of the plasma and heart and increase in the liver may be attributed to the fact that the aqueous extract of mistletoe has a major role of chemoprevention and organ protection.

Table 4.0: Effect of Aqueous Extract of Mistletoe (*Loranthus bergwensis*) leaf on the Catalase level in diclofenac induced toxicity using Wistar Albino rats.

Catalase activity (unit/mg)			
	Plasma	**Liver**	**Heart**
Group 1	0.007 ± 0.00^{b}	0.009 ± 0.00^{c}	0.009 ± 0.00^{b}
Group 2	0.006 ± 0.00^{a}	0.006 ± 0.00^{a}	0.006 ± 0.00^{a}
Group 3	0.008 ± 0.00^{c}	0.008 ± 0.00^{b}	0.007 ± 0.00^{a}

The same letter shows they are significantly different from normal control group ($p<0.05$).
Different letter shows significantly different from normal and controls ($p<0.05$).

From Table 4 above, a significant reduction ($p < 0.05$) in catalase activity in all the plasma and studied tissues of the rats treated with diclofenac in Group 2 in comparison withGroup 1, while a significant increase ($p < 0.05$) was observed in the extract treated rats in Group 3 when compared with Group 2.This study reveals the effects of aqueous extracts of Mistletoe (*Loranthus bergwensis)* leaf treatments on the catalase activity of diclofenac-induced organ toxicities in rats and the reduction in catalase activity after the administration of diclofenac is another significant finding in this study. Catalase protects cells from the accumulation of H_2O_2 by debarring it from forming H_2O and O_2 or by using it as an oxidant in which it works as a peroxidase (Dhaliwal et al. 1991). The decreased concentration of catalase is attributable to the reduction in the synthesis of the antioxidant enzyme, whose concentrations would have fallen with the administration of diclofenac to the rats. While treatment with aqueous extracts of mistletoe leaf causes a significant increase in catalase activity in all of the tissues analyzed.

Table 5.0: Effect of Aqueous Extract of Mistletoe (*Loranthus bergwensis*) leaf on Malondialdehyde (MDA) level in diclofenac induced toxicity using Wistar Albino rats.

MDA concentration (mmol/mL			
	Plasma	**Liver**	**Heart**
Group 1	1.26 ± 0.16^{a}	1.35 ± 0.92^{a}	1.11 ± 0.13^{a}
Group 2	1.49 ± 0.38^{c}	3.22 ± 0.55^{c}	2.14 ± 0.85^{b}
Group 3	1.38 ± 0.22^{b}	2.31 ± 0.78^{b}	1.28 ± 0.04^{a}

The same letter shows they are significantly different from normal control group ($p<0.05$).
Different letter shows significantly different from normal and controls ($p<0.05$).

Table 5.0 shows a significant increase ($p < 0.05$) in lipid peroxidation index (MDA) both tissues in Group 2 when compared to the normal control. The concentrations of MDA significantly decreased ($p < 0.05$) in the tissues when mistletoe extract was administered to the diclofenac induced rats of Group 3.MDA is a product of lipid peroxidation, an increase in the heart, liver and plasma MDA levels is an indication of elevated level of lipid peroxidation. Extensive lipid peroxidation leads to disorganization of the membrane by peroxidation of unsaturated fatty acids which also alters the ratio of poly-unsaturated to other fatty acids. This would lead to a decrease in the membrane fluidity and the death of cell. There is a growing consensus among workers that diclofenac induced-heart damage occurs by the production of a trichloromethyl radical from diclofenac when it is reductively dechlorinated. The trichloromethyl radical production abstracts a hydrogen atom from fatty acid to form a lipid radical that reacts with molecular oxygen. The product of such reaction is the initiation of lipid peroxidation (Matthew, 2007). From this study however, elevated plasma level of malondialdehyde which was induced by diclofenac drug in the studied organs, indicate increase in the level of production of oxygen free radicals, suggesting a possible role in atherosclerosis, leading to coronary heart disease and liver disease (Mudassir et al. 2000).Since the above mechanism is suggestive of the process of oxidative stress, it is true, therefore, that any natural product with antioxidant property will prevent or reverse lipid peroxidation; including cell membrane damage which was seen in the action of cocoa mistletoeaqueous extract when administered to diclofenac induced rats.

CONCLUSION AND RECOMMENDATION

Diclofenac is a potent cardiotoxic agent as it enhances lipid peroxidation with significant reduction in the activities of heart antioxidant capacity. It also caused cardiac dysfunction and hepatocyte injury as revealed in marked increase in liver, heart and plasma AST, ALT and ALP. The present study has also shown that diclofenac portend serious damaging effects on both heart and liver cells as evidenced by reduced activity of catalase in the studied tissues. Though diclofenac serves as a non-steroidal drug used in the relief of acute or chronic pain states caused by inflammatory and degenerative forms of rheumatism; rheumatoid arthritis and osteoarthritis, the oral administration or intake of the drug may result to increased risk of serious cardiovascular disease, including myocardial infarction, stroke and as such should be avoided.

This preliminary study has been able to demonstrate the cardiotoxic effect of diclofenac and cardioprotective potentials of aqueous extract of *Mistletoe* leaf on diclofenac-induced rats. The study shows the chemo-preventive benefit of extract of mistlletoe *(Loranthus bergwensis)* leaf on diclofenac mediated cardiac oxidative damage in rat as they significantly reduced the extent of antioxidant loss and restoration of cardiac and liver dysfunction caused by diclofenac in rat to normal.

