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Toxicological Studies Of Methanol Roots Extract Of *Ficus sycomorus.*

Abbas AY[1]*, Ladan MJ[1], Girei AM[2], and Achor M.[3]

[1]Department of Biochemistry, Faculty of Science, Usmanu Danfodiyo University, P.M.B. 2346, Sokoto, Nigeria.
[2]National Universities Commision , Abuja Nigeria
[3]Department of Pharmaceutics and Pharmaceutical Microbiology, Usmanu Danfodiyo University, P.M.B. 2346, Sokoto, Nigeria.

ABSTRACT

Ficus sycomorus (Mulberry Fig) plant have been reported to be used in Nigerian traditional medicine for the treatment of malaria and hence the need to evaluate its safety profile. Guidelines of OCED were employed for acute and sub-chronic toxicity studies of the methanol root extract of the plant in albino rats. Five (5) rats were used for the acute toxicity study as recommended by the OCED 2001 guidelines. Twenty four (24) albino rats of both sexes weighing between 100 -120 g were assigned into six groups of four rats each. The rats in groups 2, 3, 4, 5 and 6 were orally administered graded doses of the LD_{50} of the extract (20%, 40%, 60%, 80% and 100%) daily for 28 days; rats in group 1 served as control group and received distilled water for the test period. After 28 days, all the rats were decapitated and bloods collected from the individual animals were centrifuged to obtain sera for biochemical assays. The kidneys and livers of the rats were removed and preserved in 10% formalin for histopathological studies. The median lethal dose (LD_{50}) of the methanol root extract was estimated to be greater than 5000 mg/kg body weight. Methanol root extract administration for 28 days did not produce any significant effect on the hepatic and renal function indices. The results suggest that the plant is safe.

Keywords: *Ficus sycomorus, liver, kidney, extract, root malaria*

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Corresponding author

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INTRODUCTION

The use of medicinal plants to treat diseases is as old as man himself and medicinal plants have been used since ancient times to treat many illnesses [1]. Herbal medicine was the sole medical system for health care before the advent of orthodox or modern medicine [2]. Even in this present technological era, traditional medicine play a predominant role in the third world for the preservation of health of the rural majority who constitute over 70% of the total population [2]. The use of traditional remedies is common in sub-Saharan Africa, and visits to traditional healers remain a mainstay of care for many people because of preference, affordability, and limited access to hospitals and modern health practitioners [3]. In Africa more than 2,000 plants have been identified and use as herbal medicines. However, very few of these plants have been evaluated for safety [3]. Most herbal products have not been subjected to drugs approval process to demonstrate their safety and effectiveness (Raphael 2011). Some contain poisonous substances like mercury, lead, arsenic, corticoids, and some organic substances in harmful amount[4].Traditional medicines, like modern pharmaceuticals can harm, but because humans have been using herbal drugs for long time, they are considered safe and non-toxic and the toxicological effects of these agents have been mostly ignored[3]. Studies have shown that not all natural products are safe, some poisons are also natural and therefore herbal medicine can cause serious illness such as allergy, liver or kidney damage to cancer or even death [4]. It was reported that 25% of childhood blindness in Nigeria and India were associated with the use of herbal medicine [4]. At present, work conducted on traditional medicine in Africa has mainly concentrated on the collection, identification, and classification of herbal products for treatment of different ailments. However, research in the areas of safety and toxicology is lacking [3]. If the origin of herbs' toxicity is not identified, the adverse effects may be wrongly associated with other environmental exposures or some traditional belief and failure to establish the true cause of exposure also means that the patient continues taking the toxic herb [3]. Therefore, the screening of traditional remedies for safety and toxicity is recommended to protect public health.

MATERIALS AND METHODS

Chemicals and Reagents

All chemicals and reagents used in this study are of analytical grade

Ficus Sycomorus Plant

Ficus sycomorus (roots) was obtained from Sokoto metropolis, Sokoto State, Nigeria. The plant was identified at the Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria. A voucher specimen number was obtained (UDUH/ANS/0214) and specimen deposited at the herbarium.

