EVALUATION OF THE TOXICITY AND REVERSIBILITY PROFILE OF THE AQUEOUS SEED EXTRACT OF *HUNTERIA UMBELLATA* (K. SCHUM.) HALLIER F. IN RODENTS

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Abstract

Hunteria umbellata (K. Schum.) Hallier f. (family: Apocynaceae) is reputed for the folkloric management of labour, pain and swellings, stomach ulcers, diabetes, obesity, and anaemia, with no scientific report of its toxicity and reversibility profile. The present study was, therefore, aimed at investigating the *in vivo* toxicity and reversibility profile of the aqueous seed extract of *Hunteria umbellata* (*HU*). The acute oral and intraperitoneal toxicity studies of *HU* were determined in Swiss albino mice while its 90-day oral toxicity and toxicity reversibility profile on anthropometric, biochemical, haematological and histopathological parameters were also assessed using standard procedures. Results showed that the LD_{50} values for the acute oral and intraperitoneal toxicity studies for *HU* were estimated to be 1000 mg/kg and 459.3 mg/kg, respectively. Visible signs of immediate and delayed toxicities including starry hair coat, respiratory distress, and dyskinesia were observed. For the chronic oral toxicity study, *HU* administered for 90 days produced significant ($p<0.001$) reductions in the weight gain pattern and significant ($p<0.001$) and dose related increases in the relative weights of liver, stomach, spleen, testis, lungs and heart, at the 100 and 500 mg/kg of *HU*. Chronic *HU* treatment also produced significant ($p<0.05$, $p<0.001$) dose related reductions in the serum levels of fasting blood glucose, bicarbonate, urea and creatinine while causing non-significant ($p>0.05$) alterations in the serum levels of sodium, potassium, alaninine transaminase, aspartate transaminase, alkaline phosphatase, total and conjugated bilirubin, total protein and albumin. Also, chronic oral treatment with *HU* produced significant ($p<0.05$, $p<0.01$, $p<0.001$) and dose-related increases in the red cell count, packed cell volume, haemoglobin concentration, platelet count, total leucocyte count and lymphocyte differential while producing significant ($p<0.05$) reductions in neutrophil and granulocyte differentials. *HU* also produced histological features of proliferations of the stomach epithelia, lung tissues, splenic white and red pulps, and testicular spermatogenic series. Following 14 days of oral toxicity reversibility test, there was no significant ($p>0.05$) reversal in the serum levels of the biochemical and haematological parameters investigated, including the *HU*-induced histological lesions.

Overall, results of this study showed that *HU* has a relatively low oral toxicity profile but its prolonged use, particularly, at high doses should be with great caution.

Key words: *Hunteria umbellata*, Toxicity and reversibility profile, Haematology, Liver and Renal function tests; Histopathology, Rodents.

Introduction

Hunteria umbellata (K. Schum.) Hallier f. (Apocynaceae) is a medicinal plant with a long standing use in the treatment of various ailments in Nigeria and Ghana (Adegoke and Alo, 1986). Also, the use of the plant in herbal medicines has long been reported (Bevan et al., 1967). Among the Yoruba and Binis (Southwest Nigeria), it is locally known as “Abeere”.

In African folk medicine, various extracts prepared from different parts of the plant *Hunteria umbellata* (K. Schum.) Hallier f. are employed in the treatment of various human diseases such as sexually transmitted infections including yaws, stomach ulcers, pains and swellings, diabetes mellitus, dysmenorrhoea and to induce or augment labour (Adegoke and Alo, 1986; Falodun et al., 2006). Water decoction made from the dried seeds of *Hunteria umbellata* (K. Schum.) Hallier f. is highly valued in the local management of diabetes mellitus, obesity, stomach ache, pains and swellings, hypertension and as immune booster (Boone, 2006; Adeneye and Adeyemi, 2009a). Recently, Falodun et al. (2006) and Igbe et al. (2009) reported the oxytocic effect of the leaf aqueous extract and antipyretic and analgesic effect of the fresh fruit pulp of *Hunteria umbellata*, respectively. The oral

hypoglycaemic effect of the aqueous seed extract of *Hunteria umbellata* (K. Schum.) Hallier f. (*HU*) in various *in vivo* models of experimental diabetes was also recently reported (Adeneye and Adeyemi, 2009a; 2009b). In addition, the anti-obesity and hyperlipidaemic activities of *HU* have also been reported to be mediated via inhibitions of intestinal lipid absorption and *de novo* cholesterol and triglyceride syntheses (Adeneye et al, 2010).

Despite its wide application in human health, the folkloric therapeutic efficacy and the safety profile of the seed extract are yet to be scientifically validated. Therefore, the current study was designed to evaluate both the toxicity and reversibility profile of *HU* in rodents, which is strongly in line with the World Health Organization set goals on determining the safety profile of any medicinal plants before it can become acceptable for human use.

Materials and methods

Collection of plant materials

Plant collection, identification and authentication were made as previously described by Adeneye and Adeyemi (2009a).

Cold aqueous extraction

In the preparation of the cold aqueous extract of the seeds of *Hunteria umbellata* (K. Schum.) Hallier f., 100 g of the dry seeds was pulverized to white-to-light brown fine powder using domestic blender. Thirty grams of the fine powdered sample was dissolved in 500 ml of distilled water in a 1 litre Pyrex beaker and was left to stand in the refrigerator at -4 ºC for 72 h. After 72 h, the homogenate was then rigorously shaken intermittently for 6 hours and was rapidly filtered through a piece of clean white cloth. The filtrate was then transferred to an aerated oven preset at 40 ºC and completely dried until a deep brown, aromatic solid residue was obtained. The weight of the solid residue left behind was 23 g, giving a yield of 76.67% ($^{w}/_{w}$). This procedure was repeated for three more times. The residues, thus obtained, were pooled, and stored in air- and moisture-tight container which was kept in a refrigerator maintained at -4 ºC. From this, a fresh stock was reconstituted in distilled water at a concentration of 100 mg/ml (pH = 4.96), whenever needed.

Experimental animals and their care

Young adult white albino rats and Swiss albino mice (aged 8-14 weeks old) that were used in this study were obtained from the Animal House of the Lagos State University College of Medicine, Ikeja, Lagos State, Nigeria, after ethical approval was obtained. The rats were handled in accordance with international principles guiding the Use and Handling of experimental animals (United States National Institutes for Health, 1985). The rats were maintained on standard rat feed (Ladokun Feeds, Ibadan, Nigeria) and water which were made available *ad libitum*. The rats were maintained at an ambient temperature between 28-30 ºC, humidity of 55 ± 5%, and standard (natural) photoperiod of approximately 12 hour of light (06:30 h – 18:30 h) alternating with approximately 12 hour of darkness (18:30 h - 06:30 h).

Acute oral toxicity study of *HU* using Miller and Tainter method

Overnight fasted Swiss albino mice were randomly divided into eight groups with six mice in each group such that the differences within and between groups do not exceed ±20% of the average weight of the sample size. Group I mice served as the untreated control and were orally administered with 10 ml/kg of distilled water while Groups II-VIII mice were orally gavaged 125, 250, 500, 750, 1500, 1750 and 2000 mg/kg of the aqueous seed extract of *Hunteria umbellata*, respectively. The animals were closely monitored for behavioural and general signs of toxicity such as feeding and drinking pattern, restlessness, and mortality, etc. within the first 24 hrs. The median lethal dose (LD_{50}) was estimated by log-dose probit analysis of Miller and Tainter (1944) and as adopted by Agbaje et al. (2009). Surviving mice were further observed for 14 days for delayed toxicities or death.

Acute intraperitoneal toxicity studies of *HU*

Using the method of Miller and Tainter (1944) described for the acute oral toxicity, overnight fasted mice were randomly divided into five treatment groups (Groups II-VI) and given 62.5, 125, 250, 500, 750 and 1500 mg/kg of the *HU* intraperitoneally. In addition, mice in the untreated control (Group I) were given 1 ml/kg of distilled water intraperitoneally.

Chronic oral toxicity of *HU*

A total of 96 white albino Wistar rats, 6-8 weeks old and of either sex were randomly allotted to 4 groups of 12 rats of per sex per group and such that the difference within and between groups do not exceed ± 20% of the average weight of the sample size. In either sex of rats, Group I rats served as the untreated control and were orally administered with 10 ml/kg of distilled water while Groups II-IV rats were orally treated with single, daily doses of 20 mg/kg body weight (a-fifth of the pharmacologically active dose), 100 mg/kg (pharmacologically active dose), and 500 mg/kg (5 folds the pharmacologically active dose) (Tanira et al., 1988; Thanarbon et al., 2006) of *HU*, respectively, for 90 days. The rats were closely observed for the general and behavioural signs of toxicity, body weight changes and mortality. At the end of the 90-day treatment period, 6 rats from each group of treated rats of either sex were randomly anaesthetized with inhaled diethyl ether and blood samples were withdrawn directly from the heart chamber with 21 G needle mounted on a 5 ml syringe plunger (Unique Pharmaceuticals, Sango-Otta, Ogun State, Nigeria). After the samples were collected into the sample bottles, the animals were sacrificed humanely and selected internal organs such as the liver, heart, kidneys, spleen, stomach, and testes were collected. The blood and selected organs were processed for biochemical, haematological and histopathological studies.

Oral toxicity reversibility test of *HU*

After the initial sacrifice, the remain six rats per sex of the experimental animal from each treatment group were left untreated with the extract but given drinking potable water and allowed free access to feed for additional 2 weeks before they were also sacrificed humanely using same procedure described earlier.

Effect of *HU* on body weight in rats

In the course of the 90-day oral treatment, body weights of rats were regularly taken at 2 weeks interval with electronic Mettler weighing balance (Mettler Toledo Type BD6000, Mettler-Toledo GmbH, Greifensee, Switzerland). Absolute and percentage (%) weight changes were calculated in respect of the initial body weight on day 1.

Effect of *HU* on relative weights of selected vital internal organs

Rat internal organs including heart, lungs, liver, spleen, kidneys, stomach, and testis were carefully dissected out and freed from adjoining supporting connective tissues. The organs were gently rinsed in normal saline, blotted with filter paper (Whatmann's No. 1 filter paper) and weighed. Each weighed organ was grossly observed for visible lesions and thereafter standardized for 100 g body weight for the corresponding animal weight (Yemitan and Adeyemi, 2004).

