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EFFECT OF AQUEOUS STEM EXTRACT OF DISSOTIS ROTUNDIFOLIA ON PLASMA CHOLESTEROL CONCENTRATION AND ALANINE AMINO TRANSFERASE ACTIVITY OF NORMAL ALBINO RATS

Inegbedion, A.[1]; Prohp T. P.[2] and James, I. L[2]
[1]*Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Ambrose Alli University, Ekpoma, Edo, State, Nigeria.*
[2]*Department of Biochemistry, Faculty of Basic Medical Sciences, College of Health Sciences, Niger Delta University, Wilberforce Island, Bayelsa State. Nigeria.*

Correspondence concerning this article to be addressed to:
Inegbedion[1], A.

Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Ambrose Alli University, Ekpoma, Edo, State, Nigeria.

GSM: +2348075358250, Email: mails4augusta@yahoo.com

Abstract

The effect of aqueous stem extract of Dissotis rotundifolia on plasma cholesterol concentration and alanine amino transferase activity in normal rats was examined. Ten (10) healthy female rats of Wistar strain weighing between 180-200g were used in this study. The rats were housed in clean cages under standard conditions of temperature, humidity and light. They were acclimatized for a period of two weeks to the new environment, after which, they were fasted overnight and were randomly placed into two(2) groups of five rats each. Group 1 Served as control and received pelleted growers mash and distilled water ad libitum for a period of 28 days. Rats in group 2 were administered aqueous stem extract of Dissotis rotundifolia orally, two times daily (morning and evening) with the aid of a gavage at a dose of 2ml/kg body weight and also fed with pelletd growers mash and distilled water ad libitum throughout the experimental period of 28 days. At the end of the experiment, blood was collected from the tail of the animals into immobilized lithium heparin bottles for analysis. Spectrophotometric methods were used in all analyses. Exposing the rats to aqueous stem extract of Dissotis rotundifolia significantly increased ($P<0.05$) plasma alanine amino transferase activity and cholesterol concentration compared to the control. Histological analyses of tissue sections showed necrosis of the liver, kidney and heart with the central vein of the liver occluded with blood, following the administration of extract. These results show that aqueous extract of Dissotis rotundifolia may not be a potent remedy against various human diseases. Appropriate precautions should therefore be taken in the use of aqueous extract of Dissotis rotundifolia.

Keywords**:** *Dissotis rotundifolia, plasma cholesterol, alanine amino transferase, occlusion, necrosis.*

