SUB-CHRONIC TOXICITY OF EXTRACTS OF *Strophanthus hispidus* STEM BARK IN NORMAL RATS

M. Osibemhe[1*], G. O. Omaji[1], I. O. Onoagbe[2].

[1]*Department of Biochemistry and Molecular Biology, Faculty of Science, Federal University Dutsin-Ma, Katsina State, Nigeria.*

[2]*Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin - City, Edo State, Nigeria.*

*mosibemhe@fudutsinma.edu.ng

Abstract

The search for medicinally important plants with safety evidence is gaining momentum globally. *Strophanthus hispidus* (SH) is a plant used by the African natives for the management of diabetes. The aim of this study was to evaluate the sub-chronic effects of the plant extracts (aqueous and ethanol) in normal rats. Seventy-five (75) male rats (adults) were randomly distributed into 15 groups of five rats each and were kept in standard cages. Seven groups were administered orally with the aid of gavage daily doses of 100, 200, 500, 800, 1500, 2000 and 2500 mg/kg body weight of aqueous extract respectively while another seven groups were administered the respective doses of ethanol extract. The control group was administered distilled water. Administration of the extracts were carried out for 28 days. Liver-marker enzymes: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyltransferase (GGT), bilirubin and albumin and cardiac function indices: lactate dehydrogenase (LDH) and creatine kinase (CK) were analyzed. Body weight of the rats were also recorded at interval of 7 days. The results showed that the plant extract(s) caused significant ($P<0.05$) increases in liver, and heart functions enzymes whereas the liver synthetic molecules had significant ($P<0.05$) decrease in relation to the control. These alterations were in most cases seen at doses as high as 800 mg/kg or 1500 mg/kg and above. Histological results revealed mild to marked morphological changes with increasing dosage. The findings indicate that higher doses of *Strophanthus hispidus* are dangerous to the organs analyzed with the ethanol extract posing greater risk.

Keywords: Administration, African, cardiac, diabetes, gavage, liver-maker enzymes.

Introduction

The importance of toxicity study of plants intended for use as medicinal herbs for various ailments cannot be overemphasized. This became more apparent following WHO recommendations that safety should be the overriding criterion in the selection of herbal medicine for use in health care delivery (1).

Herbs and herbal formulations for the treatment of ailments have continued to receive increased attention because of the strong belief that these products are safe (2, 3). However, some plants contain toxic substances that could be hazardous to both humans and animals.

Toxicology can be defined as that branch of science that deals with poisons, and a poison can be defined as any substance that causes a harmful effect when administered, either by accident or design, to a living organism. The recently expanded and now extensive use of herbal remedies and dietary supplements has become a cause of concern for toxicologists and regulators. Not only is their efficacy frequently dubious, but their potential toxicity is largely unknown (4). According to Paracelsus "all substances can be toxic at some dose but that the right dose differentiates a poison from a remedy."

Toxicological problems associated with the use of herbal medicines are complex but have been regularly associated with serious adverse fatalities ranging from cardiovascular problems to psychiatric to neurological effects to liver toxicity or malfunction to hematologic and renal toxicity (4).

Diabetes mellitus is a group of metabolic disorders of carbohydrate metabolism in which glucose is underutilized, producing hyperglycemia (5). The increasing global prevalence of diabetes mellitus have led to the disease being described as "one of the main threats to human health in the twenty-first century".

This increasing global incidence of diabetes and its complications, despite the availability of well-known antidiabetic drugs, may be the reason for the resurgence of interest in traditional methods of combating the disease. *Strophanthus hispidus* is one of the plants used by the African natives for the management of diabetes. Preliminary studies on this plant have revealed the presence of important bioactive compounds such as flavonoids, alkaloids, tannins, etc (6) with hypoglycemic properties.

This work is therefore aimed at determining the sub-chronic toxicity effect of *Strophanthus hispidus* stem bark with specific interest on its effect in the liver and heart.

Materials and Methods

Animals

Male rats (Wistar strain) obtained from the Department of Biochemistry, University of Benin, Benin City, Nigeria were used for this study. The rats (adults) were maintained under standard animal house condition. They were fed with standard animal feed (growers mash) and water *ad libitum*. The animals were allowed to acclimatize to the new environment for two weeks. All animals were handled with proper care and humanely treated according to the internationally accepted practices for use and care of laboratory animals as contained in US guidelines (7) and local ethics committee of the University of Benin, Benin City, Nigeria.

Medicinal plant

The stems of *S. hispidus* were purchased from Ajagun Market, Ogbomosho, Oyo State, Nigeria. They were identified in the Herbarium Unit of the Department of Botany, University of Benin, Benin City, Nigeria.

Chemical and reagents

All reagents and chemicals used in this study were of analytical grade. liver/heart function enzymes were analyzed using the methods described in the respective kits.

Preparation and extraction of plant material

Preparation and extraction of the plant material was carried out according to the method described by Osibemhe & Onoagbe (2015) (6). The stems of *S. hispidus* were thoroughly washed with clean water and the barks were peeled off by incision. They were then dried under shade for two weeks and then pulverized into fine powder with the aid of a mechanical pulverizer. Measured quantities of the powdered sample were extracted separately in aqueous and 99% ethanol for 72hrs followed by periodic stirring and they were kept in a refrigerator to avoid any microbial growth. The extracts were filtered using cheese-cloth and the filtrate re-filtered using Whatman No. 42 (125mm) filter paper. The filtrates collected were lyophilized using a freeze-dryer and stored in an airtight container for further analysis.

Experimental design

A total of 75 male rats were used in this study. The rats were randomly distributed into 15 groups of five rats per group. Seven groups were administered orally with the aid of gavage daily doses of 100, 200, 500, 800, 1500, 2000 and 2500 mg/kg body weight of aqueous extract respectively while another seven groups were administered the respective doses of ethanol extract. The control group was administered distilled water. After 28 days of administration of the plant extracts, the animals were sacrificed and blood was collected for the analyses of liver and heart functions parameters. Histological examination of a cut section of the selected organs (liver and heart) was also carried out.

Blood collection

Blood samples were collected into plain container through the abdominal aorta from overnight fasted rats under chloroform anesthesia. The blood sample was centrifuged at 3000 rpm for 15 mins. After centrifugation, the sera were aspirated into clean plain sample bottles for analyses of liver/heart function parameters. The Liver Function Parameters analyzed were: Alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), Bilirubin (Direct and Total), Gamma-glutamyltransferase and Albumin whereas lactate dehydrogenase (LDH) and creatine kinase were used as indices for heart function. All analyses were carried out according to the method described in their respective kits.

BIOCHEMICAL ASSAYS

Determination of alkaline phosphatase (ALP) (8)

Principle

The alkaline phosphatase acts upon the AMP buffered sodium thymolpthalein monophosphate. The addition of an alkaline reagent stops enzyme activity and simultaneously develops a blue chromogen which is measured photometrically.
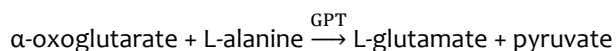
Procedure

To each of the labelled test tubes, test, standard and blank, 0.5 ml of Alkaline Phosphatase Substrate was dispensed and was equilibrated to 37℃ for three minutes. At timed intervals, 0.05 ml of sample, standard and deionized water were added to the respective test tubes. They were gently mixed and incubated for ten minutes at 37℃. At timed intervals, 2.5 ml of alkaline phosphatase colour developer was added and properly mixed. The absorbance of test and standard was read at 590 nm against the reagent blank.

Calculation

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Value of standard (IU/L)}$$

Determination of alanine aminotransferase (ALT) (9)

Principle

$$\alpha\text{-oxoglutarate + L-alanine} \xrightarrow{\text{GPT}} \text{L-glutamate + pyruvate}$$

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

Procedure

In this assay, 0.5 ml of ALT buffer was dispensed into test tubes labelled test and blank. 0.1 ml of sample and distilled water were added to the tubes labelled test and blank respectively. The mixture was incubated for exactly 30 min at 37℃. 0.5 ml of 2, 4-dinitrophenylhydrazine was added to all the tubes, mixed and allowed to stand for 20 min at 20℃. 0.5 ml of sodium hydroxide was subsequently added to the test tubes, mixed and the absorbance of sample was read against the reagent blank at 546 nm after 5 min.

Calculation

The activity of ALT was obtained from the standard table.

Determination of aspartate aminotransferase (AST) (9)

Principle

$$\alpha\text{-oxoglutarate + L-aspartate} \xrightarrow{\text{GOT}} \text{L-glutamate + Oxaloacetate}$$

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

Procedure

In this assay, 0.5 ml of AST buffer was dispensed into test tubes labelled test and blank. 0.1 ml of sample and distilled water were added to the tubes labelled test and blank respectively. The mixture was incubated for exactly 30 min at 37℃. 0.5 ml of 2, 4-dinitrophenylhydrazine was added to all tubes, mixed and allowed to stand for 20 min at 20℃. 0.5 ml of sodium hydroxide was subsequently added to the test tubes, mixed and the absorbance of sample was read against the reagent blank at 546 nm after 5 min.

Calculation

The activity of AST was obtained from the standard table provided on the leaflet.

Determination of bilirubin (Direct and Total) (10)

Principle

Colorimetric method based on that described by Jendrassik and Grof (1938) (10). Direct (Conjugated) bilirubin reacts with diazotized sulphanilic acid in alkaline medium to form a blue coloured complex. Total bilirubin is determined in the presence of caffeine, which releases albumin bound bilirubin, by the reaction with diazotized sulphanilic   acid.