Haematological assessment

Blood samples were collected directly from the heart chamber from anaesthetized rats with 12 G needle mounted on a 5 ml syringe plunger (Unique Pharmaceuticals, Sango-Otta, Ogun State, Nigeria). Blood collection into EDTA-coated sample bottles (BD Vacutainer®, BD-Plymouth, Plymouth, U.K.) was for determination of red cell count (RBC), packed cell volume (PCV), haemoglobin concentration (Hb), platelet count (PLT), erythrocyte indices, total white blood cell counts and its differentials using Automated Haematology System (Sysmex Haematology-Coagulation Systems®, Model KX-21N, Sysmex Incorporation, Kobe, Japan).

Biochemical assays

The blood samples drawn directly from the heart chamber were collected into non-heparinised and allowed to clot and then centrifuged at 5000 rpm to separate clear sera from the clotted blood samples. The clear samples were obtained for assays of the following biochemical parameters: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), fasting blood glucose (FBG), urea, creatinine, total protein, albumin, triglyceride, total cholesterol and cholesterol fractions [high density lipoprotein cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c), and very low density lipoprotein cholesterol (VLDL-c)], total (TB) and conjugated bilirubin (CB). Serum ALT, AST and ALP were measured using the enzyme kinetic method of Reitman and Frankel (1957). The readings were done at 546 nm for ALT and AST and 590 nm for ALP. One unit of ALT and AST activities were defined as the amount of protein that liberated one µmole pyruvate/ml per min and one µmole oxaloacetate/ml per min, respectively, under experimental

condition Urea was measured using modified diacetylmonoamine method, read at 546 nm (Marsh, 1965). Other biochemical determinations include triglyceride, total cholesterol and cholesterol fractions using method of Fossati and Principe (1982), while glucose was estimated using modified oxidase method of Trinder (1969). Estimation of creatinine was done by the Jaffe's reaction method (Biod and Sirota, 1948; Owen et al., 1954; Chawla, 1999). The total protein was estimated by Biuret method (Treitz, 1970) while that of albumin was determined by bromocresol green (Lowry et al., 1957). The total bilirubin and the conjugated bilirubin were determined by Jendrassik–Grof method (Spencer and Price, 1977). In addition, serum concentrations of sodium, potassium, and bicarbonate were estimated using standard procedures.

Histopathological studies

After the animals were sacrificed, postmortem examination was performed on the six randomly selected rats from each treatment and control groups with their vital organs identified and carefully dissected out *en bloc* for histopathological examinations. After rinsing in normal saline, the organs were preserved in 10% formo-saline before they were completely dehydrated in absolute (100%) ethanol. The organs were then embedded in routine paraffin blocks. From the embedded paraffin blocks, 4-5 µm thick sections of each tissue was prepared and stained with haematoxylin-eosin. These were examined under a photomicroscope (Model N-400ME, CEL-TECH Diagnostics, Hamburg, Germany) connected with a host computer. Sections were illuminated with white light from a 12V halogen lamp (100 W) after filtering with a 520nm monochromatic filter. The slides were examined for associated histopathological lesions (Thanarbon et al., 2006).

Statistical Analysis

Results were presented as mean ± S.D. for body weights, organ weights and relative organ weights while data for haematological and, biochemical indices were expressed as mean ± S.E.M. of six observations. Statistical analysis was done using two-way analysis of variance followed by post-hoc test, Student-Newman-Keuls test on SYSTAT 10.6. Statistical significance were considered at $p<0.05$, $p<0.01$, and $p<0.001$.

Results

Acute toxicity studies

The results of the acute oral and intraperitoneal toxicity tests of *HU* using the Miller and Tainter (1944) are shown in Figures 1 and 2, respectively. Treatment with *HU* did not cause mortality for up to 125 mg/kg body weight but increasing mortality was recorded with increasing doses of 250, 500, 750, 1500, 1750 and 2000 mg/kg body weight orally. However, at 2000 mg/kg body weight orally and 1500 mg/kg body weight intraperitoneally, 100% mortality was recorded. Death in each case was preceded by peri-oral tremor, followed by decreased locomotor activity and generalized tonic-clonic contraction and asphyxia. Similar pattern of behavioural toxicity was recorded in the acute intraperitoneal toxicity determination in rats given the test doses of 62.5, 125, 250, 500, 750 and 1500 mg/kg body weight.

Chronic oral toxicity studies

Effect of 90-days oral treatment with 20-500 mg/kg/day of *HU* on body weight of treated rats

Chronic treatment with 20-500 mg/kg/day caused significant ($p<0.001$) reductions in the pattern of weight gain in the 100 and 500 mg/kg of *HU*-treated rats when compared to the control values. Reductions in the weight gain pattern became noticeable from the 30th day to the 91st day of treatment in the male rats (Table 1a) while it became noticeable only from 60th day to the 91st day in the female rats (Table 1b). However, there were no significant ($p>0.05$) differences between the 20 mg/kg/day of *HU*-treated and the control rats (Tables 1a and 1b)

Effect of 14-day toxicity reversibility of *HU* on body weight of treated rats

On withdrawing *HU* treatment, there was reversal in the pattern of weight gain in the rats such that pattern of weight gain in the 20 mg/kg of *HU*-treated rats was not significantly ($p>0.05$) different from those of the control group (Tables 1a and 1b). However, the pattern of weight gain in 100 and 500 mg/kg of *HU*-treated rats was still significantly ($p<0.05$) lower than those of control and 20 mg/kg of *HU*-treated rats (Tables 1a and 1b).

Table 1a: Effect of chronic oral treatment with *HU* and toxicity reversibility on body weights of treated male rats

Treatment day	I	II	III	IV
1st	111.8 ± 51.7	118.6 ± 49.1	106.4 ± 45.3	112.1 ± 71.3
15th	116.3 ± 57.5	159.3 ± 52.0	130.3 ± 43.5	158.6 ± 75.1
30th	213.9 ± 55.8	201.7 ± 46.5	124.1 ± 39.0[f]	149.9 ± 58.3[f]
45th	247.4 ± 52.0	224.6 ± 42.5	171.3 ± 31.4[f]	188.3 ± 48.0[f]
60th	271.2 ± 37.4	282.5 ± 27.9	183.7 ± 27.1[f]	189.5 ± 56.0[f]
75th	282.5 ± 50.6	282.5 ± 29.7	199.8 ± 25.1[f]	217.3 ± 47.7[f]
91st	310.8 ± 30.7	301.8 ± 28.8	196.4 ± 26.0[f]	190.6 ± 48.4[f]
Reversibility				
15th day	385.0 ± 28.7	328.3 ± 27.7	270.7 ± 34.8[d]	251.0 ± 61.7[d]

[f] represents a significant reduction at p<0.001 when compared to the Group I (control) values
Group I = 10 ml/kg/day of distilled water, Group II = 20 mg/kg/day of *HU*, Group III = 100 mg/kg/day of *HU*
Group IV = 500 mg/kg/day of *HU*.

Table 1b: Effect of chronic oral treatment with *HU* and toxicity reversibility on body weights of treated female rats

Treatment day	I	II	III	IV
1st	107.8 ± 38.0	118.2 ± 39.3	124.6 ± 27.7	117.4 ± 36.6
15th	133.9 ± 31.6	160.5 ± 36.6	166.8 ± 19.0	152.7 ± 30.2
30th	175.5 ± 31.4	190.9 ± 34.9	182.4 ± 13.8	159.5 ± 45.3
45th	202.3 ± 37.6	186.9 ± 36.4	202.3 ± 18.8	183.4 ± 38.1
60th	231.0 ± 40.2	187.5 ± 21.5[e]	205.1 ± 19.5[e]	187.8 ± 37.0[f]
75th	258.9 ± 50.0	202.3 ± 24.3[e]	212.1 ± 17.4[e]	189.9 ± 33.8[f]
91st	293.6 ± 47.7	231.0 ± 26.5[e]	210.6 ± 13.4[e]	184.9 ± 27.9[f]
Reversibility				
15th day	299.7 ± 7.5	294.0 ± 13.2	277.0 ± 24.6	252.0 ± 17.9[d]

[d, e] and [f] represent significant reductions at p<0.05, p<0.01 and p<0.001, respectively, when compared to the Group I (control) values. Group I = 10 ml/kg/day of distilled water, Group II = 20 mg/kg/day of *HU*, Group III = 100 mg/kg/day of *HU*, Group IV = 500 mg/kg/day of *HU*'

Table 2a: Effect of chronic oral treatment with *HU* on the relative organ weight of some selected vital body organs of male rats

	I	II	III	IV
Liver	2.7 ± 0.3	2.8 ± 0.3	3.4 ± 0.6[c]	3.8 ± 0.2[c]
Stomach	0.5 ± 0.1	0.9 ± 0.1[b]	1.0 ± 0.2[c]	1.1 ± 0.3[c]
Lungs	0.7 ± 0.1	0.4 ± 0.2	0.9 ± 0.2[c]	1.1 ± 0.2[c]
Kidneys	0.7 ± 0.1	0.7 ± 0.0	1.0 ± 0.3[c]	0.5 ± 0.0
Spleen	0.3 ± 0.0	0.3 ± 0.0	0.5 ± 0.1[c]	0.5 ± 0.0[c]
Heart	0.3 ± 0.0	0.3 ± 0.0	0.5 ± 0.1[c]	0.5 ± 0.0[c]
Testis	2.3 ± 0.2	2.4 ± 0.3	2.5 ± 0.2	2.7 ± 0.3[a]

[a, b] and [c] represent significant increases at p<0.05, p<0.01 and p<0.001, respectively, when compared to control values while [d] represents a significant decrease at p<0.05 when compared to compared to Group II values
Group I = 10 ml/kg/day of distilled water, Group II = 20 mg/kg/day of *HU*, Group III = 100 mg/kg/day of *HU*
Group IV = 500 mg/kg/day of *HU*.

