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Evaluation of Possible Nephrotoxic Potential of *Gliricidia Sepium* Aqueous Leaf Extract in Wistar rats

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Abstract

Gliricidia sepium aqueous leaf extract is used in some parts of Nigeria by herbalists in the management of sickle cell disease, its anti-sickling property have been established. This study was designed to investigate the effect of ingestion of aqueous leaf extract of *Gliricidia sepium* on kidney functions in Wistar rats. Acute oral and sub-chronic toxicity studies of the leaf extract were determined according to Organization for Economic Co-operation and Development (OECD) guidelines 423 (Limit test) and 407 respectively. At the end of each experiment, the animals were sacrificed and blood samples taken for determination of electrolytes: Sodium, Potassium, Bicarbonate, Chloride; urea and creatinine were assayed using standard techniques. Kidney tissues were also processed histologically. In the oral acute toxicity study, no death or any sign of toxicities were recorded in the rats after 24 hours and up to 14 days post oral administration. There was no significant difference ($p > 0.05$) in all the parameters analysed between the control and the study groups in both acute and sub-chronic toxicity studies. Histology result of the kidney reveals normocytic normochromic cells. The present findings revealed that aqueous leaf extract of *Gliricidia sepium* is relatively safe and may not be nephrotoxic.

Keywords: Sickle cell disease, management, kidney, *Gliricidia sepium*, adverse effect, Wistar rat

Introduction

Gliricidia sepium known locally as

Agunmmaniye in Southwest, Nigeria, is a medium size leguminous tree belonging to the family Fabaceae. *Gliricidia*, which literally means “rat poison” originated in Central America and its plantation, has spread to many parts of the world specifically South Asia. The plant is used for fuel wood, animal feed, green manure, shade, living fences and as support plants (Csurches and Edwards, 1998). The root, stem bark, leaf and flower of *Gliricidia sepium* are used for various purposes in different countries. The leaf extract of *Gliricidia sepium* have been reported to possess insecticidal and nematicidal activities (Nazil *et al.*, 2008), antimicrobial (Nazil *et al.*, 2011), antihelminthic activity (Adama *et al.*, 2012), antioxidant (Akharaiyi *et al.*, 2012), and anti-sickling properties (Oduola *et al.*, 2016). Studies have shown that ingestion of *Gliricidia sepium* leaf extract in Wistar rats had no adverse effect on liver function profiles (Oduola *et al.*, 2018a) and oxidative stress factors (Oduola *et al.*, 2018b).

The kidneys play a vital role in regulating the composition and volume of extracellular fluid. Important functions of the kidneys are regulating the osmotic pressure of the body fluids, arterial blood pressure, maintenance of electrolytes and acid base balance. The kidneys are responsible for the elimination of metabolic waste products, drugs and toxic compounds; and involved in the secretion of certain hormones (Ogedegbe, 2007). Studies have revealed nephrotoxic potentials of many medicinal plants routinely administered in the treatment of various diseases in Africa and other parts of the world without knowledge of their adverse effects on the kidney, or with the

assumption they are safe. To the best of our knowledge, no study has been conducted to evaluate toxic effect of consumption of *Gliricidia sepium* leaf extract on kidney; hence this study was designed to assess possible nephrotoxic effect of *Gliricidia sepium* aqueous leaf extract on kidney function and structure in Wistar rats

Materials and Methods

Plant collection and identification

Fresh leaves of *Gliricidia sepium* were collected from Osogbo, Osun state, Nigeria. The plant was identified and authenticated at the Herbarium unit of Botany Department by Mr. G.A. Ademoriyo, Obafemi Awolowo University, Ile Ife, by comparing with established Herbarium specimen with voucher number: IFE/17460 reference number which was kept at the Herbarium.

Preparation and extraction

Fresh leaves of *Gliricidia sepium* were collected and air dried at room temperature over a period of 6 weeks. The dried leaves were ground manually using mortar and pestle. One kilogram of the grinded plant material was immersed in 5 litres of 80% methanol for 24 hours on a mixer to ensure maximum extraction by maceration technique at room temperature. This is followed by periodic stirring (Ahmed and Sani, 2013). Resulting crude extract was filtered using Whatman number 1 filter paper and the filtrate was concentrated in an oven at 40°C to obtain 63.5 g of green crude extract.

Experimental animals

Adult Wistar rats of both genders, weighing 150 g to 170 g purchased from the animal house of the Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria were used for the study. The rats were housed in well aerated cages under hygienic conditions in the animal house of Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto for the duration of the study. They were allowed to acclimatize for a period of 2 weeks before the commencement of the study. The animals were fed with pelletized growers feed (Vital®), obtained from Grand Cereal Soil Mills Limited, Jos, Nigeria. They were also allowed access to

clean drinking water ad libitum throughout the experimental period. Cleaning of the animal cages was carried out on a regular basis. The animals were maintained as described by Aniagu *et al.* (2005) in a clean metabolic cage-sand, placed in a well-ventilated room with a temperature of 26°C to 28°C, photoperiods of 12 hours' light and 12 hours' darkness. All the experimental protocols were in compliance with our Institutional Animal Ethics Committee guidelines as well as internationally accepted practices for use and care of laboratory animals as contained in US guidelines (NIH,1992) and also in accordance with the recommendations of the International Association for the Study of Pain (IASP) (Zimmerman, 1983).