Experimental Animals

Wistar albino and rats were obtained from the Animal Houses of the Department of Biochemistry and Faculty of Pharmaceutical Sciences Usmanu DanFodiyo University, Sokoto, Nigeria. The animals were housed in standard cages in Animal House, Department of Biochemistry. The animals were allowed access to feed and clean water *ad libitum* for seven days to acclimatize before commencement of research.

Preparation of the Plant Methanol Extracts

The roots were washed with tap water and air-dried under the shade. The dried samples were pulverized with pestle and mortar into small pieces and ground separately into powder. The powdered samples were then stored in plastic containers for subsequent use under room temperature. Two hundred (200) grams of the roots were soaked in 500ml of methanol for seventy two (72) hours and stirred occasionally. They were filtered with a clean muslin cloth, and the filtrates were evaporated to dryness using rotary evaporator at 45^0C. The recovered methanol extracts (filtrates) were stored in plastics containers and kept under room temperature.

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Acute oral toxicity study

A total of five (5) adult Wistar albino rats were used for the study. After acclimatization period, five groups of one rat each were dosed individually at 48 hours interval with 5000 mg/kg body weight of methanol root extract of *F.sycomorus* using the Organization for Economic and Cultural Development OECD up and down procedure [5]. The behavioral changes and other changes observed in animals were recorded according to OECD [5]. Subsequently all animals were observed for the next 14 days for any delayed toxic effects.

Sub-Chronic Toxicity study

Twenty four albino rats of both sexes weighing between 100g -120g were assigned into six groups of four rats each. The rats in groups 2, 3, 4, 5 and 6 were orally administered graded doses of the LD_{50} of the extract (20%, 40%, 60%, 80% and 100%) daily for 28 days. Rats in group one served as control group and received distilled water for the test period. The body weights of all the animals were recorded before and during the treatments period weekly. After 28 days, all the rats were decapitated and bloods collected from the individual animals were centrifuged to obtain sera for biochemical assays. The kidneys and livers of the rats were removed and preserved in 10% formalin for histopathological studies.

Biochemical assays

Serum Alanine Aminotransferase and Serum Aspartate Aminotransferase (AST) activities were ascertain using the method of Reitman and Frankel[6](Assay kit: Randox laboratories, UK). Total protein in the blood was determined by Biuret method of Gomall *et al.*[7] Total and conjugated bilirubin was determined using the method of Jendrassik and Grof [8] (Assay kit: Randox laboratories, UK), Alkaline phosphatase was estimated using Colorimetric method of Sood[9](Assay kit: Randox laboratories, UK), Albumin was determined by the dye binding technique utilizing Bromocresol green (BCG) as modified by Doumas *et al.*[10] was employed. Urea was measured using Diacetyl monoxime Method[11] (Assay kit: Randox laboratories, UK) and Uric acid by enzymatic colourimeric method[12] (Assay kit: Randox laboratories, UK). Serum ceatinine was estimated using the method of Henry [13](Assay kit: Randox laboratories, UK)

Histopathological Studies of the Liver and Kidney

A portion of the tissue was collected and immediately fixed in 10% formalin, dehydrated in gradual ethanol (50-100%), cleared in xylene and embedded in paraffin. Sections (4-5µm) were prepared and then stained with Haematoxylin and eosin (H-E) dye for photomicroscopic observations[14]

RESULTS

Acute Oral Toxicity (LD_{50}) of Root Methanol Extracts

Oral administration of a single dose of 5000mg/kg body of the methanol root extract of the *Ficus sycomorus* produced no mortality in the rats(table 1). Likewise no sign of toxicity such as lost in body weight, hair, change in eyes and behavioral pattern was observed. Furthermore, negative behavioral changes, like tremors, convulsions, salivation, diarrhea, lethargy, sleep or coma at 5000mg/kg body weight were not observed. Thus, the median lethal dose (LD_{50}) of the methanol root extract was estimated to be greater than 5000 mg/kg

Table 1: Acute Oral Toxicity of the Methanol Root Extract of the *Ficus sycomorus*