Procedure

In the determination of total bilirubin, 200 µL of sulphanilic acid (reagent 1) was dispensed into test tubes labelled test and blank. One drop of reagent 2 (sodium nitrite) was added to the test. 1000 µL of reagent 3 (caffeine) was subsequently added to both test tubes and then followed with the addition of 200 µL of the sample. The mixture was incubated for exactly 10 min at 20℃. 1000 µL of reagent 4 (tartrate) was again added to both test tubes and the mixture was incubated for 15 min. at 25℃ after which the absorbance of the sample was read against the sample blank at 578 nm. Similarly, in the determination of direct bilirubin, 200 µL of sulphanilic acid (reagent 1) was dispensed into test tubes labelled test and blank. One drop of reagent 2 (sodium nitrite) was added to the test. 2000 µL of 0.9% of NaCl was then added to both test tubes and followed with the addition of 200 µL of the sample. The absorbance of the sample was again read against the sample blank at 546 nm.

Calculation

Total bilirubin = $10.8 \times A_{TB}$

Direct bilirubin = $14.4 \times A_{DB}$

Where A_{TB} and A_{DB} represent absorbance of sample for total and direct bilirubin respectively.

Determination of gamma-glutamyltransferase (GGT) (11)

Principle

The substrate L-gamma-glutamyl-3-carboxy-4-nitroanilide, in the presence of glycylglycine is converted by gamma-GT in the sample to 5-amino-2-nitrobenzoate which can be measured at 405nm.

L-gamma-glutamyl-3-carboxy-4-nitroanilide + glycylglycine $\xrightarrow{\gamma-GT}$ L-gamma-glutamyl-glycylglycine + 5 amino-2-nitrobenzoate.
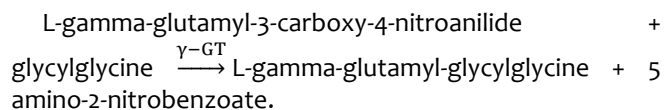
Procedure

In this assay, 1 ml of working reagent was dispensed into a test tube labelled test. 0.10 ml of sample was added. The solution was mixed and initial absorbance was taken at 405 nm and started timing simultaneously. Absorbance was then taken after 1, 2 and 3 min.

The working reagent was prepared by reconstituting one vial of GGT substrate (L-γ-glutamyl-3-carboxy-4-nitroanilide) with 3.0 ml of R1a (buffer/glycylglycine).

Calculation

The activity of GGT was obtained using the following formula:

$U/L = 1158 \times \Delta A$, where ΔA represents change in absorbance.

Determination of albumin (12)

Principle

The measurement of serum albumin is based on its quantitative binding to the indicator 3, 3′, 5, 5′-tetrabromocresol sulphonephthalein (bromocresol green, BCG). The albumin-BCG-complex absorbs maximally at 578 nm, the absorbance being directly proportional to the concentration of albumin in the sample (12).

Procedure

In this assay, 0.01 ml of sample, standard, and distilled water was dispensed into test tubes labelled test, standard and blank respectively. 3.0 ml of BCG reagent was subsequently added to all the test tubes. The mixture was incubated for 5 minutes and the absorbance of sample and standard was read against the reagent blank at 630 nm.

BCG reagent was prepared by diluting the content of one bottle of BCG concentrate with 87 ml of distilled water.

The concentration of albumin in the sample was calculated from the following formula:

Albumin conc. (g/l or g/dl) = $\frac{A_{sample}}{A_{standard}} \times$ Concentration of standard

Determination of lactate dehydrogenase (LDH) (13)

Principle

LDH catalyzes the oxidation of lactate to pyruvate in the presence of NAD, which is subsequently reduced to NADH. The rate of NADH formation measured at 340 nm is directly proportional to serum LDH-L activity.
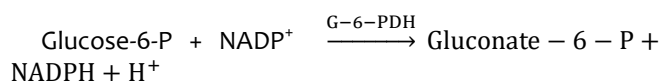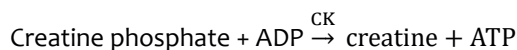
Procedure

In this assay, 1.0 ml of working reagent was dispensed into test tube labelled test and was prewarmed at 37℃ for at least 5 minutes. Subsequently, 25 µL of sample was added, mixed and incubated for 37℃ for 1 minute. The absorbance of sample was read against the reagent blank immediately at 340 nm. Absorbance reading was repeated every minute for the next 2 minutes.

The activity of LDH-L was calculated by multiplying 6592 by Δ_{ABS}/min.

Where Δ_{ABS}/min represents change in absorbance per minute.

Determination of creatine kinase (CK) (14)

Principle

$$\text{Creatine phosphate} + \text{ADP} \xrightarrow{\text{CK}} \text{creatine} + \text{ATP}$$

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{HK}} \text{Glucose} - 6 - \text{P} + \text{ADP}$$

$$\text{Glucose-6-P} + \text{NADP}^+ \xrightarrow{\text{G}-6-\text{PDH}} \text{Gluconate} - 6 - \text{P} + \text{NADPH} + \text{H}^+$$

Procedure

In this assay, 1.0 ml of enzyme/coenzyme/substrate was dispensed into a test tube labelled test. 0.02 ml of sample was added. The mixture was incubated at 37°C for 1 minute. Initial absorbance was read and the reading of the absorbance was repeated after 1, 2, and 3 min at 340 nm.

CK activity was calculated by U/L= $4127 \times \Delta A$.

Where ΔA represents change in absorbance.

Statistical analysis

Data are presented as means ± S.E.M of five independent determinations. One-Way Analysis of Variance (ANOVA) was used in comparing the means using Statistical Package for Social Sciences (SPSS) version 16.0, followed by Duncan Post Hoc Multiple Comparisons. Values lower than 0.05 were taken as statistically significant.

Results

Liver and heart functions indices:

The results of the effects of extracts of *Strophanthus hispidus* on liver and heart functions enzymes are as presented in tables 1 and 2. Significant increases (P<0.05) were observed in the activities/concentrations of the following parameters at the respective doses of the extract(s) used when compared with the control: ALT, AST, GGT (800-2500 mg/kg) (aqueous and ethanol), total bilirubin (2000-2500 mg/kg) (aqueous and ethanol), direct bilirubin (2000-2500 mg/kg) (ethanol) and (2500 mg/kg) (aqueous), ALP (500, 1500 and 2000 mg/kg) (ethanol) and (2000-2500 mg/kg) (aqueous), CK (800-2500 mg/kg) (ethanol) and (2500 mg/kg) (aqueous), LDH (1500-2500 mg/kg) (aqueous) and (2000-2500 mg/kg) (ethanol). On the contrary, albumin concentration showed significant decrease (P<0.05) at (800-2500 mg/kg) (ethanol) and (1500-2500 mg/kg) (aqueous). Histological result shows that both extracts of SH showed moderate to marked morphological changes with increasing dosage in the selected organs (plates 2-15 & 17-30), whereas the control showed normal histology (plates 1&16).

Body weight of animals

The results of weekly records of body weight of rats treated with aqueous and ethanol extracts of *Strophanthus hispidus* for 28 days are presented in tables 3 and 4. Significant progressive increase in weight was observed in the control group on days 7-28 in relation to the basal value. Similarly, the aqueous extract treated groups showed significant progressive increases on days 14-28 at 100-800 mg/kg whereas at 1500-2500 mg/kg no significant change was observed. The ethanol extract treated groups showed increases on day 14 and 21 at 200 mg/kg and 100 mg/kg respectively. 500-2500 mg/kg did not show any significant change when compared with the basal values.

Discussion

Liver, being the primary organ for the detoxification and distribution of drugs could be assessed to establish the safety of a substance (15). Because the liver performs multiple functions, no single laboratory test or even a battery of tests is sufficient to provide a complete estimate of the liver injury in every clinical situation. A broad array of biochemical tests is used to evaluate patients with suspected or established liver disease, but also for screening asymptomatic individuals (16). Five laboratory assays are commonly called liver function tests (LFTs), although they are neither specific to the liver nor true measures of liver function: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and total bilirubin (16). LFTs are used to screen people for the presence of liver disease, suggest the underlying cause, estimate the severity, assess prognosis and monitor the efficacy of therapy. Abnormal LFTs may be the first indication of subclinical liver disease and may thereby guide further diagnostic evaluation (17). In this study, ALT, AST, ALP, GGT, Bilirubin (Total & Direct) and Albumin were assessed as well as LDH and CK for the heart function indices. Aminotransferases, ALP, GGT, 5-nucleotidase, leucine aminopeptidase e.t.c are classified as LFTs that detect injury to hepatocytes (18). Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are useful indices for identifying inflammation and necrosis of the liver (19). Serum alanine aminotransferase has its highest concentration in the liver and kidney, with skeletal muscles having lesser activity of the enzyme. The activity of AST is located in the microsomal and mitochondrial portions of the liver cells as well as in the skin, skeletal and cardiac muscles, pancreas and kidney. ALT measurements are more liver specific than AST and its activity is usually greater than AST activity at early or acute hepatocellular disease (20). AST on the other hand tend to be released more than the ALT in chronic liver diseases such as cirrhosis (20). Alkaline phosphatase (ALP) is most often measured to indicate bile duct

