

Original Research Article

Biochemical Assessment of the Effects of Aqueous Stem bark Extract of *Anogeissus leiocarpus* on Wistar Strain Rats

Abstract

Aqueous stem bark extract of *Anogeissus leiocarpus* was phytochemically screened indicating the presence of alkaloids, tannins, anthraquinones, saponins and phenols. Antimicrobial activity of the aqueous extract using *Escherichia coli*, *Streptococcus pneumoniae*, *Staphylococcus aureus* and *Klebsiella pneumoniae* as test organisms showed varying zones of inhibition with 10mm zone of inhibition exhibited by *Staphylococcus aureus* and *Escherichia coli* respectively. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration of extract were 16µg/ml and 32µg/ml respectively. Treatment of experimental animals with the aqueous stem bark extract for a period of six weeks and subsequent assessment of its effect biochemically revealed that alanine aminotransferase (ALT), aspartate aminotransferase (AST), acid phosphatase (ACP), and lactate dehydrogenase (LDH) levels were significantly higher than normal suggesting certain degree of liver injury. Glucose, cholesterol, total and direct bilirubin as well as total protein levels were significantly higher than normal values with respect to animals treated with 500mg/kg body weight suggesting a dose dependent effect of the extract on the animals. Urea and creatinine levels were slightly lower than normal. Overall, the result of this study showed that the effect of the extract was more pronounced on the liver than the kidney as depicted by the photomicrographs.

Keywords: Stem bark, test organisms, zone of inhibition, dose dependent, photomicrographs

Introduction

Trees, shrubs and herbs have commonly been used in traditional medicine especially in developing countries (Sofowora, 1982). In many cultures there is this popular belief that ‘every plant grown on the surface of the earth has a medicinal property or use’. However, it is impossible to provide a complete list of these uses to which most of these plants may be put (Bojang, 2000). Despite all the progress in synthetic chemistry and biotechnology, plants are still an indispensable source of medicinals,

33 both preventive and curative. Hundreds of species of plants are recognized as having
34 medicinal value, and many of them are commonly used to treat or prevent ailments
35 and diseases .The medicinal properties of plants are often associated with one or all of
36 the parts of the plant that include bark, leaf, flower, fruit or seed (Srivastava *et*
37 *al.*,1996)

38 ***Anogeissus leiocarpus* (Guill. and Perr.)**

39 Local names: Marke (Hausa); Kojoli (Fulfulde); Annum (Kanuri); Aina orin odon
40 (Yoruba); Atara (Igbo). This plant belongs to the family Combretaceae.

41 A very graceful tree plant of up to 28m height occurring in most of the
42 savanna regions from the driest areas to the borders of the forest zone. It is easily
43 recognized by the delicate pale green leaves, rather distantly spaced along slender and
44 often drooping branchlets; the pale coloured bark often flaking off in very thin and
45 conspicuous patches; and the terminal and axillary flower heads, about 8mm across,
46 develop into persistent cones about 12mm across.

47 The branches of the tree are in ascending manner usually from lowdown and
48 often drooping at the ends. The bark is grey or pale brown and is darker with age,
49 peeling off in very thin patches which often curl at the edges. The stem exudes a
50 brown gum.

51 Fruiting period of the plant is from August to January. The fruits have scale-
52 like appearance with a diameter of about 6mm across. The fruits have 2 wings and a
53 sharp beak, collected in a rather prickly dark brown head. The wood is greenish
54 outside, dark brown at the heat and very hard (Keay, 1989).

55 **Materials and Methods**

56 Stem bark sample of *Anogeissus leiocarpus*, was collected from within the
57 premises of Modibbo Adama University of Technology Yola, Nigeria. The plant was
58 identified at the Forestry Department of School of Agriculture and Agricultural
59 Technology, Modibbo Adama University of Technology Yola, Nigeria.

60 The plant specimen was washed with tap water and then dried in an oven at
61 50⁰C over a 72 h period. The dried sample was cut into small pieces with a clean knife
62 and then ground to powder using mortar and pestle and then put in sterile
63 polyethylene bags for further use.