Table 2b: Effect of chronic oral treatment with 20-500 mg/kg/day of *HU* on the relative organ weight of some selected vital body organs of female rats

	I	II	III	IV
Liver	2.1 ± 0.2	2.9 ± 0.4	2.9 ± 0.3[a]	3.3 ± 0.4[c]
Stomach	1.2 ± 0.3	1.0 ± 0.1	0.9 ± 0.2	1.0 ± 0.1
Lungs	0.9 ± 0.0	1.0 ± 0.1	1.0 ± 0.1	1.1 ± 0.3[a]
Kidneys	0.6 ± 0.2	0.7 ± 0.2	0.3 ± 0.1[e]	0.4 ± 0.0[e]
Spleen	0.4 ± 0.0	0.6 ± 0.2[a]	0.5 ± 0.0	0.5 ± 0.0
Heart	0.4 ± 0.0	0.5 ± 0.1	0.7 ± 0.3	0.5 ± 0.0

[a] and [c] represent significant increases at p<0.05 and 0.001, respectively, when compared to control (Group I) values while [e] represents a significant decrease at p<0.01 when compared to Group I values.
Group I = 10 ml/kg/day of distilled water, Group II = 20 mg/kg/day of *HU*, Group III = 100 mg/kg/day of *HU*, Group IV = 500 mg/kg/day of *HU*.

Table 3a: Effect of 14 days of toxicity reversibility test of *HU* on the relative organ weight of some selected vital body organs of male rats

	I	II	III	IV
Liver	2.2 ± 0.1	2.6 ± 0.3	1.8 ± 0.3	2.2 ± 0.8
Stomach	0.7 ± 0.1	0.7 ± 0.2	0.7 ± 0.1	0.7 ± 0.2
Lungs	0.7 ± 0.2	0.7 ± 0.2	0.6 ± 0.1	0.7 ± 0.2
Kidneys	0.5 ± 0.0	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1
Spleen	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.1
Heart	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.1
Testis	1.4 ± 0.1	1.6 ± 0.2	1.5 ± 0.3	1.4 ± 0.2

Group I = 10 ml/kg/day of distilled water, Group II = 20 mg/kg/day of *HU*, Group III = 100 mg/kg/day of *HU* Group IV = 500 mg/kg/day of *HU*.

Table 3b: Effect of 14 days of toxicity reversibility of *HU* on the relative organ weight of some selected vital body organs of female rats

	I	II	III	IV
Liver	2.0 ± 0.0	2.1 ± 0.1	2.4 ± 0.2[a]	2.1 ± 0.1
Stomach	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.1	0.7 ± 0.0
Lungs	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.1	0.7 ± 0.0
Kidneys	0.7 ± 0.0	0.7 ± 0.0	0.4 ± 0.0[f]	0.5 ± 0.2[e]
Spleen	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.4 ± 0.0
Heart	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.4 ± 0.0

[a] represents a significant increases at p<0.05 while [e] and [f] represent significant decreases at p<0.01 and p<0.001, respectively, when compared to control (Group I) values. Group I = 10 ml/kg/day of distilled water, Group II = 20 mg/kg/day of *HU*, Group III = 100 mg/kg/day of *HU*, Group IV = 500 mg/kg/day of *HU*.

Table 4: Effect of chronic *HU* treatment on the fasting blood glucose (FBG) concentration in the treated rats on 91st day of treatment

Groups	Treatment	FBG (mg/dl) (male rats)	FBG (mg/dl) (female rats)
I	10 ml/kg/day of distilled water	78.7 ± 0.9	76.5 ± 2.8
II	20 mg/kg/day *HU*	63.8 ± 1.7[d]	52.3 ± 2.1[d]
III	100 mg/kg/day *HU*	46.8 ± 2.5[e]	40.0 ± 2.2[e]
IV	500 mg/kg/day *HU*	37.0 ± 0.7[f]	36.2 ± 1.5[f]

[d, e] and [f] represent significant decreases at p<0.05, p<0.01 and p<0.001, respectively, when compared to Group I values

Table 5: Effect of 14-days reversibility test of *HU* on the fasting blood glucose (FBG) concentration in male and female rats

Groups	Treatment	FBG (mg/dl) (male rats)	FBG (mg/dl) (female rats)
I	10 ml/kg/day of distilled water	59.0 ± 0.7	61.7 ± 1.1
II	20 mg/kg/day *HU*	60.7 ± 1.7	54.0 ± 3.3
III	100 mg/kg/day *HU*	47.3 ± 4.0[e]	49.7 ± 1.7[e]
IV	500 mg/kg/day *HU*	38.7 ± 2.0[f]	42.7 ± 5.1[e]

[e] and [f] represent significant decreases at p<0.01 and p<0.001, respectively, when compared to Group I values

Table 6: Effect of chronic oral treatment with 20-500 mg/kg/day of *HU* on the serum sodium, potassium, bicarbonate, urea and creatinine concentrations in male and female rats on 91st day of treatment

Parameters	I	II	III	IV
Male rats				
Sodium (mEq/L)	139.0 ± 2.6	140.8 ± 2.6	151.3 ± 5.1	145.7 ± 1.5
Potassium (mEq/L)	05.6 ± 0.3	06.2 ± 0.4	06.9 ± 0.5	07.7 ± 0.6
Bicarbonate (mEq/L)	32.3 ± 4.9	28.5 ± 1.7	22.5 ± 1.5[d]	20.2 ± 1.0[d]
Urea (mg/dl)	38.0 ± 1.9	37.9 ± 2.1	34.7 ± 1.8	30.7 ± 3.0[d]
Creatinine (mg/dl)	1.1 ± 0.1	1.1 ± 0.1	0.9 ± 0.1	0.8 ± 0.0[d]
Female rats				
Sodium (mEq/L)	139.0 ± 1.2	143.0 ± 0.7	140.0 ± 0.0	139.0 ± 1.9
Potassium (mEq/L)	06.9 ± 0.3	08.4 ± 0.8	07.3 ± 0.2	08.7 ± 0.9
Bicarbonate (mEq/L)	25.2 ± 3.1	21.8 ± 1.4	21.3 ± 1.7	18.7 ± 1.3[d]
Urea (mg/dl)	40.9 ± 1.4	33.5 ± 1.9[d]	30.8 ± 2.2[d]	28.4 ± 2.2[d]
Creatinine (mg/dl)	0.8 ± 0.0	0.7 ± 0.0	0.6 ± 0.0[d]	0.6 ± 0.0[d]

[d] represents a significant decrease at p<0.05 when compared to untreated control (Group I) values
Group I = 10 ml/kg/day of distilled water. Group II = 20 mg/kg/day of *HU*, Group III = 100 mg/kg/day of *HU* Group IV = 500 mg/kg/day of *HU*.

Table 7: Effect of oral toxicity reversibility of *HU* on the serum sodium, potassium, bicarbonate, urea and creatinine concentrations in male and female rats

Parameters	I	II	III	IV
Male rats				
Sodium (mEq/L)	144.0 ± 0.4	143.7 ± 0.4	141.5 ± 1.0	139.8 ± 1.9
Potassium (mEq/L)	07.9 ± 0.1	08.4 ± 0.9	09.0 ± 0.4	08.4 ± 0.9
Bicarbonate (mEq/L)	25.3 ± 1.3	25.7 ± 0.6	25.3 ± 0.8	29.5 ± 0.9[a]
Urea (mg/dl)	41.4 ± 3.5	30.0 ± 2.2[d]	25.5 ± 3.8[e]	23.4 ± 4.2[e]
Creatinine (mg/dl)	0.8 ± 0.0	0.7 ± 0.1	0.5 ± 0.0[d]	0.6 ± 0.1[d]
Female rats				
Sodium (mEq/L)	140.7 ± 0.8	139.7 ± 0.8	138.7 ± 0.6	139.7 ± 0.8
Potassium (mEq/L)	9.1 ± 0.4	8.2 ± 0.2	7.8 ± 0.4	9.7 ± 0.5
Bicarbonate (mEq/L)	28.7 ± 0.2	26.0 ± 1.9	24.3 ± 1.5	23.7 ± 0.8
Urea (mg/dl)	32.1 ± 3.1	33.6 ± 3.2	33.4 ± 2.9	32.4 ± 1.6
Creatinine (mg/dl)	0.5 ± 0.0	0.5 ± 0.0	0.6 ± 0.1	0.3 ± 0.0[d]

[d] and [e] represent significant decreases at p<0.05 and p<0.01, respectively, when compared to untreated control (Group I) values. Group I = 10 ml/kg/day of distilled water, Group II = 20 mg/kg/day of *HU*, Group III = 100 mg/kg/day of *HU*, Group IV = 500 mg/kg/day of *HU*.

Table 8: Effect of chronic oral treatment with *HU* on serum ALT, AST, ALP, TB and CB of treated rats

	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	TB (mg/dl)	CB (mg/dl)
Male					
I	30.7 ± 1.1	55.0 ± 4.8	44.7 ± 1.2	0.2 ± 0.1	0.1 ± 0.0
II	37.8 ± 1.8	54.8 ± 4.3	52.2 ± 6.7	0.2 ± 0.0	0.1 ± 0.0
III	37.2 ± 6.4	67.2 ± 5.3	71.2 ± 9.3	0.2 ± 0.1	0.0 ± 0.0
IV	36.2 ± 2.6	66.7 ± 4.9	58.5 ± 8.9	0.2 ± 0.1	0.1 ± 0.0
Female					
I	23.7 ± 2.2	59.3 ± 6.5	55.0 ± 7.5	0.3 ± 0.1	0.3 ± 0.1
II	26.3 ± 4.2	64.2 ± 5.4	67.5 ± 8.5	0.2 ± 0.1	0.1 ± 0.0
III	19.0 ± 0.9	68.7 ± 5.6	39.0 ± 2.6	0.1 ± 0.1	0.1 ± 0.0
IV	20.0 ± 4.0	61.8 ± 6.4	51.2 ± 6.9	0.1 ± 0.0	0.1 ± 0.0

Mean ± S.E.M, p>0.05, n = 6; Group I = 10 ml/kg/day of distilled water, Group II = 20 mg/kg/day of *HU*, Group III = 100 mg/kg/day of *HU*, Group IV = 500 mg/kg/day of *HU*.