obstruction. High levels of ALP exist in cells that are rapidly dividing or are otherwise metabolically active. These cells include the epithelium of the biliary tract and liver, osteoblasts, granulocytes of circulating blood, intestinal epithelium, proximal tubules of the kidney, placenta, and lactating mammary glands. ALP levels reach spectacular heights in primary biliary cirrhosis, in conditions of disorganized hepatic architecture (cirrhosis), and in diseases characterized by inflammation, regeneration, and obstruction of intrahepatic bile ductules (21). GGT is an enzyme that is present in hepatocytes and biliary epithelial cells, renal tubules, and the pancreas and intestine. The mechanisms of alteration are similar to those described for alkaline phosphatase. GGT is a microsomal enzyme, and its activity can be induced by several drugs, such as anticonvulsants and oral contraceptives (22). Elevated GGT levels can be observed in a variety of nonhepatic diseases, including chronic obstructive pulmonary disease and renal failure, and may be present for weeks after acute myocardial infarction. Because of its lack of specificity but high sensitivity for liver disease, GGT can be useful for identifying causes of altered ALP levels, or elevated levels, together with other biochemical abnormalities (22). The increase (tables 1 and 2) in ALT, AST, and the concomitant increase in GGT and ALP levels observed in this study at higher doses, is an indication of hepatocellular injury by the extracts. Bilirubin is the product of hemoglobin catabolism within the reticuloendothelial system. Heme breakdown determines the formation of unconjugated bilirubin, which is then transported to the liver. In the liver, UDP-glucuronyltransferase conjugates the water-insoluble unconjugated bilirubin to glucuronic acid, and conjugated bilirubin is then excreted into the bile (23). Unconjugated bilirubin may increase because of augmented bilirubin production or decreased hepatic uptake or conjugation or both. Serum bilirubin, urine bilirubin e.t.c are classified as tests of the liver capacity to transport organic anions and to metabolize drugs (18). In this study, the concentration of bilirubin (Total and Direct) was increased at doses as high as 2000 mg/kg and above by the extracts. This implies that *Strophanthus hispidus* at higher doses may affect the capacity of the liver to transport organic anions. Serum albumin is frequently utilized as an index of the hepatocyte's ability to carry out synthetic function. Serum albumin does not change in mild liver injury but readily declines in the face of sub-massive liver necrosis (24, 25). Although the serum albumin level can serve as an index of liver synthetic capacity, several factors make albumin concentrations difficult to interpret (25). Albumin synthesis is affected not only by liver disease but also by nutritional status, hormonal imbalance, and osmotic pressure (16). In

practice, patients with low serum concentration and no other LFT abnormalities are likely to have a nonhepatic cause for low albumin, such as proteinuria or acute or chronic inflammatory state (24). The observed decrease in albumin concentration at doses as high as 800 mg/kg and above for the ethanol extract and 1500 mg/kg and above for the aqueous extract, juxtapose with the effect of the extracts in the other LFTs' indices suggest that *Strophanthus hispidus* is deleterious to the liver at higher doses and may affect the liver synthetic capacity. Hauwa'u *et al.* (2014) (26) has reported significant increases in the activity of ALT and AST at 1000 mg/kg and bilirubin (total) at 250 mg/kg in rats treated with hexane leaf extracts of *Guiera senegalensis.* Lactate dehydrogenase (LDH) is a cytoplasmic enzyme present in major organ systems. The extracellular appearance of LDH is used to detect cell damage or cell death (27). It is released into the peripheral blood after cell death caused by ischemia, excess heat or cold, starvation, dehydration, injury, exposure to bacterial toxins, after ingestion of certain drugs, chemical poisonings etc. Therefore, monitoring serum levels of LDH has become a routine and fundamental means to monitor organ toxicity (28). Creatine Kinase (MB) is usually present in serum at low concentration; it increases after an acute infarct of myocardium and later descends at normal levels, it also increases rarely in skeletal muscle damage (28). The observed increases in LDH and CK at higher doses of the extracts in this study, suggest that extracts of *Strophanthus hispidus* may be injurious to the heart at high dose concentration. The histological examination of the organs (liver and heart) showed moderate to marked morphological changes with increasing dosage in both extracts whereas the control showed normal histology (plates 1-15 and plates 16-30) respectively.

Body weight measurement is among the data used in the assessment of both acute and subchronic toxicity. Changes in body weight serve as a sensitive indication of the general health status of animals (29). The increase in weight that was observed on day 14-28 at 100-800 mg/kg of aqueous extract and at 100-200 mg/kg body weight in the ethanol extract as against the control animals which had increases in weight on day 7-28 in comparison with their basal values, may be an indication of the extracts' acute appetite reduction (30) which was later relieved. The body weight of animals treated with sub-chronic doses of aqueous extract of *Boerhavia diffusa* was reported to increase progressively (31). Loss of appetite is often synonymous with weight loss due to disturbances in carbohydrate, protein or fat metabolisms (32). The non-significant change in weight at 500-2500 mg/kg and at 1500-2500 mg/kg exhibited by ethanol and aqueous extracts respectively suggests that higher doses of

the extracts may have effect on the feed utilization of the animals (tables 3 and 4).

Conclusion

The importance of continuing surveys for medicinally important plants with proven safety margin cannot be overemphasized. This study has provided information on the safety level of *Strophanthus hispidus* plant on the liver and heart. The results showed that higher doses of the plant extracts are inimical to the various organs analyzed. However, clinical investigations are recommended in order to establish tolerable dose of the plant extract in humans

References

1. WHO. WHO Expert Committee on diabetes mellitus technical report series. World Health Organization, Geneva. 1980.

2. Farnsworth NR, Soejarto DD. Potential consequence of plant extinction in the United States on the current and future availability of prescription drugs. Econ Bot 1985; 39: 231-240.

3. Said O, Khalil K, Fulder S, Azaizeh H. Ethnobotanical survey of medicinal herbs of the Middle Eastern region. J. Ethnopharmacol 2002; 83: 251-265.

4. Hodgson E. A Textbook of Modern Toxicology, 3rd ed. New Jersey, John Wiley & Sons, Inc., 2004: 1-11.

5. Tietz NW. Carbohydrates In: Tietz Fundamentals of Clinical Chemistry. Burtis CA, Ashwood ER, & Bruns DE, 6 ed. W. B. London, Saunders Company, 2008:380

6. Osibemhe M, Onoagbe IO. Qualitative and quantitative phytochemical evaluations of *Strophanthus hispidus* stem bark. IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) 2015; 10(2): 124-128.

7. National Institute of Health. Institutional Animal Care and use Committee Guidebook, NIH Publication. U.S. Government Printing Office, Washington, D. C., 1992: 92-345.

8. Roy AV. Rapid method for determining alkaline phosphatase activity in serum with thymolphthalein monophosphate. Clin. Chem 1970; 16(5): 431-436.

9. Reitman S, Frankel S. A colorimetric method for determination of serum glutamate oxaloacetate and pyruvate transaminases. Am. J. Clin Path 1957; 28: 56-61.

10. Jendrassik L, Grof P. Colorimetric method of determination of bilirubin. Biochem 1938; 297: 81-82.

11. Tietz NN. *Fundamentals of Clinical Chemistry* (3rd ed.) Philadelphia: W. B. Saunders, 1987: 391.

12. Doumas BT, Watson WA, Biggs HG. Albumin standards and the measurement of serum albumin with bromocresol green. Clin. Chem. Act 1971; 31(1): 87-96.

13. Amador E, Wacker WEC, Dorfman LE. Urinary lactic dehydrogenaseactivity IV. Screening test for detection of renal disease dissociated and in association with arterial hypertension. JAMA (1964; 188(7): 671-676.

14. Rec. GSCC (DGKC). Recommendation of German Society of Clinical Chemistry. J. Clin. Chem. Clin. Biochem 1977; 15: 255.

15. Gupta M, Mazumder UK, Das S. Effect of alkaloidal extract from *Clerodendron colebrookianum* on hematological parameters and hepatorenal functions in mice. Indian J. Exp. Biol 1994; 32:189-191.

16. Thierry P, Francoise I. Laboratory Testing for Liver Disease In: A Textbook of Liver Disease .Thomas DB, Michael PM, Arun JS & David Z., 6th ed. Philadelphia, Elsevier Inc, 2012.

17. Sherman M. Hepatocellular Carcinoma: epidemiology, surveillance and diagnosis. Semin Liver Dis 2010; 30: 3-16.

18. Thapa BR, Anuj W. Liver function tests and their interpretation. Indian J Pediatr 2007; 74(7): 663-671.

19. Tilkian SM, Conover MB, Tilkian AG. *Clinical Implications of Laboratory Tests*. London: Mosby Company, 1979:45

20. Whitby LG, Smith AF, Becket GJ. *Lecture Notes on Clinical Chemistry* 9th ed. Oxford, London: Blackwell Scientific Publications, 1989: 20.

21. Sacher RA, McPherson RA. (1991). *Widmann's Clinical Interpretation of Laboratory Test* (Pp. 416–443). Pennsylvania, USA.

22. Rosalki SB, Tarlow D, Rau D. Plasma gamma-glutamyl transpeptidase elevation in patients

 receiving enzyme-inducing drugs. Lancet 1971; 2: 376-7.

23. Berk PD, Noyer C. Clinical chemistry and physiology of bilirubin. Semin Liver Dis 1994; 4(4): 346-55.

24. Johnston DE. Special considerations in interpreting liver function tests. American family physician, 1999; 59(8): 2223-2230.

25. Rothschild MA, Oratz M, Schreiber SS. Serum albumin. Hepatol 1988; 8: 385-401.

26. Hauwa'u YB, Maryam I, Ja'afaru SM, Maimuna Z, Timothy B. Toxicity studies of aqueous, methanolic and hexane leaf extracts of *Guiera senegalensis* in rats. International Journal of Scientific & Engineering Research 2014; 5(10): 1338-1347.