Male Wister strain rats weighing between 100– 150g were purchased from the animal house National Veterinary Research Institute Vom in Jos, Plateau State Nigeria. The animals were stabilized using standard laboratory chaw.

Bacterial and fungal specimens were obtained from the microbiology laboratory of the Pathology Department of the Federal Medical Centre Yola Adamawa State. The test organisms were *Klebsiella pneumoniae* (Gram-negative rod), *Escherichia coli* (Gram-negative rod), *Neisseria gonorrhoeae* (Gram-negative cocci), and *Staphylococcus aureus* (Gram positive cocci). The fungal specimen is *Candida albicans*. The test organisms were kept in slants of nutrient agar.

Fifty (50g) grammes of powdered stem bark were soaked in 200ml of sterile distilled water, shaken and allowed to stand for 24 hours. The mixture was filtered using whatman No.1 (115cm) filter paper. The filtrate was concentrated over a water bath at 50°C. Dried extract was kept in sterile universal bottles and stored in a refrigerator for further use.

Twenty-five rats weighing 100 to 150g were randomly divided into five groups of five rats each. Group 1 was the normal/control and groups 2, 3, 4, and 5 were administered daily with graded doses of 200,300,400 and 500 mg/kg body weight of the aqueous stem bark extracts of *Anogeissus leiocarpus* by  intubation. Treatment was done for the period of six weeks. Experimental animals were fed *ad libitum* with laboratory chaw throughout the period of the study.

The animals anaesthetised with diethyl-ether were sacrificed and the blood collected by cardiac puncture. The blood was allowed to clot, centrifuged for 10 minutes at 3000rpm in a desktop centrifuge. Serum was collected carefully with Pasteur pipette and transferred into clean sample bottles. The serum was used to determine serum levels of AST, ALT, LDH, ACP, Alkaline Phosphatase, glucose, cholesterol, Bilirubin (Total and Direct bilirubin), albumin, total protein, urea and creatinine. Livers of all the rats were removed and washed in 10% formal-saline and then fixed in fresh 10% formal saline. Livers of rats belonging to the same group were put in the same container.

Test for Alkaloids

Test for alkaloids was carried out according to the methods described by Harborne (1973) and Trease and Evans (1978). Zero point five (0.5g) grammes of

each extract was stirred with 5ml of 1% aqueous hydrochloric acid on a steam bath before being filtered and 1ml of the filtrate was treated with a few drops of Mayer's reagent. A second 1ml portion was treated similarly with Dragendorff's reagent. Turbidity or precipitation with either of these reagents was an evidence of the presence of alkaloids.

Test for Saponins

Test for saponins was done according to the method described by Wall *et al.*, 1954. Zero point five (0.5g) grammes of the extract was shaken with water in a test tube and frothing which persisted on warming indicated the presence of saponins. This is called frothing test. To confirm the presence of saponins, 0.5g of the extract was boiled briefly with 50ml phosphate buffer pH 7.4. The mixture was allowed to cool and after filtration about 5ml of the filtrate was passed (after 3 hours) through an asbestos disc (1.5mm thick and 7mm in diameter) which had been previously soaked in 3 drops of 1% cholesterol in ether and dried. The disc was washed with 0.5ml distilled water. After drying, it was heated in 20ml of boiling oxylol for 2 hours in order to decompose the complex formed between cholesterol and any saponins. This disc was then washed in ether and after drying, it was placed on a 7% blood nutrient agar. Complete haemolysis of red blood cells around the disc after 6 hours was evidence of the presence of saponins.

Test for Tannins

Test for tannins was carried out according to the method described by Trease and Evans (1978). Five (5g) grammes of each portion of plant extract was stirred with 10ml of distilled water and then filtered and ferric chloride reagent added to the filtrate. A blue-black, green or blue-green precipitate indicated the presence of tannins.

Test for phlobatannins

Test for phlobatannins was carried out according to the method described by Trease and Evans (1978). Five (5ml) millilitres of the aqueous extract was mixed with 5ml of 1% aqueous hydrochloric acid and boiled. The appearance of a reddish green colour indicated the presence of phlobatannins.

