

# Effects of ethanol extract of *Hymenocardia acida* leaves on aluminium chloride-induced toxicity in male albino rats

## Abstract

**Background:** The current Study investigated the effects of aqueous extract of *H. Acida* leaves on Aluminium Chloride induced toxicity in male albino rats. Thiobarbituric acid reactive substances, Biochemical and Haematological parameters were assayed in order to determine the toxic effect of  $AlCl_3$  and the ameliorative effects of the extract.

**Methods:** Twenty (20) rats grouped into four ( $n=5$ ) were administered  $AlCl_3$  and the aqueous leaves extract based on the experimental design. The animals were sacrificed after the experimental period of seven (7) days, blood was collected for assay of the biochemical and haematological parameters by cardiac puncture while the liver tissues were harvested and homogenised for the determination of Thiobarbituric acid Reactive Substances (TBARS).

**Results:** ALT, AST, Bilirubin and Glucose levels were significantly increased ( $p<0.05$ ) in groups treated with  $AlCl_3$  when compared to the normal control whereas treatment with plant extracts ameliorates the increase. The RBC, Hb, PCV and Platelet levels increased significantly ( $p<0.05$ ) particularly in the groups treated with *H. Acida* aqueous extract when compared to the normal control. The TBARS level initially increased due to  $AlCl_3$  toxicity was decreased on treatment with aqueous plant extract.

**Conclusion:** It can be concluded however that the extract demonstrated potentials in ameliorating deleterious effects of aluminium chloride intoxication. Hence, the extract showed mild toxicity level considering the parameters in question.

**Keywords:** *Hymenocardia acida*, aluminium, chloride, toxicity, extract, biochemical, parameters, estimation

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**Abbreviations:** DEHP, di (2-ethylhexylphthalate); TBARS, thiobarbituric acid reactive substances; AST, serum aspartate aminotransferase; ALT, serum alanine aminotransferase; ALP, serum alkaline phosphatase; FBC, full blood count; EDTA, ethylenediaminetetraacetic Acid; ANOVA, analysis of variance

## Introduction

Traditionally, aluminium has been considered as nontoxic to humans. However, in recent years, increased attention is being focussed on possible adverse effects of aluminium on human health. Human exposure to aluminium is from its natural occurrence in the environment i.e. through food, water and air as well as from aluminium deliberately introduced into the environment by man.<sup>1</sup> Aluminium compounds are used in pharmaceuticals (antacids, analgesics, antiperspirants) in water treatment processes (as coagulant) and as metal in consumer products. Aluminium is present in virtually all plants. Foods naturally high in aluminium include potatoes, spinach and tea. Processed dairy products, flour and infant formula may be high in aluminium, if they contain aluminium compounds as food additives.<sup>2</sup> Aluminium is present in small amounts in mammalian tissues, yet there is little or scanty research work to support its physical usefulness. However, its neurotoxic effect on living organisms is becoming clear, aluminium being implicated as interfering with a variety of cellular metabolic processes in the nervous system and in other systems. Although molecular mechanisms by which aluminium exerts its neurotoxicity is yet to be established, several pieces of evidence suggest that Aluminium can interfere with cellular

metabolism in terms of biological stimulation, inhibition, or metal accumulation and compartmentation.<sup>3</sup>

There are numerous studies that have examined aluminium's potential to induce toxic effects in humans or laboratory animals exposed via inhalation, oral, or dermal exposure.<sup>4</sup> It is widely accepted that nervous system is the most sensitive target of aluminium toxicity and it may induce cognitive deficiency and dementia when it enters the brain. Besides this cardiotoxic, nephrotoxic and hepatotoxic effects has also been provoked by aluminium.<sup>5</sup> Aluminium ingestion in excessive amount leads to accumulation in target organs and has been associated with damage of testicular tissues of both humans and animals. Alteration in the histology of testis<sup>6,7</sup> deterioration in spermatogenesis and sperm quality; enhancement of free radicals and alterations in antioxidant enzymes;<sup>8</sup> interruption in sex hormone secretion<sup>9,10</sup> and biochemical changes in testis and other accessory reproductive organs<sup>11</sup> are some of the aspects suggested that Aluminium exposure causes adverse impact on male reproduction.<sup>4</sup>

Phytochemical studies of the chemistry of *H. acida* showed the presence of saponins, tannins, flavonoids, flavonols, phenols, proanthocyanidins, steroids and triterpenoids. Hydroethanolic extract of *H. acida* stem bark revealed the presence of alkaloids, glycosides, flavonoids, saponins, tannins and terpenoids. Glycosides, saponins and tannins were also detected in the aqueous extract of *H. acida* stem bark.<sup>12</sup>