INTRODUCTION

Herbal medicine has been explored and proven to be effective in the management of health requirement locally and internationally (WHO, 2002). *Dissotis rotundifolia* is a medicinal plant which is widely used *in the treatment and management of various disease conditions* (Mann *et al*., 2003). In Nigeria and Ghana, the dried leaves of *Dissotis rotundifolia* are used for the treatment of common cold, conjunctivitis, cough, diarrhoea, dysentery, fever, gonorrhea, headache, rheumatism, painful swellings, toothache, tuberculosis, while the leaves scorched by fire are placed on the sores of yaws (Burkill, 1985). The leaves and fruits of *Dissotis rotundifolia* are used as analgesic and anti-infective agents (Iwu, 1993), and the plant is a component of a commercial cough mixture known as "sirop de Dissotis" (Omulokoli *et al*., 1997). The leaves decoction is used to relieve stomach ache, diarrhoea, dysentery, cough, stop abortion, conjunctivitis, circulatory problems and venereal diseases (Gill, 1992). Researchers have reported pharmacological effects of *Dissotis rotundifolia* in various test models. These include anti-diarrhoea effects, antimicrobial and antitrypanosomiasis. In castor oil-induced diarrhea model, the ethanol extracts of the leaves of *Dissotis rotundifolia* was able to stop the release of wet faeces in rats (Abere *et al.*, 2010). Abere *et al.*, (2010) also reported that the active components of the leaves of *Dissotis rotundifolia* contain saponins, tannins and cardiac glycosides. Also some constituents of the whole plant extract of *Dissotis rotundifolia* have been characterized and the C-glycosyl flavones from the methanolic extract of the plant were isoorientin, orientin, vitexin and isovitexin (Rath *et al*., 1995). *Dissotis rotundifolia* is a creeping herb commonly found in Nigeria, and widely spread in West Africa (Wagner *et al*., 1990). It is a short lived perennial plant with bright pink flowers and ovate, fleshy leaves, prickly fruits, and a trailing or creeping growth habit (Abere *et al*., 2010, Porembski *et al*., 1996). The species *Dissotis rotundifolia* belongs to the Family (Malastomataceae), Kingdom (Plantae), Phylum (Magnoliophyta), Class (Magnoliopsida), Subclass (Rosidae) Order (Myrtales), Genus (Diss1otis) (USDA, NRC. 2010). About 140 species of genus *Dissotis* (Melastomataceae) are native to Africa (Rath *et al.*, 1995) and common names include Pinklady (English), Awede (Yoruba; Nigeria), Ebafo (Bini; Nigeria) and Nkpisi-nku (Igbo; Nigeria) (Gill, 1992). Adongogbe in Ewe (Ghana) (Darko, 2009), Adolea in Nzema (Ghana) (Darko, 2009),Kinzasu in Kiliguru (Tanzania) (Hamisy et al., 2000) and Boreadaso in Twi (Ghana) (Mshana et al., 2000). The stems of *Dissotis rotundifolia* are used ethnomedically across Africa without scientific basis or safety concerns. In this present study, the effect of aqueous stem extract of the medicinal plant, *Dissotis rotundifolia* on plasma Cholesterol concentration and alanine amino transferase activity in normal experimental rats was investigated.

MATERIALS AND METHODS

Experimental animals

Ten (10) healthy female albino (Wistar) rats weighing between 180-200g (6 weeks old) were used in this study. They were obtained from the animal house of the College of Health Sciences, Faculty of Basic Medical Science, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria. The animals were housed in clean cages under standard conditions of temperature, humidity and light. They were fed with standard diet (pelleted growers mash) and distilled water. The animals were acclimatized for a period of two weeks to the new environment (Prohp and Onoagbe, 2012).

The experimental protocols were according to our Institutional Animal Ethics Committee guidelines as well as internationally accepted practices for use and care of laboratory animals as contained in US guidelines (NIH, 1993).

Chemicals/Reagents

All reagents/chemicals used were of analytical grades.

Medicinal plant

The *Dissotis rotundifolia* whole plant was collected from the environment of Niger Delta University in College of Health Sciences, Wilberforce Island, Bayelsa State, Nigeria. And was identified by experts in the Department of Botany, University of Benin, Benin city, Edo state.

Extraction and preparation of plant extracts

The fresh stems of *Dissotis rotundifolia* after separating the leaves were washed with clean water, sun dried for a period of three weeks and cut into small pieces. They were then pulverized to powder using an electric blender weighed with the aid of a weighing balance. 280g of the sample (stem) was extracted separately in 2800ml of aqueous (distilled water) in cold percolation by macerati Tatowa, New JerseyHUmama on technique under room temperature. This was followed by periodic stirring then the macerated sample was filtered with cheese cloth to eliminate particles after 72hrs. The filtrate collected was then concentrated by applying direct heat (pasteurization method) for 4hrs to yield a brown concentrate which was allowed to cool. This was preserved in the freezer at -21^{o}C until used. To determine the actual volume (ml) to administer, 10ml of the boiled extract was introduced into a small beaker and placed in a water bath and then evaporated to dryness. This was weighed using a weighing balance. The difference in weight of the dry and clean beaker before evaporation and the beaker + 10ml of extract after evaporation was calculated acc6ordingly and recorded as 303.2mg i.e 303.2mg/10ml 0f extract.