Test for Anthraquinones

Test for anthraquinones was carried out according to the method described by Trease and Evans (1978). Borntrager's test was used for the detection of anthraquinones. Five (5g) grammes of each plant extract was shaken with 10ml benzene. After filtration, 5ml of 10% ammonia solution was added to the filtrate, the mixture was shaken and the appearance of a pink, red, or violet colour in the ammoniacal (lower) phase indicated the presence of free anthraquinones.

For combined anthraquinones, 5g of each plant extract was boiled with 10ml aqueous Sulphuric acid and filtered while hot. The filtrate was shaken with 5ml benzene and the benzene layer was separated and half its own volume of 10% ammonia solution added. The appearance of a pink, red, or violet colour in the ammoniacal (lower) phase indicated the presence of anthraquinone derivatives

Test for Cardiac Glycosides

Legal Test

The plant extract was dissolved in pyridine and a few drops of 2% sodium nitroprusside together with a few drops of 20% NaOH added. The appearance of a deep red colour which faded to brownish yellow indicated the presence of cardenolides.

Test for flavonoids

Test for flavonoids was carried out according to the method described by Sofowora (1993). Five (5ml) millilitres of dilute ammonia solution was added to 0.5g of the extract and 5ml of concentrated H_2SO_4 was added. A yellow colouration indicated the presence of flavonoids.

Test for phenols

Test for phenols was carried out according to method described by Trease and Evans (1978). Two (2ml) millilitres of Ferric chloride was added to 2ml the extract. A deep blue-green colouration indicated the presence of phenols.

Determination of antimicrobial activity of the stem bark extract

Agar diffusion method described by Edward 1983 was used.

Principle: Agar diffusion method operates on the principle that antibiotics will diffuse from a paper disc or a small cylinder into an agar medium that contains test organism. The inhibition is observed as a failure of the organism to grow in the region

of the antibiotic. It may be dependent upon such factors as the diffusion rate of the antibiotic, the medium used, the sensitivity of the test organism, and incubation condition.

Procedure The paper discs were put into the extracts whose concentration was 20µg/ml and allowed to soak for some time. The discs were then removed and dried. The dried paper discs containing the absorbed extract were impregnated onto the surface of dried agar medium that has already been inoculated with the test organisms. These were left on the work bench for prediffusion and later incubated at 37°C for 18 to 24 hours. The plates were then observed and the diameter of zone of inhibition of growth was measured. Standard antibiotic discs of 20µg/ml concentration were used in order to compare the zone of inhibition produced by both the crude extracts and standard antibiotic discs.

Determination of Minimum Inhibitory and Minimum Bactericidal

Concentrations of the plant extract

Determination of Minimum Inhibitory and Minimum Bactericidal Concentrations were determined by the method described by Nester *et al.*, 2004. Six test tubes containing serially diluted crude extracts in a nutrient broth were set. The dilutions were 64, 32, 16, 8, 4, and 0µg/ml respectively, and the sixth test tube with 0µg/ml served as the control. 1ml of test organism's suspension was introduced into each test tube and then incubated for 24hours. The dilution was repeated with 32, 16, 8, 4, and 2µg/ml respectively. The test tubes were incubated for 24hours and then observed for growth. The test tube having the lowest concentration of the extract that do not show any growth was considered the one with minimum inhibitory concentration (MIC).

The Minimum Bactericidal Concentration was determined by using those test tubes that do not show any growth in the MIC determination. One ml suspension of each of the test tubes that showed no growth was introduced into test tubes containing extract – free nutrient broth and incubated for 24hours. The test tubes were later observed for growth. Amongst the test tubes that do not show any growth, the one having the lowest concentration was regarded as the one with the minimum bactericidal concentration (MBC).

Table 2 Phytochemical components detected in the stem bark extract of *Anogeissus leiocarpus*

Alkaloids	Anthraquinones	Flavonoids	Phlobatannins	Phenols	Quinones	Saponins	Glycosides	Tannins
+	+	–	–	+	–	+	+	+

+ = Presence of phytochemical component; – = Absence of phytochemical component.