Experimental Design

Acute oral toxicity study

Acute oral toxicity study, Limit Test, was performed in accordance with the procedures outlined by the Organization for Economic Co-operation and Development Guidelines (OECD, Limit test, 423) (OECD, 2008a). Eighteen Wistar rats of both genders were used for this study. The rats were randomly divided into three groups of six rats per group with the first group serving as control. The extract was administered to the rats by intra gastric gavage using oral cannula (a feeding needle), one animal per day starting with group 2 as follows:

Group 1 (control): distilled water

Group 2: 2000mg/kg of the extract

Group 3: 5000mg/kg of the extract

The animals were observed within the first 4 hours and subsequently 24 hours for toxic symptoms. Also, behavioural parameters and mortality were closely monitored for 14 days. Lethal dose in 50% of total population (LD₅₀) was determined using OECD method as described by Aniagu *et al.* (2005).

Sub-chronic Toxicity Study

Sub-chronic toxicity study was carried out following OECD 407 guidelines (OECD, 2008b). Forty Wistar rats of both sexes were divided into five groups of eight rats per group. Group 1 served as the control and received distilled water as vehicle. Graded doses of crude extract were administered orally to the rats daily

for 30 days as follows:

- Group 1 (control): distilled water
- Group 2: 250mg/kg
- Group 3: 500mg/kg
- Group 4: 750mg/kg
- Group 5: 1000mg/kg

All the rats had free access to feed and water throughout the period of the experiment and they were observed daily for general symptoms of toxicity and mortality.

Technique for obtaining blood samples

Blood was collected by cardiac puncture from chloroform anaesthetized rats into heparinized bottles. The rats were later sacrificed using lumbar dislocation and their kidneys were removed. The blood samples collected were centrifuged at 4000 revolutions per minute (4000 rpm) for 10 minutes. The plasma of each sample was separated and transferred into cryovial and stored (frozen) at -20°C until required for analysis (Aniagu *et al.*, 2005).

Histological examination

The kidneys of the animals were fixed in 10% formalin labeled bottles after which the tissues were processed routinely using automatic tissue processor. This was immediately followed by embedding in paraffin wax. Sections of 5-6 µm thick were cut, stained with haematoxylin and eosin and examined under the light microscope (Orchard and Nation, 2012).

Determination of biochemical parameters

The biochemical analyses were performed at the

Chemical Pathology Laboratory, Faculty of Medical Laboratory Sciences, Usmanu Danfodiyo University, Sokoto. The separated Plasma were used for the assays of electrolytes Na⁺, K⁺, HCO₃⁻, Cl⁻, (Henry *et al.*, 1974) urea and creatinine (Delaney *et al.*, 2013).

Data analysis

The data obtained from this study were analyzed using the statistical package for social science (SPSS) for Windows, version 21.0 (SPSS Inc., Chicago, IL, USA). The data were represented as the mean ± standard deviation (S.D). Student T-test at 95% confidence interval was used to evaluate the significance of the difference between the mean values of the measured parameters in the respective test and control groups. A mean difference was considered significant when p < 0.05.

Results

Acute toxicity

Table 1 shows the result of oral acute toxicity (LD₅₀ determination) in Wistar rats. The result showed that *no behavioural change or death was recorded in the rats after 24 hours and up to 14 days' post oral treatment. This indicates that the LD₅₀ is greater than 5000mg/kg.* Table 2 shows the results of the effects of intake of *Gliricidia sepium* aqueous leaf extract on the kidney functions of Wistar rats in acute oral toxicity study. Table 3 shows the results of the effects of intake of *Gliricidia sepium* aqueous leaf extract on the kidney functions of Wistar rats in sub chronic toxicity study.

Table 1: Acute oral toxicity study of *Gliricidia sepium* leaf extract in Wistar rats

Groups	No. of death or abnormal behaviour (24hr)	No. of death or abnormal behaviour (72hr)	No. of death or abnormal behaviour (14 days)
Group 1(control)			
Day 1:1 st rat	0	0	0
Day 2:2 nd rat	0	0	0
Day 3:3 rd rat	0	0	0
Group 2(2000mg/kg)			
Day 1:1 st rat	0	0	0
Day 2:2 nd rat	0	0	0
Day 3:3 rd rat	0	0	0
Group 3(5000mg/kg)			
Day 1:1 st rat	0	0	0
Day 2:2 nd rat	0	0	0
Day 3:3 rd rat	0	0	0

Table 2: Effect of *Gliricidia sepium* extract on the kidney function of Wistar rats in acute toxicity study

Parameters	Group 1	Group 2	Group3	F	p-value
	Control	2000mg/kg	5000mg/kg		
Urea 0.86	5.33±1.08	5.10±0.55	5.21±0.39	0.15	
Creatinine 0.32	1.66±0.80	1.22±0.89	0.92±0.88	1.23	
Sodium 0.18	140.13±6.40	152.00±10.88	134.40±27.09	1.92	
Potassium 0.63	4.18±0.74	3.95±0.45	3.82±0.73	0.48	
Bicarbonate	21.13±8.63	18.83±9.66	13.20±12.19	0.99	0.39
Chloride	99.75±4.40	102.67±6.25	94.2±13.22	1.47	0.26

Key: n = number of rats per group; *p<0.05= statistically significant

Table 3: Effect of *Gliricidia sepium* extract on the kidney function of Wistar rats in sub- chronic toxicity study