27. Henry JB. Clinical Diagnosis and Management by Laboratory Methods. Philadelphia, WB Saunders and Company, 1979: 365.

28. Tietz NW. Fundamentals of Clinical Chemistry. Philadelphia, WB Saunders, 1976:1028.

29. El Hilaly J, Israili ZH, Lyoussi B. "Acute and chronic toxicological studies of Ajuga iva in experimental animals." Journal of Ethnopharmacology 2004; 91(1): 43–50.

30. Osibemhe M, Abdulrahman BO, Onoagbe IO. Acute Toxicity of Aqueous and Ethanolic Extracts of *Strophanthus hispidus* Stem Bark. International Journal of Biochemistry Research & Review 2016; 9(1): 1-5.

31. Orisakwe OE, Afonne OJ, Chude M A, Obi E, Dioka CE. Sub-chronic toxicity studies of the aqueous extract of *Boerhavia diffusa* leaves. J. Health Sci 2003; 49: 444-447.

32. Klaassen CD. Casarett and Doull's Toxicology: The Basic Science of Poisons, New York, USA. McGraw-Hill Press, 2001.

Table 1: Mean serum concentrations of liver and heart function parameters of normal rats administered aqueous extract of *Strophanthus hispidus* stem bark for 28 days

Group	Control	100 mg/kg	200 mg/kg	500 mg/kg	800 mg/kg	1500 mg/kg	2000 mg/kg	2500 mg/kg
ALT(U/l)	21.0±1.30 [a]	20.2±1.16 [a]	20.6±1.08 [a]	24.0±1.58[a]	35.4±2.09[b]	37.0±0.95[b]	40.8±1.02[cd]	38.6±0.60[bc]
AST(U/l)	22.40±0.09[a]	24.2±3.54[a]	26.4±0.68[a]	28.4± 3.22[a]	38.0±1.05 [b]	34.0±1.30[b]	39.6±1.17[b]	38.8±0.80[b]
ALP(U/l)	211.0±5.22[b]	196.4±7.7[a]	212.0±6.11[b]	226.0±5.44[bc]	225.6±5.44[bc]	225.22±1.66[bc]	227.42±0.67[c]	230.74±2.36[c]
GGT(U/l)	31.04±3.37[a]	29.36±3.82[a]	32.60±2.02[ab]	27.74±2.60[a]	42.24±2.04[c]	39.14±0.67[bc]	40.76±1.06[c]	41.46±0.68[c]
Bil_T(mg/dl)	2.12±0.05[a]	2.20±0.12[a]	2.28±0.20[a]	2.40±0.09[ab]	2.40±0.08[ab]	2.42±0.09[ab]	2.66±0.06[b]	2.68±0.11[b]
Bil_D(mg/dl)	1.70±0.08[ab]	1.48±0.12[a]	1.88±0.14[bc]	1.94±0.14[bc]	2.16±0.28[bc]	2.07±0.05[bc]	2.00±0.05[bc]	2.20±0.07[c]
Albumin (g/dl)	4.49±0.07[a]	4.72±0.17[a]	4.57±0.22[a]	4.51±0.10[a]	4.20±0.18[ab]	4.05±0.12[bc]	3.98±0.09[bc]	3.73±0.09[c]
LDH(U/l)	117.5±5.95[a]	118.8±5.01[a]	122.3±3.28[ab]	122.8±5.51[ab]	129.6±7.83[ab]	135.8±6.79[b]	137.1±3.84[bc]	138.43±2.95[bc]
CK(U/l)	130.3±3.53[ab]	121.6±4.04[a]	125.8±7.22[ab]	133.6±2.99[ab]	142.6±3.76[ab]	145.71±4.43[bc]	140.86±6.57[bc]	157.04±2.69[c]

Values are expressed as activities/concentrations of liver and heart functions indices and are means ± SEM of five independent determinations. Values in the same row with different superscript represent significant difference (p<0.05) from control.

Bil_T= Total Bilirubin, Bil_D=Direct Bilirubin, CK=creatine kinase.

Table 2: Mean serum concentrations of liver/ heart function parameters of normal rats administered ethanol extract of *Strophanthus hispidus* stem bark for 28 days

Group	Control	100 mg/kg	200 mg/kg	500 mg/kg	800 mg/kg	1500 mg/kg	2000 mg/kg	2500 mg/kg
ALT(U/l)	21.0±1.30 [a]	21.2±1.46[a]	21.6±1.47[a]	22.2±1.46[a]	28.6±2.25[b]	42.40±1.25[c]	41.00±1.05[c]	42.40±0.75[c]
AST(U/l)	22.40±0.09[a]	26.8±1.59[ab]	26.4±1.20[ab]	27.0±1.05[ab]	31.6±3.26[bc]	35.8±2.27[c]	40.8±0.58[d]	42.8±1.11[d]
ALP(U/l)	211.0±5.22[ab]	197.8±3.63[a]	207.8±5.70[a]	235.0±5.87[cd]	223.8±2.78[bc]	229.08±0.87[cd]	231.84±1.23[cd]	256.68±7.61[d]
GGT(U/l)	31.04±3.37[ab]	24.44±2.32[a]	31.44±3.29[b]	33.50±1.59[bc]	40.44±3.23[cd]	40.29±0.67[cd]	41.22±0.69[d]	44.00±0.73[d]
Bil_T(mg/dl)	2.12±0.05[a]	2.24±0.18[ab]	2.38±0.31[ab]	2.57 ±0.23[ab]	2.62±0.11[ab]	2.54±0.08[ab]	2.71±0.10[bc]	3.09±0.09[c]
Bil_D(mg/dl)	1.70±0.08[a]	1.74±0.41[a]	1.84±0.23[a]	2.38±0.28[ab]	1.90±0.35[a]	2.24±0.07[ab]	2.50±0.19[b]	2.94±0.04[bc]
Albumin (g/dl)	4.49±0.17[a]	4.80±0.08[a]	4.62±0.01[a]	4.66±0.02[a]	3.51±0.19[b]	3.88±0.17[bc]	3.62±0.16[c]	3.50±0.09[c]
LDH(U/l)	117.5±5.95[a]	128.1±5.50[ab]	133.7±6.69[ab]	134.8±4.80[ab]	135.9±2.25[ab]	135.80±7.39[ab]	138.43±6.91[b]	148.98±7.39[bc]
CK(U/l)	130.3±3.53[a]	145.2±5.57[ab]	144.0±3.65[ab]	145.0±2.65[ab]	149.0±6.98[b]	153.81±4.43[bc]	155.42±4.72[c]	168.50±7.49[c]

Values are expressed as activities/concentrations of liver and heart function indices and are means ± SEM of five independent determinations. Values in the same row with different superscript represent significant difference ($p < 0.05$) from control.
Bil_T= Total Bilirubin, Bil_D=Direct Bilirubin, CK=creatine kinase.

Table 3: Body weight of normal rats (g) treated with aqueous extract of *S. hispidus* stem bark for 28 days

	Basal	Day 7	Day 14	Day 21	Day 28
Control	170.8±3.14[a]	179.8±2.46[b]	193.4±1.88[c]	221.8±2.65[d]	239.6±2.37[e]
100 mg/kg	158.2±1.74[a]	165.6±2.11[a]	176.0±4.66[b]	191.2±3.70[c]	189.8±3.15[c]
200 mg/kg	147.8±2.15[a]	152.0±1.94[a]	170.4±1.81[b]	190.0±2.03[c]	188.4±0.93[c]
500 mg/kg	146.4±2.36[a]	151.8±2.24[a]	161.4±1.12[b]	158.8±2.24[b]	158.2±2.39[b]
800 mg/kg	162.0±1.92[a]	167.2±1.16[a]	171.4±2.60[b]	172.2±1.96[b]	168.8±2.56[b]
1500 mg/kg	164.8±7.29[a]	165.5±7.28[a]	167.0±6.77[a]	172.0±7.33[a]	173.5±9.86[a]
2000 mg/kg	194.3±7.49[a]	201.5±7.66[a]	207.8±8.66[a]	207.8±8.12[a]	217.5±8.65[a]
2500 mg/kg	169.3±2.17[a]	170.8±1.31[a]	174.5±2.22[a]	175.0±3.39[a]	178.5±4.63[a]

Values are weights in grams and are means ± SEM of five independent determinations. Values in the same row with different superscript represent significant difference (p<0.05) from basal.

Table 4: Body weight of normal rats (g) treated with ethanol extract of *S. hispidus* stem bark for 28 days

	Basal	Day 7	Day 14	Day 21	Day 28
Control	170.8 ± 3.14^a	179.8 ± 2.46^b	193.4 ± 1.88^c	221.8 ± 2.65^d	239.6 ± 2.37^e
100 mg/kg	181.2 ± 6.12^a	188.6 ± 6.72^a	200.8 ± 7.94^{ab}	219.8 ± 5.71^{bc}	221.0 ± 5.78^c
200 mg/kg	182.8 ± 3.81^a	188.0 ± 2.04^a	202.0 ± 3.93^b	220.6 ± 1.50^c	219.8 ± 2.08^c
500 mg/kg	185.4 ± 3.23^a	182.6 ± 4.06^a	183.4 ± 3.02^a	185.0 ± 5.26^a	182.8 ± 4.36^a
800 mg/kg	178.6 ± 1.45^a	179.6 ± 1.02^a	177.8 ± 2.63^a	176.4 ± 2.11^a	174.0 ± 2.28^a
1500 mg/kg	178.5 ± 1.76^a	182.3 ± 5.99^a	189.3 ± 6.02^a	180.3 ± 8.33^a	181.0 ± 4.10^a
2000 mg/kg	156.3 ± 4.77^a	158.0 ± 5.57^a	164.3 ± 7.41^a	163.0 ± 11.74^a	162.3 ± 8.17^a
2500 mg/kg	155.3 ± 3.54^a	157.5 ± 3.40^a	159.3 ± 4.98^a	160.8 ± 4.11^a	157.0 ± 1.78^a

Values are weights in grams and are means ± SEM of five independent determinations. Values in the same row with different superscript represent significant difference ($p<0.05$) from basal.

