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Research Paper

Lipid profile and protective effects of aqueous extracts of some leguminous plant sprouts against doxorubicin-induced organs impairment in female wistar rats

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Abstract. Doxorubicin is one of the anthracycline drugs used in the treatment of cancer but with an attendant cardiotoxicity. Hence, this study aims to investigate the lipid profile and the protective effects of aqueous extract of seedlings of three leguminous plants: honey beans, big white beans (*Vigna unguiculata*) and African yam beans (*Spenostylis stenocarpa*) on doxorubicin induced organs damage in female Wistar albino rats. The sprouts of the three beans were obtained after 4 weeks of planting and the respective aqueous extracts were obtained and used to treat female rats that were induced with different concentrations of doxorubicin per unit body weight. The animals were sacrificed under chloroform anesthesia after induction and treatment for 28 days. The total cholesterol, HDL-cholesterol, LDL-Cholesterol, triglycerides and lipid peroxidation were determined from plasma, liver, heart and kidney using standard methods. The results showed that doxorubicin significantly and progressively lowered the cardiac tissues triglycerides, high density lipoprotein-cholesterol (HDL-cholesterol) and total cholesterol, while significantly causing elevation in low density lipoprotein-cholesterol (LDL-Cholesterol) and malondialdehyde (MDA) concentrations when compared to the control group, while 10% aqueous extract of the various sprouts produced significantly, the reversal to the control level in concentration dependent manners. These results however showed that doxorubicin is a potential organ impaired drug, while the aqueous sprouts extracts produce protective effects on the organs which support the folkloric use of aqueous extract of the plant in the management of suspected patients with organs impairment.

Keywords: Leguminous-plants, aqueous-extract, tissue-toxicity, lipid-profile, organ-protection.

INTRODUCTION

Cancer chemotherapy has made remarkable advances in the treatment of both solid and hematologic malignancies, allowing in many patients the hope for a cure of their cancer. However, these therapies are not without their complications. A wide range of chemotherapy agents used in the treatment of breast cancer has been associated with cardio-toxicity (Floyd *et al*., 2005; Monsuez *et al*., 2010). Some of these chemotherapy substances cause acute cardiac depression as they lower heart rate, contractility and conduction, and in certain causes even cardiac arrest. These substances include barbiturates (thiopental) or halogenated hydrocarbons (halothane, metoxyflurane and enflurane), even at concentrations used in surgery. However, many of drugs are administered chronically and are cardiotoxic and may trigger the development of cardiac injury even when used appropriately. The anthracyclines and related compounds (doxorubicin, daunorubicin, idarubicin, epirubicin, and the anthraquinone mitoxantrone) are some of the most frequently implicated agents (Singal and Iliskovic, 1998). The anthracycline anticancer drug doxorubicin is an effective and frequently used chemotherapeutic agent for various malignancies (Monsuez *et al*., 2010; Floyd *et al*., 2005). Its major adverse effect is cardiotoxicity, which may limit its use. Doxorubicin cardiomyopathy, once

developed, carries a poor prognosis and is frequently fatal (Singal and Iliskovic, 1998; Monsuez *et al*., 2010).

The present study therefore attempts to assess the lipid profile and protective effects of aqueous extract of seedlings of three leguminous plants: honey beans, big white beans (*Vigna unguiculata*) and African yam beans (*Spenostylis stenocarpa*) on doxorubicin induced organs damage in female Wistar albino rats.

MATERIALS AND METHODS

Sample collection and preparation

Plant material

Leguminous seed grains (honey beans, big white beans (*Vigna unguiculata*) and African yam beans (*Spenostylis stenocarpa*) were bought at the Oja-Oba market in Ado-Ekiti, Nigeria and were authenticated at Department of Plant Science, Ekiti State, University, Ado-Ekiti, Nigeria. The various seeds were planted differently at the research garden near the College of Medicine, Ekiti State University and the sprouts were obtained at the expiration of 4 weeks.

Extract preparation

The sprouts of plants were collected and air dried under shade and ground into powder with Marlex Excella laboratory blender and preserved. 10% aqueous extracts of the sprouts were prepared.

Experimental protocol

The study was performed on sixty five female Wistar albino rats housed in ventilated cages in the Animal House of College of Medicine, Ekiti State University, Ado-Ekiti, Nigeria. Animals were divided into thirteen groups of five rats each; they were acclimatized for two weeks before administration of different dosages of the drugs and treatments with the extracts.