Parameters	Group 1	Group 2	Group3	Group4	Group 5	F	p-value
	Control	250mg/kg	500mg/kg	750mg/kg	1000mg/kg		
Urea (Mmol/L)	5.34±1.08	3.90±2.02	4.18±1.43	4.21±1.09	3.60±1.18	1.73	0.17
Creatinine (Mg/dl)	1.66±0.80	1.86±0.56	1.51±0.50	1.39±1.18	2.22±1.07	0.87	0.49
Sodium (Mmol/L)	140.13±6.40	126.40±13.89	138.14±11.57	133.43±7.46	142.62±13.25	2.11	1.11
Potassium (Mmol/L)	4.18±0.74	4.42±0.37	4.27±0.43	4.49±0.61	4.35±0.78	0.30	0.88
Bicarbonate (Mmol/L)	21.13±8.63	24.00±2.35	24.00±1.29	19.71±8.83	23.33±0.82	0.68	0.61
Chloride (Mmol/L)	99.75±4.40	88.60±9.42	98.57±7.59	96.28±8.06	100.00±7.79	2.23	0.09

Key: n = number of rats per group; *p<0.05= statistically significant.

Histological examination

Figures 1 to 3 represent photomicrographs of kidney tissues of control and experimental rats in oral acute toxicity study

Figures 4 to 8 represent photomicrographs of kidney tissues of control and experimental rats in sub-chronic toxicity study

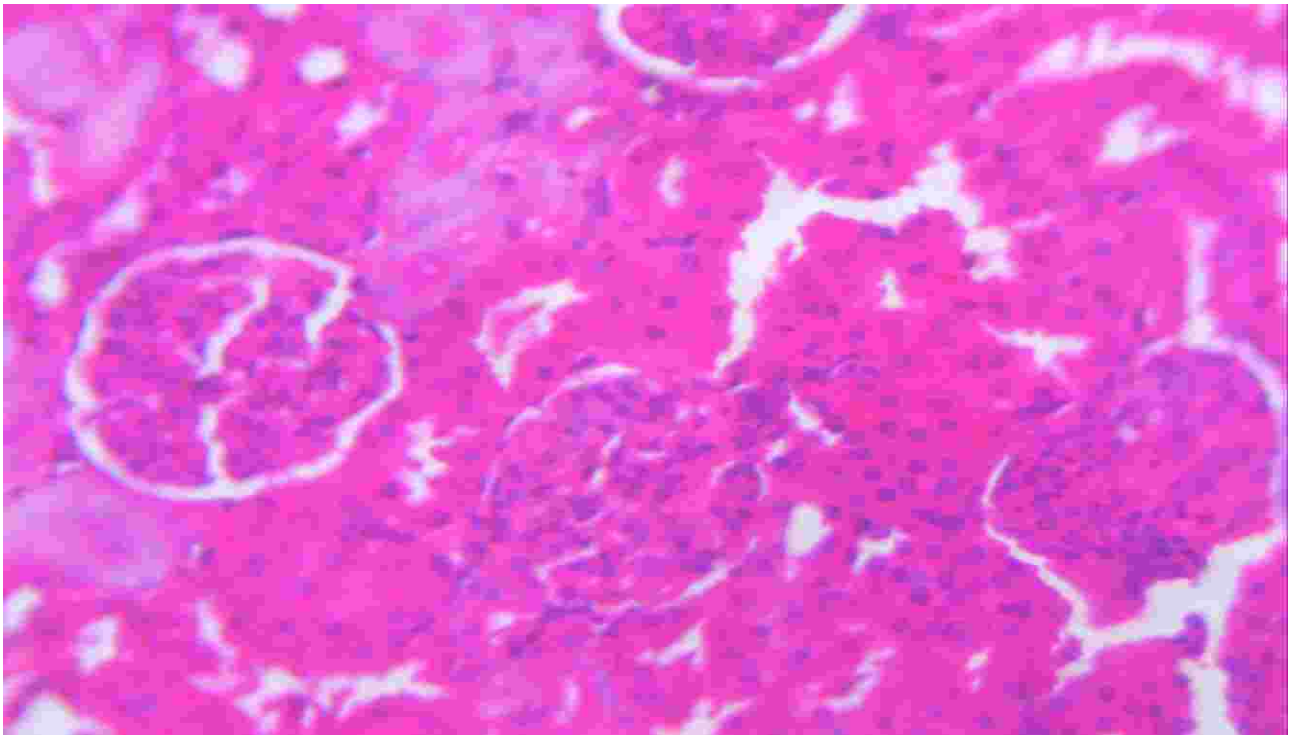


Figure 1: Photomicrograph of kidney tissue in control rats showing normocytic normochromic cells in acute oral toxicity study (H&E; A X 100).