In an investigation to determine the different chemical constituents of *H. acida*, a di (2-ethylhexylphthalate (DEHP) and homoorientin

were isolated. Igoli and Gray<sup>13</sup> have reported the isolation of five triterpenoids from *H. acida* stem bark; these triterpenoids are friedelan-3-one, betulinic acid, lupeol, b-sitosterol, stigmasterol and the fatty acid, oleic acid. Preliminary studies of the chemistry of *H. acida* showed the presence of saponins. Similarly, from the stem bark a cyclopeptide alkaloid hymenocardine has been isolated. This alkaloid was isolated together with five triterpenoids as mentioned earlier, but in this case no oleic acid;<sup>14</sup> all the plant parts contain tannins; the stem bark being richest. Lupane-type triterpenes, lupeyldocosanoate has been isolated from the bark of *H. acida*, along with lupeol and b-sitosterol. The conformational space of lupeyldocosanoate explored by molecular dynamics calculations, showed amphiphilic “horseshoes” conformations which can explain indirect anti-malarial and anti-inflammatory activities.<sup>15</sup>

## Materials and methods

### Sample collection and preparation

The leaves of the plant (*Hymenocardia acida*) were collected from Wukari, Wukari LGA Taraba State, Nigeria. The leaves were examined to be free from diseases and only healthy plant parts were selected. The leaves were thoroughly washed with clean water and dried under shade for 14 days to reduce moisture content. The dried leaves were pulverized using a laboratory blender.

### Sample extraction

One hundred gram (100g) of the powdered sample was soaked in 500 distilled water (1:5w/v) for exactly 48hrs. The extracts were filtered out first using a clean white sieving mesh and then using the Whatman No. 1 filter paper. The filtrates were concentrated using a thermostat water cabinet at 40°C for 7 days. The concentrated extracts were then transferred to air-tight containers, corked and preserved in the refrigerator at 4°C until required.

### Animals specimen

Twenty (20) male albino rats of 100-150g were obtained from the animal house of the Department of Biochemistry, Federal University Wukari, Nigeria. They were kept in clean cages (plastic bottom and wire mesh top), maintained under standard laboratory conditions (Temperature 25± 5°C, Relative humidity 50-60%, and a 12/12h light/dark cycle) and were allowed free access to standard diet and water *ad libitum*. All experiments were conducted in compliance with ethical guide for care and use of laboratory animals of the Faculty of Pure and Applied Sciences, Federal University Wukari, Nigeria.

### Experimental design

The rats were randomly divided into four groups (n = 5):

1. Group 1 Control received only normal feed and water daily.
2. Group 2 received 100mg/kg bw Aluminium chloride daily.
3. Group 3 received 100mg/kg bw aqueous extract of *Hymenocardia acida* leaves an hour after administration of 100mg/kg bw of Aluminium chloride.
4. Group 4 received 100mg/kg bw aqueous extract of *Hymenocardia acida* leaves only.

After the experimental period, animals were sacrificed and venous blood was collected by cardiac puncture and Liver was harvested. Blood samples were collected into EDTA tubes for the plasma and plain sample tubes containing no anticoagulant for the serum. Serum was obtained by centrifuging at 3000 rpm for 5 min.

### Tissue preparation

Weighed liver and kidney samples were homogenised separately in 10 parts (w/v) of ice-cold 50m MTris-HCl, (pH 7.4) using a homogeniser. The homogenates were centrifuged at 3,000 rpm for 15min and the supernatants were collected. The homogenates were centrifuged and the supernatant was examined for Thiobarbituric acid R reactive R substance (TBARS).

## Determination of biochemical parameters

**Thiobarbituric acid reactive substances (TBARS):** Hepatic lipid peroxidation was determined as thiobarbituric acid reactive substances as described by Torres et al.<sup>16</sup> Lipid peroxidation generates peroxide intermediates which upon cleavage release malondialdehyde, a product which react with thiobarbituric acid. The product of the reaction is a coloured complex which absorbs light at 535nm. The extinction coefficient,  $1.56 \times 10^5 \text{ M}^{-1} \text{ Cm}^{-1}$  was used in the calculation of TBARS and values were expressed as nmol/ml.