Group 1	Positive Control (animals given feed and water)
Group 2	Negative control (20 mg/kg doxorubicin induced animals)
Group 3	Negative control (40 mg/kg doxorubicin induced animals)
Group 4	Negative control (60 mg/kg doxorubicin induced animals)
Group 5	20 mg/kg doxorubicin induced animals + 10% honey beans sprout extract
Group 6	40 mg/kg doxorubicin induced animals + 10% honey beans sprout extract
Group 7	60 mg/kg doxorubicin induced animals + 10% honey beans sprout extract
Group 8	20 mg/kg doxorubicin induced animals + 10% big white beans sprout extract
Group 9	40 mg/kg doxorubicin induced animals + 10% honey beans sprout extract
Group 10	60 mg/kg doxorubicin induced animals + 10% honey beans sprout extract
Group 11	20 mg/kg doxorubicin induced animals + 10% Africa yam beans sprout extracts
Group 12	40 mg/kg doxorubicin induced animals + 10% Africa yam beans sprout extracts
Group 13	60 mg/kg induced animals + 10% Africa yam beans sprout extracts

Chemicals/reagent kits

All chemicals are of analytical grade, the doxorubicin used was obtained commercially. All the diagnostic kits are products of Fortress Chemical Ltd. England.

Preparation of organs homogenate

The animals were quickly dissected under chloroform anesthesia; the blood for plasma and organs (Heart, Kidney and Liver) were collected. 10% of each organ homogenate was then prepared in 6.7mM potassium phosphate buffer, (pH 7.4) using the top driven homogenizer. The homogenate was centrifuged at 10,000 rpm for 10 min at 4°C to obtain a clear supernatant which was stored at 8°C and used for measurement of biochemical parameters.

Biochemical assays

Fortress standard diagnostic kits from UK were used to determine total cholesterol, HDL-cholesterol, LDL-Cholesterol, triglycerides as malondialdehyde was determined using Varshney and Kale (1990) method from the plasma and organs homogenate.

Statistical analysis

The experimental results of the analyses were obtained in triplicates. Various formulae were used to calculate the individual parameters. The means and standard deviations of the triplicates results were determined which were then used to construct the bar charts using Microsoft Office Excel 2007.

