



## Evaluation of the Acute and Sub-acute Toxicity of the Ethanolic Extract of *Cassia sieberiana* Root in Male Wistar Rats

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### Abstract

The roots of *Cassia sieberiana* (African laburnum) is a common aphrodisiac in Northern Nigeria that is used in traditional medicine to enhance sexual function and treat erectile dysfunction in men without much consideration for its safety and side effects. This study evaluated the toxicity of oral administration of ethanol extract of *C. sieberiana* on male rats. Twenty (20) male wistar rats were randomly distributed into control group and three treatment groups made up of five (5) rats each, and orally administered crude extract of *C. sieberiana* roots for 21 days at 100, 200 and 400 mg/kg body weight respectively. The body weight, organ weight, liver function, kidney function, antioxidant and malondialdehyde levels were determined. The results revealed that LD<sub>50</sub> of the extract was greater than 5000 mg/kg b.w. Additionally, no significant differences ( $p > 0.05$ ) were found in the respective body weight, liver function parameters, antioxidant and lipid peroxidation markers of treated groups with reference to the control group. There was a significant increase in the weight of the pancreas of the treated groups compared to the control. The test rats administered 100 and 200 mg/kg b. w. recorded a significant reduction ( $p < 0.05$ ) in serum potassium ions concentration. These results therefore suggest that oral administration of *C. sieberiana* did not induce any significant alterations or toxic effect in the male rats. *C. sieberiana* ethanolic extract can be considered safe. However, further investigation on histopathology of vital organs and chronic toxicity study is still required to ensure the safety of the plant

**Keywords:** Antioxidant, Biochemical parameters, Kidney, Liver, Toxicity

### 1.0. Introduction

The use of plant-based materials as a herbal remedy for sexual disorders especially for erectile dysfunction and sexual enhancement has increased in recent years. This is evident in increased demand for aphrodisiac herbs from traditional herbal practitioners due to their perceived safety profile and easy availability. Aphrodisiac herbs can improve sexual performance, thus enhancing sexual satisfaction and self-esteem in human relations [1]. Although natural products are commonly recognized as safe and used as self-medication, the toxicity of many herbal remedies has been evaluated [2, 3]. Many pharmacological compounds synthesized by plants have been implicated in toxicity and damage to vital organs including liver and kidney [4]

*Cassia sieberiana* also called African laburnum, belonging to the Fabaceae family is a savannah plant found in many Africa countries including Nigeria [5]. *C. sieberiana* plant parts contain various phytochemicals such as alkaloids, saponins, tannins, flavonoids that are important in treatment of many diseases [6]. The leaves and root bark decoction are used to treat erectile dysfunction [7]. Boiled leaves are squeezed and applied on burns. The leaves are also used to treat arthritis, malaria and fever [8]. Furthermore, the roots are used to alleviate body pain and dysmenorrhoea [5]. However, very little information is available on the safety profile of *C. sieberiana* roots. Based on that, this study evaluated the acute and sub-acute toxicity of its ethanolic crude extract on male wistar rats.

## 2.0. Materials and Methods

### 2.1. Plant collection and authentication

Roots of *Cassia sieberiana* were collected in Dutsin-Ma Local Government Area of Katsina State, Nigeria in May 2023. The plant was identified in the Department of Plant Biology, Bayero University Kano with voucher number 0065.

### 2.2. Extract preparation

The fresh *C. sieberiana* roots collected were air-dried for two weeks in the laboratory, ground to powder. Seven hundred and fifty grams (750 g) of the powdered sample was macerated in 5.25 L of ethanol for 48 h with stirring at interval. The resulting suspension was filtered with muslin cloth and then Whatman No 1 filter paper. The filtrate were concentrated using a rotary evaporator under reduced pressure and kept in a desiccator.

### 2.3. Experimental animals

Twenty (20) male wistar rats weighing 119-225 g were used and kept in the animal house of the Department of Biochemistry and Molecular Biology, Federal University Dutsin-Ma, Nigeria. The animals were allowed to acclimatize and maintained under standard conditions (12 h light/12 h dark cycle). The animals were fed with commercial pellet and water *ad libitum*. All animal experiments were carried out according to the guide for the care and use of laboratory animals published by the [9].

