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Original Article

Nephroprotective effects of the aqueous root extract of *Harungana madagascariensis (L.)* In acute and repeated dose acetaminophen renal injured rats

Adeneye AA[1*], Olagunju JA[2], Benebo AS[3], Elias SO[4], Adisa AO[5], Idowu BO[5], Oyedeji MO[5], Isioye EO[5], Braimoh OB[5], Oladejo OO[5], Alana EO[5]

[1]Department of Pharmacology, [2]Department of Medical Biochemistry, [3]Department of Pathology and Forensic Medicine, [4]Department of Physiology, Faculty of Basic Medical Sciences, Lagos State University College of Medicine, Ikeja, Lagos State, Nigeria
[5]Department of Biochemistry, College of Health Sciences, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria

Summary: In African traditional medicine, decoctions from different parts of *Harungana madagascariensis* (L.) are highly valued in the treatment of various human diseases including drug related renal disease. In the current study, effects of pretreatments with single daily oral 100 – 500 mg/kg/day of the root aqueous extract of *Harungana madagascariensis* were investigated in acute and repeated dose acetaminophen nephrotoxic rats for 24 hours and 14 days, respectively, using renal function parameters – serum urea (UR), uric acid (UA) and creatinine (CR). Effects of the extract pretreatments on the hematological and renal histological profile in acetaminophen nephrotoxic rats were also evaluated. Results showed that treatment with intraperitoneal acetaminophen for 24 hours and 14 days induced significant ($p<0.05$, $p<0.01$, $p<0.001$) elevations in the serum concentrations of UR, UA and CR, varying degrees of tubular necrosis on histology and varying degrees of alterations in the hematological parameters in acute and repeated dose acetaminophen nephrotoxic rats, respectively. However, pretreatments with graded oral doses of the extract significantly ($p<0.05$, $p<0.01$, $p<0.001$) attenuated elevations in the serum concentrations of UR, UA and CR, and improved diffuse tubular necrosis in both models of acetaminophen nephrotoxicity. The extract also significantly ($p<0.05$, $p<0.01$, $p<0.001$) improved packed cell volume (PCV), hemoglobin (Hb), and total leucocyte count (TLC) levels but non-significant ($p>0.05$) increase in the mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), in the repeated acetaminophen model. Thus, the overall results showed that *Harungana* extract protects against acetaminophen nephrotoxicity.

Industrial relevance: The industrial relevance of the study cannot be overemphasized since minor and major toxicities induced by acute and chronic drug treatment could be very important for human health. Also, the discovery and ultimate development of suspected lead compounds could constitute a major scientific breakthrough in the understanding of the exact etiopathogenesis and therapeutics of acetaminophen nephropathies.

Keywords: *Harungana madagascariensis* (L.), root aqueous extract, acetaminophen nephrotoxicity, renal function parameters, hematological profile, Wistar rats

Introduction

Acetaminophen remains one of the most effective, over-the-counter chemotherapeutic analgesic-antipyretic agents belonging to the para-aminophenol class of the non-steroidal anti-inflammatory drugs (NSAIDs) (Jackson-Robert II and Morrow, 2001). Its acute or chronic high doses are reported to produce hepatotoxicity, but impairment of renal function by acetaminophen as the main untoward effect is becoming increasingly reported (Perneger et al., 1994; McLaughlin et al., 1998; Fored et al., 2001). Acetaminophen nephropathy is characterized by alterations in urine volume, in glutathione status, creatinine clearance and increase products of lipid peroxidation.

Acetaminophen nephropathy is closely associated with a significant decrease in the renal tissue concentration of glutathione and nitric oxide overproduction (Abdel-Zaher et al., 2007). Research into the etio-pathological basis of acetaminophen nephrotoxicity has recently been encouraged (Henrich et al., 1996). However, despite recognition of acetaminophen nephrotoxicity and concerted scientific efforts directed into developing therapeutic or prophylactic agents to protect against acetaminophen nephrotoxicity, conventional chemotherapeutic options available to either treat or prevent its development, are still limited. In the absence of reliable and effective modern nephroprotective drugs and available traditional medicines employed for the disease treatment, concerted efforts are currently channelled toward