Table 9: Effect of toxicity reversibility of *HU* on serum ALT, AST, ALP, TB and CB of treated rats

	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	TB (mg/dl)	CB (mg/dl)
Male					
I	21.7 ± 0.4	62.3 ± 4.3	55.6 ± 16.0	0.5 ± 0.2	0.2 ± 0.1
II	24.0 ± 0.8	64.0 ± 2.3	45.6 ± 10.3	0.5 ± 0.1	0.5 ± 0.1
III	23.8 ± 2.0	65.0 ± 2.5	52.5 ± 4.0	0.6 ± 0.3	0.6 ± 0.3
IV	23.7 ± 2.8	61.8 ± 5.6	58.5 ± 13.6	0.5 ± 0.0	0.3 ± 0.0
Female					
I	24.7 ± 2.3	65.0 ± 2.6	36.9 ± 16.8	0.5 ± 0.1	0.5 ± 0.1
II	35.3 ± 1.7	62.7 ± 2.2	28.9 ± 11.0	0.4 ± 0.0	0.3 ± 0.0
III	30.3 ± 4.1	65.0 ± 4.5	28.1 ± 10.6	0.3 ± 0.1	0.2 ± 0.1
IV	27.3 ± 3.2	63.3 ± 2.5	24.1 ± 6.5	0.7 ± 0.1	0.3 ± 0.1

Mean ± S.E.M, p>0.05, n = 6; Group I = 10 ml/kg/day of distilled water, Group II = 20 mg/kg/day of *HU*, Group III = 100 mg/kg/day of *HU*, Group IV = 500 mg/kg/day of *HU*.

Table 10: Effect of chronic oral treatment of 20-500 mg/kg of *HU* on serum total protein and albumin

Group	Treatment	total protein (mg/dl)	albumin (mg/dl)
Male			
I	10 ml/kg of distilled water	4.8 ± 0.3	3.1 ± 0.2
II	20 mg/kg *HU*	4.9 ± 0.4	3.3 ± 0.2
III	100 mg/kg *HU*	4.4 ± 0.5	2.7 ± 0.4
IV	500 mg/kg *HU*	4.4 ± 0.1	2.7 ± 0.1
Female			
I	10 ml/kg of distilled water	6.6 ± 0.3	3.1 ± 0.2
II	20 mg/kg *HU*	6.8 ± 0.2	3.5 ± 0.2
III	100 mg/kg *HU*	6.8 ± 0.0	3.6 ± 0.3
IV	500 mg/kg *HU*	6.6 ± 0.4	3.4 ± 0.4

Mean ± S.E.M, p>0.05, n = 6

Table 11: Effect of toxicity reversibility test of *HU* on serum total protein and albumin

Group	Treatment	total protein (mg/dl)	albumin (mg/dl)
Male			
I	10 ml/kg of distilled water	6.7 ± 0.2	4.2 ± 0.3
II	20 mg/kg *HU*	6.8 ± 0.2	3.5 ± 0.1
III	100 mg/kg *HU*	7.2 ± 0.5	3.6 ± 0.4
IV	500 mg/kg *HU*	6.8 ± 0.1	3.6 ± 0.2
Female			
I	10 ml/kg of distilled water	7.2 ± 0.0	4.0 ± 0.1
II	20 mg/kg *HU*	7.0 ± 0.1	3.8 ± 0.2
III	100 mg/kg *HU*	7.2 ± 0.2	4.1 ± 0.2
IV	500 mg/kg *HU*	7.6 ± 0.3	4.1 ± 0.0

Mean ± S.E.M, $p>0.05$, n = 6.

Table 12: Effect of 90-days oral treatment with 20-500 mg/kg of *HU* on the full blood count of treated male rats

	I	II	III	IV
RBC (x 10^6/µl)	08.7 ± 0.1	09.1 ± 0.3	08.6 ± 0.4	09.2 ± 0.2
Hb (g/dl)	14.4 ± 0.6	15.6 ± 0.2	16.0 ± 0.2^b	16.7 ± 0.1^b
PCV (%)	49.2 ± 1.4	50.0 ± 0.7	51.6 ± 0.2^a	55.9 ± 0.8^c
PLT (x10^3/µl)	672.3 ± 39.8	969.7 ± 28.6^a	1291.7 ± 94.9^b	1611.8 ± 95.9^c
MCV (fl)	56.8 ± 1.1	56.7 ± 0.5	57.1 ± 0.8	59.3 ± 0.4^a
MCH (pg)	16.7 ± 0.8	18.1 ± 0.3^a	19.3 ± 0.4^a	20.0 ± 0.5^b
MCHC (g/dl)	29.4 ± 1.3	32.0 ± 0.5^a	33.6 ± 0.6^b	35.2 ± 1.0^c
TLC (x10^3/µl)	14.3 ± 1.0	16.9 ± 0.8	17.7 ± 0.7^a	19.3 ± 0.4^c
lymp (%)	79.4 ± 2.0	82.4 ± 1.6^a	88.7 ± 1.6^c	90.6 ± 0.9^c
Neut (%)	11.4 ± 1.1	11.7 ± 1.2	07.9 ± 0.7^d	07.7 ± 0.7^d
Gran (%)	09.2 ± 1.0	08.9 ± 0.4	03.5 ± 1.7^e	01.7 ± 0.3^e

$^{a, b}$ and ^{c} represent significant increases while $^{d, e}$ and ^{f} represent significant decreases at $p<0.05$, $p<0.01$ and $p<0.001$, respectively, when compared to Group I values. Group I = 10 ml/kg/day distilled water, Group II = 20 mg/kg/day of *HU*, Group III = 100 mg/kg/day of *HU* , Group IV = 500 mg/kg/day of *HU*.

Table 13: Effect of 90-days oral treatment with 20-500 mg/kg of *HU* on the full blood count of treated female rats

	I	II	III	IV
RBC (x 10^6/µl)	07.2 ± 0.2	07.9 ± 0.4	09.6 ± 0.2^c	09.9 ± 0.2^c
Hb (g/dl)	12.7 ± 0.7	14.5 ± 0.4^a	16.0 ± 0.5^c	16.3 ± 0.4^c
PCV (%)	40.8 ± 0.8	44.6 ± 1.6^a	45.4 ± 1.3^a	50.1 ± 1.6^b
PLT (x10^3/µl)	671.0 ± 18.4	1356.0 ± 114.4^a	1802.2 ± 38.1^b	1878.8 ± 41.9^c
MCV (fl)	55.1 ± 0.5	57.8 ± 0.6^b	58.3 ± 0.3^b	57.9 ± 0.9^b
MCH (pg)	18.6 ± 0.2	19.6 ± 0.3^b	19.5 ± 1.0^b	19.6 ± 0.1^b
MCHC (g/dl)	33.7 ± 0.6	34.0 ± 0.5	34.8 ± 0.1^b	36.8 ± 0.4^c
TLC (x10^3/µl)	08.2 ± 1.6	10.6 ± 0.3	14.8 ± 0.8^b	17.8 ± 0.7^c
lymp (%)	77.7 ± 2.7	89.7 ± 1.5^a	92.6 ± 0.8^c	93.4 ± 0.4^c
Neut (%)	11.7 ± 1.0	07.7 ± 0.8^f	05.4 ± 0.6^f	05.0 ± 0.5^f
Gran (%)	10.6 ± 1.7	02.5 ± 0.8^f	02.0 ± 0.2^f	01.7 ± 0.5^f

$^{a, b}$ and ^{c} represent significant increases while $^{d, e}$ and ^{f} represent significant decreases at $p<0.05$, $p<0.01$ and $p<0.001$, respectively, when compared to Group I values. Group I = 10 ml/kg/day distilled water, Group II = 20 mg/kg/day *HU*, Group III = 100 mg/kg/day *HU* , Group IV = 500 mg/kg/day *HU*.

Table 14: Effect of 14-day toxicity reversibility study of *HU* on the full blood count of treated male rats

	I	II	III	IV
RBC (x 10^6/µl)	08.0 ± 0.1	08.2 ± 0.3	09.0 ± 0.1[b]	10.0 ± 0.3[c]
Hb (g/dl)	14.0 ± 0.2	14.3 ± 0.3	15.4 ± 0.3[b]	16.8 ± 0.2[c]
PCV (%)	44.4 ± 0.7	45.7 ± 1.1	52.8 ± 1.5[a]	56.1 ± 1.0[a]
PLT (x10^3/µl)	893.7 ± 13.5	955.8 ± 16.6[a]	1097.2 ± 25.9[b]	1234.2 ± 07.0[c]
MCV (fl)	55.8 ± 0.6	58.7 ± 0.5[a]	60.0 ± 1.1[a]	65.2 ± 1.1[a]
MCH (pg)	17.6 ± 0.2	18.2 ± 0.1	18.1 ± 0.4	19.3 ± 0.1[a]
MCHC (g/dl)	31.5 ± 0.3	31.5 ± 0.6	32.1 ± 0.7	35.9 ± 0.4[a]
TLC (x10^3/µl)	07.9 ± 0.7	14.3 ± 1.1	19.2 ± 1.3[b]	19.5 ± 1.8[b]
lymp (%)	78.6 ± 1.5	82.3 ± 0.7	84.4 ± 1.4[a]	89.5 ± 2.0[b]
Neut (%)	10.2 ± 0.9	11.2 ± 0.5	10.5 ± 0.7	07.1 ± 1.8[d]
Gran (%)	11.2 ± 0.9	06.9 ± 0.8[d]	5.2 ± 1.0[d]	03.5 ± 0.3[f]

[a, b] and [c] represent significant increases while [d, e] and [f] represent significant decreases at $p<0.05$, $p<0.01$ and $p<0.001$, respectively, when compared to Group I values. Group I = 10 ml/kg/day distilled water, Group II = 20 mg/kg/day of *HU*, Group III = 100 mg/kg/day of *HU* , Group IV = 500 mg/kg/day of *HU*.