RESULTS AND DISCUSSION

The effects of the aqueous extracts of the sprouts of

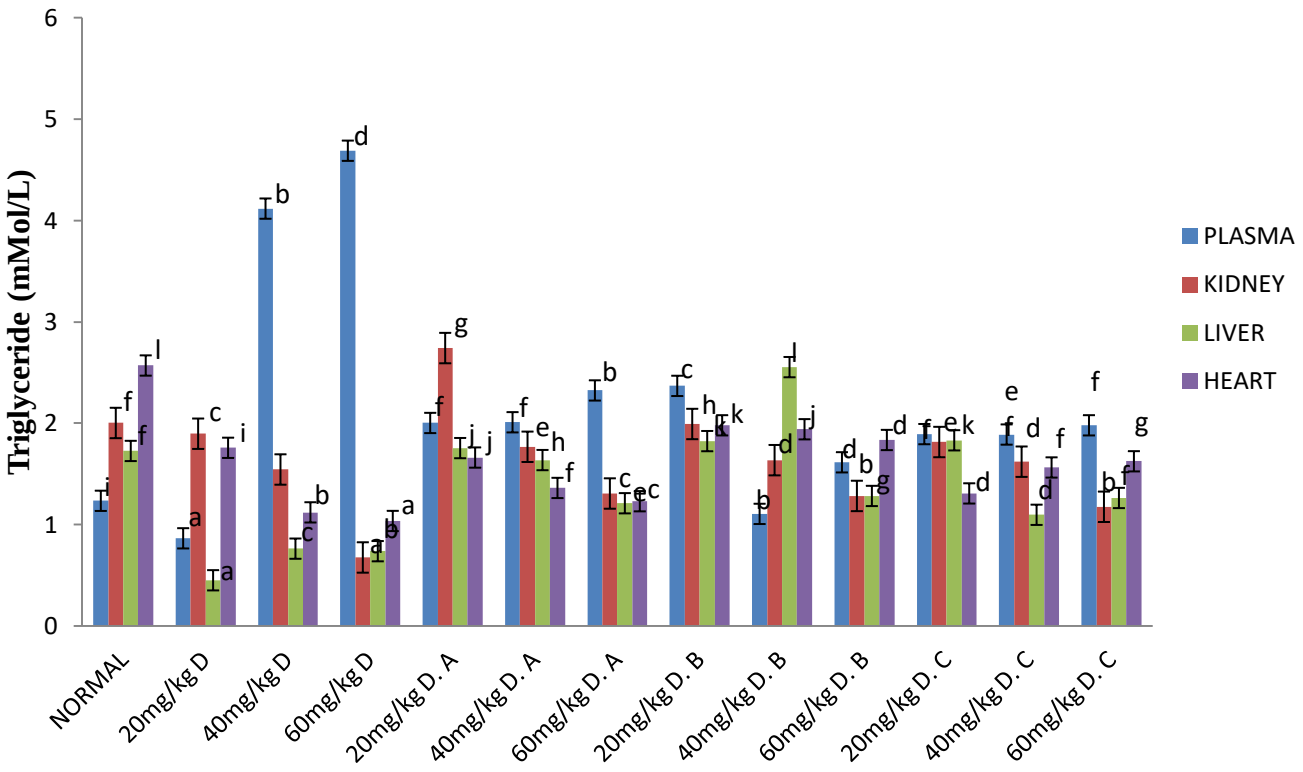

Figure 1. Effect of the Aqueous extracts of the sprouts of different edible leguminous plants on the concentration of Triglyceride (mMol/dl) in Doxorubicin-induced cardiac toxicity in Female Wistar Albino rats. Each value is a mean ± S.E.M., n = 3. Values not sharing a common superscript (a–j) differ significantly with each other ($p < 0.05$) in all the groups.

Key:
A = Honey beans
B = *Vigna unguiculata* (Big white beans)
C = *Spenostylis stenocarpa* (Africa yam beans)
20 mg/kg D= 20 mg/kg of Doxorubicin
40 mg/kg D= 40 mg/kg of Doxorubicin
60 mg/kg D= 60 mg/kg of Doxorubicin
20 mg/kg D.A = 20 mg/kg of Doxorubicin + 10% Aqueous extract of A
40 mg/kg D.A = 40 mg/kg of Doxorubicin + 10% Aqueous extract of A
60 mg/kg D.A = 60 mg/kg of Doxorubicin + 10% Aqueous extract of A
20 mg/kg D.B = 20 mg/kg of Doxorubicin + 10% Aqueous extract of B
40 mg/kg D.B = 40 mg/kg of Doxorubicin + 10% Aqueous extract of B
60 mg/kg D.B = 60 mg/kg of Doxorubicin + 10% Aqueous extract of B
20 mg/kg D.C = 20 mg/kg of Doxorubicin + 10% Aqueous extract of C
40 mg/kg D.C = 40 mg/kg of Doxorubicin + 10% Aqueous extract of C
60 mg/kg D.C = 60 mg/kg of Doxorubicin + 10% Aqueous extract of C

different edible leguminous plants of honey beans, big white beans (*Vigna unguiculata),* Africa yam beans *(Spenostylis stenocarpa*) on the concentration of triglyceride (mMol/dl) in doxorubicin-induced cardiac toxicity in female Wistar albino rats were studied in this work. Significant differences were observed in triglyceride concentration at ($p < 0.05$) in all the groups in both plasma and the studied organs after induction with doxorubicin; treatments with aqueous extracts of the sprouts also produced significant increase in the tissues triglyceride with corresponding decrease in the plasma triglyceride as shown in Figure 1 The results of total cholesterol were shown in Figure 2 which showed slight increase towards the control when treated with sprouts extracts after slight reduction with doxorubicin induction. The LDL-cholesterol in Figure 3 showed significant increase in the plasma, kidney and heart after induction with doxorubicin with significant reduction to normal when treated with sprouts aqueous extract, the reverse was observed in the liver. The results of HDL-cholesterol was significantly increased in the plasma and kidney but reduced in liver and heart during induction, but significantly increased in liver and heart with corresponding reduction in plasma and kidney as seen in Figure 4 while the results of MDA (Figure 5) showed that treatments with the 10% aqueous extracts of the three