**Serum glucose:** Glucose oxidase catalyses the oxidation of glucose to produce hydrogen peroxide and gluconic acid. The hydrogen peroxide, in the presence of the enzyme peroxidase is broken down and the oxygen given off reacts with 4-aminophenazone and phenol to give a pink colour. The absorbance can be read 340nm using the spectrophotometer.

**Serum aspartate aminotransferase (AST):** Aspartate aminotransferase was determined as described by Reitman et al.,<sup>17</sup> using assay kits (Randox Laboratories Ltd, UK).

**Serum alanine aminotransferase (ALT):** Alanine aminotransferase was determined as described by Reitman et al. [17] using assay kits (Randox Laboratories Ltd, UK).

**Serum alkaline phosphatase (ALP):** Serum alkaline phosphatase was determined as described by Klein et al.<sup>18</sup> Using assay kits (Randox Laboratories Ltd, UK).

**Serum bilirubin:** This was determined calorimetrically according to the method described by Jendrassik et al.,<sup>19</sup> using assay kits (Randox Laboratories Ltd, UK).

**Potassium ion:** The amount of potassium is determined by using sodium tetraphenylboron in a specifically prepared mixture to produce a colloidal suspension.<sup>20</sup> The turbidity of which is proportional to potassium concentration in the range of 2-7mEq/L.

**Full blood count (FBC):** This was carried out using Abacus 380 Auto haematology analyzer.

### Statistical analysis

The results were analyzed by one-way ANOVA, using SPSS statistical package version 21. All data were expressed as Mean ± SD and difference between groups considered significant at p<0.05.

## Results and discussion

### Results

The liver function tests revealed significant increase (p< 0.05) increase in the activities of ALT, AST and ALP as well as bilirubin and glucose concentrations. However, the levels of these parameters significantly decreased (p< 0.05) owing to treatment with the extract at the respective groups when compared to normal control (Table 1).

Full blood count results shows that AlCl<sub>3</sub> intoxication caused significant decrease in Hb, PCV and platelets concentration. Treatment

of intoxicated rats with the extract caused significant increase in these parameters. However, the differences between the intoxicated groups treated with the extract and the normal group treated with the extract was statistically non-significant ( $p > 0.05$ ). Non-significant changes in WBC level were observed across the groups (Table 2). It was observed

that administration of  $AlCl_3$  caused significant increase ( $p < 0.05$ ) in the levels of thiobarbituric acid reactive substances (TBARS) in the control group compared to normal. However, extract treatment caused significant decrease ( $p < 0.05$ ) in the levels of TBARS in the extract treated groups (Table 3).

**Table 1** Results of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Bilirubin, Glucose (GLU)

Group	Normal Control	$AlCl_3$ Control	$AlCl_3$ + HA	N. + HA
ALT (U/L)	18.43±01.49 <sup>a</sup>	47.00±02.15 <sup>c</sup>	26.50±03.65 <sup>b</sup>	26.54±05.91 <sup>b</sup>
AST (U/L)	32.00±10.25 <sup>a</sup>	283.75±56.48 <sup>d</sup>	100.50±10.85 <sup>c</sup>	58.75±12.37 <sup>b</sup>
Bilirubin (mg/dl)	16.20±05.58 <sup>b</sup>	22.88±06.64 <sup>b</sup>	14.33±03.47 <sup>a</sup>	15.50±05.31 <sup>ab</sup>
Glucose (mmol/L)	04.90±01.22 <sup>a</sup>	07.68±06.19 <sup>b</sup>	04.33±02.38 <sup>a</sup>	04.33±02.82 <sup>a</sup>
ALP (U/L)	38.58±10.10 <sup>a</sup>	207.00±51.56 <sup>c</sup>	119.25±69.36 <sup>b</sup>	118.5±154.60 <sup>b</sup>
K <sup>+</sup> (mg/dl)	09.50±05.00 <sup>a</sup>	12.00±0.00 <sup>a</sup>	11.75±0.50 <sup>a</sup>	12.00±0.00 <sup>a</sup>

Alkaline Phosphatase (ALP) and Potassium ion (K<sup>+</sup>).

Each value represent mean of five rats ± SD, HA = *Hymenocardia acida*.

Groups with same superscript in the row are not significantly different.