Table 15: Effect of 14-day reversibility test of *HU* on haematological parameters of treated female rats

	I	II	III	IV
RBC (x 10^6/µl)	07.6 ± 0.1	08.2 ± 0.1[a]	10.0 ± 0.2[b]	11.7 ± 0.1[c]
Hb (g/dl)	13.2 ± 0.8	15.1 ± 0.2[a]	16.4 ± 0.2[c]	16.8 ± 0.1[c]
PCV (%)	44.5 ± 0.8	47.6 ± 1.0[a]	53.3 ± 1.2[b]	57.3 ± 0.4[b]
PLT (x10^3/µl)	797.7 ± 79.9	1048.0 ± 55.5[a]	1175.7 ± 16.4[b]	1543.0 ± 115.3[c]
MCV (fl)	58.8 ± 0.5	57.2 ± 0.2	61.5 ± 1.5[a]	66.1 ± 0.2[b]
MCH (pg)	17.4 ± 0.9	18.4 ± 0.0[a]	21.4 ± 0.9[b]	22.6 ± 0.4[b]
MCHC (g/dl)	29.6 ± 1.7	32.2 ± 0.2[a]	34.2 ± 0.5[b]	41.1 ± 0.1[c]
TLC (x10^3/µl)	09.7 ± 0.6	16.4 ± 0.3[b]	20.1 ± 0.6[c]	21.3 ± 1.4[c]
lymp (%)	84.1 ± 3.6	88.9 ± 0.4	92.1 ± 0.6[a]	92.7 ± 0.6[a]
Neut (%)	09.5 ± 1.8	05.6 ± 0.1[d]	05.2 ± 0.7[e]	05.6 ± 0.6[d]
Gran (%)	06.3 ± 1.9	04.9 ± 0.5	03.0 ± 0.5	01.8 ± 0.3[d]

[a, b] and [c] represent significant increases at $p<0.05$, $p<0.01$ and $p<0.001$, respectively, while [d] and [e] represent significant decreases at $p<0.05$ and $p<0.01$, respectively, when compared to Group I values.
Group I = 10 ml/kg/day distilled water, Group II = 20 mg/kg/day *HU*, Group III = 100 mg/kg/day *HU*, Group IV = 500 mg/kg/day *HU*.

Effect of 90-days oral treatment with 20-500 mg/kg/day of *HU* and its reversibility test on the relative organ weights of treated rats

Chronic oral treatment with *HU* caused a significant ($p<0.001$) and dose related increases in the relative weights of liver, stomach, spleen, testis, lungs and heart in the male rats (Tables 2a). At the oral dose of 500 mg/kg/day of *HU*, there was a significant ($p<0.05$) reduction in the relative kidney weight when compared to the 100 mg/kg/day of *HU*-treated values in the male rats (Table 2a). In the female rats, *HU* treatment caused significant ($p<0.05$, $p<0.001$) and dose-related increases in the relative weights of liver and lungs, a significant ($p<0.01$) decrease in the relative kidney weight while causing non-significant ($p>0.05$) decreases in the relative weights of stomach and heart (Table 2b). However, following withdrawal of *HU* treatment for 14 days, there was no significant ($p>0.05$) difference in the relative organ weight among the control and treated groups of rats (Tables 3a and 3b) except in the 100 mg/kg of *HU*-treated rats that still had significant ($p<0.05$) increase in the relative liver weight and significant ($p<0.001$) decreases in the relative kidney weight (Table 3b). Similarly, there was still a significant ($p<0.01$) decrease in the relative kidney weight of 500 mg/kg of *HU*-treated female rats after the treatment withdrawal (Table 3b).

Effect of 90-days oral treatment with 20-500 mg/kg/day of *HU* and reversibility test on the fasting blood glucose of treated rats

Prolonged oral treatment with *HU* caused significant and dose-related ($p<0.05$, $p<0.01$ and $p<0.001$) reductions in the fasting glucose levels in both sexes of treated rats, with the most significant hypoglycaemic effect recorded in rats treated with 500 mg/kg/day of *HU* by reducing the glucose level from 78.7 ± 0.9 mg/dl to 37.0 ± 0.7 mg/dl and 76.5 ± 2.8 mg/dl to 36.2 ± 1.5 mg/dl in the male and female rats, respectively (Table 4). Despite the withdrawal of oral treatment with *HU* for 14 days, there was persistence in the significant ($p<0.01$, $p<0.001$) dose-related hypoglycaemic effect at 100 mg/kg and 500 mg/kg of the extract when compared to those of control and 20 mg/kg of *HU*-treated groups (Table 5).

Effect of 90-day oral treatment with 20-500 mg/kg/day and toxicity reversibility of *HU* on the serum sodium, potassium, bicarbonate, urea and creatinine concentrations of treated rats

Tables 6 and 7 show the effect of chronic oral treatment with *HU* and its reversibility on the serum concentration of sodium, potassium, chloride, bicarbonates, urea and creatinine in the treated rats. *HU* treatment produced significant ($p<0.05$) dose-related reductions in the serum levels of bicarbonate, urea and creatinine while causing no significant ($p>0.05$) alterations in the serum levels of sodium and potassium (Table 6). Upon *HU* treatment withdrawal, the significant ($p<0.05$ and $p<0.01$) dose-dependent reductions in the serum level of urea still persisted in the male rats while there was no significant ($p>0.05$) alteration in the level of urea in the female rats (Table 7). In a similar pattern, *HU* withdrawal did not significantly ($p>0.05$) affect the serum levels of sodium and potassium (Table 7). However, withdrawal of *HU* resulted in a reversal to about that of the control values in the serum level of bicarbonates in the female rats while there was a significant ($p<0.05$) increase in the serum level of bicarbonates in the male (Table 7).

Effect of 90-day oral treatment with 20-500 mg/kg/day of *HU* and toxicity reversibility on the serum alanine transaminase, aspartate transaminase, alkaline phosphatase , total and conjugated bilirubin concentrations of rats

Chronic treatment with 20-500 mg/kg *HU* did not significantly ($p>0.05$) affect the serum concentrations of ALT, AST, ALP in both sexes of treated rats (Table 8). Similar effect was recorded in the toxicity reversibility tested rats (Table 9).

Effect of 90-day oral treatment with 20-500 mg/kg/day of *HU* and its toxicity reversibility test on the serum total protein and albumin concentrations of treated rats

Chronic oral treatment with 20-500 mg/kg *HU* did not significantly ($p>0.05$) alter the serum concentrations of TP and ALB in both sexes of treated rats (Table 10). Similar effect was recorded in the toxicity reversibility tested rats (Table 11).

Effect of 90-day oral treatment with 20-500 mg/kg/day of *HU* on the full blood counts of treated rats

Treatment with *HU* significantly and dose-dependently increased the haemoglobin concentration (Hb), packed cell volume (PCV), platelet counts (PLT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), total leucocyte count (TLC), and lymphocyte while causing significant and dose-related ($p<0.05$ and $p<0.01$) reductions in neutrophils and granulocyte (Tables 12 and 13). These results suggest that *HU* could have neutropaenic and granulopaenic effect despites its significant haematopoietic effect.

Effect of 14-days reversibility test of *HU* on the full blood counts of treated rats

Haematological data in Tables 15 and 16 indicated that despite the withdrawal of *HU* treatment for 14 days, the significant ($p<0.001$) elevations in the measured haematological parameters induced by *HU* treatment still persisted in both sexes of treated rats. Also, the significant reductions in % Neut and % Gran remained sustained after 14 days of the extract withdrawal (Tables 14 and 15).

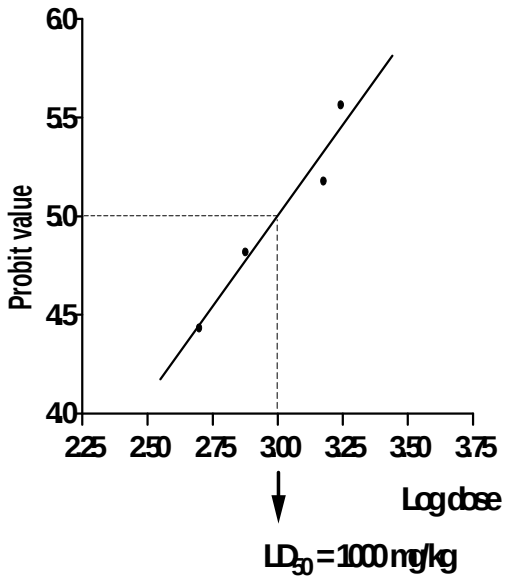

Figure 1: Graph of log dose vs. probit for the acute oral toxicity of *HU*.

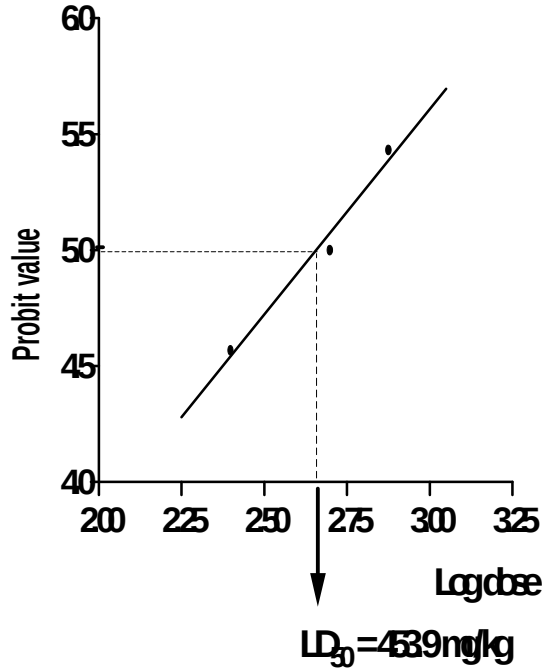

Figure 2: Graph of log dose vs. probit value for the acute intraperitoneal toxicity of *HU*.