Blood Collection

The rat was restrained while the tail was cleansed with a ball of cotton wool soaked in methylated spirit. The tail of rat was gently and repeatedly massaged towards the tip following a vaseline smear. The red tip of the tail was then slightly and carefully incised with a new and sterilized blade and further massaged gently as the blood trickled into immobilized fluoride oxalate sample tubes. Cotton wool soaked in methylated spirit was again used to cleanse the incised area of the tail.

Blood samples collected were subjected to centrifugation for 10 minutes at 3,000 g to obtain the plasma for cholesterol and alanine amino transferase assays. Analysis was carried out immediately after centrifugation (Prohp and Onoagbe, 2012).

Experimental procedure

Ten (10) healthy female rats of Wistar strain weighing between 180-200g were used in this study. The rats were housed in clean cages under standard conditions of temperature, humidity and light. They were acclimatized for a period of two weeks to the new environment, after which, they were fasted overnight and were randomly placed into two (2) groups of five rats each. Group 1 Served as control and received pelleted growers mash and distilled water ad libitum for a period of 28 days. Rats in group 2 were administered aqueous stem extract of Dissotis rotundifolia orally, two times daily (morning and evening) with the

aid of a gavage at a dose of 2ml/kg body weight and also fed with pelleted growers mash and distilled water ad libitum throughout the experimental period of 28 days. At the end of the experiment, blood was collected from the tail of the animals into immobilized lithium heparin bottles for analysis. Spectrophotometric methods were used in all analyses.

Administration of aqueous stem extract of *Dissotis rotundifolia*

Aqueous stem extract of *Dissotis rotundifolia* were administered to the experimental rats in group 2 orally, twice daily (morning and evening) throughout the duration of the experiment (28 days) with the aid of a gavage at a dose of 2ml/kg body weight.

Biochemical Assays

Cholesterol concentration and Alanine amino transferase (ALT) activity were estimated as outlined in the procedure described by Randox Laboratories manual, United Kingdom.

Principle

Cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase.

Alanine amino transferase is measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4-dinitophenylhydrazine.

Histological Assessment of Tissues in Treated Normal Rats

The experimental albino rats were sacrificed on the twenty-eight day (28^{th}) day and dissected to collect the hearts, livers and kidneys for histological studies. The tissue samples were immediately fixed by immersion into 10% formalin. The fixed tissue samples were later washed, cleared in xylene and embedded in paraffin wax and then cut into 5 micron sections using a microtone. The sections were then examined on slides using the light microscope after staining with haematoxylin and eosin dyes and interpreted by an expert histologist.

Statistical Analysis

Analysis was done by one way analysis of variance (ANOVA) using SPSS version 17.0. Values that were less than 0.05 were accepted as significant.

RESULTS

Results of the analyses of plasma samples for cholesterol concentration and alanine amino transferase activity in normal rats treated with aqueous stem extract of *Dissotis rotundifolia* are presented in tables 1 & 2. Aqueous stem extract of Dissotis rotundifolia significantly increased ($P<0.05$) plasma alanine amino transferase activity and cholesterol concentration compared to the control. Figures 1 to 3 are photomicrographs of the livers, kidneys and hearts sections. The aqueous extract caused adverse effects in the livers, kidneys and hearts, while the central vein of the liver was occluded with blood.

Table 1: Mean plasma cholesterol concentration (mg/dl) of normal rats administered with aqueous stem extract of *Dissotisrotundifolia*

Groups/Days	Day 0	Day1	Day 6	Day 12	Day18	Day 24	Day 28
Normal control	6O.47±7.71[a]	47.60±4.87[a]	71.64±1.74[a]	57.72±1.05[a]	56.07±3..17[a]	67.05±6..65[a]	64.78±3..55[a]
Test	53.06±6.18[a]	56.70±8..36[a]	82.01±5.12[a]	83.88±1..61[b]	43.63±4.18[b]	78.84±5.28[a]	83.47±6.21[b]

Values are mean ± S.E.M. of 3 separate determinations from 6 rats. Mean in the same column with different superscript letters are significantly different (P<0.05) when compared to normal control.