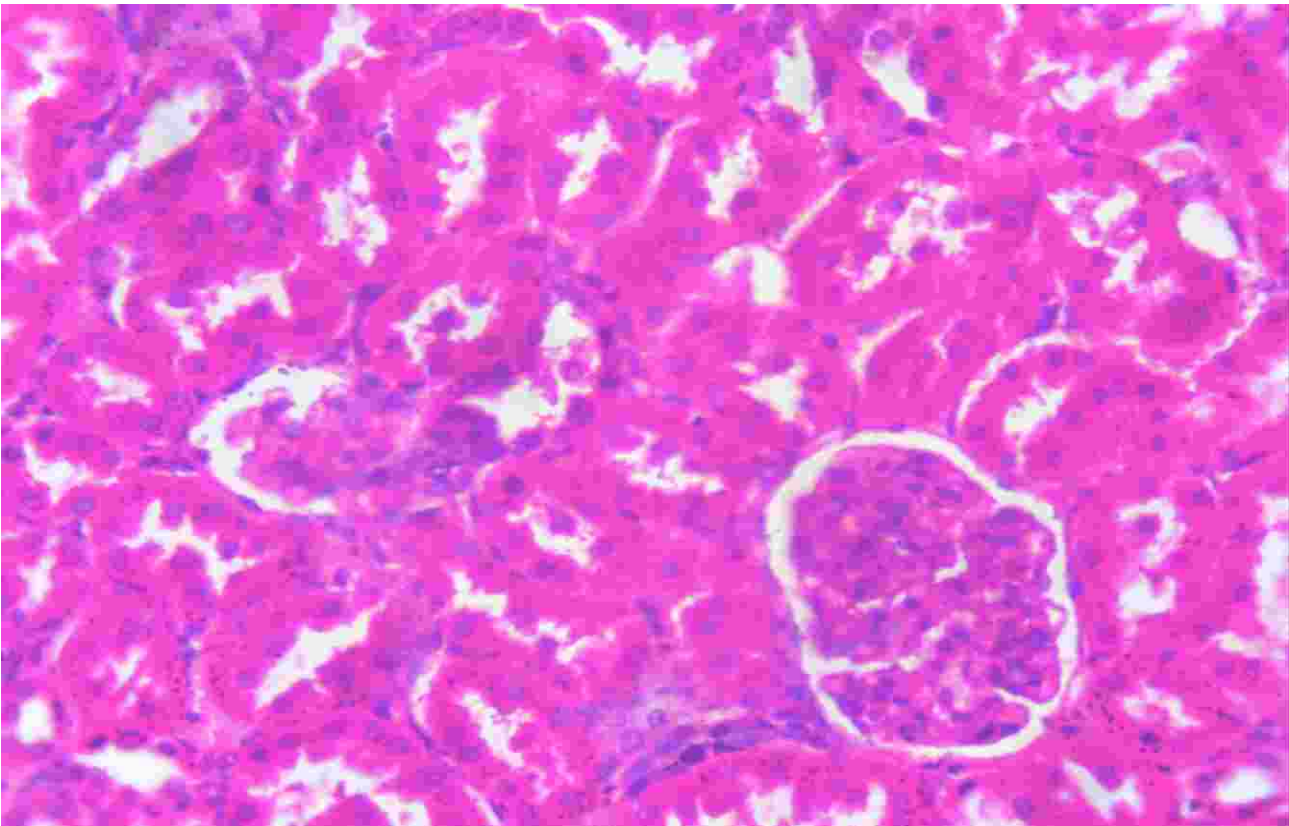


Figure 2: Photomicrograph of kidney tissue in group 2 (2000mg/kg) rats showing normocytic normochromic cells in acute oral toxicity study (H&E; A X 100).

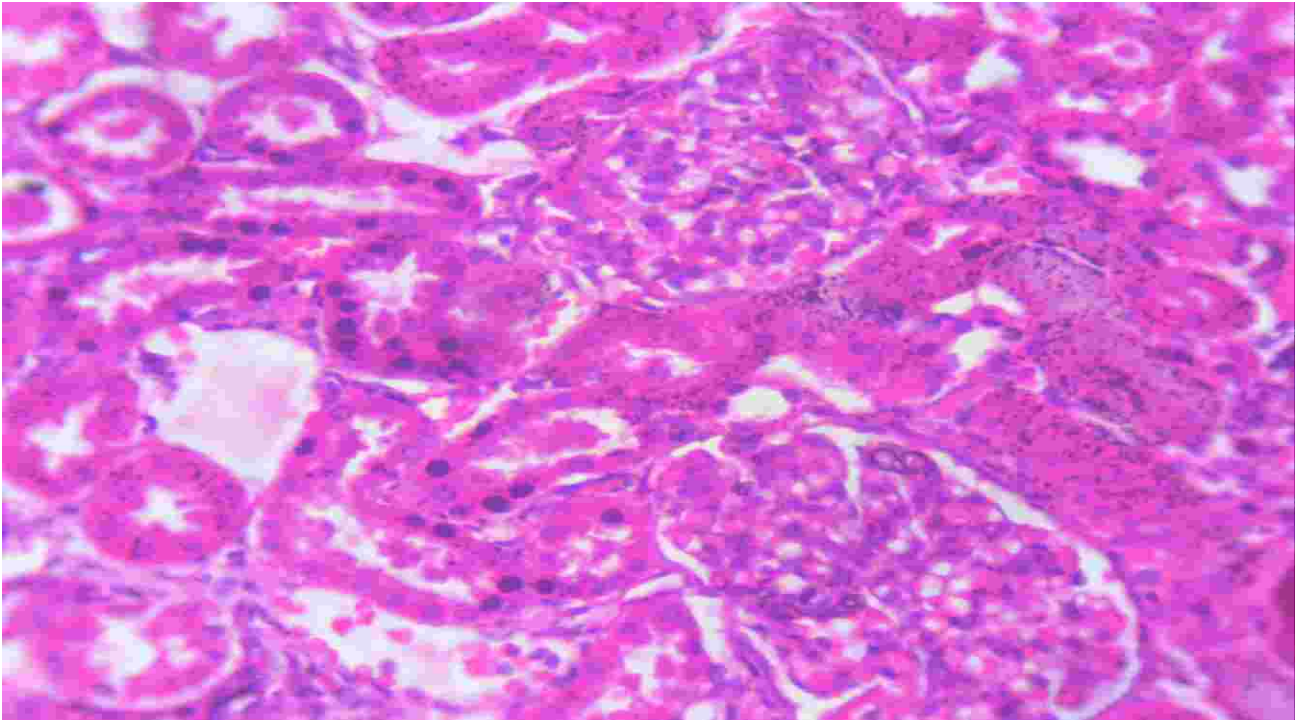


Figure 3: Photomicrograph of kidney tissue in group 3 (5000mg/kg) rats showing normocytic normochromic cells in acute oral toxicity study (H&E; A X 100).