***Corresponding Author:**
Tel: +234-802-0690-946
Fax: +234-702-800-2756
E-mail: adeneye2001@yahoo.com
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exploring complementary or alternative chemotherapy in the disease treatment and/or prevention. Different parts of the plant *Harungana madagascariensis* (L.) (family: Hypericaceae) are employed in African folk medicine for the treatment of a wide spectrum of veterinary and human diseases. For example, the red juice from the plant leaves and stem bark are reputed for arresting post-partum or post-abortal bleedings in Sierra Leone, while the unopened buds are equally reputed for treating puerperal infection in Liberia (Olagunju et al., 2000). In Ghana, the plant stem bark is employed in treating skin diseases and as dressing materials for wounds (Irvine, 1961; Adjanohoun, 1981). Among the Yoruba herbalists, the plant which is locally known as "elepo" is equally employed for the treatment of drug-related liver and kidney poisonings. The boiled water decoction of the plant root is believed to neutralize the toxic activities of ingested poisons and restores deranged hepatic and renal functions to normal (personal communication). However, despite the ancestral use of the plant water decoction in the management of drug-related renal lesions, there is paucity in the scientific validation of this therapeutic use. Therefore, the current study was designed to investigate the protective effects of 100 - 500 mg/kg/day/oral route of the aqueous root extract of *Harungana madagascariensis* in acute dose (800 mg/kg/intraperitoneal route) and repeated dose (200 mg/kg/day/intraperitoneal route) acetaminophen nephrotoxic adult male Wistar rats for 24 hours and 14 days, respectively. Doses of acetaminophen and *Harungana* aqueous root extract used for the acute and repeated dose models were determined from results of preliminary studies earlier conducted.

Materials and Methods

Collection of Plant Materials

About 300 g of fresh roots of *Harugana madagascariensis* were collected from the deciduous forest in Kiire Village in Lagelu Local Government of Osun State, in the month of July, 2007. Plant collection, identification, authentication and specimen referencing was done as described by Olagunju et al. (2000). The collected fresh materials were cleaned, rinsed in distilled water and sorted, leaving about 290 g. This was then air-dried completely at room temperature (30 ± 2 ºC), protected from heat and direct sunlight for about 3 weeks.

Preparation of Root Aqueous Extract of Harungana madagascariensis

About 200 g of the dried plant root was ground to fine powder using Laboratory Hammer mill. 100g of the powdered sample was homogenized in 1 L of 0.9% (w/v) NaCl and stirred at 4 ºC for 24 hours. The suspension was filtered through cotton gauze and the resulting filtrate was clarified by centrifugation at 500 revolutions per minute for 20 minutes at room temperature. The filtrate obtained was then lyophilized to yield 13.6 g (yield: 13.6 %). The lyophilized powder was stored in air- and moisture-tight container which was stored in a dessicator prevented from direct heat and sunlight. From this, a fresh stock was reconstituted in 0.9% (w/v) NaCl to a final concentration of 425 mg/mL, whenever needed.

Experimental Animal and their care

A total of 30 of 12 - 16 weeks old, male Wistar rats were used for the acute and repeated dose experiments, respectively. The animals were inbred rats obtained from the rat Colony of the Animal House of the Lagos State University College of Medicine, Ikeja, Lagos State, in the month of September, 2007. 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 (Livestock Feeds, Ikeja, Lagos State) and potable 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 hours of light (06:30 hour – 18:30 hour) alternating with approximately 12 hours of darkness (18:30 hour - 06:30 hour).

Experimental Induction of Acute Acetaminophen-induced Nephrotoxicity

Fasted rats were randomly divided into 5 groups of 6 rats each such that the weight difference within and between group does not exceed ± 20% of the average weight of the total rats. Group I rats that served as the untreated control were administered single daily dose of 10 mL of distilled water orally and intraperitoneally, while group II rats that served as the treated or model control were administered single daily dose of 10 mL distilled water and 800 mg/kg via the oral and intraperitoneal routes, respectively. Groups III, IV and V were pretreated with single oral dose of 100, 200 and 500 mg/kg of *Harungana madagascariensis* extract 1 hour before the intraperitoneal administration of 800 mg/kg of acetaminophen (Emzor Paracetamol®, Emzor Pharmaceuticals, Isolo, Lagos State, Nigeria).