Table 2: Mean plasma Alanine amino transferase (µ/l) of normal rats administered with aqueous stem extract of *Dissotis rotundifolia*

Groups/Days	Day 0	Day 1	Day 6	Day 12	Day 18	Day 24	Day 28
Normal control	68.34±7.56[a]	61.10±6.72[a]	46.11±5.60[a]	33.01±4.60[a]	57.34±8.28[a]	62.34±4.53[a]	68.11±7.10[a]
Test	78.76±31.75[a]	115.10±26.00[b]	87.34±25.18[b]	81.34±29.75[b]	125.34±8.78[b]	110.33±9.66[b]	121.10±21.10[b]

Values are mean ± S.E.M. of 3 separate determinations from 6 rats. Mean in the same column with different superscript letters are significantly different (P<0.05) when compared to normal control.

HOTOMICROGRAPH OF HISTOLOGICAL FINDINGS AFTER 28 DAYS OF ADMINISTRATION OF AQUEOUS STEM EXTRACT OF *DISSOTIS ROTUNDIFOLIA*.

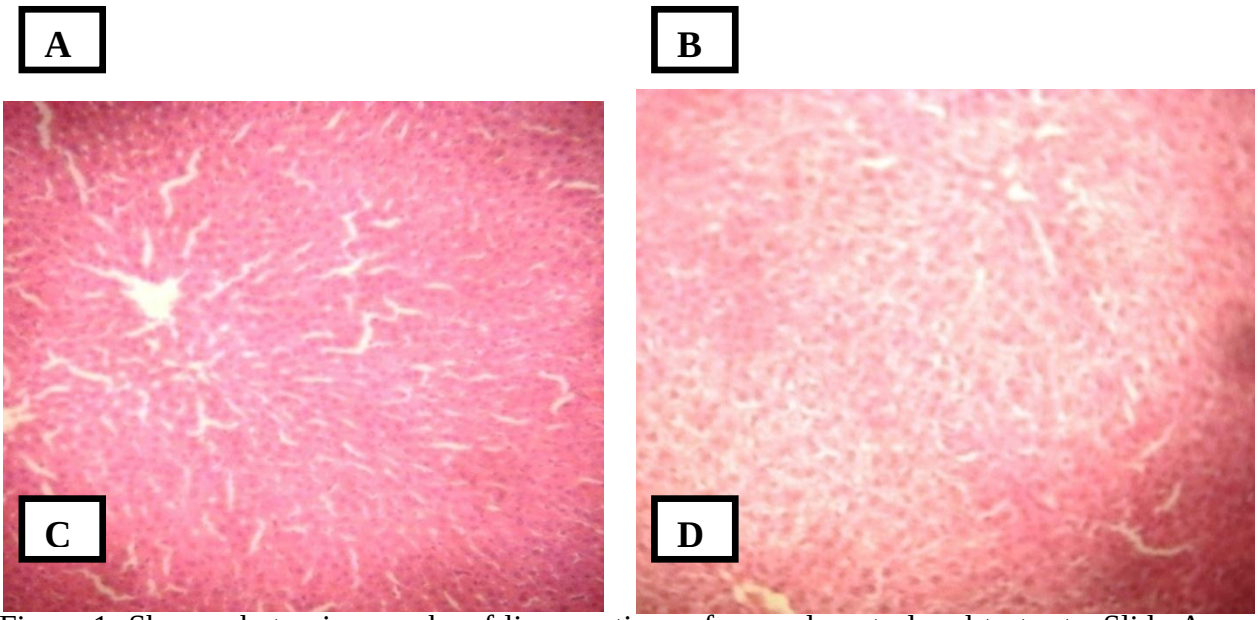

Figure 1: Shows photomicrographs of liver sections of normal control and test rats. Slide A (Control) shows normal hepatic stroma with sinusoid and central vein; while slides B, C and D show necrosis in the hepatic stroma. The central vein of slide B is occluded with blood. Sections Show the histology of the liver stained with Haematoxylin and Eosin. X100