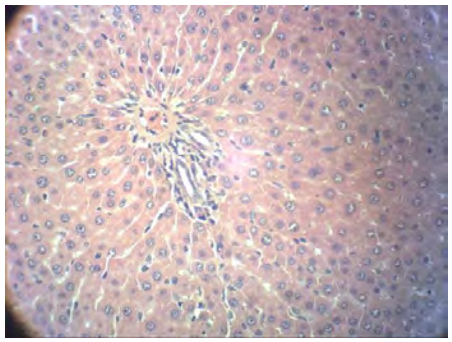

Figure 3a: A cross-sectional representation of normal rat liver (Haematoxylin & Eosin stain, x400 magnification) showing the central hepatic vein and porta triads surrounded by normal hepatocytes

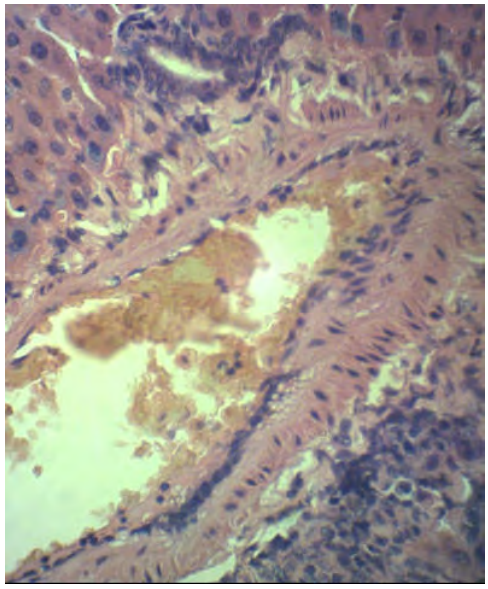

Figure 3b: A cross-sectional representation of 20 mg/kg *HU*-treated rat liver (Haematoxylin & Eosin stain, x400 magnification) showing minimally congested central hepatic vein with mild lymphocytic infiltration surrounded by hepatocytes with picnotic nuclei

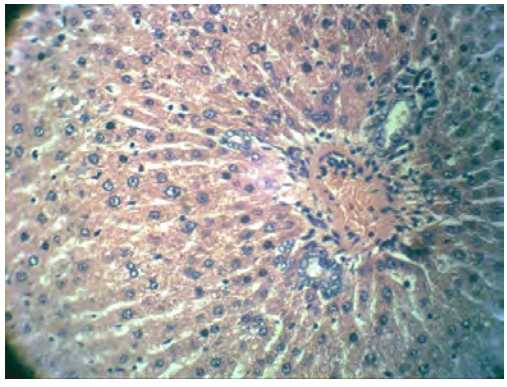

Figure 3c: A cross-sectional representation of 100 mg/kg *HU*-treated liver (Haematoxylin & Eosin stain, x400 magnification) showing moderately congested central hepatic vein and bile ductal proliferation surrounded by hepatocytes with picnotic nuclei indicating actively dividing (mitotic) hepatocytes

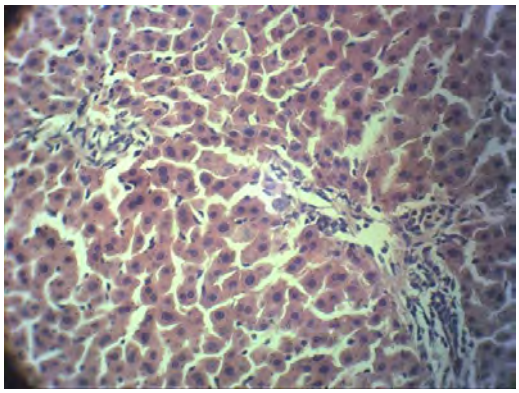

Figure 3d: A cross-sectional representation of 500 mg/kg *HU*-treated liver (Haematoxylin & Eosin stain, x400 magnification) showing mild lymphocyte infiltrated porta triad and bile ductal proliferation surrounded by hepatocytes with picnotic nuclei indicating actively dividing hepatocytes

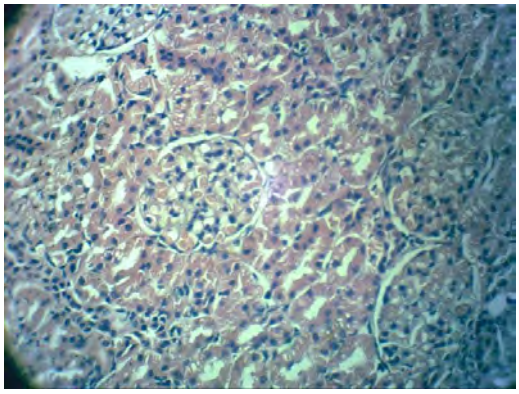

Figure 4a: A cross-sectional representation (Haematoxylin & Eosin, x400 magnification) showing normal glomeruli and renal tubules

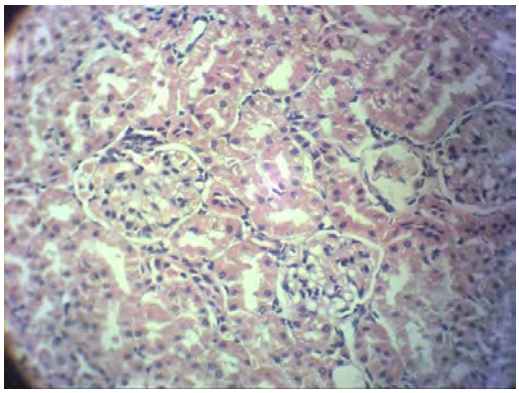

Figure 4b: A representative section of 20 mg/kg *HU*-treated kidney (Haematoxylin & Eosin stain, x400 magnification) showing normal glomeruli and normal tubules

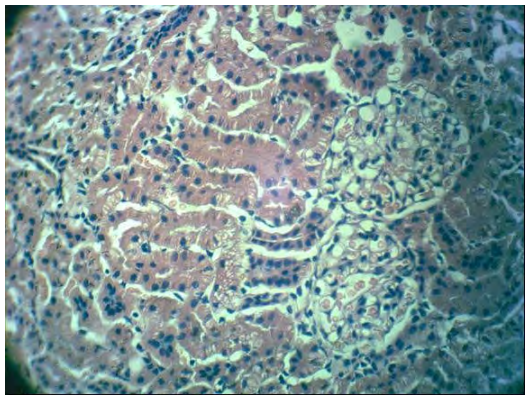

Figure 4c: A sectional representative of 100 mg/kg *HU*-treated rat kidney (Haematoxylin & Eosin stain, x400 magnification) showing minimal glomerular and tubular congestion indicating mild glomerulonephritis

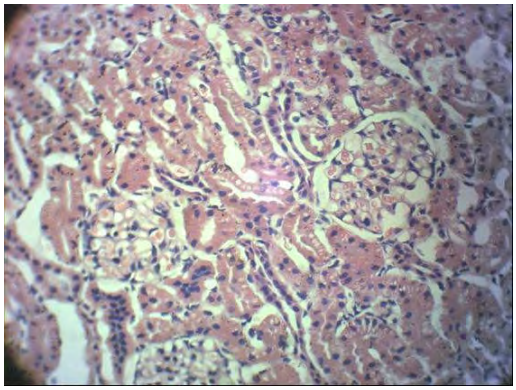

Figure 4d: A sectional representative (Haematoxylin & Eosin stain, x400 magnification) of 500 mg/kg *HU*-treated rat kidney showing moderate glomerular and tubular congestion indicating some degree of glomerulonephritis

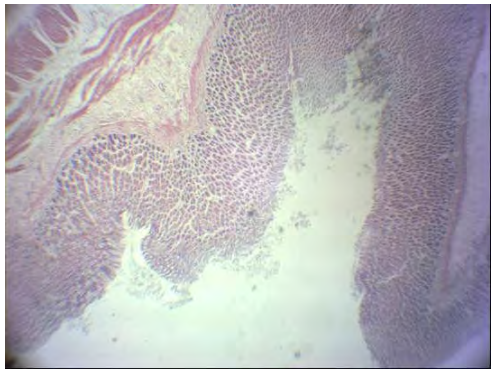

Figure 5a. A sectional representative (Haematoxylin & Eosin stain, x400 magnification) of the body of normal rat stomach showing the circular and longitudinal muscle layers, submucosa and folded mucosa.

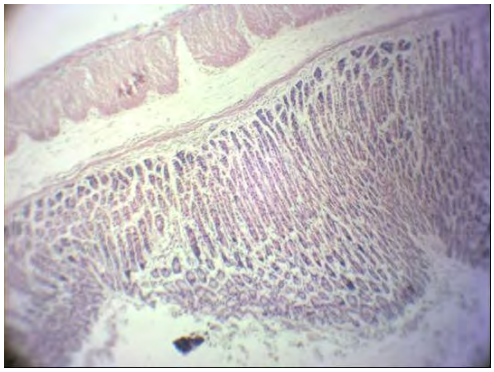

Figure 5b: A sectional representative (Haematoxylin & Eosin stain, x400 magnification) of the body of 20 mg/kg *HU*-treated rat stomach showing mild hypertrophy of the muscularis propria, submucosa and mucosa.

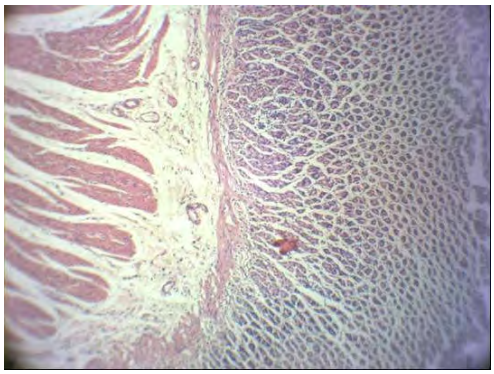

Figure 5c: A sectional representative (Haematoxylin & Eosin stain, x400 magnification) of the body of 100 mg/kg *HU*-treated stomach showing moderate muscularis propria, submucosal hypertrophy and mucosal hyperplasia

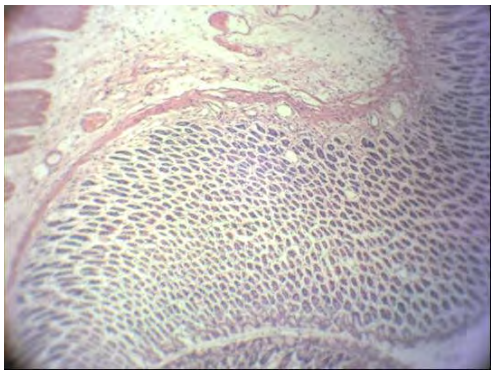

Figure 5d: A sectional representative (Haematoxylin & Eosin stain, x400 magnification) of 500 mg/kg *HU*-treated stomach showing moderate muscularis propia, submucosa hypertrophy and mucosal hyperplasia.