Experimental Induction of Repeated Dose Acetaminophen-Induced Nephrotoxicity

Equally, rats for this phase of the study consisted of 30 adult male Wistar rats. Fasted rats were randomly allotted to 5 groups of 6 rats in each group, such that the difference within and between groups does not exceed ±20% of the average weight of the rats. Acetaminophen nephrotoxicity was induced using same procedure described for acute acetaminophen nephrotoxicity except that the daily extract and acetaminophen treatments were for 14 days.

Measurement of serum urea, uric acid and creatinine in acute and repeated dose acetaminophen nephrotoxic rats

On termination of acute and repeated dose acetaminophen-induced nephrotoxicity experiments 24 hours post-induction and day 14 of the experiments, respectively, the rats were fasted overnight. Twenty-four hours post-induction and on day 15, respectively, the rats were sequentially anesthetized with inhaled diethyl ether for about 30 - 40 seconds. The rats were restricted on the dissecting board and about 4 - 5 mL of whole blood for blood lipids assay were

collected directly from the heart chambers by cardiac puncture with a 21 Gauge needle mounted on 5 mL syringe (Unique Pharmaceuticals, Sango Otta, Ogun State, Nigeria). Each blood sample obtained for each rat was collected into a well labeled 10 mL capacity plain sample bottle. The blood samples were allowed for complete clotting for about 3 – 5 hours before they were centrifuged with Uniscope Laboratory Centrifuge (Model SM 112, Surgifriend Medicals, England) at 2000 revolution per minute for 15 minutes. This was aimed at separating the sera from clotted blood cells. The sera were carefully separated into new, well labeled, corresponding plain sample bottles at room temperature 23 - 26 ºC. The sera were assayed for serum urea, uric acid and creatinine. Serum creatinine and blood urea were assayed using Randox Diagnostic kits (Randox Laboratories Ltd., Crumlin, U.K.) by method of Varley and Alan (1984).

Measurement of hematological parameters in acute and repeated dose nephrotoxic rats

Prior to termination of the experiments 24 hours and 14th day post-induction, respectively, the rats were fasted overnight but distilled water was made available *ad libitum*. Blood samples were collected by cardiac puncture under diethyl ether anesthesia, using 21 gauge (21G) needles mounted on a 5 mL syringe (Hindustan Syringes and Medical Devices Ltd., Faridabad, India) into Ethylene Diamine Tetra-acetic Acid (EDTA)-coated sample bottles for full blood count (FBC), which included DLC, Hb, PCV, MCV, MCH, MCHC, PL and TLC. The collected blood samples were analyzed using Automated Hematology System (Sysmex Hematology-Coagulation Systems®, Model KX-21N, Sysmex Incorporation, Kobe, Japan).

Histopathological studies of rat kidneys

After the animals were sacrificed, postmortem examination was performed. The rat kidneys were identified and carefully dissected out *en bloc* for histopathological examination. After rinsing in normal saline, sections were taken from each harvested kidney. The tissue was fixed in 10% formo-saline, dehydrated with 100% ethanol solution and embedded in paraffin. It was then processed into 4 - 5 µm thick sections stained with hematoxylin-eosin and observed under a photomicroscope (Model N - 400ME, CEL-TECH Diagnostics, Hamburg, Germany).

Data Analysis

Data were express as mean ± standard error of the mean (SEM) and analyzed using two ways analysis of variance on statistical software package, SYSTAT 10.6. Post-hoc test was also conducted using Student's t-test. Values of $p<0.05$, $p<0.01$, $p<0.001$ were taken to be statistically significant.

Results and Discussion

In recent time, the safety of the chronic use of acetaminophen at therapeutic dose has generated a lot of hot debate (Watkins et al., 2006). Acetaminophen overdose has been associated with significant glutathione depletion and consequent lipid peroxidation. As a consequence of lipid peroxidation, intracellular accumulation and covalent bonding of its highly reactive metabolite, N-acetyl-*para*-benzoquinone-imine (NAPQI), hepatocyte malfunction and death often result. Similar effect is often recorded for renal tissues. The selective renal accumulation of non-steroidal anti-inflammatory nephrotoxins including acetaminophen in animal and human is thought to result in a chain of biochemical reactions which culminate in acute or chronic nephropathies (Schnellman, 2001). In addition, acetaminophen has been reported to promote hepatocyte and renal apoptosis (Ray et al., 2000; Boulares et al., 2002).