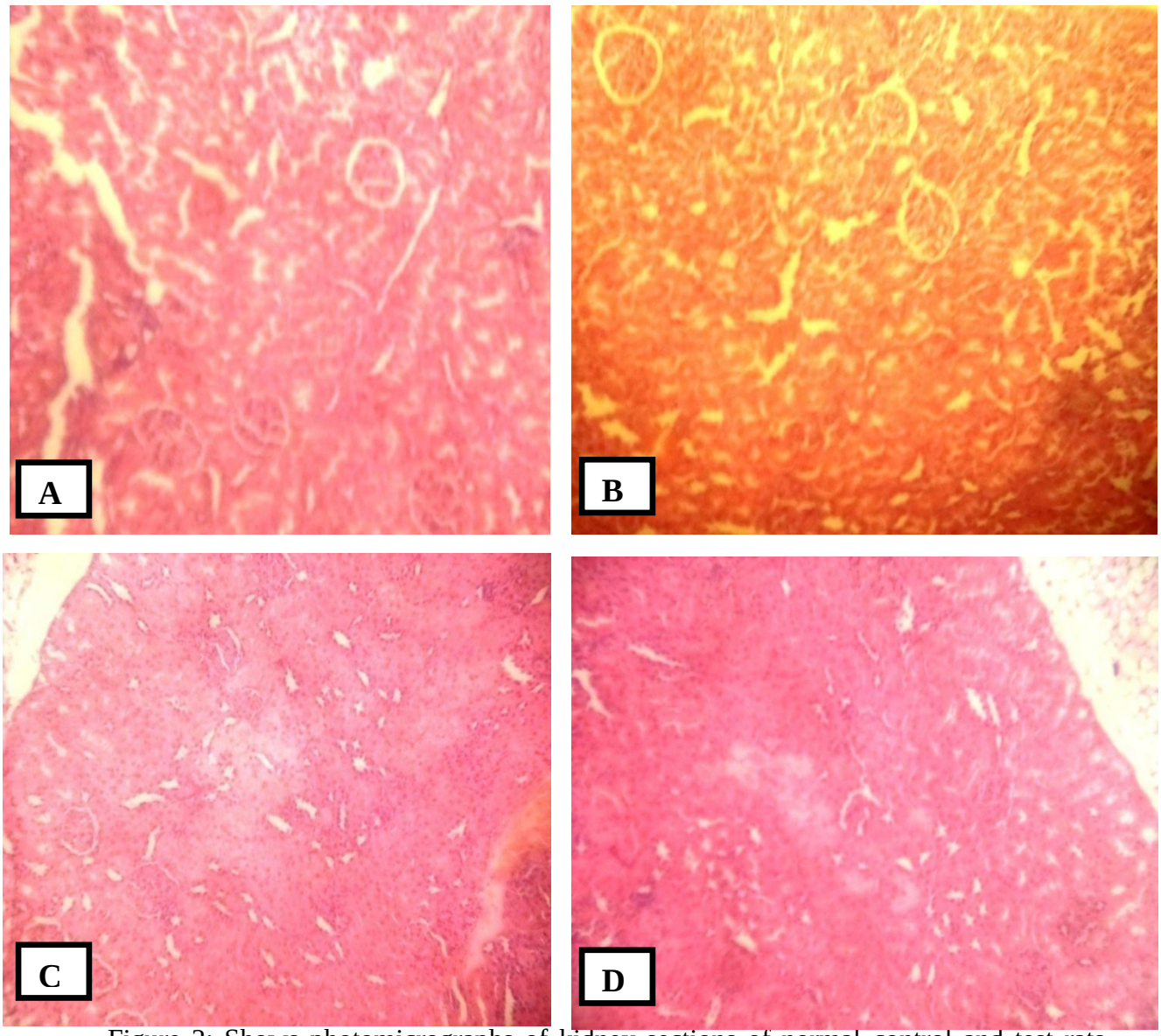

Figure 2: Shows photomicrographs of kidney sections of normal control and test rats. Slide A (control) and B shows normal kidney stroma with Bowman's capsule with glomerulus (renal corpuscle) and renal tubules; while slide C and D shows gray portions of necrosis. Sections show the histology of the Kidney stained with Haematoxylin and Eosin. X100