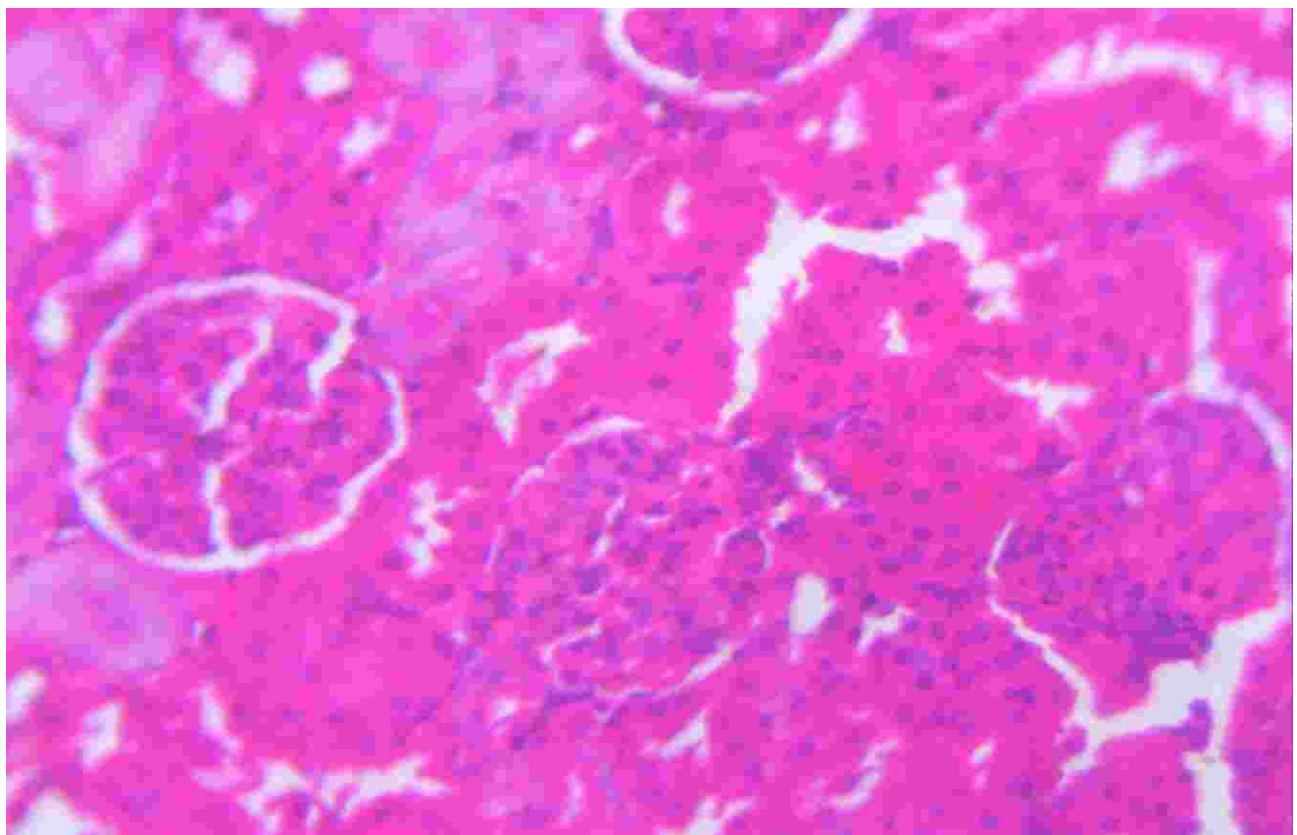


Figure 4: Photomicrograph of kidney tissue in control rats showing normocytic normochromic cells in sub-chronic toxicity study (H&E; A X 100).