Acetaminophen overdose (acute or chronic) is often associated with a wide range of metabolic disorders including serum electrolytes, urea and creatinine derangements. As such, elevations in the serum concentrations of these parameters, particularly, serum urea and creatinine are considered reliable, well documented parameters for investigating drug-induced nephrotoxicity in animals and man (Adelman et al., 1981).

Blood urea nitrogen is derived in the liver from proteins/amino acids from diet or tissue sources and is normally excreted in the urine. In renal disease, the serum urea accumulates (resulting in uremia) because the rate of serum urea production exceeds the rate of clearance (Mayne, 1994). Other causes of uremia include high protein diet, increased catabolism due to starvation, tissue damage, sepsis or steroid treatment and absorption of amino acids and peptides from digested blood after hemorrhage into the gastrointestinal lumen or soft tissue (Mayne, 1994). Creatinine, on the other hand, is mostly derived from endogenous sources by tissue creatine breakdown. The plasma creatinine concentrations in normal individuals are usually affected by a number of factors such as the muscle mass, high protein diet, and catabolic state (Mayne, 1994). Thus, serum urea concentration is often considered a more reliable renal function predictor than serum creatinine.

In the present study, results obtained showed that acute and repeated dose acetaminophen nephrotoxicities were reliably established with 800 mg/kg/day and 200 mg/kg/day of intraperitoneal acetaminophen, as evidenced by significant ($p<0.05$, $p<0.01$, $p<0.001$) elevations in the serum urea, uric acid and creatinine in acetaminophen-treated control (group II) rats when compared to untreated control (group I) rats (Tables 1 and 2). Establishment of acute and repeated dose acetaminophen nephrotoxicities were also corroborated by the histological findings which showed glomeruli with loss of surrounding Bowman's capsule and varying degrees of tubular necrosis (Fig. 2 and 4) when compared to untreated control (group I) rats which showed normal glomerulus with intact Bowman's capsule and tubular brush borders (Fig. 1). However, oral pretreatment with graded oral doses of the root extract of *Harungana madagascariensis* significantly attenuated the elevated serum concentrations of these parameters, in dose related pattern

Table 1. Effect of graded oral doses of *Harungana madagascariensis* aqueous root extract on serum urea (UR), uric acid (UA) and creatinine (CR) in acute acetaminophen induced nephrotoxic rats

Treatment Group	UR (mmol/L)	UA (µmol/L)	CR (µmol/L)
I	6.48 ± 0.37	115.67 ± 10.50	68.33 ± 5.03
II	11.77 ± 2.08[b]	111.67 ± 28.22	100.17 ± 15.68[b]
III	5.60 ± 0.23[c]	145.83 ± 30.43	62.33 ± 5.43[d]
IV	5.62 ± 1.15[c]	104.00 ± 18.63	56.33 ± 4.63[d]
V	3.90 ± 0.41[d]	35.65 ± 12.43[d]	46.83 ± 6.41[d]

[b] represents significant increase at $p<0.001$ when compared to negative control (Group I) values while [c] and [d] represent significant decrease at $p<0.01$ and $p<0.001$, respectively when compared to model control (Group II) values

I = 10 mL/kg of distilled water via the intraperitoneal and oral routes, respectively
II = 10 mL/kg of distilled water *per os* + 800 mg/kg via the intraperitoneal route of acetaminophen
III = 100 mg/kg *per os* of *Harungana madagascariensis* + 800 mg/kg of intraperitoneal acetaminophen
IV = 200 mg/kg *per os* of *Harungana madagascariensis* + 800 mg/kg of intraperitoneal acetaminophen
V = 500 mg/kg *per os* of *Harungana madagascariensis* + 800 mg/kg of intraperitoneal acetaminophen

Table 2. Effect of 14 days of oral doses of *Harungana madagascariensis* aqueous root extract on serum urea (UR), uric acid (UA) and creatinine (CR) in repeated dose acetaminophen induced nephrotoxic rats

Treatment Group	UR (mmol/L)	UA (µmol/L)	CR (µmol/L)
I	7.13 ± 0.95	85.53 ± 27.47	53.67 ± 3.53
II	13.42 ± 2.02[a]	135.67 ± 20.21[b]	151.83 ± 3.936[b]
III	9.25 ± 1.65[c]	169.17 ± 33.34	55.17 ± 2.50[d]
IV	7.77 ± 0.75[c]	104.33 ± 19.63[c]	51.67 ± 2.81[d]
V	6.93 ± 0.33[c]	85.83 ± 8.55[d]	50.00 ± 2.76[d]