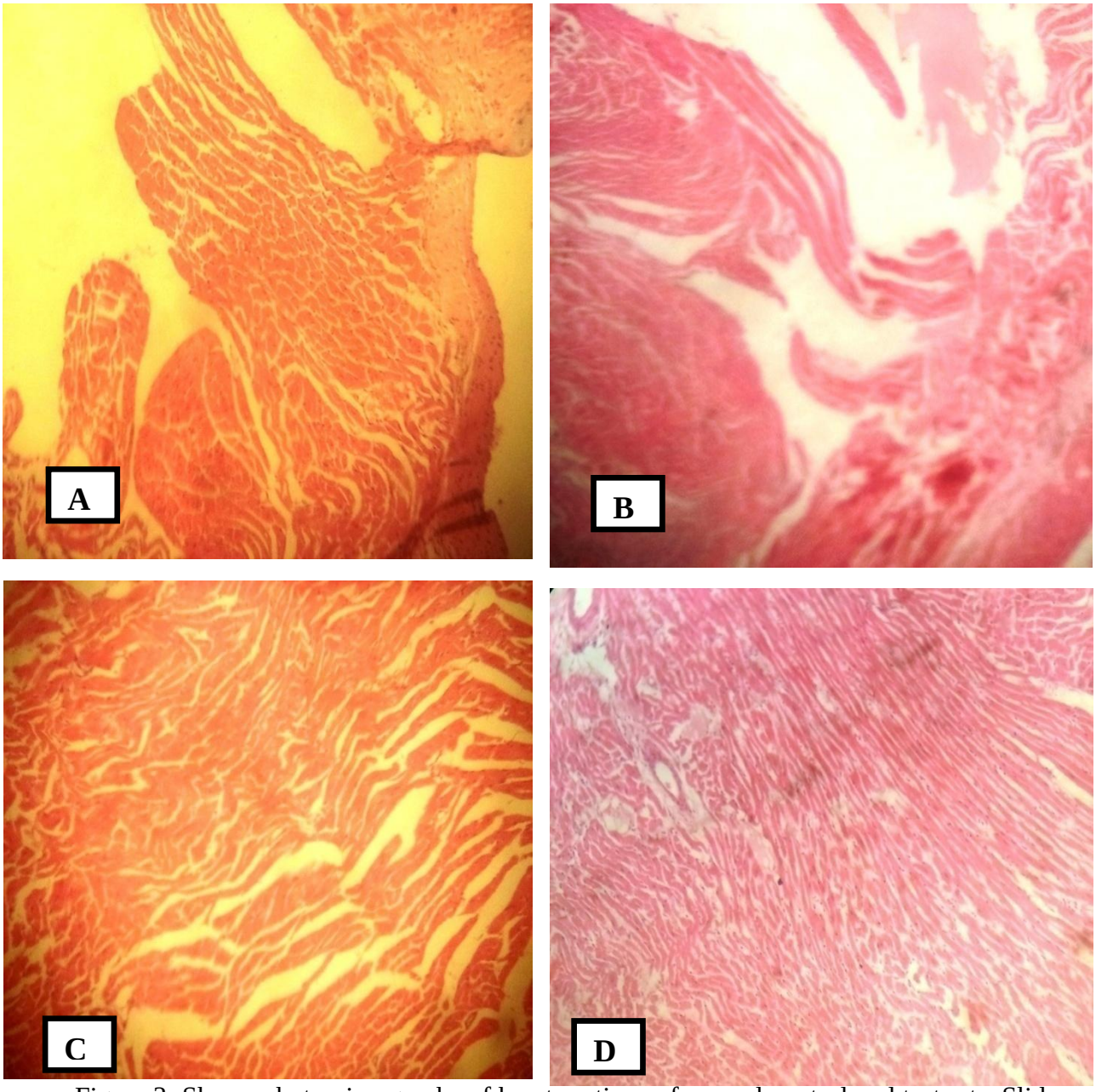

Figure 3: Shows photomicrographs of heart sections of normal control and test rats. Slide A and C show normal longitudinal and transverse section of branching cardiac muscles and inter-ventricular wall. There are presence of necrosis in slides B and D. Sections show the histology of the heart stained with Haematoxylin and Eosin. X100

DISCUSSION

The efficacy and safety of a number of herbs in the treatment of diseases have been demonstrated by correlated clinical studies (Shansi-baghbanan et al, 2014). Kono et al (1992) and Imai et al (1995) suggested that drinking either green or black tea may lower blood cholesterol concentration and blood pressure thereby providing some protection against cardiovascular disease. Studies by Oyeyemi *et al* (2015) provided evidence that the aqueous extracts of leaves and seeds of *Persea americana,* showed a significant decrease in plasma cholesterol level, indicating that the extracts of the leaves and seeds of *Persea americana* may be useful in the treatment of hypertension and other cardiovascular diseases. Results

obtained from this study showed that aqueous stem extract of *Dissotis rotundifolia* significantly ($p<0.05$) increased plasma cholesterol concentration in test rats compared to the control rats. Implying that it has hypercholesterolemic property and so may not protect against cardiovascular diseases. The protective effect of medicinal herbs have been ascribed to the antioxidants, polyphenols and other active ingredients in plants (Shun Chan et al. 2008). *Dissotis rotundifolia* contain alkaloids, tannins, saponins, cardiac glycosides, isoorientin, orientin, vitexin and isovitexin (Rath *et al*., 1995; Abere *et al*., 2010) and is therefore expected to protect against cardiovascular diseases. The significantly ($p<0.05$) increased