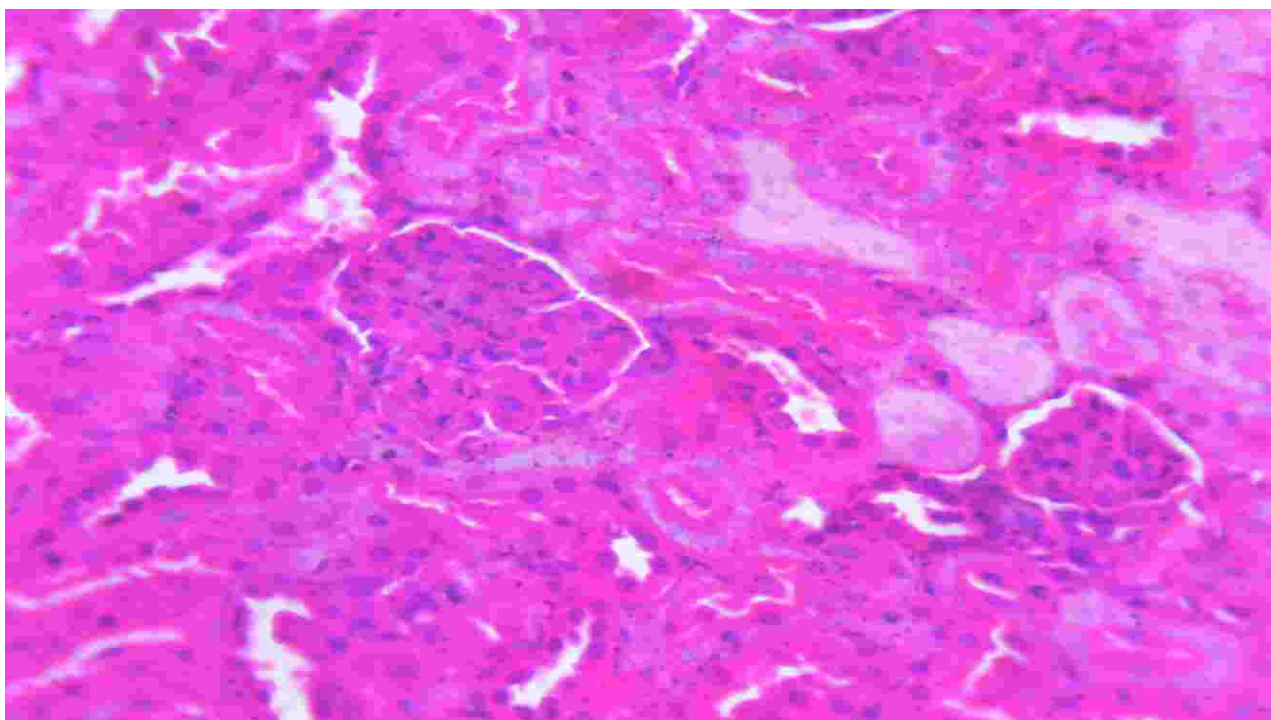


Figure 5: Photomicrograph of kidney tissue in group 2 rats (250mg/kg) showing normocytic normochromic cells in sub-chronic toxicity study (H&E; A X 100).

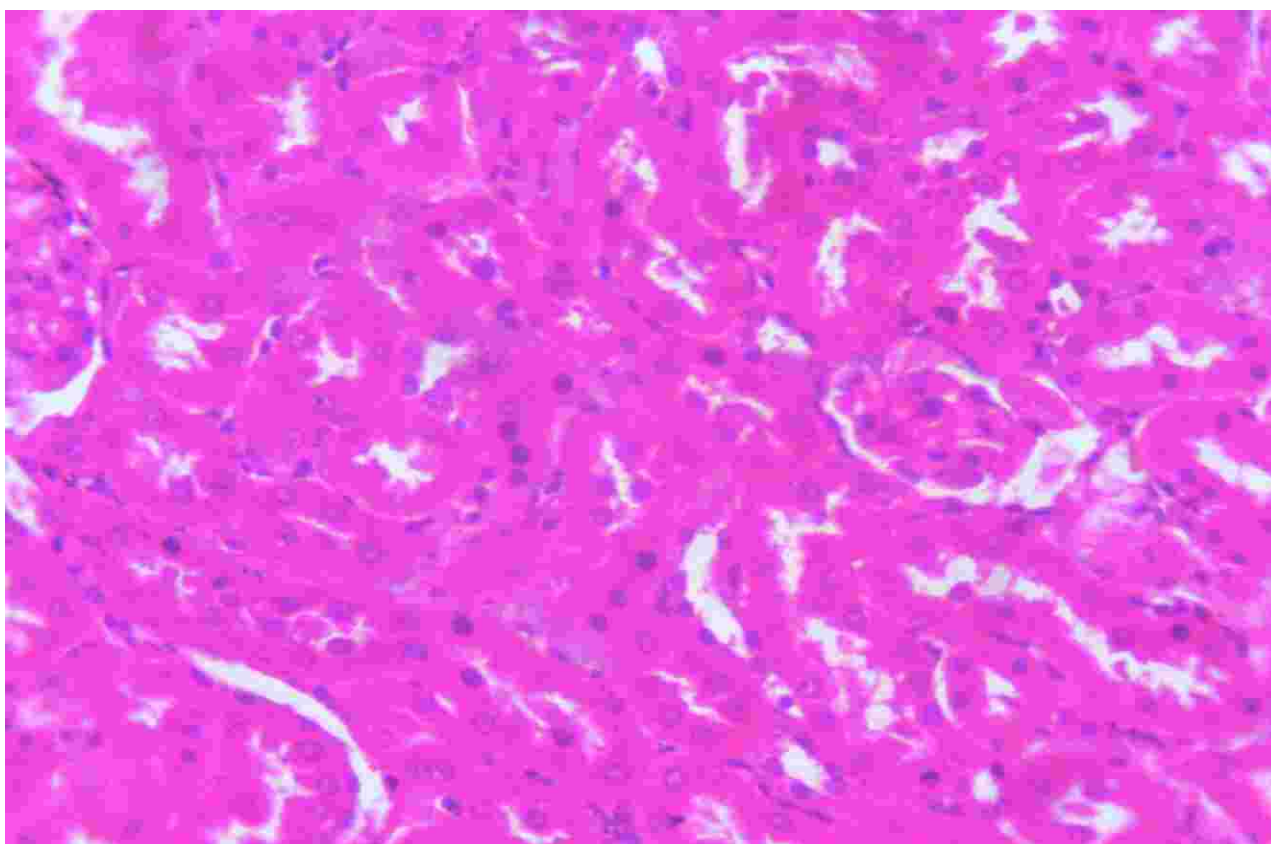


Figure 6: Photomicrograph of kidney tissue in group 3 rats (500mg/kg) showing normocytic normochromic cells in sub-chronic toxicity study (H&E; A X 100).

