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RESEARCH PAPER

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Possible Effects of *A. Congensis* Extract on Hepato and Renal Organs of Albino Rat

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ABSTRACT

Purpose: The study was conducted to investigate the possible effects of *A. congensis* extracts on hepato and renal organs of albino rat.

Research questions:

Is the extract safe for consumption?

What is the LD50 of the extract?

What is the effect on liver enzymes?

What effect does the extract has on urea and creatinine levels of the test animals?

Significance of the study: There is a widespread misconception that natural always means safe and a common belief that remedies from natural origin are harmless and carry no risk. This work defined that herbal medicines are expected to have side effect which may be of an adverse nature.

Method: This study was carried out between February 2019 to October 2020. *A. congensis* was collected from Emekuku area of Imo State, Nigeria. The albino rats were purchased from Animal Science and Production Department, Michael Okpara University of Agriculture, Umudike, Umuahia Abia State, Nigeria. The possible effects of the extracts on hepato and renal organs of the albino rats was determined using automated machine.

Result: *In sub-acute study, Aspartate aminotransferase (AST) and alkaline phosphatase (ALP) levels were significantly affected at all the doses of the extracts. There was no significant increase in the creatinine but there was increase in urea when a high dose of root extract was given.*

Conclusion: *There was no evidence of drug-induced symptoms at all the doses of the extract administered but the results revealed a tendency to cause kidney problem when high dose is used on a long term.*

Keywords: *Alstonia, Hepato-renal, Urea, Creatinine, Extraction and Toxicity.*

INTRODUCTION

Perhaps the most commonly used indicators of liver (hepatocellular) damage are the alanine aminotransferase (ALT) and aspartate aminotransferase (AST), formerly referred to as the SGPT and SGOT. These are enzymes normally found in liver cells that leak out of these cells and make their way to the blood when liver cells are injured. The ALT is felt to be a more specific indicator of liver inflammation as AST is also found in other organs such as the heart and skeletal muscle (Sushruta et al 2006). The liver and heart releases alanine aminotransferase (ALT) and an elevation in plasma concentration are an indicator of liver damage. The liver and heart release AST and ALT and an elevation in plasma concentration are an indicator of liver and heart damage (Wasan, et al. 2001). The alkaline phosphatase is the most frequently used test to detect obstruction in the biliary system. Elevation of this enzyme may be found in a large number of disorders as common as gallstone disease, alcohol abuse, and drug-induced hepatitis, or in less common disorders such as primary biliary cirrhosis (PBC) or biliary tumors, Wasan et al [2001]. Although this enzyme is found both in the liver and bile, and leaks into the bloodstream in a manner similar to that described for the ALT and AST, alkaline phosphatase is also found in other organs such as bone, placenta, and intestine.

Bilirubin is the main bile pigment in humans which, when elevated causes the yellow discoloration of the skin called jaundice. Bilirubin is formed primarily from the breakdown of a substance called heme found in red blood cells. It is taken up from the blood, processed, and then secreted into the bile by the liver. There is normally a small amount of bilirubin in the blood in healthy individuals ($<17\mu\text{mol/L}$), Wasan et al [2001]. Conditions which cause increased formation of bilirubin, such as destruction of red blood cells, or decrease its removal from the blood stream as in liver dysfunction, may result in an increase in the level of bilirubin in the blood, Wasan et al [2001]. Levels greater than $50\mu\text{mol/L}$ usually are noticeable as jaundice.

Albumin is a major protein which is formed by the liver. Although there are many factors which can affect the level of albumin circulating in the blood, chronic liver disease causes a decrease in the amount of albumin produced, and therefore the level of albumin in the blood is reduced, Wasan et al [2001]. Albumin is also part of most automated chemistry screening panels.

Blood urea nitrogen and serum creatinine requires blood samples. Renal function test measures the level of urea, creatinine and certain dissolved salts that can help determine whether or not the kidneys are functioning properly and paint a picture of the overall health.

A blood urea nitrogen (BUN) test measures the amount of nitrogen in the blood that comes from the waste product urea. Urea is made when protein is broken down in the body. Urea is made in the liver and passed out of the body in the urine. A BUN test is done to see how well

the kidneys are working. If the kidneys are not able to remove urea from the blood normally, the BUN level rises. Heart failure, dehydration, or a diet high in protein can also make the BUN level higher. Liver disease or damage can lower the BUN level, Wasan et al [2001].