Dose (mg/kg)	Groups	No. of Animals	No. of Death
5000	I	1	0
5000	II	1	0
5000	III	1	0
5000	IV	1	0
5000	V	1	0

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Sub-Chronic Toxicity Studies

Liver Function Indices

Table 2 below showed mean values of serum alanine aminotransferase (ALT), alkaline phosphate (ALP), aspartate aminotransferase (AST), total protein, albumin and direct and total bilirubin of rats orally administered methanol root extract of *Fycus sycomorus* (groups 2 to 6) and the control group (1).The mean values of the activities of the liver function enzymes (AST and ALT) obtained from the experimental groups (2 to 6) were not significantly (P>0.05) increase compared to the control group (group 1). The levels of TP and ALB also do not significantly (P>0.05) decrease when experimental groups are compared with the control. The TB and DB were not significantly (P>0.05) affected when experimental groups are compared with the control group. However, there was significant (P<0.05) increase in ALP activity in experimental group.

Table 2: Liver Function Indices of Rats Administered Methanol Root Extract of *Ficus sycomorus*

Group	Dose (%LD_{50})	ALT (U/L)	ALP (U/L)	AST (U/L)	TP (g/dl)	ALB (g/dl)	TB (mg/dl)	DB (mg/dl)
I	0	27.40±0.67	473.93±16.00	56.20±0.92	5.56±0.22	2.54±0.07	3.10±0.17	0.40±0.06
II	20	23.80±1.41	502.32±43.14	43.90±4.78a	5.50±0.20	2.64±0.05	2.70±0.38	0.23±0.03
III	40	20.83±0.60	441.60±71.92	52.27±0.89b	5.07±0.15	2.68±0.02	2.67±0.18	0.33±0.03
IV	60	10.00±0.21	742.03±37.11*	214.43±7.66	4.90±0.26	2.67±0.05	3.33±0.61	0.27±0.03
V	80	19.73±0.15	743.57±30.29*	32.03±0.49	5.90±0.06	2.67±0.02	3.97±0.23	0.33±0.03
VI	100	9.80±0.12	731.40±25.93*	33.20±1.14	5.44±0.23	2.83±0.01	2.50±0.21	0.40±0.06

Values are expressed as Mean± SEM (n=3). Values in the experimental groups are not significantly (P>0.05) different compared to the control, *except ALP were there is significant (P<0.05) increase compared to control group.

Group I: (Control group) received normal saline
Group II: received 20% LD_{50} of the methanol root extract once daily for 28 days
Group III: received 40% LD_{50} of the methanol root extract once daily for 28 days
Group IV; received 60% LD_{50} of the methanol root extract once daily for 28 days
Group V; received 80% LD_{50} of the methanol root extract once daily for 28 days
Group VI; received 100% LD_{50} of the methanol root extract once daily for 28 days

Kidney Function Indices

Table 3: Kidney Function Indices of Rats Administered Methanol Root Extract of *Ficus sycomorus*

Groups	Dose (% LD_{50})	Urea (mg/dl)	Uric Acid (mg/dl)	Creatinine (mg/dl)
I	0	17.18±0.95	14.49±139	4.40±0.53
II	20	20.63±0.92	10.39±1.10	3.65±0.24
III	40	14.01±0.95	14.06±1.79	3.53±0.06
IV	60	15.85±3.17	22.37±1.31	2.59±0.31
V	80	10.84±0.95	13.91±0.32	2.78±0.35
VI	100	15.35±0.50	11.93±1.09	2.65±0.02

Values are expressed as Mean± SEM (n= 3). Values in the experimental groups are not significantly increase (P>0.05) compared to the control group.