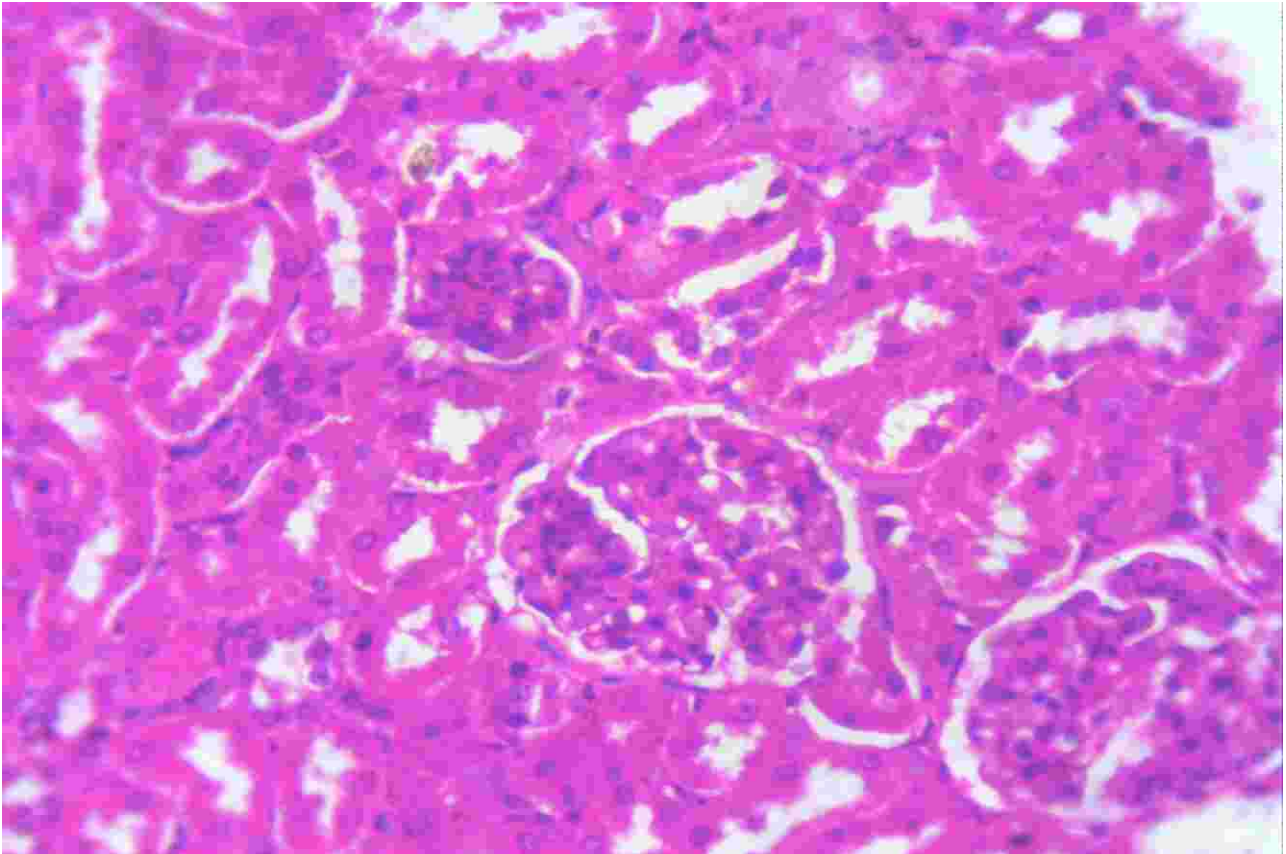


Figure 7: Photomicrograph of kidney tissue in group 4 rats (750mg/kg) showing normocytic normochromic cells in sub-chronic toxicity study (H&E; A X 100).