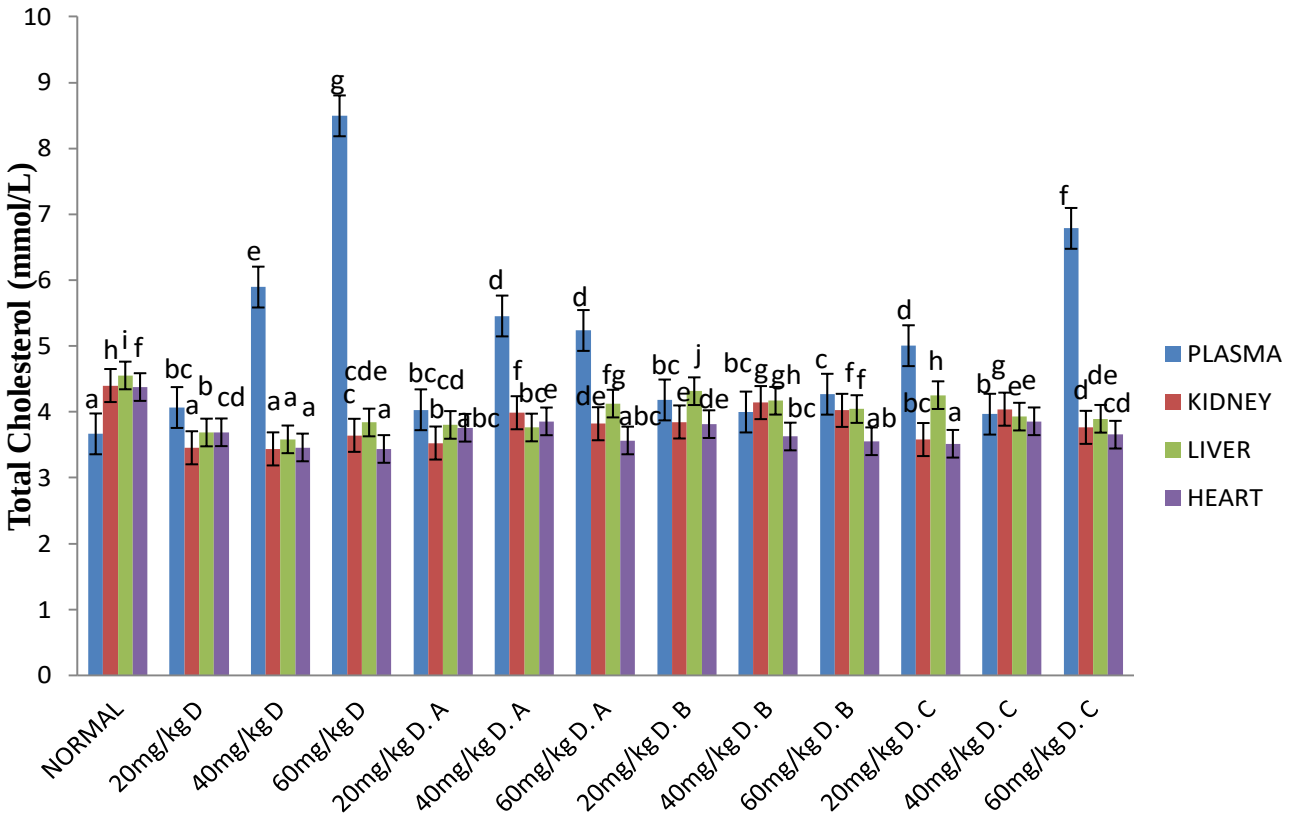

Figure 2. Effect of the Aqueous extracts of the sprouts of different edible leguminous plants on the concentration of Total Cholesterol (mmol/L) in Doxorubicin-induced cardiac toxicity in female Wistar albino rats. Each value is a mean ± S.E.M., n = 3. Values not sharing a common superscript (a–j) differ significantly with each other (P < 0.05) in all the groups.

sprouts on 20 mg/kg, and 40 mg/kg concentrations of doxorubicin produced significant increase in MDA in plasma, kidney, liver and heart while the reverse was the case for 60 mg/kg concentration of doxorubicin

Figures 1 and 2 depict the levels of triglycerides and total cholesterol in normal and doxorubicin-administered rats in the organs examined. The plasma levels of triglyceride and cholesterol were estimated at the beginning and the end of the administration of the drugs and the three extracts respectively. Administration of doxorubicin caused a significant (P < 0.05) increase in the levels of triglycerides and cholesterol in the plasma but a significant decrease in the other organs (kidneys, heart and liver), compared with positive control rats. This is related to the findings of Mohammed *et al*. (2009) in isoproterenol-induced myocardial infarction in rats. This shows the hyperlipidemic effect of doxorubicin. Hypercholesterolemia has been identified as a primary risk factor in the development of cardiovascular disease (CVD). This implies that, preventing or reducing the serum levels is associated with reducing risk of CVD (Onyeneke *et al*., 2008). However, after the rats' treatment with the extracts, significant improvement in the levels of triglycerides and cholesterol were observed in the plasma by the extracts of the three sprouts (honey beans, big white beans (*Vigna unguiculata*) and African yam bean (*Spenostylis stenocarpa*) used except in the heart which showed no significant change in the levels of cholesterol and triglycerides. There has been a growing body of evidence from epidemiologic, clinical and laboratory data indicating that elevated triglyceride levels are an independent risk factor for cardiovascular disease (Jeyadevi *et al*., 2012). Hypertriglyceridemic patients are at a risk for cardiovascular disease which often develops as lipoprotein profile characterized by elevated triglyceride, LDL and low HDL cholesterol which causes myocardial membrane damage. Hypertriglyceridemia observed in doxorubicin is clinically reported in isoproterenol treated rats and ischemic heart disease (Jeyadevi *et al*., 2012). Treatment with extracts prevented the elevation of triglycerides and cholesterol in the plasma, signifying that the myocardial membrane is intact and not damaged. This is also reported by Jeyadevi *et al*. (2012) in the study on isoproterenol treated rats. The study suggests that the intake of any of the extracts decrease the absorption of triglycerides and cholesterol, and these findings are in accordance with the fact that green tea intake also decreases the absorption of triglycerides and cholesterol (El-Sayed and Eslam, 2014). The observed significant fall in the cardiac concentrations