The results of this study shows protective effect on heart function including the liver which might be due to the presence of some bioactive compound in the plant extract. Further investigation should be conducted to identify this bioactive compounds present in Mistletoe leaf aqueous extract.

REFERENCES

1. Adám-Vizi V, Seregi A. Receptor independent stimulatory effect of noradrenaline on Na,K-ATPase in rat brain homogenate. Role of lipid peroxidation. Biochem Pharmacol, 1982; 31: 2231-6.
2. Bort R, Ponsoda X, Jover R, Gomez-Lechon MJ, Castell JV. Diclofenac toxicity to cardiac tissues: a role for drug metabolism in cell toxicity. J Pharmacol Exp Ther, 1998; 288: 65−72.
3. Dhaliwal H, Krishenbaum LA, Randhawa AK, Singal PK. Correlation between antioxidant changes during hypoxia and recovery on oxygenation. Am J Physiol, 1991; 261: H632-H638.
4. Hudson M, Rahme E, Richard H, Pilote L. Risk of congestive heart failure with nonsteroidal anti-inflammatory drugs and selective cyclooxygenase 2 inhibitors: a class effect? Arthritis and Rheumatism, 2007; 57: 516−23.
5. Hulsen H, Dosen C, Mechelke F. Differences in the in vitro effectiveness of preparations produced from mistletoe of various trees. Arzneimittelforschung, 1986; 36: 433-36.
6. Kearney PM, Baigent C, Godwin J, Halls H, Emberson JR, Patrono C. Do selective cyclo-oxygenase-2 inhibitors and traditionalnon-steroidal anti-inflammatory drugs increase the risk ofatherothrombosis? Metal-analysis of randomized trials. Br Med J, 2006; 332: 1302−08.
7. Luczkiewics M, Cissowski W, Kaiser P. Comparative analysis of phenolic acids in mistletoe plants from various hosts. Acta Poloniae Pharmaceutica, 2001; 58: 373–79.

8. Mahady GB. Medicinal plants for the prevention and treatment of coronary heart disease ETHNOPHAMACOL, 2005; II. http://www.eolss.net/Eolss-sampleAllchapter.aspx
9. Matthew OW, Blessing CD. Hepatoprotecive effects of Garcinia Kola seed against heptoxicity induced by carbon tetrachloride in rat. BIOKEMISTRI, 2007; 19(1): 17-21.
10. Mayo Clinic, "Heart-healthy living, Heart disease". Med. Education and Research, 2014.
11. McGettigan P, Henry D. Cardiovascular risk and inhibition of cyclooxygenase: a systemic review of the observational studies of selective and nonselective inhibitors of cyclooxygenase-2. J Am Med Assoc, 2006; 296: 1633−44.
12. Mudassir AK, Abdul B. Increased malondiadehyde level in coronary heart disease. JPMA, 2000; 50: 261.
13. Obi FO, Usenu LA, Osayande JO. Prevention of carbon tertrachlorideinduced hepatotocixity in the rat by H.rosainensis anthocyanin extract administered in ethanol. Toxicology, 1998; 131: 93-8.
14. Ogunmefun OT, Fasola TR, Saba AB, Oridupa OA. The Ethnobotanical, Phytochemical and Mineral Analyses of *Phragmanthera Incana* (Klotzsch), A Species of Mistletoe Growing on Three Plant Hosts in South-Western Nigeria. Int. J. Biomed Sci, 2010; 9(1): 33–40.
15. Oseni OA, Olagboye SA, Idowu ASK. Potassium Bromate Induced Renal Toxicity in Wistar Albino Rats: Effects of Aqueous Extract of Nutmeg (*Myristica fragrans* Houtt). British Journal of Medicine & Medical Research, 2015; 5(12): 1547-56.
16. Rahman MF, Siddiqui MK, Jamil K. Effects of vepacide (Azadirachta indica) on aspartate and alanine aminotransferase profiles in sub-chronic study with rats. J. Hum. Exp. Toxicol, 2001; 20: 243–49.
17. Reinke LA, Lai EK, McCay PB. Ethanol feeding stimulatestrichloromethyl radical formation fromcarbon tetrachloride in liver. Xenobiotics, 1988; 18: 1311-18.
18. Singh K, Ram M, Kumar A. Detetmination of Alanine amino transaminase (AST/GPT) in clinical samples. Hort, 2010; 851: 81-8,
19. Sinha AK. Colorimetric assay of catalase. Anal Biochem, 1972; 47: 389-94.
20. Stein GM, Berg PA. Mistletoe extract induced effects on immunocompetent cells: in vitro studies. Anticancer Drugs, 1997; 8: 539–41.
21. Varshney R, Kale RK. Effects of Calmodulin Antagoniss. Int. J Rad. Biol, 1990; 58: 733-43.
22. Von Euler HV, Josephson K. European Journal of Organic Chemistry - EUR J ORG CHEM, 1972; 452(1): 158-81.

23. Waksman JC, Brody A, Phillips SD. Nonselective nonsteroidal anti-inflammatory drugs and cardiovascular risk: are they safe? Ann Pharmacother, 2007; 41: 1163−73.