### 2.4. Acute toxicity studies

The oral acute toxicity study of ethanolic extract of *C. sieberiana* was evaluated according to the method described by [10]. Nine (9) adult male wistar rats were randomly divided into three groups consisting of 3 rats each in the phase I of the experiment. The rats were administered 10, 100 and 1000 mg/kg p.o body weight of *C. sieberiana* ethanol extract respectively. The rats were observed for 24 h for any sign of toxicity (behavioural changes), including death. In the phase II, four (4) rats were divided into four (4) groups consisting of 1 rat each ( $n = 1$ ). The rats were administered 1000, 1600, 2900 and 5000 mg/kg p.o. body weight of the plant extract respectively. They were observed intermittently for the next 24 h and 48h after dosing for behavioural changes associated with toxicity, including mortality. The behavioural changes closely observed

for were as follows: convulsion, restlessness, difficult breathing, diarrhoea, sleep, and coma.

### 2.5. Subacute toxicity studies

Twenty (20) male *wistar* rats were utilized in this phase of the study, the rats were distributed into four (4) groups of  $n = 5$ . The test rats received 100, 200, and 400 mg/kg body weight, respectively, of *C. sieberiana* ethanolic extract for twenty-one (21) days, while the control group received 10 ml/kg of distilled water.

### 2.6. Biochemical examination

At the end of the experimental period, the rats were sedated with chloroform and sacrificed. Blood samples were collected from abdominal aorta using a 5 ml syringe, after which the liver, kidney and pancreas were harvested. The organs were weighed using a digital weighing balance. The blood sample was allowed to clot and centrifuged to obtain the serum. The procedures described by Thefeld et al [11] was used to determined serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity while serum alkaline phosphatase (ALP) activity was evaluated using the method of Schlebusch et al. [12]. Total protein (TP) level was determined using the method of Gomall [13], albumin was evaluated using the method of Doumas et al. [14], direct bilirubin (DB) and total bilirubin level was determined by method of Walter and Gerard [15] and Annino [16]. To evaluate the renal function indices, Agappe Diagnostic Kit was used following the method of Kassirer [17], Allen et al [18], Henry et al. [19], Schonfeld et al. [20] and Tietz [21] to determine urea, creatinine, potassium ( $K^+$ ), chloride ( $Cl^-$ ), and bicarbonate ( $H_2CO_3^-$ ) concentration respectively. Superoxide dismutase (SOD) activity was determined using the method of Misra and Fridovich [22] Catalase (CAT) activity was evaluated following the protocol of Cohen et al. [23]. Reduced glutathione (GSH) concentration was determined using the method of Ellman [24] while malondialdehyde (MDA) level was estimated using the method of Buege and Aust [25].

### 2.7. Statistical analysis

All the values were expressed as mean  $\pm$  SEM ( $n = 5$ ). Data was analysed by One-way analysis of variance (ANOVA) using a statistical package for social science (SPSS, version 16). The value of  $p < 0.05$  was considered to be statistically significant.

### 3.0. Results

#### 3.1. Acute toxicity of ethanol extract of *Cassia sieberiana* roots

The acute toxicity test using Lorke method at oral dose limit of 5000 mg/kg of the ethaolic extract of *C. sieberiana* did not result in mortality in the male wistar rats. No lethal effect or behavioural changes such as convulsion, restlessness, difficult breathing, diarrhoea, sleep, and coma were observed over the 48 h period. Therefore, the extract may be safe at the administered doses and the oral LD<sub>50</sub> considered higher than 5000 mg/kg.

#### 3.2. Sub-acute toxicity of ethanol extract of *Cassia sieberiana* roots

##### 3.2.1. Effect on body weight

Effects of sub-acute oral treatment with ethanol root extract of *C. sieberiana* on body weight of male rats are shown in Table 1. There were no significant differences ( $p > 0.05$ ) in the body weight of rats administered the different doses of the extract when compared to the control group throughout the 21 days period of administration.