[a,] and [b] represent significant increase at $p<0.05$ and $p<0.001$, respectively, when compared to negative control (Group I) values while [c] and [d] represent significant decrease at $p<0.05$ and $p<0.001$, respectively when compared to model control (Group II) values

I = 10 mL/kg of distilled water via the intraperitoneal and oral routes, respectively
II = 10 mL/kg of distilled water *per os* + 200 mg/kg via the intraperitoneal route of acetaminophen
III = 100 mg/kg *per os* of *Harungana madagascariensis* + 200 mg/kg of intraperitoneal acetaminophen
IV = 200 mg/kg *per os* of *Harungana madagascariensis* + 200 mg/kg of intraperitoneal acetaminophen

Table 3. Effect of graded oral doses of *Harungana madagascariensis* aqueous root extract on full blood counts in acute acetaminophen-induced nephrotoxic rats

Parameters	Group I	Group II	Group III	Group IV	Group V
PCV (%)	35.58 ± 0.77	34.80 ± 1.74	36.08 ± 1.78	37.30 ± 1.56	37.65 ± 0.61
Hb (g/dl)	11.43 ± 0.28	11.10 ± 0.52	11.65 ± 0.48	12.05 ± 0.40	12.30 ± 0.23
TLC (x 10^3/µL)	8.23 ± 1.36	7.68 ± 1.62	8.62 ± 1.72	11.23 ± 0.88	5.18 ± 0.46
DLC					
Lymph (%)	65.70 ± 8.78	78.32 ± 1.18	80.13 ± 3.73	62.62 ± 9.78	68.57 ± 1.07
Neut (%)	18.53 ± 3.57	15.18 ± 1.84	13.53 ± 3.55	23.07 ± 5.84	22.72 ± 2.25
Gran (%)	15.77 ± 5.70	6.50 ± 1.32[b]	6.30 ± 1.64[b]	15.47 ± 3.99	8.72 ± 2.41[b]
MCV (fL)	58.83 ± 0.83	58.50 ± 0.21	58.55 ± 1.02	57.22 ± 0.34	57.18 ± 0.85
MCH (pg)	20.33 ± 1.59	18.68 ± 0.18	18.98 ± 0.42	18.45 ± 0.24	18.67 ± 0.26
MCHC (g/dL)	34.47 ± 2.28	31.93 ± 0.26	32.42 ± 0.42	32.18 ± 0.28	32.67 ± 0.32
PLC (x 10^3/µL)	593.17±19.96	516.33±81.51	573.67±71.79	665.50±87.61	761.67±53.69[a]

[a] represents significant increase at $p<0.05$ when compared to negative control (Group I) values while [b] represents significant decrease at $p<0.05$ when compared to negative control (Group I) values

I = 10 mL/kg of distilled water via the intraperitoneal and oral routes, respectively
II = 10 mL/kg of distilled water *per os* + 800 mg/kg via the intraperitoneal route of acetaminophen
III = 100 mg/kg *per os* of *Harungana madagascariensis* + 800 mg/kg of intraperitoneal acetaminophen
IV = 200 mg/kg *per os* of *Harungana madagascariensis* + 800 mg/kg of intraperitoneal acetaminophen
V = 500 mg/kg *per os* of *Harungana madagascariensis* + 800 mg/kg of intraperitoneal acetaminophen

Table 4. Effect of graded oral doses of *Harungana madagascariensis* aqueous root extract on full blood counts in repeated dose acetaminophen induced nephrotoxic rats