Creatine is a chemical produced by amino acids in the body, creatine functions to supply energy to the muscles and other cells. As creatine performs its function in the body it produces a waste product called creatinine. The creatinine then travels through the blood stream until it reaches the kidney, at which point it is filtered out of the blood and passed out of the body in the urine, [Crook, 2006]. The kidneys are wholly responsible for the removal of creatinine from the body. Therefore a creatinine blood test or a creatine blood test can provide a lot of information about kidney functioning. If creatinine blood test results show abnormally high levels of creatinine in the blood, then the kidneys are likely not working at full capacity (Crook, 2006). Considering the work of the liver enzymes, this study was carried out to determine the effect of the extract on the liver enzymes and how the level of the creatinine are affected by the extract which in turn determines the working of the kidney in waste removal from the body.

MATERIALS AND METHODS

Extraction of Plant Materials

Fifty grams (50 g) of the pounded dried plant materials (leaf, bark and root powder) were weighed and extracted with 400 ml of aqueous (distilled water) and 400 ml of ethanol using Tedong [2007] extraction method. The processes were run for 2 hours each, after which the samples were evaporated to dryness using water bath. The dried extracts were weighed and kept in a well labeled sterile specimen bottles and stored in a refrigerator at 4 degree celsius until is required.

Acute Toxicity/Lethal dose (LD₅₀) test: The medium lethal dose of the crude extracts of leaf, bark and root of *A. congensis* were determined by Lorkes [1983] method using the oral routes with the assistance of Pharmacist Solomon Nwafuru of Federal Medical Centre, Owerri. The acute oral toxicity study was conducted in compliance with OECD guideline 425, which stipulate the use of only three animals, Jonsson et al [2013]. The test was divided into two stages.

Stage One: Determination of the toxic range of the leaf, bark and root extracts of *Alstonia congensis*. Mice were divided into 9 groups of 3 animals in each group. Each group received a dose (10, 100, 1000mg/kg) of the ethanolic extracts of leaf, bark and root suspended in distilled water respectively. The doses were administered orally and the treated animals observed for 72hrs for number of deaths.

Stage Two: Determination of lethality of leaf, bark and root extracts.

The doses used in this stage were determined from the number of deaths per dose recorded in the stage one test. Since no death occurred in the stage one test, three different higher doses: 1600mg/kg, 2900mg/kg and 5000mg/kg were administered to another group of animals at one dose per animal. The treated animals were monitored for number of deaths for 24hrs and continued to 72hrs. The LD₅₀ in this test is determined by calculating the geometric mean of the test and most toxic doses.

$$LD_{50} = \sqrt{\text{minimum toxic dose} \times \text{maximum tolerated dose}}$$

Sub-Acute Toxicity Studies

For the sub-acute toxicity studies, hepato and renal parameters were determined in relation to the control treatment. These include AST, ALP, ALT, blood urea, creatinine using albino rat as the test animal. These investigations were carried out to determine the effect of the extract on the internal organs of test animals. A low (400mg/kg) and high 800mg/kg were used in this study.

RESULTS

Pre-treatment Acute toxicity/lethality test

The result of the lethality and acute toxicity studies of the leaf, bark and root extract of *Alstonia congensis* in a naïve rat is shown on tables 1,2 and 3.

Table 1. Acute toxicity (LD₅₀) test of the crude leaf extract of *A. congensis*.

Stages	Doses mg/kg	Mortality
Stages 1	10	0/3
	100	0/3
	1000	0/3
Stages 2	1600	0/1
	2900	0/1
	5000	0/1

Table 2. Acute toxicity (LD₅₀) test of the crude bark extract of *A. congensis*.

Stages	Doses mg/kg	Mortality
Stages 1	10	0/3
	100	0/3
	1000	0/3
Stages 2	1600	0/1
	2900	0/1
	5000	0/1

Table 3. Acute toxicity (LD₅₀) test of the crude root extract of *A. congensis*

Stages	Doses mg/kg	Mortality
Stages 1	10	0/3
	100	0/3
	1000	0/3
Stages 2	1600	0/1
	2900	0/1
	5000	0/1

At respective doses of 10, 100 and 1000mg/kg, all the three animals given the extracts of leaf, bark and root survived beyond the two weeks of observation without any sign of illness. When the extract was increased to 1600, 2900 and 5000 all the animals equally survived. All the rats that received the doses (10, 100, 1000, 1600, 2900 and 5000mg/kg) of the extract survived beyond the 2weeks of observation. The medium lethal dose toxicity value (LD₅₀) of the extract must be above 5000mg/kg. There were no gross physical and behavioral changes including rigidity, sleep, diarrhea, depression, abnormal secretion and hair erection within the observation period.