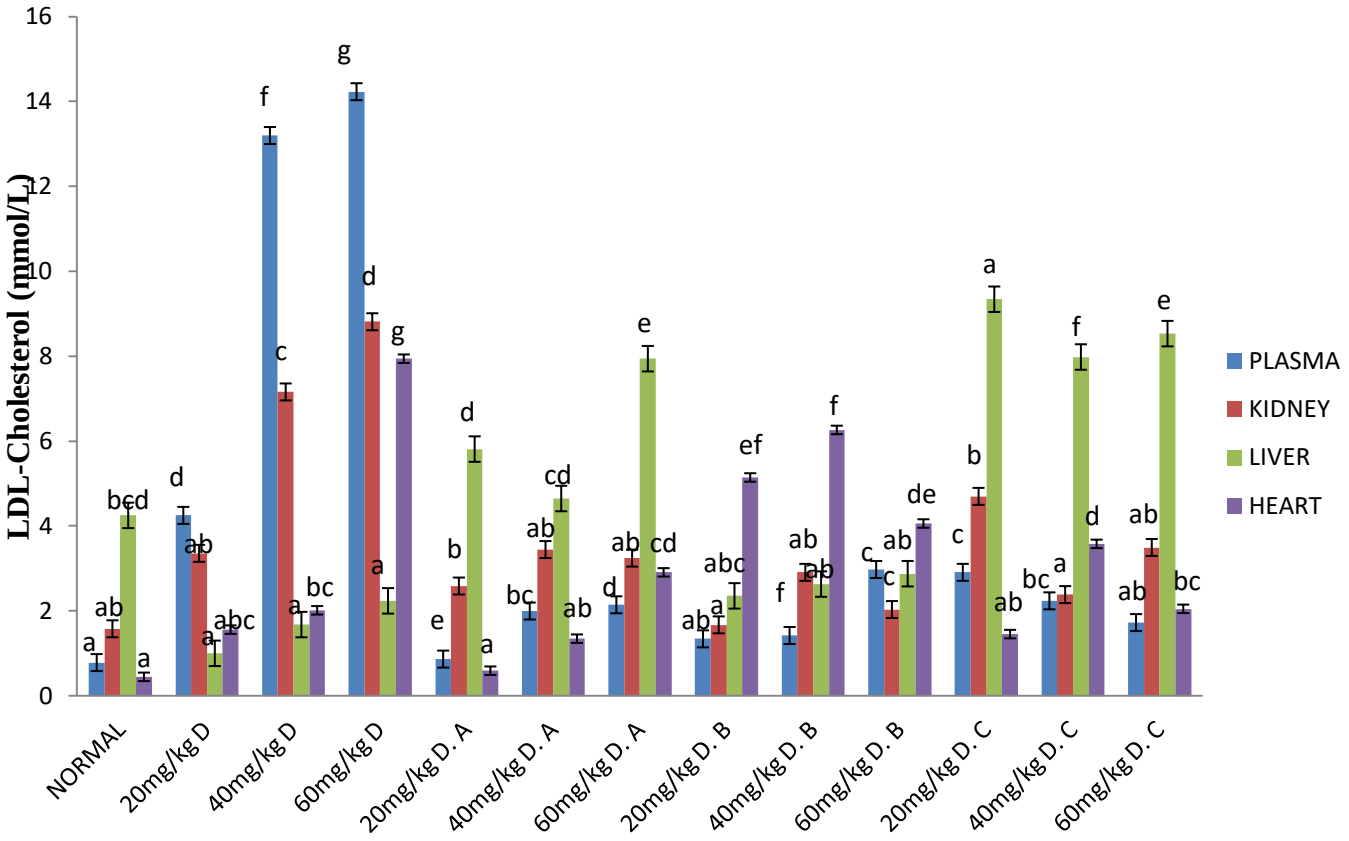

Figure 3. Effect of the aqueous extracts of the sprouts of different edible Leguminous plants on the concentration of LDL-Cholesterol (mmol/L) in Doxorubicin-induced cardiac toxicity in Female Wistar Albino rats. Each value is a mean ± S.E.M., n = 3 Values not sharing a common superscript (a–j) differ significantly with each other (P < 0.05) in all the groups.

of total cholesterol and triglyceride fractions could be due to depressed hepatic gluconeogenesis by doxorubicin, although this claim remains a speculation until it is subjected to further scientific validation by the key enzymes regulating this pathway. Triglycerides, which influence lipid deposition and clotting mechanisms, are reduced significantly in the groups treated with the extracts. A positive relationship between gluconeogenesis and lipogenesis has been well documented in literature (Harris and Crabbs, 1982). Any drug that interferes with gluconeogenesis has also been reported to also interfere with lipogenesis.

Data illustrated in Figures 3 and 4 show the effects of doxorubicin on LDL and HDL cholesterols in doxorubicin-induced cardiotoxicity in female rats. This profile is used to access the risk of cardiovascular disease and is altered in the serum or plasma of various disease states as demonstrated in diabetes (Betteridge, 1994; Nwangwu *et al*., 2009). LDL is one of the lipoprotein components of the blood. It transports cholesterol mainly to the arterial wall. This results in the buildup of