Parameters	Group I	Group II	Group III	Group IV	Group V
PCV (%)	37.17 ± 1.02	32.57 ± 1.86[c]	34.77 ± 0.85	39.67 ± 0.61[b]	40.85 ± 1.73[b]
Hb (g/dl)	11.70 ± 0.40	10.57 ± 0.57	10.60 ± 0.36	12.90 ± 0.21[a]	13.60 ± 0.59[a]
TLC (x 10^3/µL)	5.53 ± 1.04	5.52 ± 1.12	9.70 ± 1.10[a]	10.48 ± 1.52[a]	6.97 ± 1.07
DLC					
Lymph (%)	69.87 ± 4.20	59.50 ± 2.86	56.42 ± 5.60	57.45 ± 4.28	63.87 ± 4.02
Neut (%)	20.32 ± 4.07	25.02 ± 3.92	28.38 ± 3.99	31.55 ± 5.48	21.43 ± 3.37
Gran (%)	9.82 ± 1.07	15.48 ± 3.21	15.20 ± 2.96	11.00 ± 2.22	14.70 ± 1.47
MCV (fL)	60.00 ± 1.25	60.62 ± 1.39	62.07 ± 1.89	65.80 ± 1.10	66.87 ± 4.95
MCH (pg)	18.92 ± 0.67	16.05 ± 0.37	18.88 ± 0.33	19.92 ± 0.56	21.85 ± 3.79
MCHC (g/dL)	31.45 ± 0.53	30.23 ± 1.04	30.47 ± 0.54	32.20 ± 0.34	36.35 ± 2.77
PLT (x 10^3/µL)	702.83±20.47	857.83±169.34	946.00±196.44	702.33±76.75	835.33±53.7

[a] and [b] represent significant increases at $p<0.05$ and $p<0.001$ while [c] represents a significant decrease at $p<0.05$ when compared to positive controls (Group II) values, respectively
I = 10 mL/kg of distilled water via the intraperitoneal and oral routes, respectively
II = 10 mL/kg of distilled water *per os* + 200 mg/kg via the intraperitoneal route of acetaminophen
III = 100 mg/kg *per os* of *Harungana madagascariensis* + 200 mg/kg of intraperitoneal acetaminophen
IV = 200 mg/kg *per os* of *Harungana madagascariensis* + 200 mg/kg of intraperitoneal acetaminophen
V = 500 mg/kg *per os* of *Harungana madagascariensis* + 200 mg/kg of intraperitoneal acetaminophen

(Tables 1 and 2). The biochemical results were also confirmed by the histological findings which showed preservation of the glomeruli and the surrounding Bowman's capsule and mildly swollen tubules (Fig. 2 and 5). The protection offered by the extract could have been due to the presence of any of the active principles contained in the extract. Literature has shown *Harungana madagascariensis* to contain high concentrations of glycosides, flavonoids, alkaloids, saponins and tannins (Okoli et al., 2002). Taking into account that flavonoids, particularly quercetin, in other nephroprotective medicinal plants have been reported of inhibiting xenobiotic-induced nephrotoxicity in experimental animal models (Devipraya and Shyamaladevim, 1999) due to their potent anti-oxidant or free radicals scavenging effects (Annie et al., 2005). In addition, alkaloids have also been reported to strongly inhibit lipid peroxidation induced in isolated tissues via its antioxidant activity (Lanhers et al., 1991, Kumaran and Karunnakaran, 2007). Any of these or their combination could be responsible for the observed effect. In view of the above, one of the possible mechanisms of action of the extract could be via its antioxidant and/or free radical scavenging activities. However, this hypothesis requires validation. Oxidative stress occurs in cells when there is disruption of cellular redox balance (Liu et al., 1999). Acetaminophen –induced oxidative stress results in lipoperoxidation, protein thiols oxidation, mitochondrial endoplasmic reticulum injury, altered calcium homeostasis and irreversible DNA damage characterized by protein adduct formation (Corcoran and Ray, 1992; Cohen et al., 1998). Several antioxidant systems occur in the body, which include superoxide dismutase, glutathione peroxidase, catalase, glutathione, vitamin C and vitamin E (Sie, 1993; Liu et al., 1999).

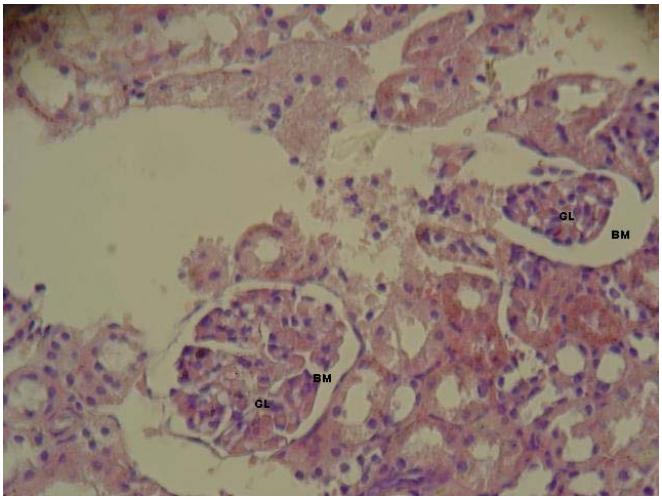

Figure 1. is a representative section of a normal kidney showing normal tubular brush-borders and intact glomerulus (**GL**) and surrounding Bowman's capsule (**BM**) (x400 magnification, hematoxylin and eosin staining)