The result of the effect of *A. congensis* extracts on AST and ALT of the test animals were shown in table 4 and fig 1.

Table 4. Showing the effects of the extract on AST and ALT.

Liver enzyme	Dosage	Mean values	Significance
AST (μ /l)	Normal (control)	24.67 \pm 3.88	-
	400mg/kg Leaf	27.33 \pm 1.75	P > 0.05
	800mg/kg Leaf	27.67 \pm 3.88	P > 0.05
	400mg/kg Bark	30.33 \pm 4.23	P < 0.05
	800mg/kg Bark	31.00 \pm 3.41	P < 0.05
	400mg/kg Root	32.00 \pm 3.29	P < 0.05
	800mg/kg Root	33.00 \pm 3.46	P < 0.05
	20mg/kg Artesunate	32.50 \pm 4.09	-
ALT (μ /l)	Normal (control)	34.17 \pm 2.99	-
	400mg/kg Leaf	35.50 \pm 3.39	P > 0.05
	800mg/kg Leaf	36.83 \pm 4.22	P > 0.05
	400mg/kg Bark	37.67 \pm 4.50	P > 0.05
	800mg/kg Bark	38.17 \pm 3.31	P > 0.05
	400mg/kg Root	38.17 \pm 5.19	P > 0.05
	800mg/kg Root	40.00 \pm 5.10	P < 0.05
	20mg/kg Artesunate	40.50 \pm 3.62	-

The results are expressed as mean \pm SEM
 P>0.05 is not significant,
 n = 10, P<0.00 is significant.

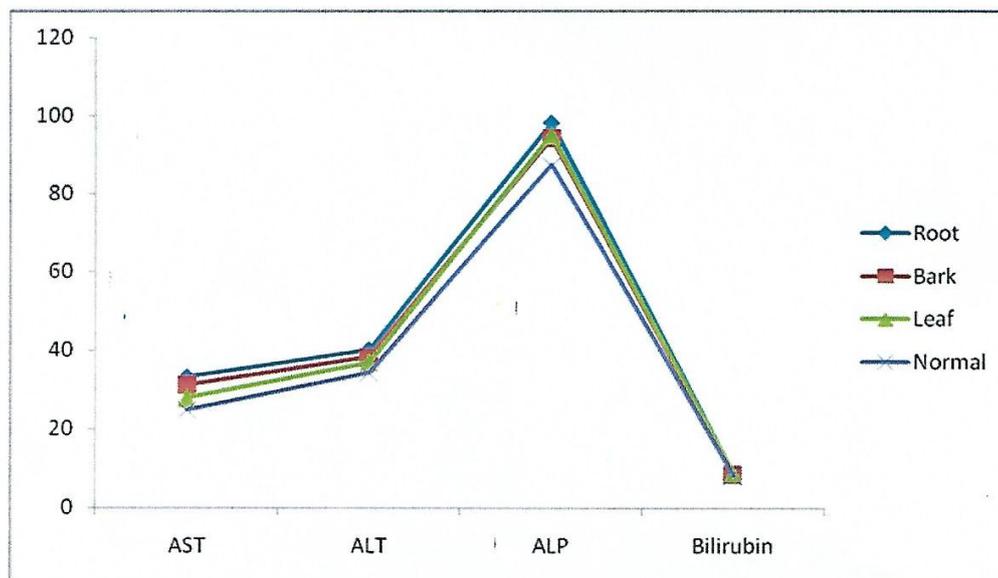


Figure 1. Effect of extract on liver enzymes.

The AST and ALT of the test animals before the treatment were 24.67 ± 3.88 and 34.17 ± 2.99 respectively.

When 400mg/kg and 800mg/kg of leaf extracts were given, the values for AST and ALT were 27.33 ± 1.75 , 35.50 ± 3.39 and 27.67 ± 3.88 , 36.83 ± 4.22 respectively.