insoluble lipid on the wall of the arteries thereby reducing blood flow and increases the pressure on the wall as well as the heart. The deposition of the cholesterol on the arterial wall results to a condition known as arteriosclerotic plaque which is the major cause of cardiovascular disease. Cardiovascular Diseases (CVD) are the leading cause of death in developing countries (Latunde-Dada, 1990). Apparently from the Figures 3 and 4; there were significant increase ($p \leq 0.05$) in levels of LDL in the plasma, kidney and heart of the various dosages of doxorubicin induced rats (Group 2 to 4) and a significant decrease was recorded in the liver of the induced rats when compared to normal Group1. Similarly, there were significant increase ($p \leq 0.05$) in levels of HDL in the plasma and kidney of the various dosages of doxorubicin induced rats, with significant fall observed in the liver and the kidney of the induced rats when compared to control group. LDL carries mostly cholesterol; this is what is left over from the VLDLs after most of the triglycerides are gone. It also delivers cholesterol to cells (away from liver). This is responsible for the decreased level of LDL cholesterol recorded in the liver. The significant increase in the plasma LDL in this study shows adverse effect of the drug because if LDLs are taken up by blood cells, it ultimately leads to atherosclerosis. Unlike LDL, HDL scavenges cholesterol from arterial plaques and dying cells and ultimately returns cholesterol to liver. But the