Alkaloids, anthraquinones, phenols, saponins, glycosides and tannins were detected in the stem bark while flavonoids, phlobatannins, and quinones were not detected in the stem bark of *Anogeissus leiocarpus*

Table 3 Zone of inhibition of the growth of the test organisms (mm)

<i>Anogeissus leiocarpus</i>	<i>S. aureus</i>	<i>K. pneumoniae</i>	<i>N. gonorrhoeae</i>	<i>E. coli</i>
Zone of inhibition	10	-	-	10

- = Resistance of the test organism on the antimicrobial substance

Table 4 Minimum Inhibitory Concentration of Test substance

<i>Anogeissus leiocarpus</i>	<i>S. aureus</i>	<i>K. pneumoniae</i>	<i>N. gonorrhoeae</i>	<i>E. coli</i>
Min. Inhibitory Conc	16	32	32	16

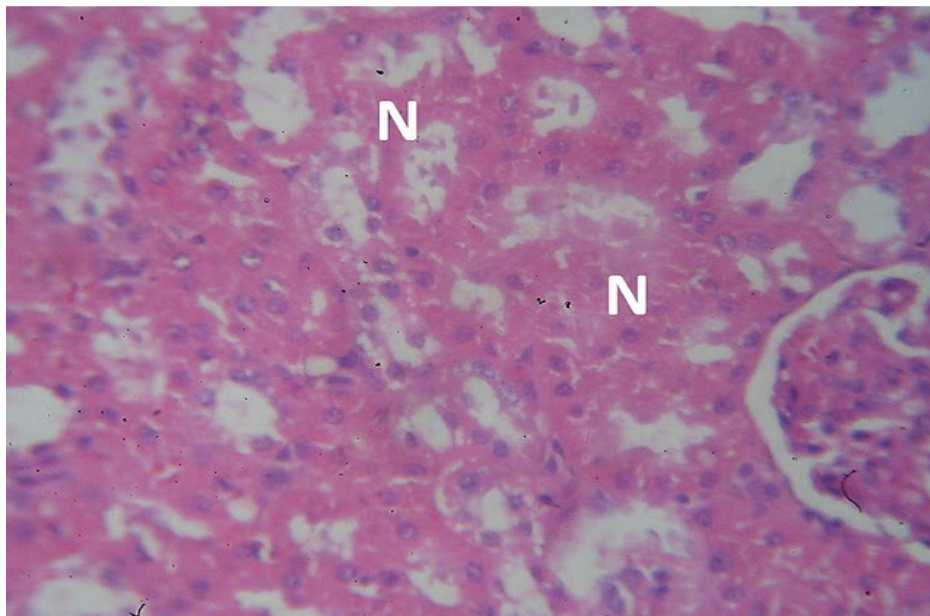
206 **Table 5 Effects of aqueous stem bark extract of *Anogeissus leiocarpus* on experimental animals**

Treatment.	Group1 Normal	Group2 200mg/kg	Group3 300mg/kg	Group4 400mg/kg	Group5 500mg/kg
AST U/I	12.1±0.4	12.9±0.3	13.9±0.3#	14.3±0.5#	13.9±0.3#
ALT U/I	29.1±0.5	30.4±0.5	32.7±0.3#	33.5±0.1#	34.0±0.2#
ACP U/I	20.9±0.2	23.2±0.1#	24.4±0.0#	24.9±0.2#	22.4±0.3#
ALP U/I	18.6±0.2	13.1±0.1*	14.5±0.2*	14.7±0.4*	21.3±0.5#
LDH U/I	105.9±1.6	337.5±0.7#	253.7±1.8#	416.6±0.9#	347.7±1.1#
Glucose mg/dl	60.4±2.1	65.7±5.8	63.7±4.4	64.5±2.8	79.3±1.6#
Cholesterol mmol/l	3.1±0.1	3.0±0.2	3.9±0.3	4.4±0.0#	4.8±0.2#
Total bilirubin µmol/l	0.9±0.0	0.9±0.0	1.2±0.1	1.6±0.2	1.9±0.3#
Direct bilirubin µmol/l	0.6±0.0	0.9±0.0	1.0±0.0	1.0±0.1	1.4±0.2#
Albumin g/l	37.8±0.7	40.7±0.5	38.6±1.0	39.5±1.0	38.9±0.9
Total protein g/l	36.7±3.3	47.9±0.5#	15.5±0.3*	32.0±0.6	45.1±0.9#
Urea mmol/l	6.2±0.8	3.0±0.2*	5.3±0.1	4.9±0.1	3.4±0.0*
Creatinine mg/dl	0.2±0.0	0.2±0.0	0.1±0.0*	0.1±0.0*	0.2±0.0