For 400 and 800mg/kg of bark, the values of AST and ALT were 30.33 ± 4.23 , 37.67 ± 4.50 and 31.00 ± 3.41 and 38.17 ± 3.31 respectively.

The values at 400mg/kg and 800mg/kg of root were 32.00 ± 3.29 , 38.17 ± 5.19 and 33.00 ± 3.46 , 40.00 ± 5.10 respectively.

The AST of the test animals were significantly increased on treatment with all the doses of the extracts of bark and root but was not significantly affected by treatment with leaf. When 800mg/kg of root was given the ALT was significantly ($P < 0.05$) increased.

The results of the effect of extracts of *A. congensis* on ALP and bilirubin of the test animals were shown in table 5 and figure 2.

Table 5. Showing the effect of extracts of *A. congensis* parts on ALP and Bilirubin of test animals.

Enzyme	Dosage	Mean values	Significance
ALP (μ /l)	Normal control	87.17 ± 7.91	-
	400mg/kg Leaf	94.50 ± 2.88	$P < 0.05$
	800mg/kg Leaf	95.00 ± 3.35	$P < 0.05$
	400mg/kg Bark	94.00 ± 2.19	$P < 0.05$
	800mg/kg Bark	94.33 ± 3.45	$P < 0.05$
	400mg/kg Root	98.33 ± 6.19	$P < 0.05$
	800mg/kg Root	98.00 ± 6.45	$P < 0.05$
	20mg/kg Artesunate	95.00 ± 4.73	-

BILIRUBIN (mg/dl)

Normal control	8.10 ± 1.12	-
400mg/kg Leaf	8.20 ± 1.39	$P > 0.05$
800mg/kg Leaf	8.70 ± 0.92	$P > 0.05$
400mg/kg Bark	8.70 ± 0.90	$P > 0.05$
800mg/kg Bark	8.40 ± 0.75	$P > 0.05$
400mg/kg Root	7.60 ± 1.02	$P > 0.05$
800mg/kg Root	8.40 ± 0.52	$P > 0.05$
20mg/kg Artesunate	7.90 ± 0.89	-

The results are expressed as mean \pm SEM

$P < 0.05$ is significant,

$n = 10$

$p > 0.05$ is not significant

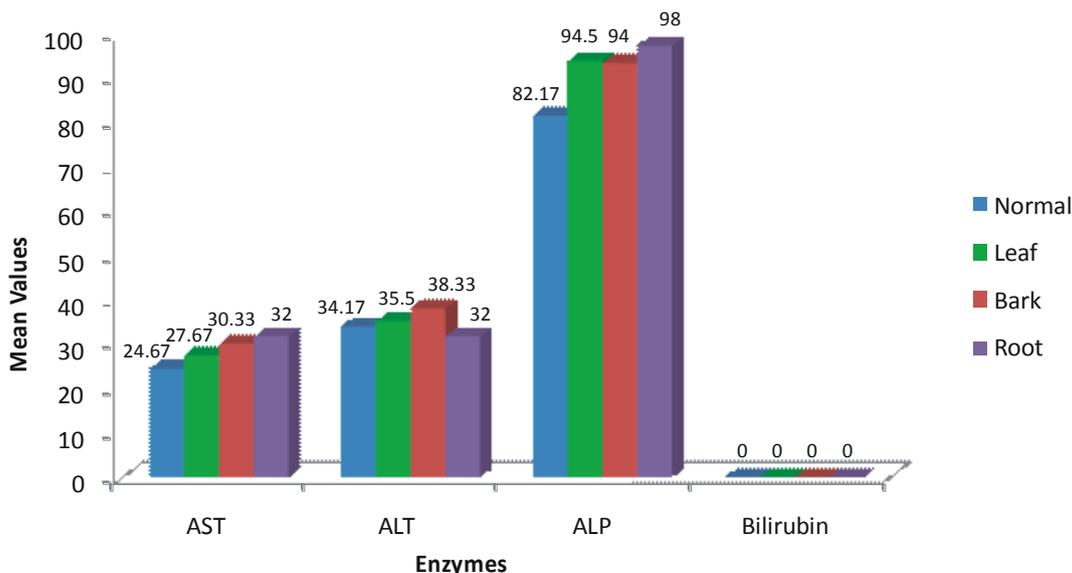


Figure 2. Effect of 400mg/kg of extracts on liver enzymes.