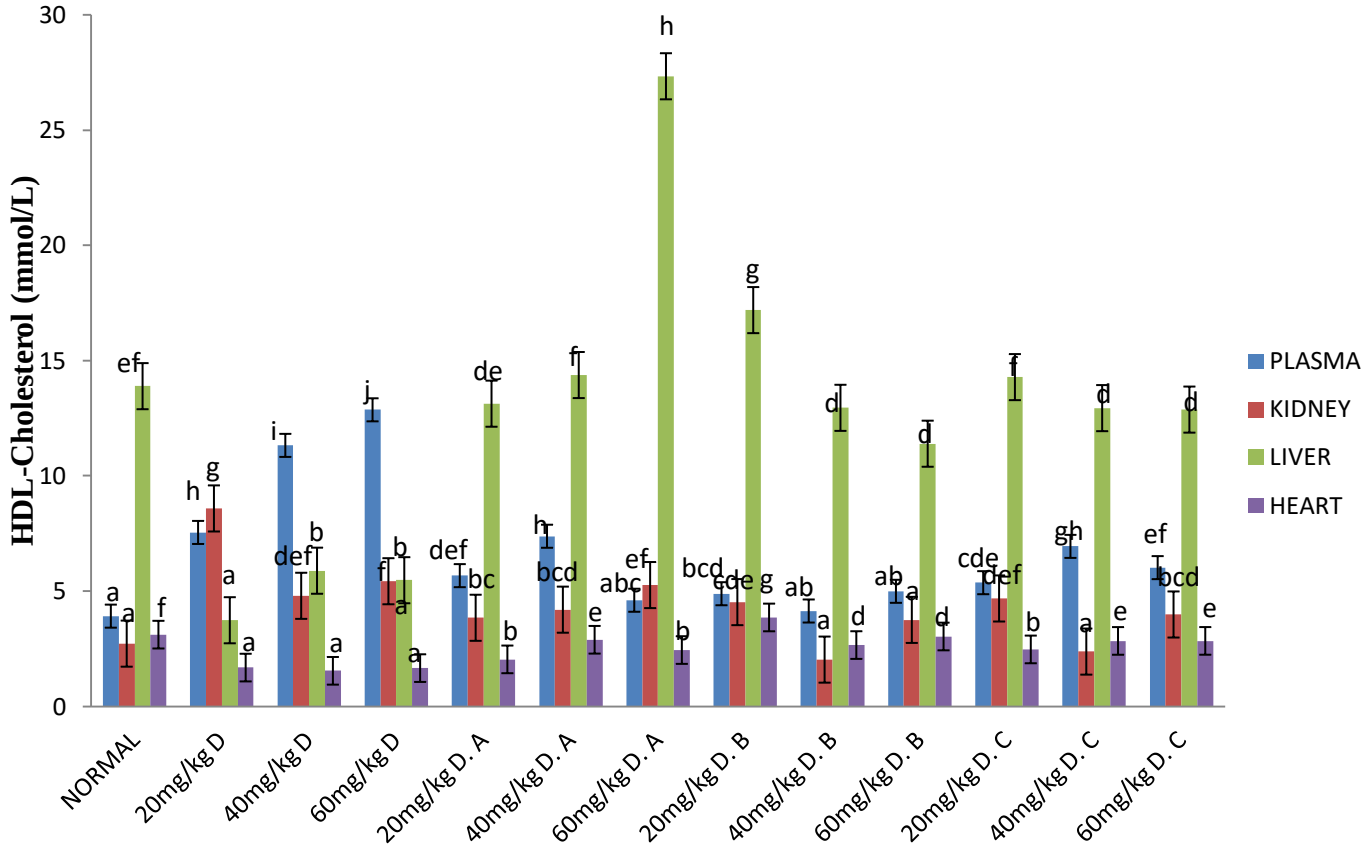

Figure 4. Effect of the Aqueous extracts of the Seedlings of different edible Leguminous plants on the concentration of HDL-Cholesterol (mmol/L) in Doxorubicin-induced cardiac toxicity in Female Wistar Albino rats. Each value is a mean ± S.E.M., n = 3. Values not sharing a common superscript (a–j) differ significantly with each other (P < 0.05) in all the groups.

reverse was the case on the effect of the drug (doxorubicin) because; there is still an increase in the plasma HDL and significant reduction in the liver. This means that the drug does not allow the scavenging of cholesterol from arterial plaques and dying cells. Similar results were reported by El-Sayed and Eslam (2014) on the effect of green tea on obese rats. Induced rats administered with the 10% aqueous extract of the seedlings showed significant decrease in both LDL and HDL levels in the plasma and kidney, with significant rise in liver and heart when compared to control negative groups. Rats administered with white beans extract showed the highest significant decrease in HDL in the plasma, while rats administered with African yam beans and honey beans extract showed the highest significant decrease in the level of LDL cholesterol when compared to control negative groups. LDL cholesterol, which is a strong atherogen since it favours lipid deposition in tissues including blood vessels, is significantly reduced in animals that were administered with the three extracts. From the observations in this study, the extracts can be useful for treatment of various complications resulting in hypercholesterolemia, cardiotoxicity and hypertension.

MDA level which is a measure of lipid peroxidation in tissues is also indicative of various disease conditions. The increase in the amount of MDA in the heart may result in the destruction of membrane integrity (Kempaiah and Srinivasan, 2005; Tauseef *et al*., 2007). Rats in groups induced with different dosages of doxorubicin (20, 40 and 60 mg/kg) body weight showed significant ($P < 0.05$) decrease in plasma, liver and kidney malondialdehyde levels when compared with normal control rats (Figure 5).