Groups with different superscripts in the same row are significantly different

**Table 2** Results of Full blood count

Group	Normal Control	$AlCl_3$ Control	$AlCl_3$ + HA	N. + HA
WBC (x 10 <sup>9</sup> /L)	8.55±6.04 <sup>a</sup>	8.78±5.69 <sup>a</sup>	6.35±1.82 <sup>a</sup>	8.19±1.80 <sup>a</sup>
RBC (x 10 <sup>6</sup> /μL)	6.90±1.04 <sup>ab</sup>	5.26±1.37 <sup>a</sup>	7.42±0.25 <sup>b</sup>	6.18±0.13 <sup>ab</sup>
Hb (g/dL)	13.75±2.69 <sup>b</sup>	10.60±0.72 <sup>a</sup>	12.98±0.43 <sup>ab</sup>	11.95±0.61 <sup>ab</sup>
PCV (%)	47.33±06.65 <sup>c</sup>	30.78±04.83 <sup>a</sup>	38.35±01.51 <sup>b</sup>	44.21±01.74 <sup>bc</sup>
Plt(x 10 <sup>3</sup> /μl)	410.50±65.17 <sup>c</sup>	229.25±20.21 <sup>a</sup>	363.75±16.54 <sup>b</sup>	349.75±77.75 <sup>b</sup>

Each value represent mean of five rats ± SD, HA = *Hymenocardia acida*.

Groups with same superscript in the row are not significantly different.

Groups with different superscripts in the same row are significantly different.

**Table 3** Results of Thiobarbituric acid reactive substances (TBARS)

Group	TBARS (nmol/ml)
N. Control	0.05±0.030 <sup>a</sup>
$AlCl_3$ Control	0.24±0.010 <sup>d</sup>
$AlCl_3$ + HA Aq.	0.09±0.007 <sup>b</sup>
N. + HA Aq.	0.11±0.028 <sup>c</sup>

Each value represent mean of five rats ± SD, HA, *Hymenocardia acida*. Groups with same superscript in the column are not significantly different. Groups with different superscripts in the column are significantly different.

## Discussion

Herbal medicines are now receiving greater attention as an alternative to clinical therapy leading to increase in their demands.<sup>21</sup> In the rural communities of developing countries, the exclusive use of herbal drugs to treat various diseases is still very common and is prepared most often and dispensed by herbalists without formal training. Experimental screening method is therefore important in order to establish the active components present, ascertain the efficacy and safety of the herbal products.<sup>22</sup>

The liver and kidneys have demonstrated to play crucial roles in various metabolic processes and are, therefore, particularly exposed to the toxic effects of exogenous compounds<sup>23</sup> such as  $AlCl_3$ . AST and ALT are common liver enzymes because of their higher concentrations in hepatocytes, but only ALT is remarkably specific for liver function.<sup>24</sup>

Therefore, an elevation in plasma concentration of ALT is an indication of liver damage.<sup>25</sup> AST is mostly present in the myocardium, skeletal muscle, brain and kidneys.<sup>26,27</sup> Thus, the

liver and heart release AST and ALT and an elevation in plasma concentration are an indicator of liver and heart damage.<sup>28,24</sup> In this study, a significant ( $p < 0.05$ ) decrease in both ALT and AST values were observed in the animals treated with aqueous extract of *H. acida*. This clearly demonstrated that the extracts and their mixture had neither nephrotoxic nor deleterious effects on the heart.<sup>29</sup> Alkaline phosphatase, ALP, is a plasma and endoplasmic reticulum membrane-bound enzyme. Transient increase of this enzyme may be noticeable in all types of liver problems. There was great increase in the level of this enzyme in all the groups as compared to the normal control.

Serum bilirubin levels could be expressed as total bilirubin comprising of conjugated and non-conjugated or as direct bilirubin comprising only of the conjugated and an increase in bilirubin level could be attributed to three major causes such as haemolysis, biliary obstruction and liver cell necrosis.<sup>30</sup> A decrease in bilirubin level observed in the animals that received aqueous extract of *H. acida*.

The study showed that the extract of *H. acida* was able to effectively reduce the glucose level in rats as compared to the normal control and the  $AlCl_3$  control. The *H. acida* aqueous extract can suppress elevated levels of the blood glucose, which acts as an essential trigger for both liver and kidney to revert to their normal metabolic homeostasis.<sup>31</sup> The Potassium levels for the controls and treated were within the same range. This may be due to the fact that the extracts have cellular protection properties, with a normal extracellular Potassium level, or it may be due to the fact that these electrolytes are not directly associated with the development of hypertension.<sup>32</sup> The increase in TBARS induced by  $AlCl_3$  was prevented by the supplementation of the aqueous extract of *H. acida*. This finding showed that the aqueous extract of *H. acida* possesses antioxidant property.