Group I: (Control group) received normal saline
Group II: received 20% LD_{50} of the methanol root extract once daily for 28 days
Group III: received 40% LD_{50} of the methanol root extract once daily for 28 days
Group IV; received 60% LD_{50} of the methanol root extract once daily for 28 days
Group V; received 80% LD_{50} of the methanol root extract once daily for 28 days
Group VI; received 100% LD_{50} of the methanol root extract once daily for 28 days

Table 3 indicated the results of serum uric acid, urea and Creatinine of rats orally administered methanol root extract of *fycus sycomorus* (20%, 40%, 60%, 80% and 100% LD_{50}). The mean values revealed no

significant (P>0.05) increase on serum uric acid, urea and Creatinine between the experimental groups and the control group. In fact all the three parameters decreases as a result of treatment with the root extract.

Histopathological Observations

Liver Lesion Score

The lesion scores of the rats administered methanol root extract of *fycus sycomorus* in the experimental groups (2 to 6) and the control group (1) is presented in table 4 and plates 1, 2, , 4, 5 and 6. Oral administration of the extract revealed no degeneration of the hepatic cells and no necrosis were observed in the experimental groups compared to control group. Congestions were observed though not significant.

Table 4: Liver lesion Scores of the Rats Administered Methanol Root Extract of *Ficus sycomorus*

Group	Treatment (% LD_{50})	Lesion Score (Mean Rank)		
		Congestion	**Degeneration**	**Necrosis**
I	0	7.00	-	-
II	20	7.00	-	-
III	40	13.50	-	-
IV	60	11.50	-	-
V	80	7.00	-	-
VI	100	11.00	-	-

Mean Ranking of the lesion scores for the experimental and control groups using Kruskal-Wallis Test. Values in experimental and control groups are not significantly different (P>0.05).

Group I: (Control group) received normal saline

Group II: received 20% LD_{50} of the methanol root extract once daily for 28 days

Group III: received 40% LD_{50} of the methanol root extract once daily for 28 days

Group IV; received 60% LD_{50} of the methanol root extract once daily for 28 days

Group V; received 80% LD_{50} of the methanol root extract once daily for 28 days

Group VI; received 100% LD_{50} of the methanol root extract once daily for 28 days

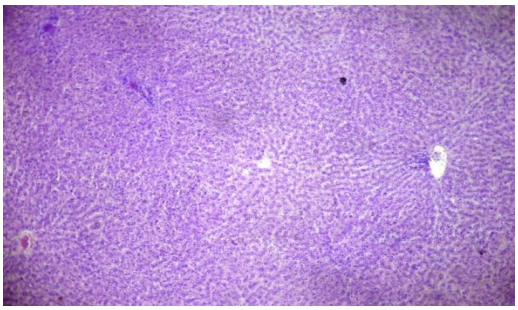

Plate 1: Photomicrograph of section of the liver of group 1 showing normal liver architecture . HE X100

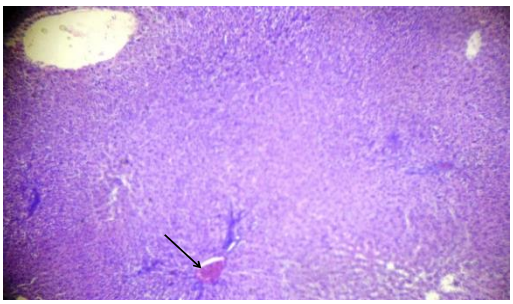

Plate 2: Photomicrograph of section of the liver of group 2 showing mild vascular congestion (arrow). HE X100

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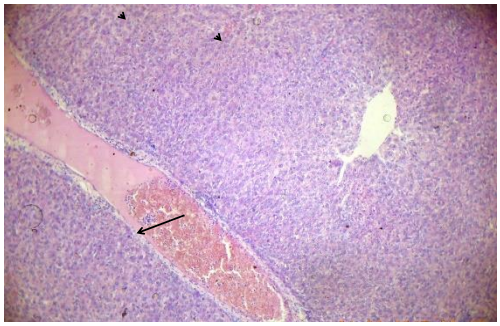

Plate 3: Photomicrograph of section of the liver of group 3 showing severe vascular congestion (arrow) and mild hemorrhages (arrowheads). HE X 100