**Table 1:** Body weight of wistar male rats fed ethanol extract of *Cassia sieberiana* roots.

Groups	Body Weight (g)			
	Initial	Day7	Day14	Day21
Control	202.62 ± 11.23 <sup>a</sup>	217.06 ± 13.23 <sup>a</sup>	229.80 ± 14.07 <sup>a</sup>	236.72 ± 12.00 <sup>a</sup>
100 mg/kg	167.24 ± 14.12 <sup>a</sup>	187.00 ± 14.70 <sup>a</sup>	192.12 ± 15.86 <sup>a</sup>	214.76 ± 16.55 <sup>a</sup>
200 mg/kg	175.64 ± 13.92 <sup>a</sup>	178.48 ± 13.19 <sup>a</sup>	187.58 ± 13.54 <sup>a</sup>	201.14 ± 13.17 <sup>a</sup>
400 mg/kg	175.84 ± 16.45 <sup>a</sup>	198.46 ± 17.77 <sup>a</sup>	203.72 ± 19.57 <sup>a</sup>	218.62 ± 19.47 <sup>a</sup>

Data are represented as mean ± SEM, n = 5 determinations. Values with different alphabet in the same row indicate a significant difference ( $p < 0.05$ ).

##### 3.2.2. Effects on organs weight

The effects of the extract on organs' weight are presented in Table 2. The extract did not significantly increase organ weight of liver and kidney ( $p > 0.05$ )

in groups that received the varying doses of the extract compared to the control. However, the extract significantly ( $p < 0.05$ ) increased the weight of the pancreas in group administered with 200 and 400 mg/kg of the extract as compared to the control.

**Table 2.** The weights of organs of male wistar rats fed ethanol extract of *Cassia sieberiana* roots

Groups	Weight of organs (g)		
	Liver	Kidney	Pancreas
Control	6.74 ± 0.41 <sup>a</sup>	1.52 ± 0.37 <sup>a</sup>	0.40±0.04 <sup>a</sup>
100 mg/kg	6.60 ± 0.56 <sup>a</sup>	1.34 ± 0.11 <sup>a</sup>	0.44±0.05 <sup>ab</sup>
200 mg/kg	5.80 ± 0.10 <sup>a</sup>	1.38 ± 0.11 <sup>a</sup>	0.64±0.06 <sup>c</sup>
400 mg/kg	6.88 ± 0.50 <sup>a</sup>	1.40 ± 0.10 <sup>a</sup>	0.60±0.08 <sup>bc</sup>

Data are represented as mean ± SEM, n = 5 determinations. Values with different alphabet in the same column indicate a significant difference ( $p < 0.05$ ).

##### 3.2.3. Effects on liver function parameters

The liver function indices of rats fed the extract are presented in Table 3. No significant difference was observed in the liver function parameters and activities of serum AST, ALT and ALP when the

control group was compared with the group administered various doses of the extract. Similarly, serum total protein, albumin, direct bilirubin, and total bilirubin were not statistically significant when compared to the control.

**Table 3.** Liver function parameters of rats administered ethanol extract of *Cassia sieberiana* roots.

Groups	Parameters						
	ALT (u/I)	AST (u/I)	ALP (u/I)	TP (g/dL)	Albumin (g/dL)	DB (mmol/l)	TB (mmol/l)
Control	9.20±3.35 <sup>a</sup>	25.40±7.65 <sup>a</sup>	11.62±1.99 <sup>a</sup>	5.16±0.28 <sup>a</sup>	1.64±0.10 <sup>a</sup>	4.92±0.58 <sup>a</sup>	13.98±0.62 <sup>a</sup>
100 mg/kg	8.40±2.73 <sup>a</sup>	25.00±7.11 <sup>a</sup>	10.96±1.50 <sup>a</sup>	5.0±0.38 <sup>a</sup>	1.90±0.18 <sup>a</sup>	4.86±0.32 <sup>a</sup>	12.32±0.80 <sup>a</sup>
200 mg/kg	13.80±1.36 <sup>a</sup>	37.80±2.99 <sup>a</sup>	10.06±1.25 <sup>a</sup>	4.78±0.26 <sup>a</sup>	1.56±0.30 <sup>a</sup>	4.62±0.48 <sup>a</sup>	13.70±0.77 <sup>a</sup>
400 mg/kg	12.40±2.87 <sup>a</sup>	33.40±7.33 <sup>a</sup>	10.56±0.79 <sup>a</sup>	4.80±0.22 <sup>a</sup>	1.70±0.19 <sup>a</sup>	6.82±1.11 <sup>a</sup>	13.38±1.26 <sup>a</sup>

Values are mean ± SEM, n=5 animals in each group, values with different letter in a column differ significantly from each other (p < 0.05)

### 3.2.4. Effects on kidney function parameters

The effect of *C. sieberiana* on kidney function parameters are shown in Table 4. Administration of the extract to the experimental animals produced a non-significant difference in the levels of creatinine

urea, chloride (Cl<sup>-</sup>), and bicarbonate (H<sub>2</sub>CO<sub>3</sub><sup>-</sup>) when compared to the control group. However, there was a significant decrease (p < 0.05) in the levels of serum electrolyte potassium (K<sup>+</sup>) among the groups administered with 100 and 200 mg/kg body weight doses of the extract as compared to the control group.