The RBC, Hb, PCV and Platelet levels increased considerably particularly in the groups treated with *H. Acida* aqueous extract when compared to the normal control. An increase in the levels of these haematological parameters was indicative that the extracts have the potential to stimulate erythropoietin release in the kidney known to enhance RBC production (erythropoiesis).<sup>33,34</sup> Similar observation has been made on a number of plants.<sup>34–36</sup> The white blood cells serve as scavengers that destroy the microorganisms at infection sites, removing foreign substances and debris that results from dead or injured cells.<sup>37</sup> Consequently, the level is known to rise as body defence in response to toxic environment.<sup>38</sup> In this study, WBC count exhibited decrease in the group treated with  $AlCl_3$  + *H. acida*.

## Conclusion

It can be concluded however, that the extract demonstrated potentials in ameliorating deleterious effects of aluminium chloride intoxication. Hence, the extract showed mild toxicity level considering the parameters in question.

## Acknowledgments

None.

## Conflicts of interest

The authors declare no conflict of interest.

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None.

## References

- Buraimoh AA. Effects of Aluminium Chloride Exposure on the Cerebral Cortex of Adult Wistar Rats Were Not Transferable to the Offspring. *American International Journal of Contemporary Research*. 2012;2(8):294–303.
- WHO. Guidelines for drinking water quality. 2nd ed. World Health Organization, Geneva, Switzerland;1998.
- Zattal PF, Nicolini M, Corain B. Aluminium (III) toxicity and blood–brain barrier permeability: IN Aluminium in Chemistry, biology and medicine cortina international. Verona and Reven Press, New York, USA; 1991. p. 97–112.
- Pandey G, Jain GC. A Review on Toxic Effects of Aluminium Exposure on Male Reproductive System and Probable Mechanisms of Toxicity. *International Journal of Toxicology and Applied Pharmacology*. 2013;3(3):48–57
- Geyikoglu F, Turkez H, Bakir TO, et al. The genotoxic, hepatotoxic, nephrotoxic, haematotoxic and histopathological effects in rats after aluminium chronic intoxication. *Toxicol Ind Health*. 2012 29(9):780–791.
- Buraimoh AA, Ojo SA, Hambolu JO, et al. Histological study of the effects of aluminium chloride exposure on the testis of Wistar rats. *American International Journal of Contemporary Research*. 2012;2(5):114–122.
- Kutlubay R, Oguz EO, Can B, et al. Vitamin E protection from testicular damage caused by intraperitoneal aluminium. *Int J Toxicol*. 2007;26(4):297–306.
- Yousef MI, Salama AF. Propolis protection from reproductive toxicity caused by aluminium chloride in male rats. *Food Chem Toxicol*. 2009;47(6):1168–1175.
- Shahraki MR, Zahedi AS, Sarkaki AR. The Effect of Aluminum Injection in Lateral Ventricle on Sex Hormones in Male Rat. *Shiraz E Medical Journal*. 2004;5(2):1–10.
- Guo CH, Lin CY, Yeh MS, et al. Aluminum–induced suppression of testosterone through nitric oxide production in male mice. *Environment Toxicol Pharm*. 2005;19(1):33–40.
- Chinoy NJ, Momin R, Jhala DD. Fluoride and aluminium induced toxicity in mice epididymis and its mitigation by vitamin C. *Fluoride*. 2005;38(2):115–121.
- Ukwe CV. Evaluation of the anti–ulcer activity of aqueous stem bark extract of *Hymenocardia acida*. *Nigerian Journal of Pharmaceutical Research*. 2004;3(1):86–89.
- Igoli JO, Gray IA. Friedelanone and other triterpenoids from *Hymenocardia acida*. *International Journal of Physical Sciences*. 2008;3(6):156–158.
- Mpiana PT, Mudogo V, Kabangu YF, et al. Antisickling Activity and Thermostability of Anthocyanins Extract from a Congolese Plant, *Hymenocardia acida* Tul. (*Hymenocardiaceae*). *International Journal of Pharmacology*. 2009;5(1):65–70.
- Mahmout Y, Mianpeurem T, Dolmazon R, et al. Amphiphile triterpenoids from *Hymenocardia acida* Tul. Phytoantimalarial and anti–inflammatory activities. *Emecolloque sur la pharmacopée et la Médecine Traditionnelles Africaines*. 2008;15:178–186.
- Torres SH, De Sanctis JB, De L, et al. Inflammation and nitric oxide production in skeletal muscle of type II diabetic patients. *J Endocrinol*. 2004;181(3):419–427.
- Reitman S and Frankel S. A colorimetric method for the determination of sGOT and sGPT. *American Journal of Clinical Pathology*. 1957;28(1):56–63.
- Klein B, Read PA, Babson LA. Rapid Method for the Quantitative Determination of Serum Alkaline Phosphatase. *Clin Chem*. 1960;6(3):269–275.
- Jendrassic L, Groff P. Quantitative determination of total and direct bilirubin. *Biochem*. 1938;297:81.
- Terri AE, Sesin PG. Determination of serum potassium by using sodium tetraphenylboron. *American Journal Clinical Pathology*. 1958;29:86–90.
- Mythilypriya R, Shanthi P, Sachdanandam P. Oral acute and subacute toxicity studies with Kalpaamruthaa a modified indigenous preparation on rats. *Journal of Health Sciences*. 2007;53(4):351–358.
- Chakarvath BK. Herbal medicines safety and efficacy guidelines. *Regul Affairs J*. 1993;4:699–701.
- Bihde RM, Ghosh S. Acute and subchronic (28–day) oral toxicity study in rats fed with novel surfactants. *AAPS PharmSci*. 2004;6(2):1–10.
- Crook MA. Clinical Chemistry and Metabolic Medicine. 7th ed. Hodder Arnold, London, UK; 2006. 426 p.
- Horton R, Moran LA, Ochs R, et al. Principles of Biochemistry. 2nd ed. Prentice Hall, USA; 1996.
- McIntyre N, Rosaki S. Investigations biochimiques des affections hépatiques. Pharmazie, Australia; 1987. p. 294–309.
- Witthawaskul P, Ampai P, Kanjanapothi D, et al. Acute and subchronic toxicities of saponin mixture isolated from *Scheffleraleucantha* Viguier. *Journal of Ethnopharmacology*. 2003;89(1):115–121.
- Wasan KM, Najafi S, Wong J, et al. Assessing plasma lipid levels, body weight and hepatic and renal toxicity following chronic oral administration of a water soluble phytostanol compound. *J Pharm Pharm Sci*. 2001;4(3):228–234.
- Ogbonnia SO, Mbaka GO, Nkemhule FE, et al. Acute and Subchronic Evaluation of Aqueous Extracts of *Newbouldialaavis* (*Bignoniaceae*) and *Nauclealatifolia* (*Rubiaceae*) Roots used Singly or in Combination in Nigerian Traditional Medicines. *British Journal of Pharmacology and Toxicology*. 2014; 5(1):55–62