HISTOLOGICAL RESULTS
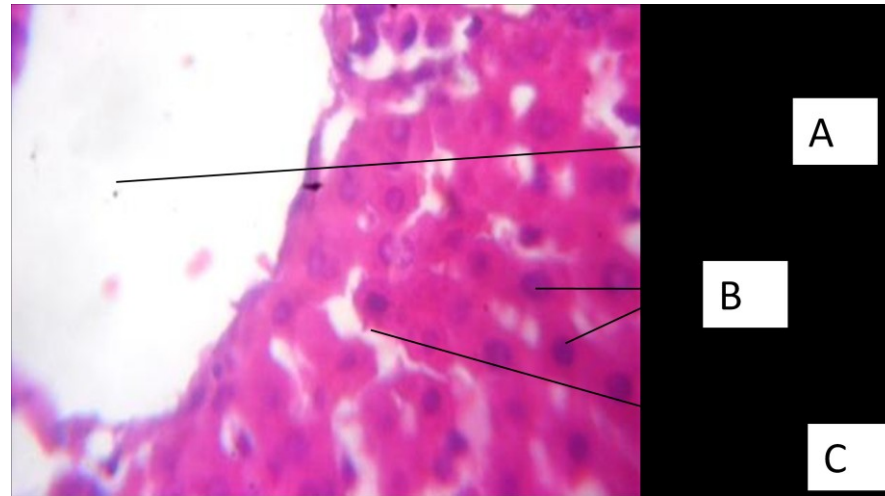
Plate 1

Figure 1. Photomicrograph of liver section of normal control (normal histology)(plate 1). (H&E Stain, x 400) (A =portal vein B= hepatocytes , C =sinusoids)

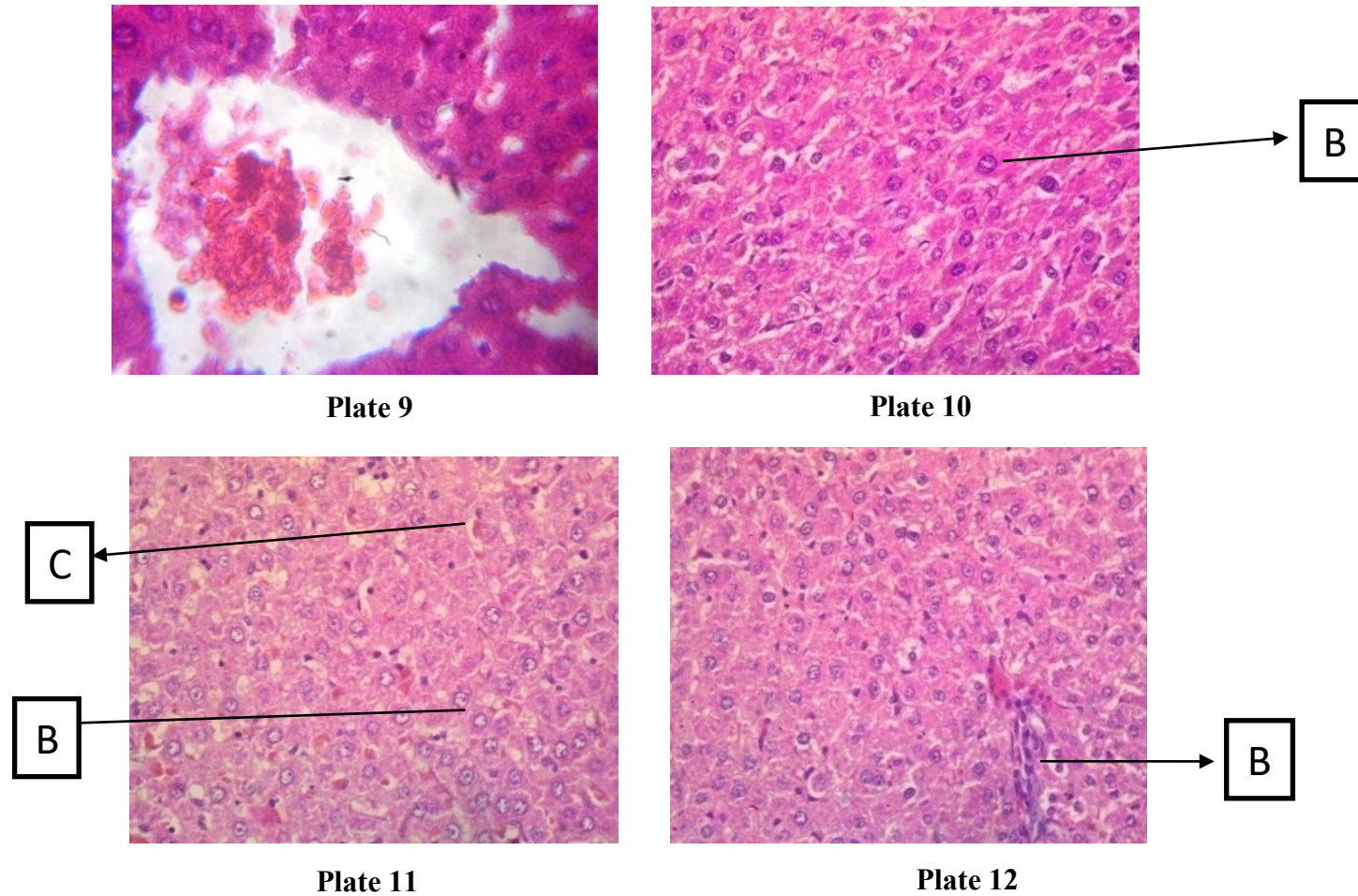Plate 2

Plate 3
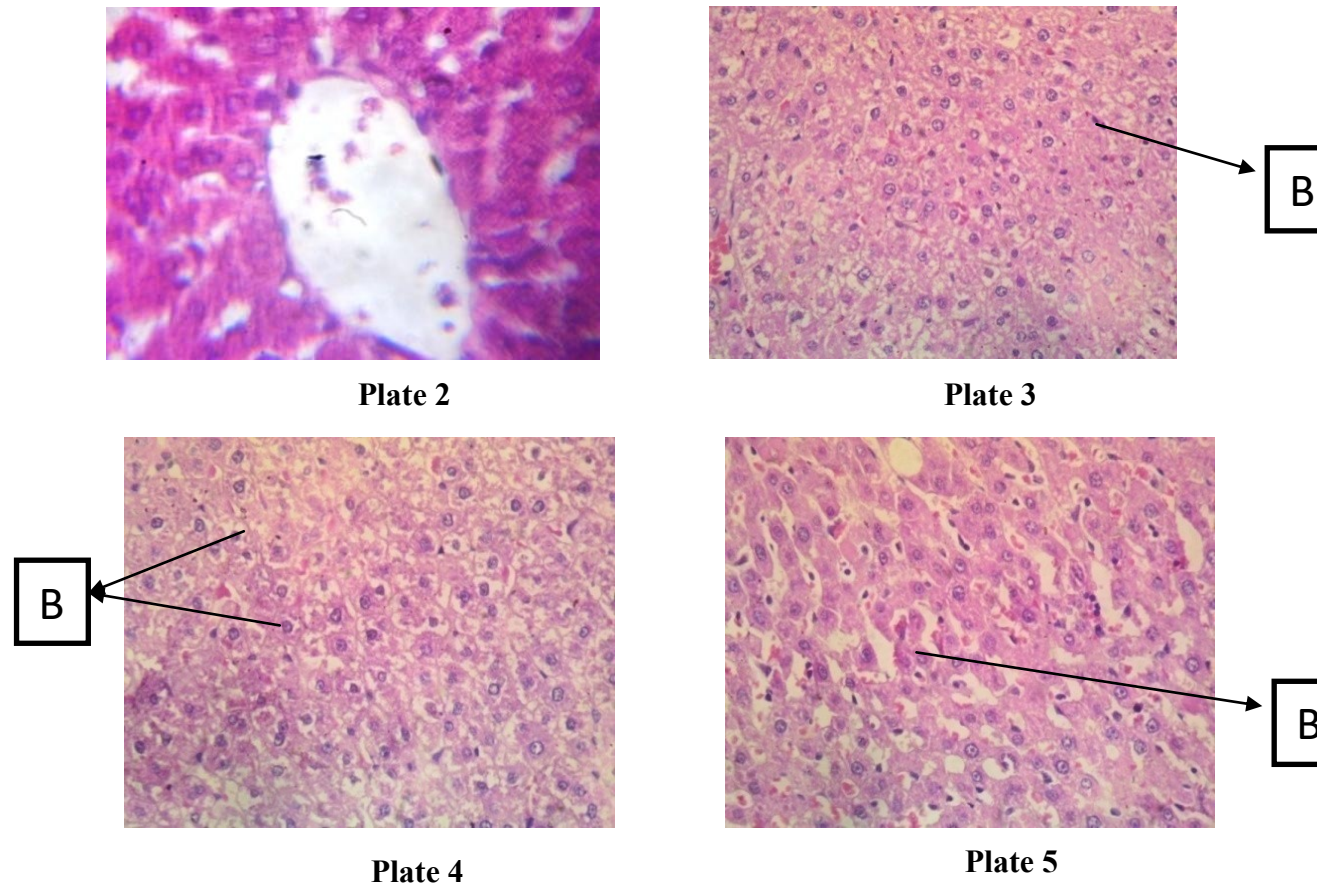
Plate 4

Plate 5

Figure 2. Photomicrographs of liver sections of aqueous extract (100, 200, 500 800, 1500, 2000, & 2500 mg/kg bw): (no prominent hepatocytes change) (plate 2), (widespread moderate vacuolar change of hepatocytes; mild KCH) (plate 3), (Widespread marked vacuolar change of hepatocytes) (plate 4), (Multiple foci of moderate thinning of hepatic cords; moderate KCH; multiple random foci of moderate single-cell hepatocellular necrosis) (plate 5)