**Table 4.** Kidney function parameters of rats administered ethanol extract of *Cassia sieberiana* roots.

Groups	Parameters				
	Urea (mg/dl)	Creatinine (meq/l)	K <sup>+</sup> (mmol/l)	Cl <sup>-</sup> (mg/dl)	H <sub>2</sub> CO <sub>3</sub> <sup>-</sup> (mg/dl)
Control	17.80±3.93 <sup>a</sup>	0.94±0.08 <sup>a</sup>	32.38±2.97 <sup>b</sup>	32.60±2.79 <sup>a</sup>	102.40±8.62 <sup>a</sup>
100 mg/kg	31.00±6.96 <sup>a</sup>	0.96±0.74 <sup>a</sup>	16.52±1.86 <sup>a</sup>	31.80±3.20 <sup>a</sup>	109.60±10.47 <sup>a</sup>
200 mg/kg	29.00±10.65 <sup>a</sup>	0.88±0.09 <sup>a</sup>	20.70±4.98 <sup>a</sup>	29.80±5.05 <sup>a</sup>	101.80±7.12 <sup>a</sup>
400 mg/kg	17.00±2.98 <sup>a</sup>	1.04±0.13 <sup>a</sup>	22.70±2.53 <sup>ab</sup>	32.20±4.14 <sup>a</sup>	93.80±4.27 <sup>a</sup>

Data are represented as mean ± SEM, n = 5 determinations. Values with different alphabet in the same row indicate a significant difference (P < 0.05). K<sup>+</sup> = potassium, Cl<sup>-</sup> = chloride, H<sub>2</sub>CO<sub>3</sub><sup>-</sup> = bicarbonate.

### 3.2.5. Effects on antioxidant enzymes and MDA level

Effects of *C. sieberiana* on the activity of antioxidant enzymes and MDA levels are presented in Table 5. The activity of antioxidant enzymes such as SOD,

CAT and GSH were not significantly altered as compared to the control group. In addition, there was no significant differences (p > 0.05) in the levels of MDA when the extract treated group was compared to the control.

**Table 5.** Antioxidant enzymes and MDA of rats administered ethanol extract of *Cassia sieberiana* roots

Groups	SOD (u/ml)	CAT (u/ml)	GSH (µg/ml)	MDA (nmol/ml)
Control	20.44±2.14 <sup>a</sup>	12.22±1.17 <sup>a</sup>	22.42±1.94 <sup>a</sup>	122.32±9.70 <sup>a</sup>
100 mg/kg	23.60±1.73 <sup>a</sup>	14.26±1.24 <sup>a</sup>	25.10±1.92 <sup>a</sup>	101.34±23.27 <sup>a</sup>
200 mg/kg	20.32±0.98 <sup>a</sup>	13.82±1.48 <sup>a</sup>	23.62±1.45 <sup>a</sup>	120.46±18.21 <sup>a</sup>
400 mg/kg	19.52±0.93 <sup>a</sup>	12.42±1.47 <sup>a</sup>	24.92±1.73 <sup>a</sup>	106.94±21.02 <sup>a</sup>

Data are represented as mean ± SEM, n = 5 determinations. Values with different alphabet in the same column indicate a significant difference (p < 0.05). SOD = superoxide dismutase, CAT = catalase, GSH = reduced glutathione, MDA = malondialdehyde.

## 4.0. Discussion

The worldwide used of herbal plants for the treatment of many diseases has been linked to their

natural bioactive components, and thus are considered to be have little or no toxicity [26]. These bioactive compounds are pharmacologically important at specific doses or quantity [27].

However, intake of herbal medicines without adequate knowledge of its efficacy and safety can result in toxicity of major organs and tissues [28]. Thus, toxicity studies are important in establishing the safety or detrimental effects of medicinal plants regardless of the duration of its usage. The LD<sub>50</sub> of *C. sieberiana* extract was found to be above 5000 mg/kg body weight, suggesting that the plant has high safety margin and maybe considered relatively nontoxic. The LD<sub>50</sub> in this study is in line with that reported by [29].