30. Tilkian M, Sarko CBM, Tilkian GA. Clinical Implication of Laboratory Tests. 2nd ed. The C.V Mosby Co. St Louis, Missouri, USA; 1979.
31. El-Soud NHA, Khalil MY, Hussein JS, et al. Antidiabetic Effects of *Fenugreek* Alkaliod Extract in Streptozotocin Induced Hyperglycemic Rats. *Journal of Applied Sciences Research*. 2007;3(10):1073–1083
32. Hall JE, Guyton AC. Textbook of medical physiology. 10th ed. Saint Louis, Mo: Elsevier Saunders, USA; 2006. p.795–98.
33. Polenakovic M, Sikole. Is erythropoietin a survival factor for red blood cells? *J Am Soc Nephrol*. 1996;7(8):1178–1182.
34. Elsner ST, Ramirez JR, Sanz RF, et al. A cross talk between hypoxia and TGF-beta orchestrates erythropoietin gene regulation through SPI and SMADS. *J Mol Biol*. 2004;36(1):9–24.
35. Mbaka GO, Adeyemi OO, Oremosu AA. Acute and sub-chronic toxicity studies of the ethanol extract of the leaves of *Sphenocentrumjollyanum* (*menispermaceae*). *Agriculture and Biology Journal of North America*. 2010;1(3):265–272.
36. Mbaka GO, Owolabi MA. Evaluation of haematinic activity and subchronic toxicity of *Sphenocentrumjollyanum* (*menispermaceae*) seed oil. *European Journal of Medicinal Plants*. 2011;1(4):140–152.
37. Guilhermino L, Soares AM, Carvalho AP, et al. Acute effects of 3, 4-Dichloroaniline on blood of male wistar rats. *Journal of Chemosphere*. 1998;37(4):619–632.
38. Ngogang JY. Haematinic activity of *Hibiscus cannabinus*. *African Journal of Biotechnology*. 2005;4(8):833–837.