Plate 6
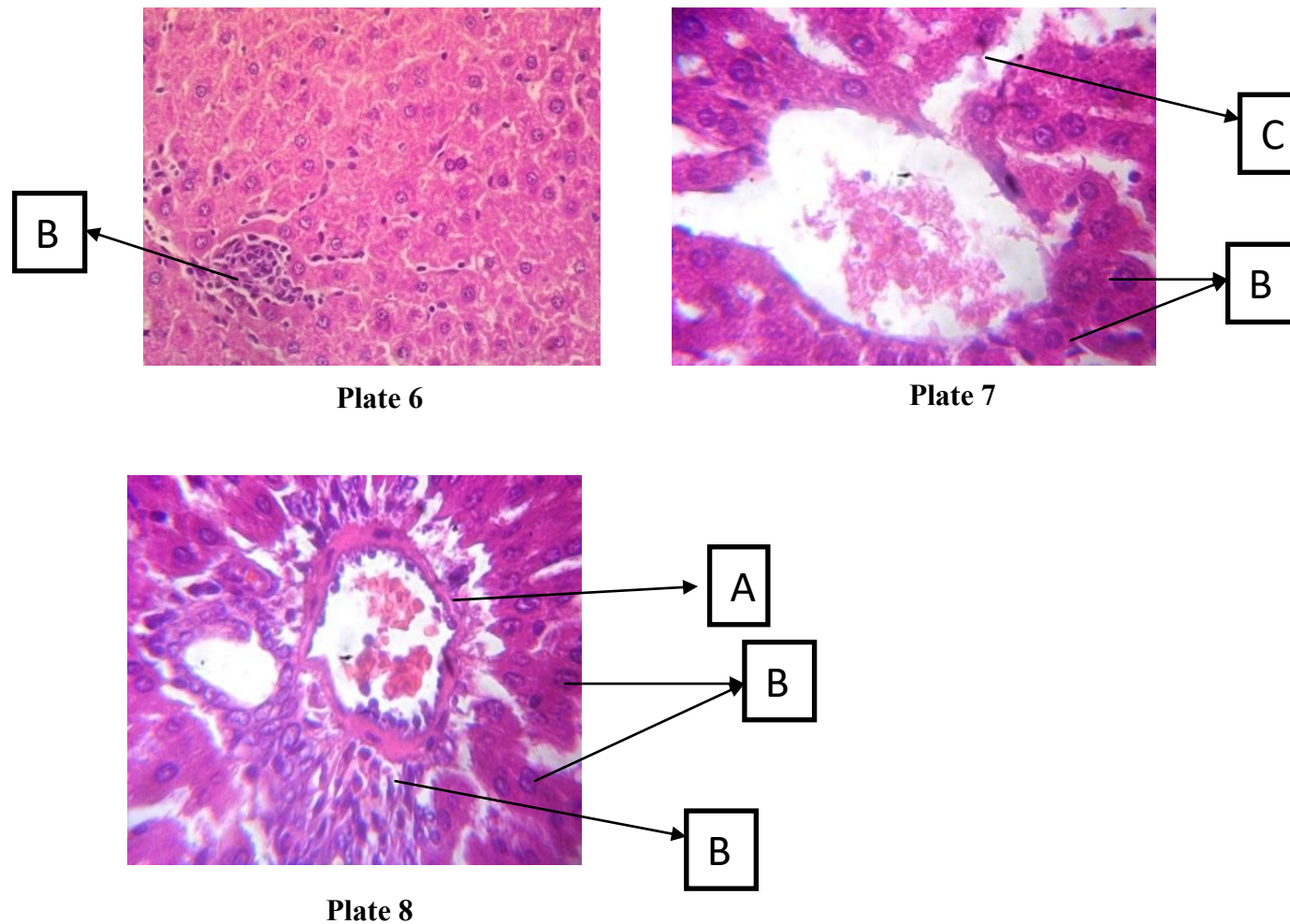
Plate 7

Plate 8

Figure 3. (Closely packed hepatic cords; mild KCH; multiple random foci of moderate single-cell hepatocellular necrosis; multiple aggregates of MNCs [bottom left of photomicrograph]) (plate 6), (mild KCH & dilation of sinusoid) (plate 7), (A, thickened walled portal vein, mild KCH & sinusoids with pools of inflammation) (plate 8) respectively.(H&E Stain, x 400).

Plate 9   **Plate 10**

Plate 11   **Plate 12**

Figure 4. Photomicrographs of liver sections of ethanol extract (100, 200, 500 800, 1500, 2000 & 2500 mg/kg bw):(no prominent hepatocytes change) (Plate 9), (mild vacuolar change of hepatocytes) (plate 10), (marked congestion of hepatic sinusoids; moderate KCH) (plate 11), (mild vacuolar change of hepatocytes) (plate 12)