207 Values represent the mean ±SEM (n=3). P<0.05; *=Significantly lower than normal;#=Significantly higher than normal/control

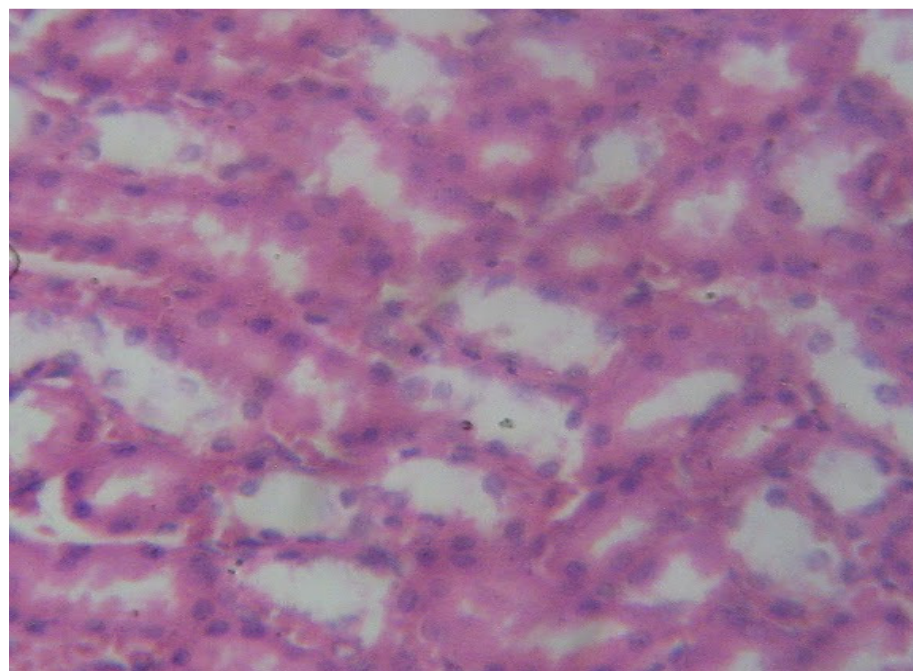
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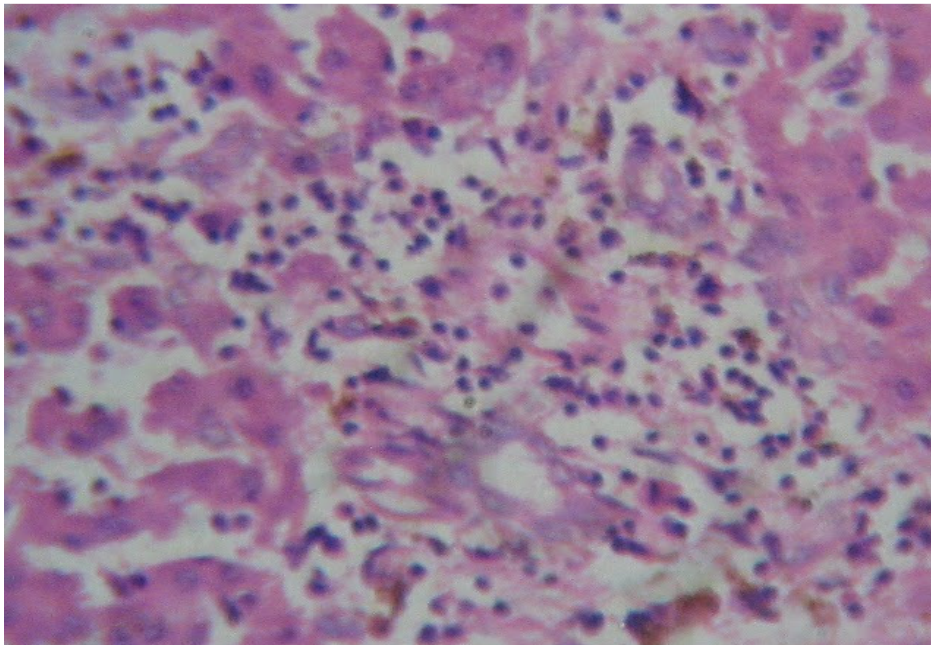
211 Plate 1: Kidney: Area of renal tubular necrosis (N) (x760). Kidney section of group 4 of
 212 *Anogeissus leiocarpus* treated experimental animals showing areas of renal tubular necrosis
 213 as compared to normal (Plate 2 below).
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216 Plate 2: Kidney: Normal renal tubular epithelium (x760)