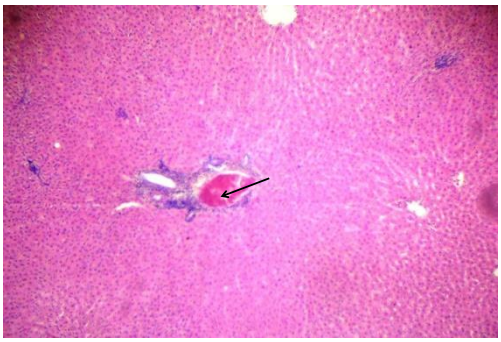

Plate 4.Photomicrograph of section of the liver of group 4 showing vascular congestion (arrow). HE X 100

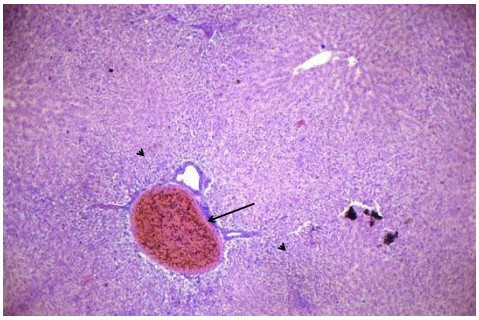

Plate 5: Photomicrograph of section of the liver of group 5 showing vascular congestion (arrow) and microvacuolation (arrowheads). HE X100

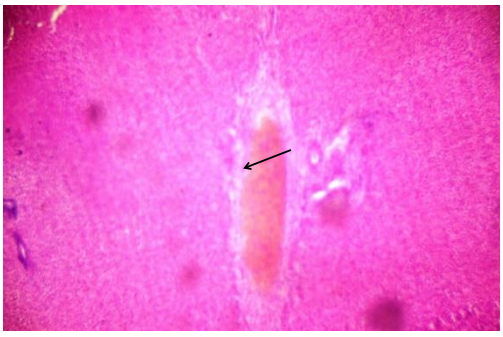

Plate 6: Photomicrograph of section of the liver of group 6 showing vascular congestion (arrow). HE X 100

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Kidney Lesion Score

Table 5 and plates 7. 8, 9, 10, 11 and 12 indicated the lesion scores of the rats administered methanol root extract of *Fycus sycomorus* in the experimental groups (2 to 6) and the control group (1). Oral administration of the extract revealed no degeneration of the kidney cells, however congestion and necrosis were observed in the experimental groups compared to control group.

Table 5: Kidney Lesion Score of the Rats Administered Methanol Root Extract of *Ficus sycomorus*

Group	Treatment (% LD_{50})	Lesion Score Mean Rank		
		Congestion	**Degeneration**	**Necrosis**
I	0	10.83	-	2.50
II	20	8.00	-	2.70
III	40	10.83	-	7.00
IV	60	8.00	-	6.33
V	80	8.00	-	9.00
VI	100	11.33	3.5	10.00

Mean Ranking of the lesion scores for the Experimental and Control Groups using Kruskal-Wallis Test. Values for necrosis in experimental and control group significantly different (P>0.05).

Group I: (Control group) received normal saline

Group II: received 20% LD_{50} of the methanol root extract once daily for 28 days

Group III: received 40% LD_{50} of the methanol root extract once daily for 28 days

Group IV; received 60% LD_{50} of the methanol root extract once daily for 28 days

Group V; received 80% LD_{50} of the methanol root extract once daily for 28 days

Group VI; received 100% LD_{50} of the methanol root extract once daily for 28 days

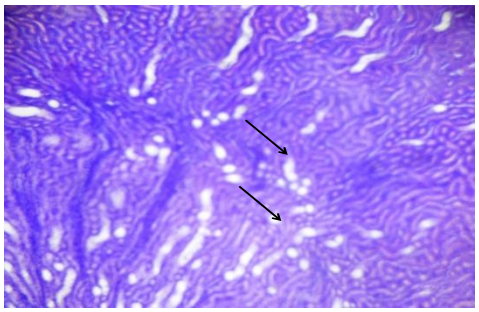

Plate 7: Photomicrograph of section of the Kidney of group 1 showing mild vacuolation (arrows) in medullary portion of the kidney. HE X100