Plate 13
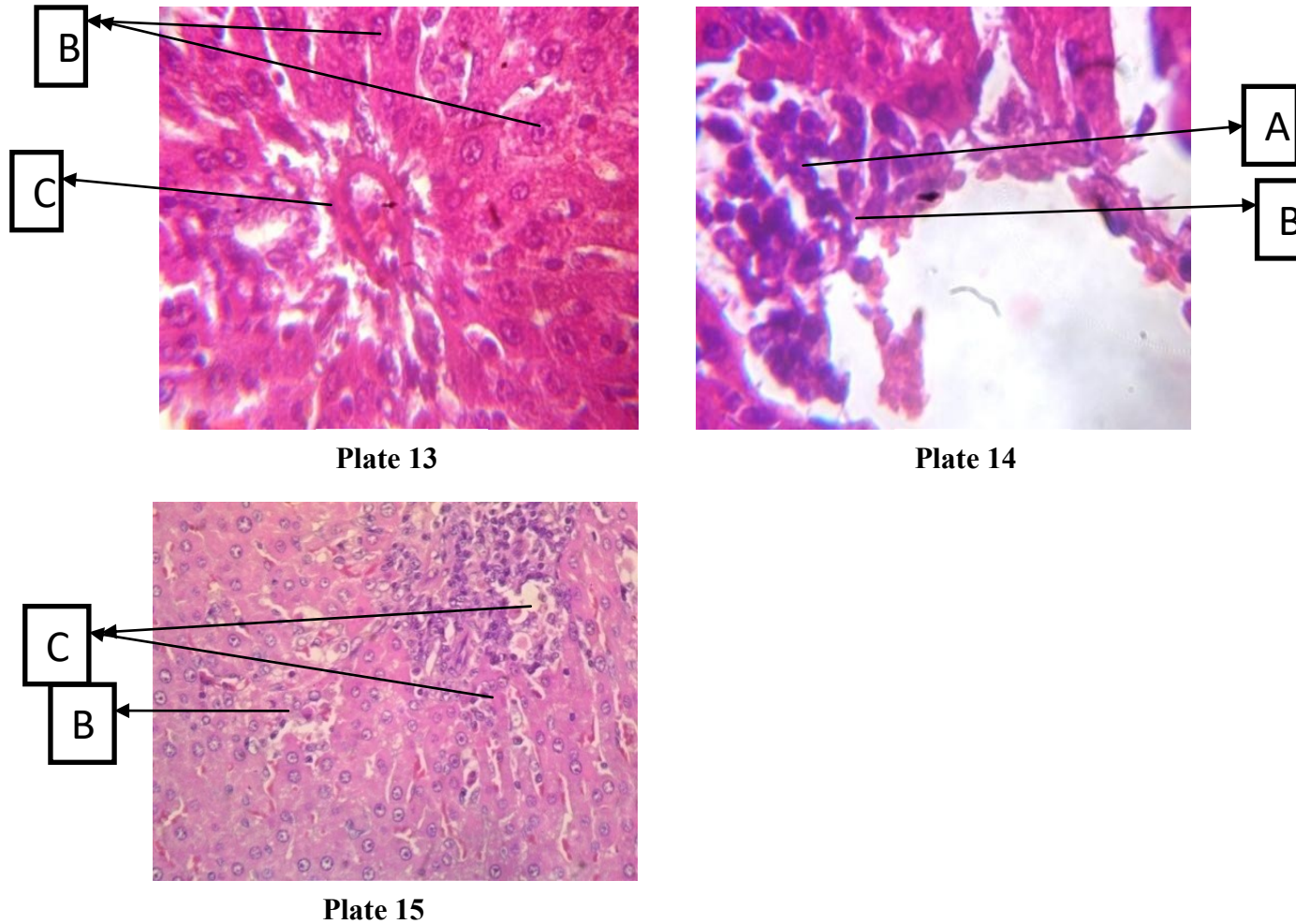
Plate 14

Plate 15

Figure 5. (mild KCH and thickened walled portal vein) (plate 13), (Pools of inflammatory cells around the central vein, bulcerated portal vein   wall.) (plate 14), ((moderate hepatocellular necrosis; periportal aggregates of MNCs; marked congestion of hepatic sinusoids)(plate 15) respectively. H&E Stain, x 400).
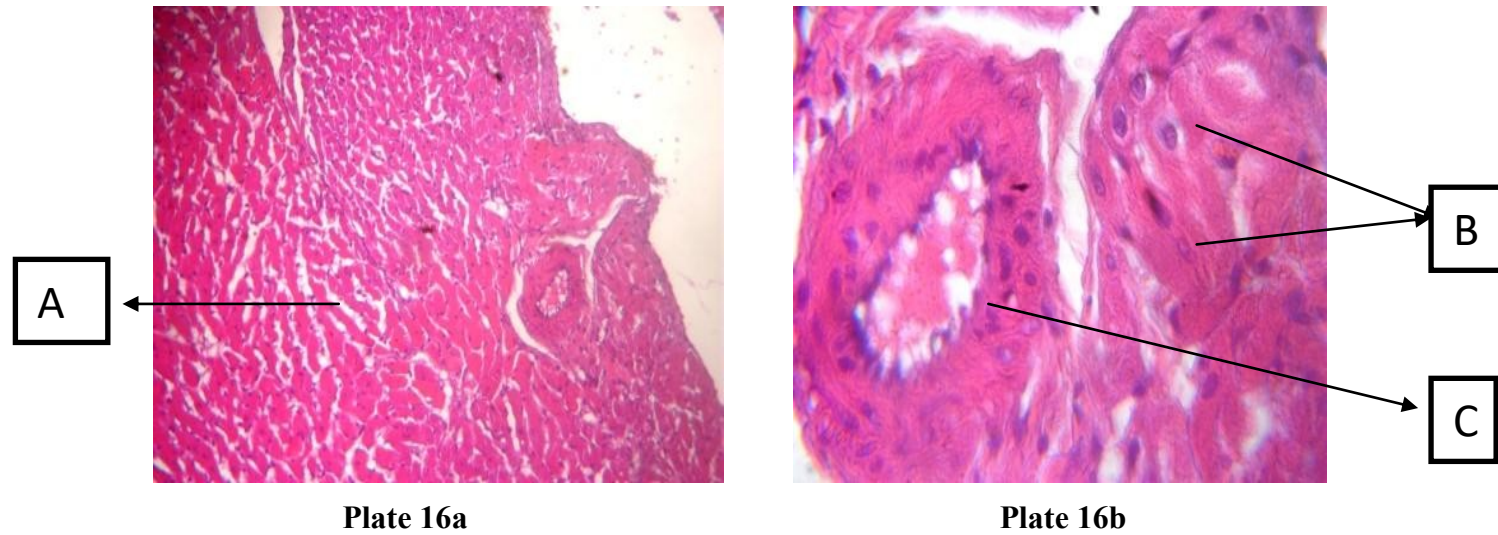
Plate 16a   **Plate 16b**

Figure 6. Photomicrographs of heart section of normal control (normal histology)(plate 16). (H&E Stain, x 100 (16a) and 400 (16b) respectively) (A=bundles of myocardial fibres, B =interstitial space, C =coronary artery)
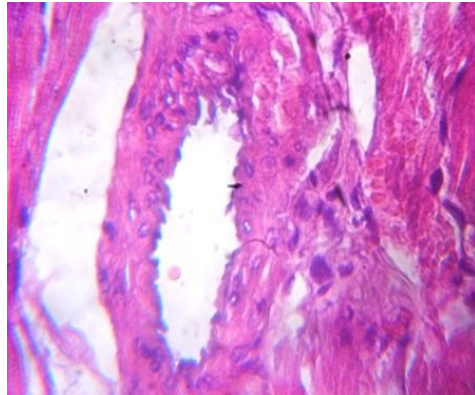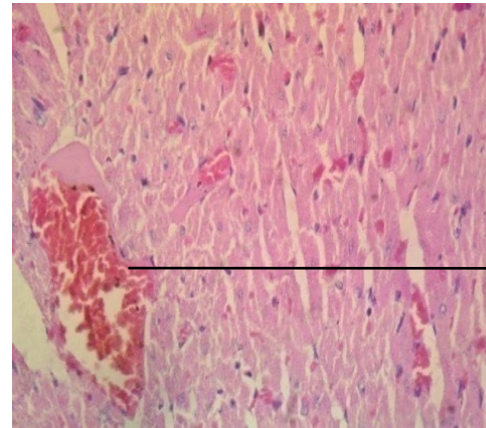
Plate 17
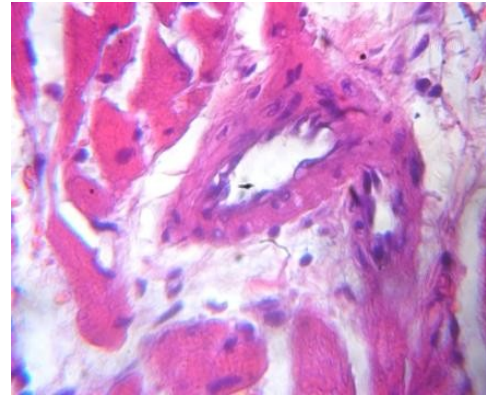
Plate 18
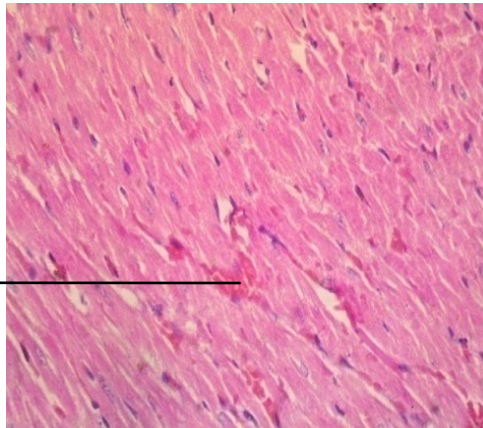
Plate 19

Plate 20

Figure 7. Photomicrographs of heart sections of aqueous extract (100, 200, 500, 800, 1500, 2000 & 2500 mg/kg): (no prominent change) (plate 17), (no prominent change) (plate 18), (moderate congestion of coronary blood vessels and inter-myocardial capillaries)(plate 19), (marked congestion of coronary blood vessels and inter-myocardial capillaries) (plate 20)
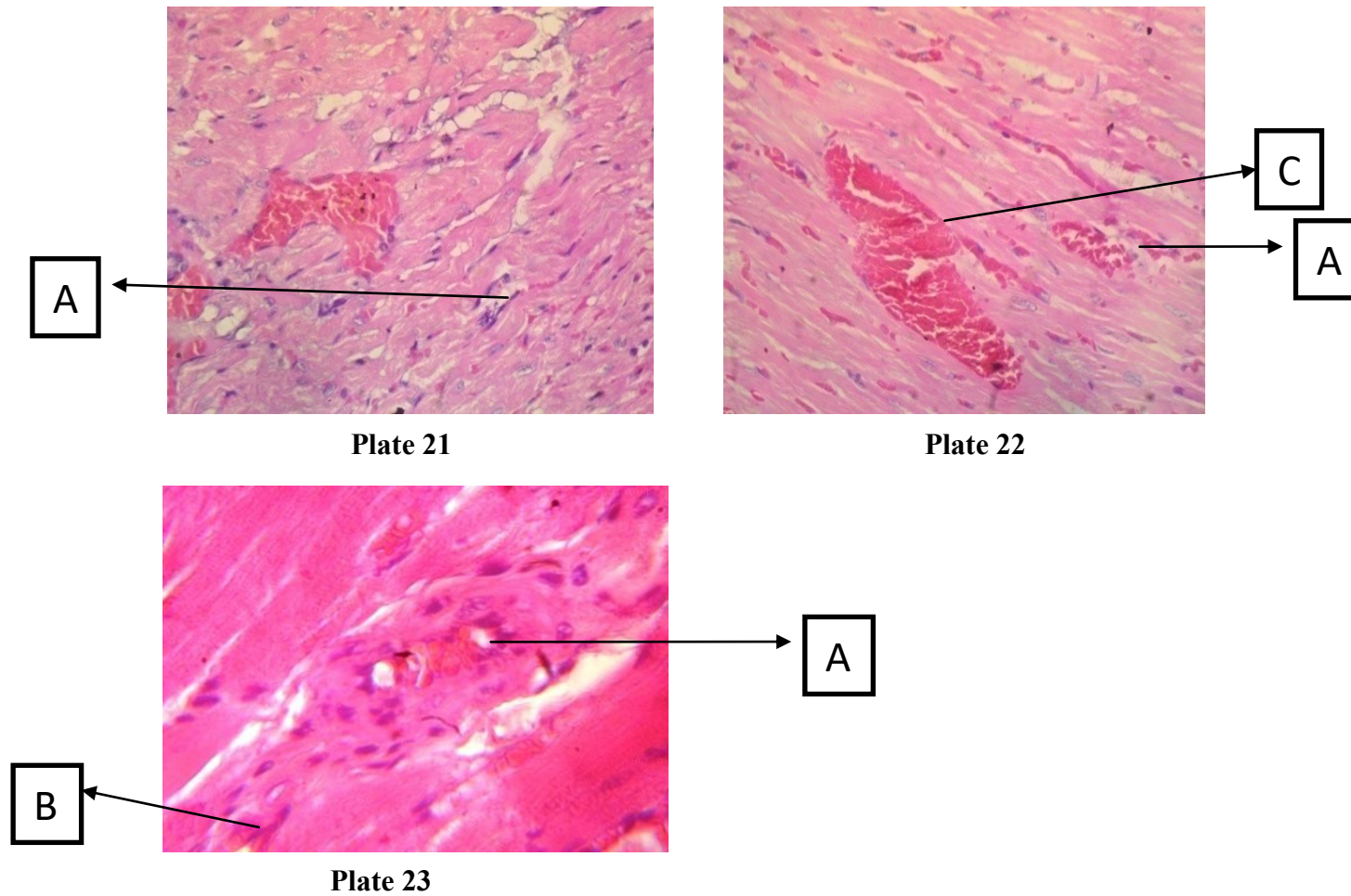
Plate 21