This absence of stimulatory effect on thiobabituric acid reacting species TBARS generation is in agreement with the work of Fraga *et al.* (1990), which did not show lipid peroxidation of cerebral or hepatic membranes after exposure to low doses of aluminum. But a significant increase in MDA level was observed in the heart. The figure also shows that the effect of the doxorubicin is not dose dependent in the liver, plasma and kidney because no significant difference was observed in doxorubicin induced groups. The decrease recorded in this study is against the previous studies that showed that aluminum exposure can increase lipid peroxidation rates (Fraga *et al*., 1990; Chugh *et al.*, 1995, 1996; Deloncle *et al.*, 1999; Osman *et al.*, 2010) and this effect seems to be dose dependent (Fraga *et al*., 1990). On the contrary, the

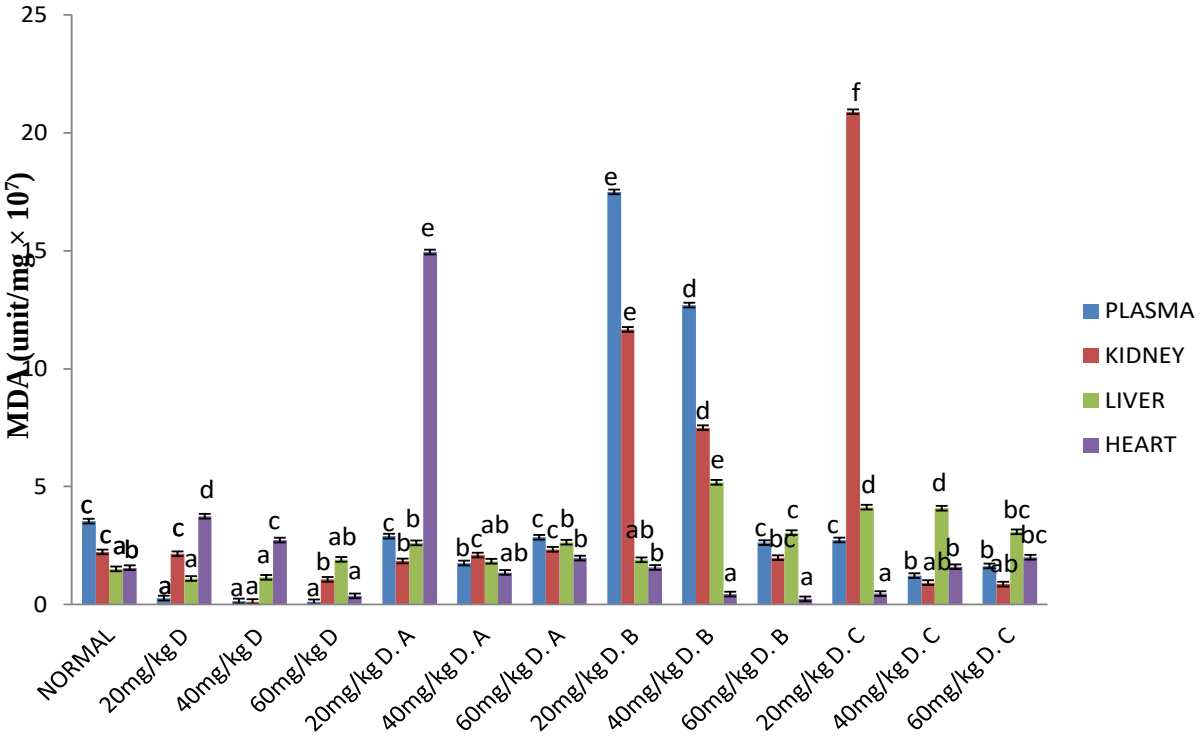

Figure 5. Effect of the aqueous extracts of the sprouts of different edible leguminous plants on the extent of lipid peroxidation measuring the malondialdehyde (MDA ($\times 10^7$)) concentration units/mg in Doxorubicin-induced cardiac toxicity in female Wistar albino rats. Each value is a mean ± S.E.M., n = 3. Values not sharing a common superscript (a–j) differ significantly with each other (P < 0.05) in all the groups.

observed increase in the amount of MDA in the plasma and decrease in the amount of MDA in the heart in all the groups treated with the various extract indicate that extracts effectively protect membrane integrity when compared with the negative control of the respective dosage. In this study, doxorubicin did not change lipid peroxidation rates in kidney.

CONCLUSION

The present study revealed that administration of doxorubicin not only caused impairment in cardiac functions but also caused marked impairment in renal function along with significant oxidative stress in the kidneys. The results also showed protective effects of the extracts on major organs functions which might be due to the presence of some bioactive compound in plants extracts.

The studied sprouts can be utilized to mitigate the scourge of this accompanied side effect in the use of this drug, doxorubicin for treatment of cancer.

Ethical approval

The author hereby declares that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

Competing interests

Author declared that no competing interests exist in this study.

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