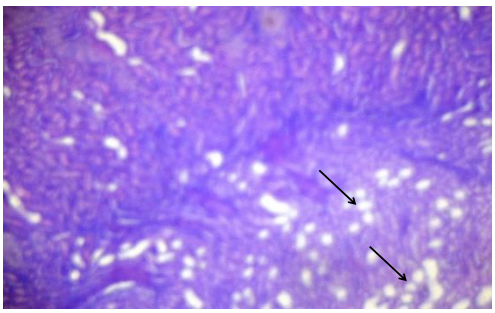

Plate 8: Photomicrograph of section of the Kidney of group 2 showing moderate vacuolation (arrows) in medullary portion of the kidney. HE X100

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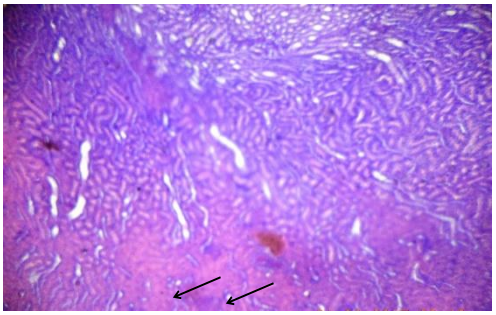

Plate 9 : Photomicrograph of section of the Kidney of group 3 showing moderate cortical necrosis (arrows). HE X100

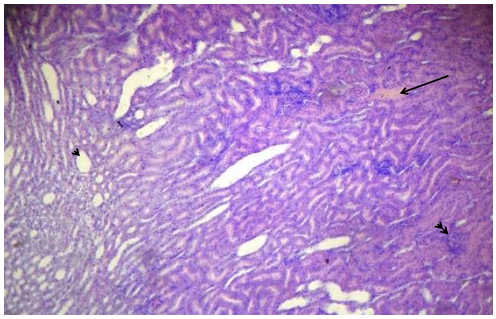

Plate 10: Photomicrograph of section of the Kidney of group 4 showing vacuolation (arrow head), glomerular degeneration (double arrowhead) and tubular necrosis (arrow). HE X100

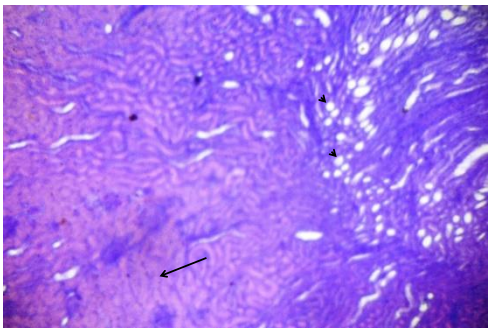

Plate 11: Photomicrograph of section of the Kidney of group 5 showing cortical necrosis (arrow) and moderate vacuolation (arrowheads) in medullary portion of the kidney. HE X100

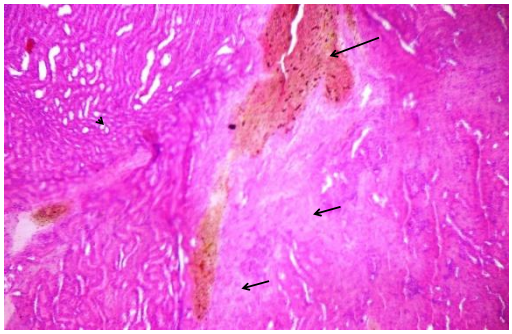

Plate 12: Photomicrograph of section of the Kidney of group 6 showing, severe cortical necrosis (short arrows), hyaline degeneration (long arrow) and mild vacuolation (arrowhead) in medullary portion of the kidney. HE X100

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DISCUSSION

The oral administration of 5000mg/kg body weight of the methanol root extract of *Ficus sycomorus* revealed no mortality, this is an indication that the plant has very low toxicity[15, 16] .This suggests that the LD_{50} is greater than 5000mg/kg and can be classified as practically non-toxic using the Hamburger's classification of range of LD_{50}. Also, no negative behavioral changes, such as restlessness, excitement, convulsions or coma at 5000 mg/kg body weight. LD_{50} greater than 5000 mg/kg body weight is considered safe based on recommendations of Organization for Economic Cooperation and Development (OECD) guidelines.