Plate 22

Plate 23

Figure 8. (marked congestion of coronary blood vessels and inter-myocardial capillaries; a few foci of vacuolar change of cardiomyocytes) (plate 21), (Marked congestion of coronary blood vessels; a few foci of myocardial haemorrhages) (plate 22), (mild vascular congestion with pools of inflammatory cells) (plate 23) respectively.(H&E Stain, x 400).
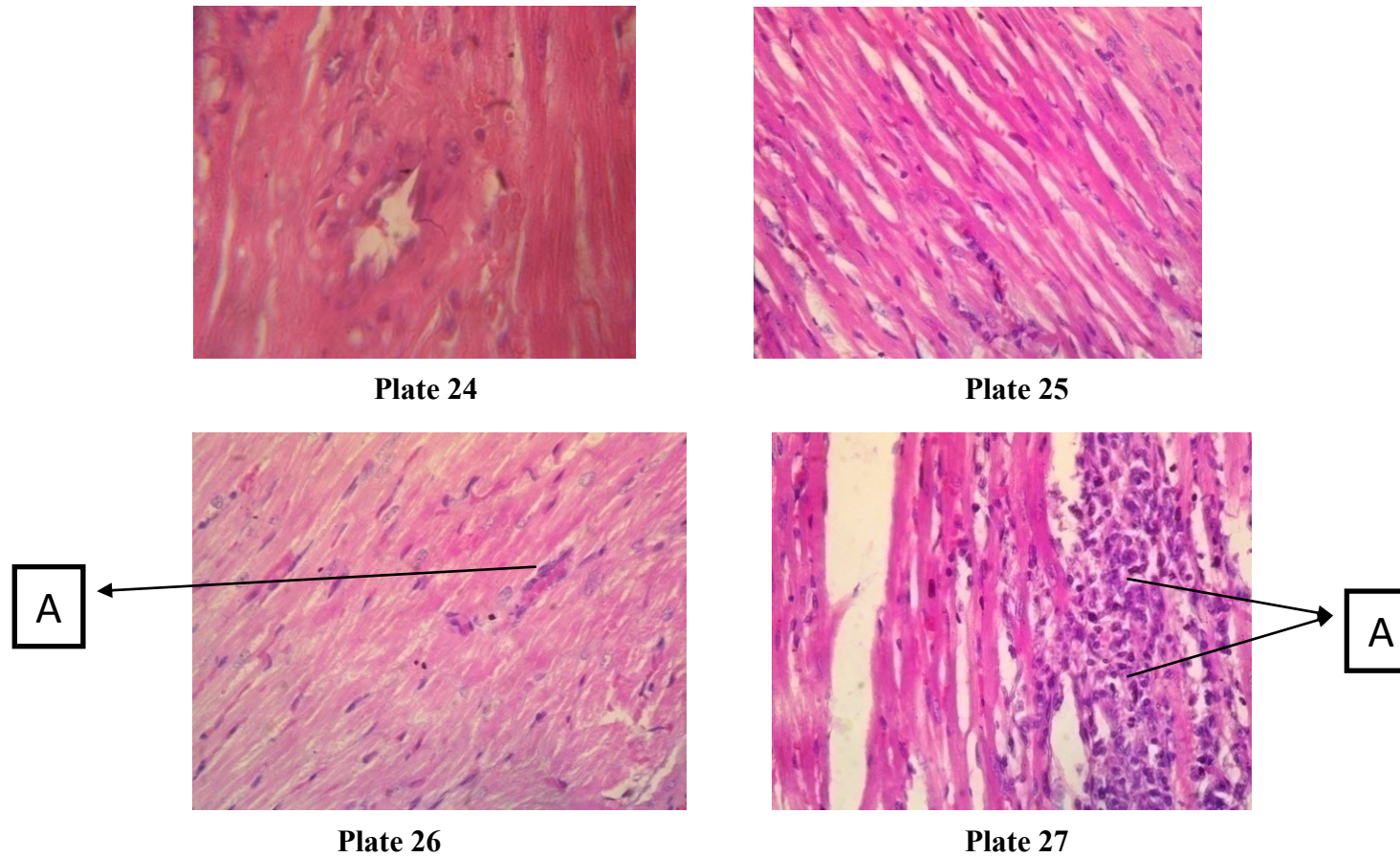
Plate 24

Plate 25

Plate 26

Plate 27

Figure 9. Photomicrographs of heart sections of ethanol extract (100, 200, 500 800, 1500, 2000 & 2500 mg/kg): (no prominent change) (plate 24), (no prominent change) (plate 25), (Moderate swelling of endothelial cells of myocardial blood vessels) (plate 26), (A few foci of hypertrophic cardiomyocytes; a large focus of aggregates of macrophages in the myocardium [right of photomicrograph]) (plate 27)
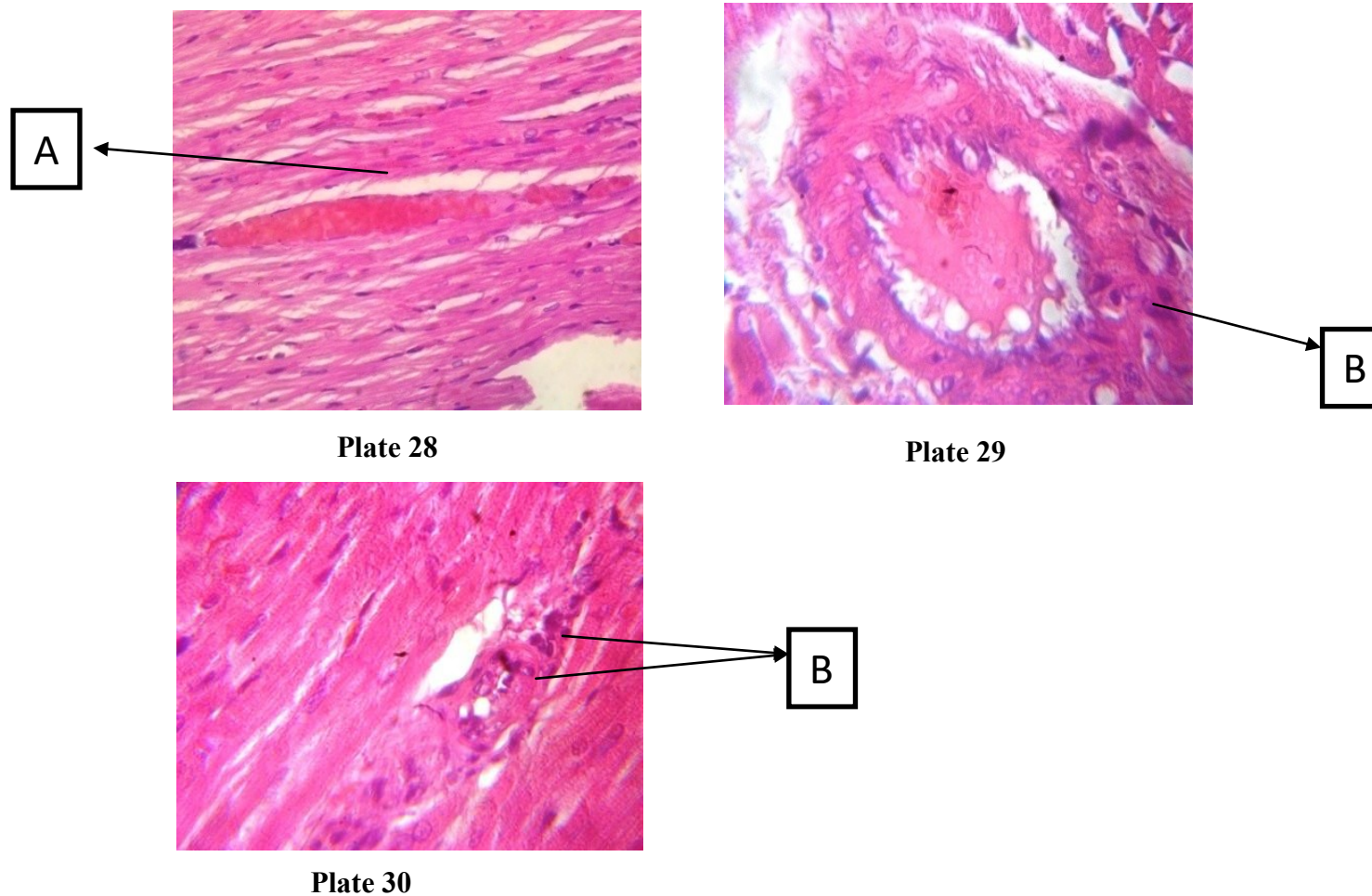
Plate 28

Plate 29

Plate 30

Figure 10. (A few foci of hypertrophic cardiomyocytes (brightly eosinophilic excessively long strap of cardiomyocyte); moderate swelling of endothelial cells of myocardial blood vessels (plate28), (mild perivascular infiltrate of chronic inflammatory cells) (plate 29), (mild perivascular infiltrate of chronic inflammatory cells) (plate 30) respectively. (H&E Stain, x 400).