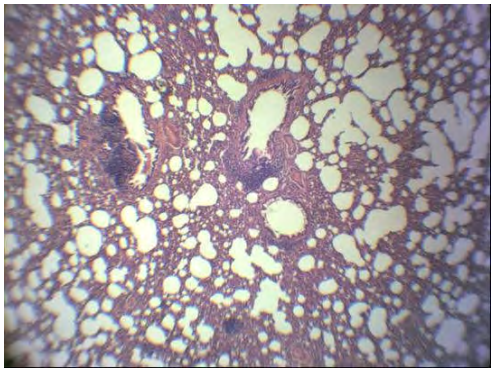

Figure 6a: A cross-section of normal rat lungs (Haematoxylin & Eosin stain, x100 magnification) showing the bronchioles, pulmonary interstitia and alveoli

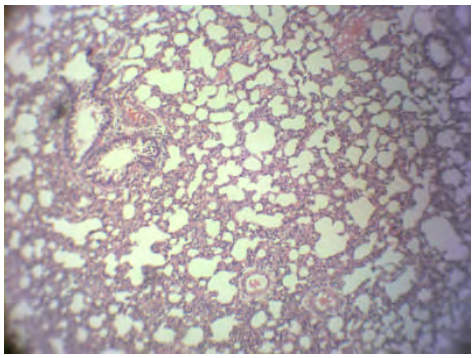

Figure 6b: A cross-section of 20 mg/kg *HU*-treated rat lungs (Haematoxylin & Eosin stain, x40 magnification) showing congested pulmonary vessels, normal bronchioles and alveolar sacs and mild pulmonary interstitial proliferation and congestion

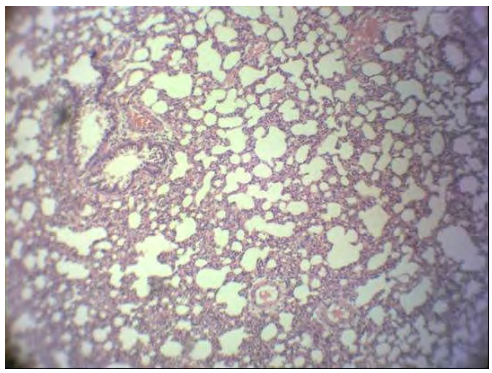

Figure 6c: A cross-section of 100 mg/kg *HU*-treated rat lungs (Haematoxylin & Eosin stain, x40 magnification) showing congested bronchioles and pulmonary vessels and mild pulmonary interstitial proliferation and congestion but normal alveoli.

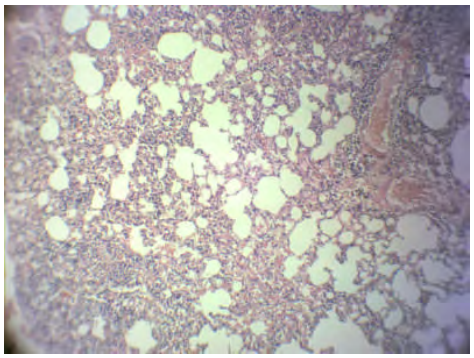

Figure 6d: A cross-section of 500 mg/kg *HU*-treated rat lungs (Haematoxylin & Eosin stain, x40 magnification) showing normal bronchioles and alveoli and moderately congested pulmonary interstitial and pulmonary vessels.

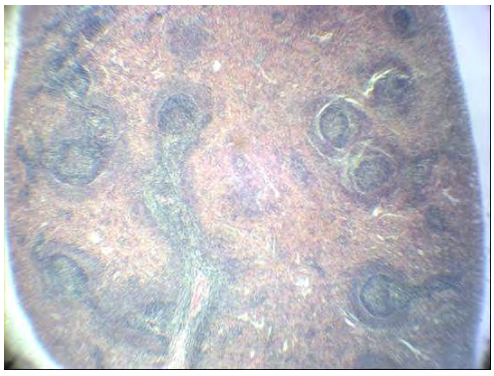

Figure 7a: A sectional representation of normal rat spleen (Haematoxylin & Eosin, x40 magnification) showing normal splenic white pulps

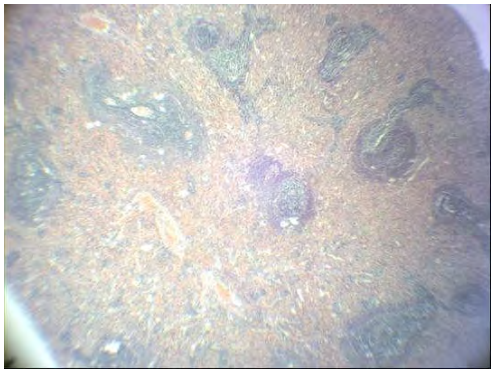

Figure 7b: A sectional representation of 20 mg/kg *HU*-treated rat spleen showing proliferation of splenic white pulp and mildly congested splenic vessels

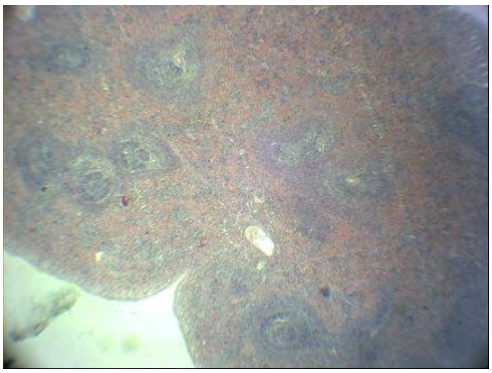

Figure 7c: A sectional representation of 100 mg/kg *HU*-treated rat spleen showing splenic white pulp proliferation.

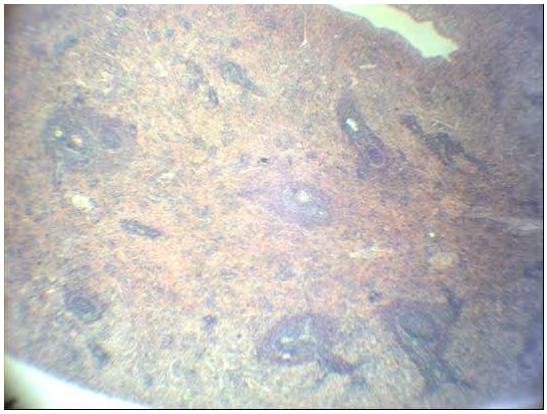

Figure 7d: A sectional representation of 500 mg/kg *HU*-treated rat spleen showing proliferation of splenic white pulp and increased vascularization of the splenic red pulp.

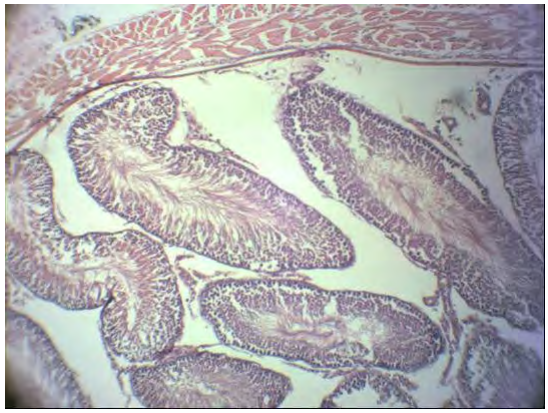

Figure 8a: A cross-section of normal rat testis (Haematoxylin & Eosin stain, x100 magnification) showing the tunica albuginae, seminiferous tubules lined by spermatogenic series, Sertoli and Leydig's cells.

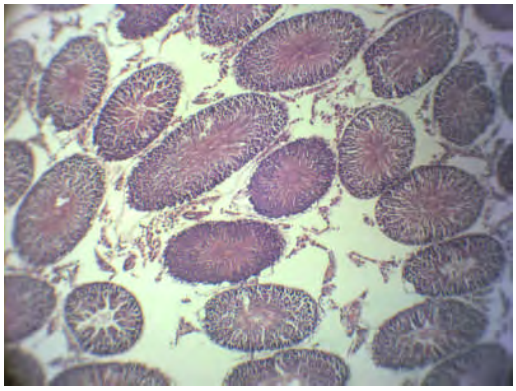

Figure 8b: A cross-section of 20 mg/kg *HU*-treated rat testis (x100 magnification) showing seminiferous tubules lined by mildly dense spermatogenic series, Sertoli and Leydig's cells.

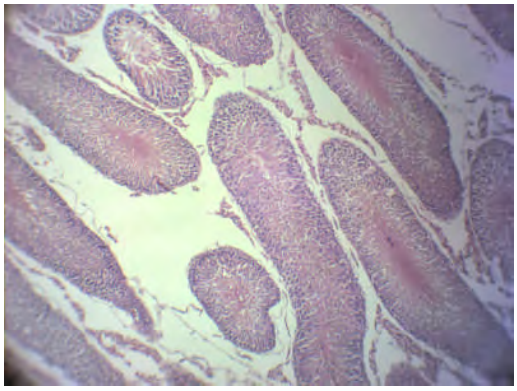

Figure 8c: A cross-section of 500 mg/kg *HU*-treated rat testis (Haematoxylin & Eosin stain, x100 magnification) showing seminiferous tubules lined by moderately dense spermatogenic series, Sertoli and Leydig's cells.

Effect of 90-days oral treatment with 20-500 mg/kg/day of *HU* on the histopathology of selected vital organs of treated rats

Histopathological effect of chronic HU treatment on liver

Figures 3a-3d are representative sections of normal rat liver, 20 mg/kg *HU*-treated, 100 mg/kg *HU*-treated and 500 mg/kg *HU*-treated rat livers, respectively. Prolonged oral *HU* treatment produced mild-to-moderate central hepatic congestion with varying degree of lymphocytic infiltration and hepatocytes with picnotic nuclei, indicating varying degree of hyperplasia in the hepatocytes of *HU*-treated livers (Figures 3b-3d).

Histopathological effect of chronic HU treatment on kidneys of rats

Figures 4a-4d are representative sections of normal rat kidney, 20 mg/kg *HU*-treated, 100 mg/kg *HU*-treated and 500 mg/kg *HU*-treated rat kidneys, respectively. Prolonged *HU* treatment at 20 mg/kg produced no obvious histological lesion (Figure 4b). At the oral doses of 100 mg/kg and 500 mg/kg, *HU* produced mild and moderate glomerular and tubular congestions indicating glomerulonephritis (Figures 4c-4d).