The ALP and Bilirubin of the test animals before the treatment were 87.17 ± 7.91 and 8.10 ± 1.12 respectively.

On treatment with 400mg/kg and 80mg/kg of leaf extract, ALP and Bilirubin values were 94.50 ± 2.88 , 8.20 ± 1.39 and 95.00 ± 3.35 , 8.70 ± 0.92 respectively.

When 400mg/kg and 800mg/kg of bark were given the values of ALP and Bilirubin were 94.00 ± 2.19 , 8.70 ± 0.90 and 94.33 ± 3.45 , 8.40 ± 0.75 respectively.

The values when 400mg/kg and 800mg/kg of root were given 98.33 ± 6.19 , 7.60 ± 1.02 and 98.00 ± 6.45 , 8.40 ± 0.52 respectively. All the extracts in their doses significantly (<0.05) increased the ALP of the test animals, but the Bilirubin level was not significantly affected.

The results of the effect of extracts of *A. congensis* on urea and creatinine of the test animals were shown in table 6 and figure 3.

Table 6. The effects of the extracts of *A. congensis* parts on urea and creatinine.

	Dosage	Mean values	Significance
UREA (mg/dl)	Normal (control)	24.58 ± 2.44	-
	400mg/kg Leaf	24.78 ± 2.64	$P > 0.05$
	800mg/kg Leaf	24.85 ± 3.23	$p > 0.05$
	400mg/kg Bark	22.75 ± 2.50	$P > 0.05$
	800mg/kg Bark	24.13 ± 2.99	$P > 0.05$
	400mg/kg Root	25.45 ± 2.46	$P > 0.05$
	800mg/kg Root	27.80 ± 1.66	$P < 0.05$
	20mg/kg Artesunate	27.15 ± 1.48	-
		11.20 ± 1.69	

CREATININE (mg/dl)	Normal (control)		
	400mg/kg Leaf	10.30 ± 2.57	P>0.05
	800mg/kg Leaf	12.40 ± 2.59	P>0.05
	400mg/kg Bark	10.60 ± 3.62	P>0.05
	800mg/kg Bark	12.10 ± 2.07	P>0.05
	400mg/kg Root	11.10 ± 1.49	P>0.05
	800mg/kg Root	11.50 ± 2.16	P>0.05
	20mg/kg Artesunate	10.60 ± 1.22	

The results are expressed as mean ± SEM

P>0.05 is not significant, p<0.05 is significant, n = 10

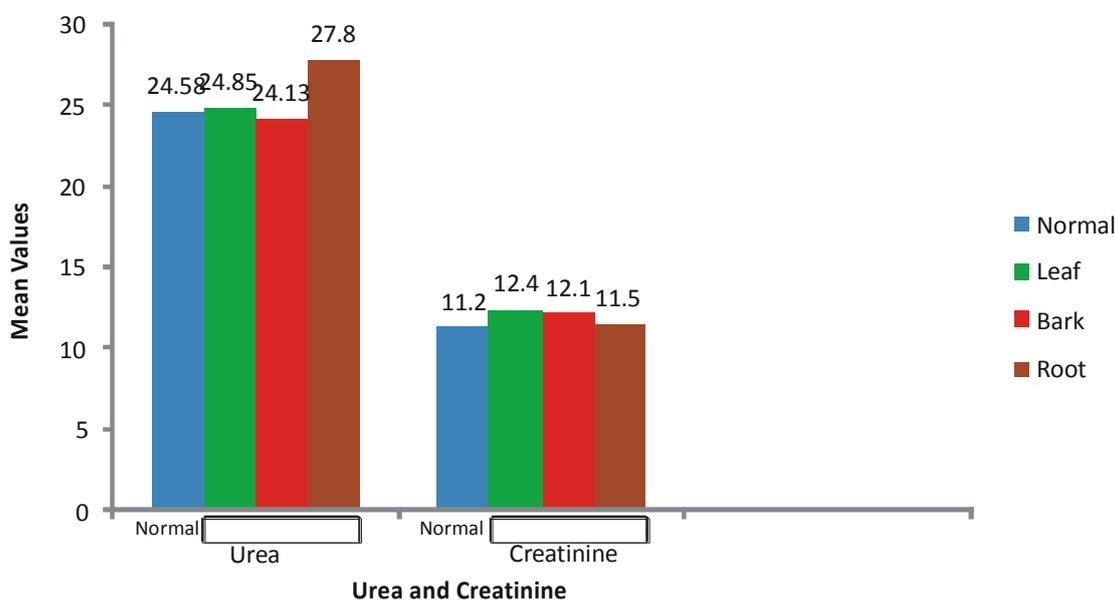


Figure 3. Effect of 800mg/kg of extracts on Urea and Creatinine.