plasma cholesterol concentration observed in this study with the administration of aqueous stem extract of *Dissotis rotundifolia* shows it may not protect against cardiovascular diseases. The reason for this is however not clear. The histological findings from this study showed that the aqueous stem extract of *Dissotis rotundifolia* caused necrosis of the liver, kidneys and heart, and the central vein of the liver was occluded with blood. Liver damage is associated with cellular necrosis (Subramaniam et al, 2015; (Murli Krishna, 2017). It implies that extract of *Dissotis rotundifolia* caused injury to the liver, kidneys and heart of test animals. Aspartate aminotransferase and alanine aminotransferase activities are used as parameters for detecting liver injury (Kallies et al, 1964; Subramaniam et al, 2015).In liver injury, serum levels of biochemical markers like transaminases are elevated (Mumoli et al, 2006). The increased alanine aminotransferase activity obtained from this work indicates damage to the liver, suggesting that aqueous stem extract of *Dissotis rotundifolia* may not be hepato-protective. This is in consonance with the work done by Offor *et al*, (2015) in their study on the effect of ethanol leave-extract of *Pterocarpus santalinoides* on liver enzymes of albino rats. They reported that ethanol leave-extract of *Pterocarpus santalinoides* significantly ($p<0.05$) decreased the activities of aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase thus indicating that ethanol leaf-extract of *Pterocarpus santalinoides* could be hepato-protective. Findings from this work showed that aqueous stem extract of *Dissotis rotundifolia* caused adverse effect on the liver and as such, may not be safe for therapeutic use. Therefore, its intake should not be abused.

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