Histopathological effect of chronic HU treatment on stomach of rats

Figures 5a-5d are representative sections of normal rat stomach, 20 mg/kg *HU*-treated, 100 mg/kg *HU*-treated and 500 mg/kg *HU*-treated rat stomach, respectively. Prolonged *HU* treatment at 20 mg/kg produced mild hypertrophy of the muscularis propria (Figure 5b). At the oral doses of 100 mg/kg and 500 mg/kg, *HU* produced moderate muscularis propria, submucosal and mucosal hypertrophy (Figures 5c-5d) when compared to that of normal rat stomach (Figure 5a).

Histopathological effect of chronic HU treatment on lungs of rats

Figures 6a-6d are representative sections of normal rat lungs, 20 mg/kg *HU*-treated, 100 mg/kg *HU*-treated and 500 mg/kg *HU*-treated rat lungs, respectively. Prolonged *HU* treatment at 20 mg/kg produced mild interstitial proliferation (Figure 6b). At the oral doses of 100 mg/kg and 500 mg/kg, *HU* produced mild-to-moderate interstitial proliferation (Figures 6c-6d) when compared to that of normal lungs (Figure 6a).

Histopathological effect of chronic HU treatment on rat spleen

Prolonged *HU* treatment caused splenic white and red pulp proliferations in the spleens of treated rats in a dose-related fashion (Figures 7b-7d) when compared to normal spleen (Figure 7a).

Histopathological effect of chronic HU treatment on rat testes

Prolonged *HU* treatment at 20 mg/kg produced mild interstitial proliferation (Figure 8b). At the oral doses of 100 mg/kg and 500 mg/kg, *HU* produced mild-to-moderate interstitial proliferation (Figures 8c-8d) when compared to that of normal lungs (Figure 8a).

Histopathological effect of HU treatment on rat heart muscle

Chronic oral treatment with 20-500 mg/kg of *HU* did not produce any significant histological lesions on the heart muscles when compared to normal heart muscles.

Effect of 14-days toxicity reversibility on the histopathology of selected vital organs of rats

Histological examination of selected vital organs after 14 days of reversibility test showed no reversal in the earlier documented histological lesions which were associated with chronic *HU* treatment.

Discussion

Acute oral toxicity studies showed that *HU* was orally tolerated for up to 1000 mg/kg body weight. According to the American Society for Testing and Materials (1987), any chemical substance with LD_{50} estimate less than 2000 mg/kg/oral route but greater than 1000 mg/kg/oral could be considered to be slightly toxic, although Clarke and Clarke (1977) consider any compound with an estimated LD_{50} equal to or greater than 1000 mg/kg/oral to be safe. Based on the latter recommendation, *HU* can, thus, be considered relatively safe on acute exposure. Similarly, the intraperitoneal LD_{50} of *HU* was estimated to be 453.9 mg/kg body weight. Substances with intraperitoneal LD_{50} value of 500-5000 mg/kg body weight in toxicity rating are classified as being slightly toxic. Thus, *HU* can be considered slightly toxic on acute exposure. The acute oral toxicity of *HU* could be due to some of the toxic phytocomponents in *HU* such as tannin and saponin. Saponin has been documented to induce haemolysis and behavioural toxicity in rats treated with extracts containing high concentration of saponin (Ajagbonna et al., 1999). It is, therefore, possible that saponin and/or other phytocomponents present in *HU* to be responsible for the observed behavioural toxicity of the extract.

In the chronic oral toxicity study, *HU* at the oral doses of 100 mg/kg and 500 mg/kg produced significant reductions in the pattern of body weight gain over the 90 days of treatment, indicating the inherent weight losing potential of the extract. Reductions in body weight gain and internal organ weights are simple but strong and sensitive indices of toxicity after exposure to toxic substances (Teo et al., 2002). The observed significant weight loss could have been mediated via appetite inhibiting or lipid lowering effect or other mechanisms. The presence of saponins in high concentrations has earlier been reported to induce weight loss in animal exposed to such plants due to its appetite-inhibiting effect (Ajagbonna et al., 1999). Thus, the presence of this phytocomponent in *HU* could account for the weight losing effect of *HU*. In respect of relative organ weight, *HU* produced variable effect on the selected organs. In the male and female rats, *HU* produced increases in the relative organ weight of all the selected organs except in the relative kidney weight of the female rat where *HU* produced significant reduction. The increases in the relative organ weight are strong indications of either organ hyperplasia/hypertrophy (a physiological enlargement of an organ) or organomegally (a diseased enlargement of an organ). However, the presence of the histological features of rapid cellular proliferation within the organs particularly the liver, lungs, stomach and testis, strongly strengthen the former assertion.

Blood is an important index of physiological and pathological status in man and animals and the parameters usually measured are total red blood counts and its indices, haemoglobin, packed cell volume, total white blood cell count and its differential counts, platelets count and biochemical parameters such as liver and renal function tests (Raza et al., 2002; Oduola et al., 2007). The normal range of these parameters can be altered by the ingestion of some toxic plants (Abatan and Arowolo, 1989; Ajagbonna et al., 1999; Adedapo et al., 2004). In the present study, *HU* treatment for 90 days produced significant elevations in the measured haematological parameters indicating the haematopoietic effect of *HU*. Thus, the significant elevations in RBC, PCV and Hb strongly suggest that *HU* could be useful in the management of anaemia. However, the significant

thrombocytosis produced by *HU* treatment suggests that while its haemopoietic effect could be beneficial in the management of anaemia, the resultant thrombocytosis could invoke predisposition to thrombotic stroke and ishaemic heart disease. The relative lymphocytosis and the splenic lymphoid proliferation induced by *HU* indicate its lymphopoetic effect and possible immunostimulatory potential. This result may be due to the immune response of the rats to the extract, which led to the mobilization of immune-competent cells. The implication of this finding is that *HU* could be immunogenic and this finding appears to be in agreement with that previously reported in rabbits (Ibeh et al., 2007). On the other hand, the *HU*-induced neutropaenia and granulocytopaenia are indicative of its cytotoxic effect on neutrophil and granulocyte lineages. The neutrophils are the first line of defence in any microbial infection and are often significantly elevated in acute inflammatory conditions (Guyton and Hall, 2000) while lymphocytes on the other hand produce antibodies that bind to pathogens to enable their destruction and are more involved in defence against intracellular microbes and tumour cells (Ganong, 2001). The neutropaenic and granulocytopaenic effects of *HU* could suggest that the extract could have damaging effect on this first line of body defence while its lymphocyte-forming effect suggest that *HU* could have immune boosting effect. The non reversal in the haematopoietic effect of *HU* despite the extract withdrawal indicates that the haematopoietic effect of the extract is long lasting.

The liver is one of the most important organs in the body and is responsible for breaking down all ingested xenobiotics. Liver function tests conducted through blood assays provide in-depth information about the state of the liver, describing its functionality (e.g. albumin, total proteins), cellular integrity (e.g. aminotransaminases) and its link with the biliary tract (e.g. gamma-glutamyl transferase and alkaline phosphatase) (Boyde and Latner, 1961; Adeoye and Oyedapo, 2004). ALT is the enzyme produced within the cells of the liver, recording increases in conditions where liver cells have been inflammed or undergone cell death (Boyde and Latner, 1961; Adeoye and Oyedapo, 2004). As the cells are damaged, the ALT leaks into the bloodstream leading to a rise in its serum concentrations (Adedapo et al., 2004). However, of these hepatic enzymes, ALT is the most sensitive and reliable marker of hepatocellular injury since AST is known to be present in abundance in the cardiac muscles, skeletal muscles, kidneys and testes, and ALP abundant in the growing bone (Friedman et al., 1996). As a result, any disease state affecting any of these extrahepatic tissues significantly elevates the serum levels of these enzymes (Friedman, 1996). From the results of chronic oral toxicity study of *HU*, serum analyses of the treated rats showed that *HU* treatment caused no significant alterations in the serum ALT and AST levels despite elevation in the serum ALP concentration indicating that *HU* has no deleterious effect on liver functions. This is a strong indication of the oral safety of *HU* on liver function. Similarly, the effect of *HU* on serum electrolytes, urea and creatinine which are reliable markers of renal function is also a strong indication of the extract's safety on the renal function and the possible inherent nephroprotective potential of the extract despite the minimal and reversible histological lesions associated with chronic *HU* use. However, reduction in the serum bicarbonate level indicates the tendency of chronic *HU* use to be associated with metabolic acidosis. The presence of flavonoids and alkaloids in *HU* could account for these protective effects since these phytocomponents have been documented to confer protection on the liver and kidneys through prevention of tissue lipid peroxidation which is mediated their anti-oxidant and free-radical scavenging activities (Fraga et al., 1987; Laughton et al., 1989; Sanz et al., 1994).

Apart from liver enzymes, serum proteins and albumin assays are also used as reliable and sensitive indicators of liver function status since they are synthesized and metabolized in the liver (Ganong, 2001). Reductions in their levels are reflective of the hepatocellular damage, particularly in chronic liver disease, while their levels are elevated during the anabolic phase of protein metabolism, ingestion of high protein diets or in certain cancerous states like multiple myeloma (Mayne, 1996; Guyton and Hall, 2000). The profound increase in the serum levels of these proteins couple with the histological lesion of hepatic hyperplasia may indicate the anabolic effect of *HU*. The profound increase in the relative testicular weight and the histological evidence of enhanced spermatogenesis in the testes of *HU*-treated rat not only strengthen the anabolic theory of *HU* but also indicate the fertility-enhancing of the extract in although further studies will be required in this area.

The proliferative effect of *HU* on the body organs particularly the stomach, liver, lungs, testes and the spleen is indicative of the potential tumorogenesis of *HU*, particularly when used on long-term basis. Thus, the chronic toxicity results indicate that *HU* should be used with great caution, particularly, when used on long-term basis.

Results of the oral toxicity reversibility test suggest that despite the withdrawal of *HU* treatment for 14 days, most of the toxic biological effects and histological lesions induced by *HU* remain irreversible strongly indicating that the extract may contain toxic phytocomponents with prolonged half-lives. In the same vein, the results of the oral toxicity reversibility results indicate that *HU* should be used with great caution, particularly, when used on long-term basis as the toxicities emanating from the chronic use of *HU* may not readily become reversible on short-term withdrawal.

In conclusion, *HU* can be considered relatively safe on acute and chronic exposures, although its prolonged use should be with a great caution as it could have tumour-promoting tendency.

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