The adverse treatment effect of a drug on an animal can be deduced by changes in body weight. The body weight of animals exposed to certain substance over a period of time may decrease, indicating the harmful nature of the substance. However, studies seems to suggest that body weight changes can also result from physiological adaptation responses to the substance by the animal rather than to toxic effect [30]. This may lead to increased or decreased appetite and thus a higher or lower caloric intake by the animal. The non-significant increase in body weight in this study indicate that the extract did not alter the caloric intake of the male rats. This is in agreement with study of Donkor et al. [31] who observed no significant change in body weight between the control and treated groups administered aqueous root extract of *C. sieberiana*.

The liver, kidney and other biochemical parameters are important in assessing the safety of herbal medicines. The assessment of organ weight provide available insight to damage caused by toxic substances [32]. A non-significant change in organ weight of liver and kidney was observed in this study. This result is in consonance with the work of [29] who also reported no significant differences in the weight of liver and kidney. However, administration of the extract at the dose of 200 and 400 mg/kg significantly increased the weight of the pancreas as compared to the control. Studies seems to suggest that an increase in organ weight may be as a result of hypertrophy or hyperplasia [33]. Therefore, organ weight data should be interpreted with histopathological findings.

Liver injury caused by toxic agents can result in increased levels of liver function enzymes. Biomarkers of level function become elevated in serum following liver damage [32]. No statistically significant elevation in the biomarkers (ALT, AST and ALP) relative to the control were observed in this study. A reduction in these biomarkers suggest reduced synthetic function as observed in liver

damage or disease [33]. Furthermore, there were also no alterations in the levels of total protein, albumin and bilirubin. The result indicate that the extract of *C. sieberiana* did not alter protein synthetic and excretory function of the liver. The present study data are in agreement with Evenamede et al. [29].

Renal injury caused by exposure to toxic substances can hinder the ability of the kidney to effectively remove metabolic waste and regulate serum electrolytes. Waste product of protein metabolism like urea and creatinine are usually elevated when kidney function is impaired [3]. The result of this study showed a non-significant difference in urea and creatinine levels. In addition, the results revealed a non-significant change in serum bicarbonate and chloride ion concentration respectively. However, there was a significant reduction in serum potassium ion concentration of rats administered 100 mg/kg and 200 mg/kg of *C. sieberiana*. Similar results have been reported by Donkor et al. [31].

The most relevant and widely used biochemical parameters in assessing oxidative stress include antioxidant enzymes (SOD, CAT) and GSH. These oxidative stress markers have been shown to decrease in conditions relating to oxidative stress caused by exposure to toxic substances. Consequently, administration of the extract at the various doses did not significantly affect the activities of the antioxidant enzymes and GSH when compared to the control. These biomarkers have been shown to decrease in conditions relating to oxidative stress caused by exposure to toxic substances. These enzymes are considered first line antioxidant defence system [34]. SOD protects cells from hazardous substances by catalysing the conversion of superoxide anion to hydrogen peroxide and molecular oxygen, while CAT convert hydrogen peroxide to water and oxygen [35]. It is well known that GSH protect cells from deleterious effect of reactive oxygen species, and it's used as a co factor by several peroxidase enzymes [34].

Furthermore, no significant change was observed in MDA levels in the group of rats administered *C. sieberiana* root extract compared to the control. MDA is a product of lipid peroxidation and has been associated with oxidative stress. MDA levels are increased in the presence of toxic compounds that might have generated a number free radicals, with their reactions cumulating in oxidative stress and lipid peroxidation [36]. The observed non-significant changes antioxidant enzymes and MDA levels may

be an indication that the extract of *C. sieberiana* did not enhance the risk of oxidative stress.

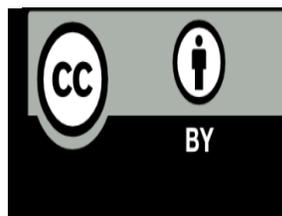
## 5.0. Conclusion

The results show that ethanolic root extract of *C. sieberiana* is relatively safe with mean lethal dose higher than 5000 mg/kg body weight. *C. sieberiana* root did not result in significant alterations in the levels of biochemical indices tested. However, there is need for caution due to the observed changes in the weight of the pancreas. Further investigation on histopathology of vital organs is recommended to better understand the safety profile of the plant. The extract should also be assessed for chronic toxicity.