The results of the effect of extracts of *A. congensis* leaf, bark and root on Urea and Creatinine of the test animals were shown in table 6 and figure 3. The ALP and Bilirubin of the test animals before the treatment were 24.58 ± 2.44 and 11.20 ± 1.69 respectively. On treatment with 400mg/kg and 80mg/kg of leaf extract, urea and creatinine values were 24.78 ± 2.64 , 10.30 ± 2.57 and 24.85 ± 3.23 , 12.40 ± 2.59 respectively.

When 400mg/kg and 800mg/kg of bark were given the values of urea and creatinine were 22.75 ± 2.50 , 10.60 ± 3.62 and 24.13 ± 2.99 , 12.10 ± 2.07 respectively.

The values when 400mg/kg and 800mg/kg of root were given 25.45 ± 2.46 , 11.10 ± 1.49 and 27.80 ± 1.66 , 11.50 ± 2.16 respectively.

The level of urea in the blood was significantly (<0.05) increased when 800mg/kg of root was given.

DATA ANALYSIS

Data were analysed using computer software SPSS, Version 16. Results of the study were expressed as a mean ± standard error of the mean (m ± SEM). Statistical significance was determined by one way analysis of variance (ANOVA) with multiple comparison tests (Post

Hoc/Tukeys test/HSD) to compare parameters within groups. All data were analysed at a 95% confidence interval ($\alpha = 0.05$).

DISCUSSION

The potential adverse effects of the medicinal plants preparations on internal organs have been a major concern in the use of herbal preparations. Based on this, the study was conducted to investigate the adverse effect of *A. congensis* preparations in hepato and renal organ of albino rat.

Acute toxicity test was carried out on laboratory animals, in this particular case on albino rats receiving different doses of the substances in question. Oral administration of the aqueous extracts of *A. congensis* did not show any signs of toxicity nor did they produce lethality in rats up to 5000 mg/Kg. As this dose is minimal, it is very unlikely to associate the death of the animal with the extract, therefore, this indicated that the LD50 of the extract must be above 5000 mg/kg body weight. In this present study, *A. congensis* preparations showed a mild increase in ALT.

According to, Nyblom et al [2006], when AST and ALT are greater than three times normal, the differential can include among other things drug induced level liver cancer but in this study the AST and ALT levels were not three times greater than normal. The mild increase in ALT which occurs in cases of pancreatitis, heart attack, infectious mononucleosis and shock, Burtis et al [2001] was observed in this study, which may be as a result of ingestion of the extracts. The present work showed elevated ALP which occurs in diseases that impair bile formation (cholestasis) and also in hepatitis, heart attack and gall stones, Burtis et al [2001], which implies that the extract may be deleterious to the liver when taken for long period and in high dose.

From this study, there was an increase in the level of urea in the blood of the treated animals when 800mg/kg of root extract was given. This agrees with the work carried out by Oze et al [2006], that the increase in urea and creatinine depended on the dose and duration of the extract. This shows that continuous use of the high dose of root extract may affect the kidney which may slow the removal of urea from the blood by the kidney which in turn becomes poisonous to the body and may lead to heart attack which will eventually result to death.

CONCLUSION

The effect of the extract on the internal organs of the treated animals was deleterious when high dose was used for long term treatment. It is advisable not to use this plant extract for long time in high doses until the extract is standardized. It would therefore be worthwhile to standardize the extract to know the dose and the time frame in order not to cause any adverse effect on the internal organs.

SUMMARY

The high dose of the extracts should not be used for long time as it may likely affect the liver and kidney. The dosage range should not exceed 4 to 7 days to avoid any deleterious effect on the internal organ. The extract may be preferable for chronic infections where orthodox medicine failed.

ETHICAL APPROVAL

Ethical clearance was given by Michael Okpara University of Agriculture Umudike, Umuahia Abia State Nigeria.

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