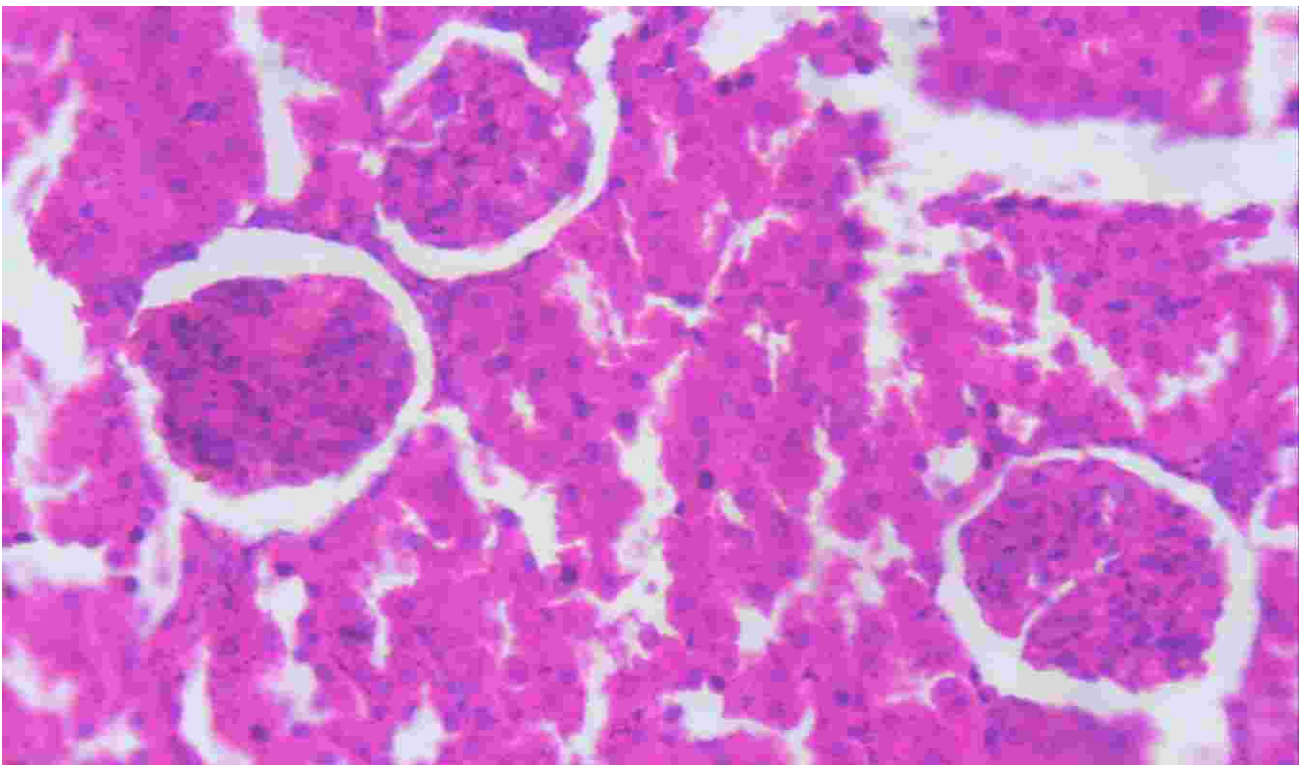


Figure 8: Photomicrograph of kidney tissue in group 5 rats (1000mg/kg) showing normocytic normochromic cells in sub-chronic toxicity study (H&E; A X 100).

Discussion

The main physiological function of the kidney is the maintenance of fluid and electrolytes homeostasis in the body, formation of urine, regulation of acid-base balance, excretion of waste products of protein metabolism and unwanted substances from plasma, regulation of hormone level in the blood, and protein conservation (Ogedegbe, 2007). Kidneys are susceptible to toxic substances because of high surface area and flow, high metabolic activity and active secretion and reabsorption mechanisms (Ogedegbe, 2007). A significant increase in the use of herbal remedies especially in patients with kidney diseases may increase the probability of renal injuries (Nethathe, 2014). Due to the renal vulnerability to toxic substances, the improper use of herbal remedies might lead to various kidney conditions such as electrolyte abnormalities, proteinuria, acute and chronic kidney diseases and even kidney failure (Luyckx, 2012; Raskin *et al.*, 2002).

From this study, in the acute oral toxicity study, no death or signs of toxicity were recorded in experimental animals, groups 2 (2000mg/kg) and 3 (5000mg/kg) after 24 hours and up to 14 days, this indicates that the LD₅₀ of *Gliricidia sepium* leaf extract is greater than 5000mg/kg. According to Kennedy *et al.* (1986) substances with LD₅₀ values higher than 5000mg/kg, by oral route are regarded as being safe or practically non-toxic, hence *Gliricidia sepium* leaf extract may be safe for human consumption. Electrolytes take part in many metabolic and homeostatic processes in the body. These processes include biochemical and enzymatic reactions, neurotransmission, conduction of nerve signals, adequate functioning of hormones, muscle contraction, bone composition, fluid and acid-base balance, and cardiovascular functions (Delaney *et al.*, 2013). Nephrotoxic effects of some plants by inducing electrolytes imbalance have been documented. Studies have shown that herbs such as *Glycyrrhiza glabra* and *Areca catechu* caused kidney damage via alteration of serum potassium levels (Danis *et al.*, 2015; Velickovic *et al.*, 2011; and Kang *et al.*, 2007). From human studies, it has been documented that licorice root contained glycyrrhizic acid which caused

hyperaldosteronism like syndrome with hypokalemia, metabolic alkalosis and high blood pressure. In high doses and long application, Licorice caused sodium retention and high blood pressure and led to hypokalemia and rhabdomyolysis with acute renal failure (Danis *et al.*, 2015; Velickovic *et al.*, 2011). Chinese weight loss herbal remedies containing aristolochic acid; have been reported to cause urothelial carcinoma. Aristolochic acid is a nitrophenanthrene carboxylic acid which forms DNA adducts in renal as well as other tissues after metabolic activation. The DNA adducts result in genotoxic mutations resulting in urothelial carcinoma as well as the characteristic renal interstitial fibrosis and extensive loss of cortical tubules (Singh and Prakash, 2011). From this study, the serum levels of electrolytes (sodium, potassium, chloride and bicarbonate), in experimental rats were not significantly different from control rats in both acute and sub-chronic toxicity studies. Ingestion of *Gliricidia sepium* had no adverse effect on serum electrolytes of experimental rats, and hence might not cause electrolyte imbalance in the consumers. Normal serum levels of urea and creatinine are good indicators of a normal functioning kidney and increase in the serum are markers of kidney dysfunction and are widely accepted and commonest parameters to assess kidney functions (Davis and Bredt, 1994). In this study, there was no significant difference in urea and creatinine levels between the experimental and control rats in both acute and sub-chronic toxicity studies. Histopathological examination of the kidney tissues from both experimental and control rats showed normal structure and absence of any gross pathological lesion in organs. In conclusion, *Gliricidia sepium* aqueous leaf extract produced no adverse effect in Wistar rats, hence it may not be nephrotoxic in humans.

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Conflict of interest

There was no conflict of interest in whatever form.

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