## References

- [1] Sundaram V, Mohammed S, Srinivasan MR., Johnson J, Suepaul R, Pargass I, et al. Acute and subacute toxicity evaluation of hydroalcoholic extract from the stem bark of Bois Bande (*Parinari campestris* Aubl.1772) in rats. *BMC Pharmacol Toxicol.* 2021; 22:51.
- [2] Raji RO, Muhammad HL, Abubakar A, Shekwoyan Maikai SS, Raji HF. Acute and sub-acute toxicity profile of crude extract and fractions of *Gymnema sylvestre*. *Clin. Phytosci.* 2021; 7:56.
- [3] Saleem U, Aminb S, Ahmad B, Azeem H, Anwar F, Mary S. Acute oral toxicity evaluation of aqueous ethanolic extract of *Saccharum munja* Roxb. roots in albino mice as per OECD 425 TG. *Toxicol. Rep.* 2017; 4(2017):580–585.
- [4] Yahaya T, Shehu K, Isah H et al. Toxicological evaluation of the leaves of *Guiera senegalensis* (J. F. Gme), *Cassia occidentalis* (Linn), and *Ziziphus mauritiana* (Lam). *Benisuef Univ J Basic Appl Sci.* 2019; 8:14
- [5] Archer M, Agyei TA, Mintah SO, Atta P, Junior A, Kumadoh D, Asiedu-Larbi J. Medicinal Uses of *Cassia Sieberiana*; A Review. *IJSBAR.* 2019; 48 (2):161-180
- [6] Mshelia HE, Sani J, Abdullahi S, Umaru ML, Abiodun DJ. Phytochemical screening, free radical scavenging and antibacterial activity of *Cassia sieberiana* root bark extracts. *JPB.* 2017; 14(1):75-82.
- [7] Singh R, Singh S, Jeyabalan G, Ali A. An Overview on Traditional Medicinal Plants as Aphrodisiac Agent. *J. Pharmacogn Phytochem.* 2012; 1 (4):43-56
- [8] Yakubu MT, Adebisi AI, Nurudeen QO. Aphrodisiac effects of aqueous extracts of *Pausinystalia yohimbe*, *Cassia sieberiana* and *Cissus populnea* roots in paroxetine-induced sexual dysfunction male rats: a comparative study. *Pac. J. Med. Sci.* 2019; 20 (1):30-42.
- [9] National Research Council. Guide for the care and use of laboratory animals. 8th ed. National Academy Press. 2011. <https://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals.pdf>
- [10] Lorke D. A new Approach to practical oral toxicity testing. *Arch. Toxicol.* 1983; 54(1):275-287
- [11] Thefeld W, Hoffmeister H, Busch EW, Koller PU, Vollmar J. Reference values for the determination of GOT, GPT and ALP in serum with optimal standard methods. *Dtsch. Med. W ochenschr.* 1974; 99:343 - 351.
- [12] Schlebusch H, Rick W, Lang H, Knedel M, (1974). Standards in the activities of clinically important enzymes. *Dtsch med Wochenschr.* 1974; 99:(15),765-766.
- [13] Gomall AJ. Determination of Serum Proteins by means of the biuret reaction. *J Biol Chem.* 1949; 177C:751.
- [14] Doumas BT, Watson WA, Biggs HG. Albumin standards and the measurement of serum albumin with bromocresol green. *Clinica chimica acta*, 1971; 31(1):87-96.
- [15] Walter M, Gerard H. Ultramicromethod for the determination of conjugated and total bilirubin in serum or plasma. *Microchem J.* 1980; 15:231–236
- [16] Annino JS. Clinical chemistry principles and procedures, 2nd edn. Little Brown and Co, Boston, 1960. 203
- [17] Kassirer JP. Clinical evaluation of kidney function-- glomerular function. *N Engl J Med.* 1971; 285(7): 385-9. doi: 10.1056/NEJM197108122850706.
- [18] Allen LC, Michalko K, Coons C. More on cephalosporin interference with creatinine determinations. *Clin chem.* 1982; 28 (3):555-556.
- [19] Henry RJ, Cannon DC, Winkelman JW. Clinical Chemistry Principles and Techniques. 2nd edition, Harper and Row, 1974; 525.
- [20] Schönfeld RG, Lowellen CS. A Colorimetric method for determination of serum chloride. *Clin Chem.* 1964; 10:533-539.
- [21] Tietz NW. Textbook of Clinical Chemistry. 2nd ed. W.B. Saunders Co., 1994; 717-723