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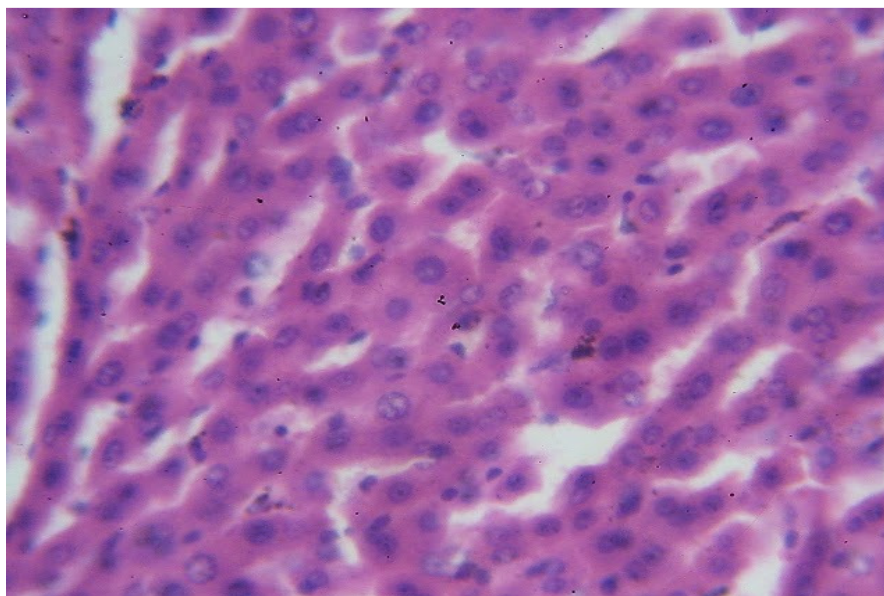
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Plate 3: Liver portal triad: Infiltration of neutrophils and macrophages into connective tissue (x760). Liver section of group 3 of *Anogeissus leiocarpus* showing the portal triad: Showing infiltration of neutrophils and macrophages into connective tissue when compared with the normal (Plate 18 below).



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Plate 4: Liver: Normal (x760)

Result and Discussion

Alkaloids, tannins, saponins, phenols, anthraquinones, phlobatannins, and flavonoids were the phytochemicals that can possibly contribute to the antimicrobial and physiological effects associated with the plants extracts. The phytochemicals possibly contribute to the toxic and/or antioxidant/protective effects especially for the ones present in substantial amounts.

Some of the phytochemicals observed in this study have been implicated in the treatment of certain ailments and diseases such as diarrhoea, syphilis, and gonorrhoea (Maspalma, 1997). The effectiveness of the use of stem bark of *Anogeissus leiocarpus* for the treatment of diarrhoeal disease in children by the local traditional healers could be due to the fact that they contain high concentrations of phytochemicals such as alkaloids and saponins. This tend to agree with what was reported by Ghoshal *et al.*,1996 that alkaloids have antidiarrhoeal effect which is probably due to their effect on transit time in the small intestine.