Liver in an important organ present in vertebrates and some other animals whose functions among others include glycogen storage, plasma protein synthesis and detoxification. It is prone to many diseases such as infections, hepatitis, alcohol liver damage, fatty liver, cirrhosis, cancer, drug damage etc. Liver damage causes swelling and necrosis of hepatocytes which results in release of cytosolic enzymes such as ALT, AST and ALP into the circulating blood [17, 18].

Biochemical analyses are useful in toxicity studies for providing information about *in vivo* effects of test substance [19]. The liver enzymes, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) have been established as markers of hepatocellular injury while alkaline phosphatase (ALP) and total bilirubin are markers of cholestasis in which their levels are elevated in biliary obstruction [18,20]. Albumin is a protein synthesize by the liver. It is the main constituent of total protein (the remaining from globulins). Albumin levels are decreased in acute liver disease, such as hepatitis, cirrhosis etc.

In this study, the extract did not exhibit a significant ($P>0.05$) effect on ALT and AST at all treatment doses relative to the control. The non-significant changes in ALT and AST in rats at 20%, 40%, 60%, 80% and 100% LD_{50} of methanol root extract of *Ficus sycomorus* suggest that the root extract does not affect hepatocyte function in rats or induce any cytotoxic damage to the liver. Serum levels of AST and ALT become elevated whenever disease processes affect liver cells. Significant ($P<0.05$) elevation in ALP activity in treated groups without commensurate increase in ALT and AST activities might indicate that the ALP activity increase is from non-hepatic isoenzymes Also, there was no significant decrease ($P>0.05$) in the level of albumin in the experimental groups compared to the control group. Serum level of albumin decreases when the liver is damage being site of albumin production [21]. The kidney is the primary organ for clearance and excretion of biochemical waste products including drugs and drug products from the body. Renal function indices such as urea, creatinine and uric acid can be used to assess the functional integrity of the nephrons of the animals[17] and are considered as good indicators of kidney function. From the results of this study, *Ficus sycomorus* caused decrease non-significant ($P>0.05$) difference in urea, uric acid and creatinine levels (Table 3)**.** Urea, uric acid and creatinine are metabolic waste products that need to be excreted by the kidney. Therefore, marked increase in serum urea and creatinine are indications of functional damage to the kidneys. Therefore, this study further indicated non toxicity of the plant.

The histopathological observations of the control and experimental groups generally support the results obtained from serum enzyme analyses. The photomicrograph of the liver and kidney sections of normal control (group I), showed normal hepatocytes and normal kidney tissues with no histopathological lesion. Photomicrograph of liver sections of rats in group II showed normal liver tissues, the histopathological architecture of liver sections of rats in group III showed mild congestion, Histopathological observations of liver sections of rats in group IV normal hepatocytes (but one severe vascular congestion) which may be due handling induced stress. Group V animals showed normal hepatocytes with one mild congestion and photomicrograph of liver sections of rats in group VI showed normal liver tissues with moderate congestion.

The kidney sections of rats in group II showed mild tubular degeneration and cortical necrosis, likewise the kidney sections of group III showed mild congestion and cortical necrosis. The kidney of the rats in group IV showed mild necrosis and degenerations. The kidney sections of group V rats showed mild congestion as well as degeneration. Also the kidney sections of rats in group VI showed mild degeneration and cortical necrosis. The mild necrosis, degeneration and congestions observed in the kidneys of the experimental groups are insignificant and might be due to stress in the animal during sacrifice. Generally, the results of the liver and kidney micrographs do not indicate toxicity of the plant extract on the animals.

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CONCLUSION

The results of the current study indicated that the plant , *Ficus sycomorus* is relatively safe and nontoxic and can be further exploited in the treatment of Malaria

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