- [22] Misra HP, Fridovich I. The univalent reduction of oxygen by reduced flavins and quinines. *J Biol Chem.* 1972; 247:188-192.
- [23] Cohen G, Dembiec D, Marcus J. Measurement of catalase activity in tissue extracts. *Anal. Biochem.* 1970; 34:30-38.
- [24] Ellman GL. Tissue sulfhydryl groups. *Arch Biochem Biophys.* 1959; 82:70-77.
- [25] Buege JA, Aust SD. Microsomal lipid peroxidation. *Methods Enzymol.* 1998; 51:303-310.
- [26] Jitäreanu A, Trifan A, Vieriu M, Caba IC, Mârțu I, Agoroaei L. Current Trends in Toxicity Assessment of Herbal Medicines: A Narrative Review. *Processes*, 2023; 11(1):83.
- [27] Njinga NS, Kola-Mustapha AT, Quadri AL, Atolani O, Ayanniyi RO, Buhari MO, et al. Toxicity assessment of sub-acute and sub-chronic oral administration and diuretic potential of aqueous extract of *Hibiscus sabdariffa* calyces. *Heliyon.* 2020; 6 (2020):e04853
- [28] Ojatula AO, Afolabi FO. Aphrodisiac Plant *Pausinystalia yohimbe* Induces Myocardial Histological Normalcy in Wistar Rats. *J. Appl. Sci. Environ. Manage.* 2022; 26(8):1385-1389
- [29] Evenamede KS, Kpegba K, Idoh K, Agbonon A, Simalou O, Boyode P, et al. Comparative study of the toxicity of hydroethanolic extracts of the root and stem barks of *Cassia sieberiana* D.C. on Wistar rats. *J. Appl. Biol. Biotechnol.* 2019; 7(03):47-52.
- [30] Arsad SS, Esa NM, Hamzah H, Othman F. Evaluation of acute, subacute and sub chronic oral toxicity of *Rhaphidophora decursiva* (Roxb.) Schott extract in male Sprague Dawley rats. *J. Med. Plants Res.* 2013; 7(41):3030-3040.
- [31] Donkor K, Okine LN, Abotsi WK, Woode E. Acute and sub-chronic toxicity studies of aqueous extract of root bark of *Cassia Sieberiana* DC in rodents. *J Appl Pharm Sci.* 2014; 4(4):084-089.
- [32] Alkahtani S, Hasnain S, Algamy H, Aljarba NH, AlKahtane A. Acute and sub-acute oral toxicity *Lagerstroemia speciosa* in Sprague-Dawley rats. *Saudi J Biol. Sci.* 2022; 29 (2022):1585–1591.
- [33] Ugwah- Oguejiofor CJ, Okoli CO, Ugwah MO, Umaru M, Ogbulie CS, Mshelia HE, et al. Acute and sub-acute toxicity of aqueous extract of aerial parts of *Caralluma dalzielii* N. E. Brown in mice and rats. *Heliyon.* 2019; 5(2019):e01179.
- [34] Jomova K, Alomar SY, Alwasel SH, Nepovimova E, Kuca K, Valko M. Several lines of antioxidant defense against oxidative stress: antioxidant enzymes, nanomaterials with multiple enzyme-mimicking activities, and low-molecular-weight antioxidants. *Arch Toxicol.* 2024; 98: 1323–1367
- [35] Ebohon O, Irabor F, Omoregie ES. Sub-acute toxicity study of methanol extract of *Tetrorchidium didymostemon* leaves using biochemical analyses and gene expression in Wistar rats. *Heliyon.* 2020; 6(6):e04313.
- [36] Aouachria S, Boumerfeg S, Benslama A, Benbacha F, Guemmez T, Khennouf S, et al. Acute, sub-acute toxicity and antioxidant activities (in vitro and in vivo) of *Reichardia picroide* crude extract. *J Ethnopharmacol.* 2017; 208:105–116.



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