The effects of the crude plant extract on the test organisms as observed in this research work showed that the diameter of zones of inhibition produced by. More so, it is believed that each active principle of component parts making up the recipe complement one another in the fight against bacterial infection. These observations could be attributed to the various antimicrobial (phytochemical components) substances detected in this study which have been implicated by various authors as strong antimicrobial agents which are toxic to microorganisms. Typical amongst the phytochemical components are phenols and phenolic compounds which have been reported by Mason and Wasserman (1987) as compounds whose toxicity to microorganisms include enzyme inhibition by the oxidized compounds possibly through reaction with sulphhydryl groups or through more non specific interactions with protein.

Furthermore, none of the individual crude extracts and the combined form of the extracts had any antifungal effects on *Candida albicans*. These results therefore suggest that only antibacterial substances could be present in the four plants. The Minimum Inhibitory Concentration (MIC) for each extract was determined against the four test organisms. *Anogeissus leiocarpus* has an MIC of 16µg/ml each for *Staphylococcus aureus* and *Escherichia coli*, and 32µg/ml each for *Klebsiella pneumoniae* and *Neisseria*

gonorrhoeae. The effectiveness of each extract on the growth of the test organisms has manifested itself here as it did earlier in antimicrobial susceptibility test where the zones of inhibition were observed to be 10µg/ml for *Staphylococcus aureus* and *Escherichia coli* and no zone of inhibition for *Klebsiella pneumoniae* and *Neisseria gonorrhoeae*.

It has been observed in this study that whenever there is an elevation of AST activity, a corresponding elevation of ALT activity will be followed. The AST: ALT ratio (with elevated levels of both enzymes) is approximately 1:1 which is a pointer for a possible primary liver disease. This finding was in agreement with the earlier report by the American **Society** of Clinical Pathologists (ASCP, 2003). Although AST is localized in the liver, heart, muscles, kidneys, brain, pancreas, spleen and lungs, and ALT is localized in the liver, heart, muscles, and kidneys, however, the histological studies of the liver and kidney tissues actually predicts some degree of cell damage. The elevated level of Lactate Dehydrogenase (LDH) activity indicated some degree of cell damage to those tissues where the enzyme is localized. Also high serum LDH indicates liver congestion (Devlin, 1986). In fact, the level of this enzyme would suggest liver damage even though the myocardium was the most probable one.

This research work has shown increased serum glucose levels in the treated groups of the experimental animals as compared to normal/ control groups, even though the elevation was not statistically significant ($p < 0.05$). The rise in serum glucose levels above normal/control could be due to the effects of the crude extracts which might have affected the secretion of insulin by the islets of langerhans of the pancreas thereby reducing the rate of uptake of glucose by the body cells. Another possible cause might be due to leakage of glycogen into the blood as a result of liver cell injury.

This study has revealed that there was increase in serum level of cholesterol in the treated groups of experimental animals which was statistically significant ($p < 0.05$) when compared with the normal/control groups.

Serum urea and creatinine levels of the experimental animals showed no significant increase as compared to normal/control groups. Serum bilirubin indicated significant increase only in group five of the experimental animals. This rise in the levels of bilirubin could be as a result of the prolonged administration of the aqueous stem bark extracts which could contain some antimicrobial agents and antimicrobials or antibiotics

are among the substances that could cause increase blood levels of bilirubin. Low levels of albumin signify liver cell damage or kidney disease. This observation was in harmony with the reports on low albumin levels by Mohamadi-Nejad *et al.*, 2002.

Histological examination of the tissues (liver and kidney) of the experimental animals revealed that the cells were damaged to some extent by the extracts. The deleterious effects of the extracts were observed to be more pronounced on the liver than the kidney. The finding that the liver cells were more damaged than the kidney cells would suggest that the liver cells were more susceptible to the crude extract than the kidney. It is also possible that since the crude extracts will first go the liver for detoxification, the hydrophilic conjugate may be less potent and will not have a drastic effect on the kidney, thus causing less damage to the kidney as observed in this study.

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