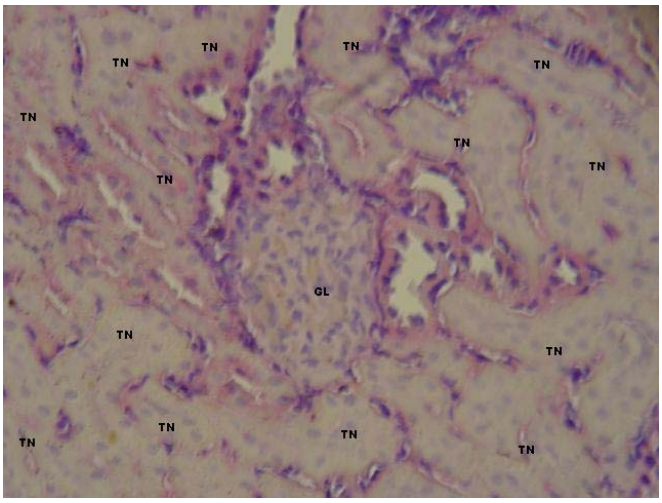

Figure 2. is a representative of an acute 800 mg/kg/day of intraperitoneal acetaminophen-treated rat kidney showing a glomerulus (**GL**) with loss of surrounding Bowman's capsule diffuse tubular necrosis (**TN**) (x400 magnification, hematoxylin and eosin staining)

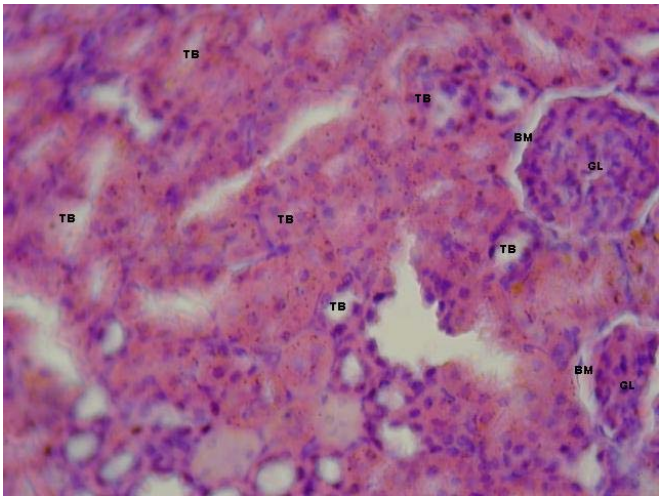

Figure 3. a representative of oral 500 mg/kg/day of *Harungana* root extract-treated acetaminophen-induced nephrotoxic rat kidney showing a glomerulus (**GL**) with an intact Bowman's capsule (**BM**) and diffuse mildly swollen tubules (**TB**) (x400 magnification, hematoxylin and eosin staining)

Glutathione redox cycle is known to be an important intracellular antioxidant system in the body because of the vital role glutathione plays in maintaining cell metabolism and integrity (Yu and Anderson, 1997). Significant reduction in the renal tissue concentrations of glutathione has been associated with drug induced nephrotoxicity including acetaminophen nephrotoxicity (Halliwell and Gutteridge, 1989; Visarius et al., 1996). Although renal glutathione concentrations were not assayed in the present study, it is possible for *Harungana* extract to be mediating its protection against acetaminophen nephrotoxicity by either protecting or replenishing renal glutathione storage. It is also possible for the *Harungana* extract to be mediating its renal antioxidant activities by enhancing the antioxidant defense enzymes mediated by superoxide dismutase, an important anti-lipoperoxidation enzyme in the body. However, further evaluation into the exact antioxidant mechanism(s) of the extract would be required to validate these hypotheses.

The vital function that blood cells perform, together with the susceptibility of this highly proliferative tissue to intoxication by xenobiotics, makes the hematopoeitic system unique as a target organ. Accordingly, it ranks with liver and kidney as one of the most important considerations in the risk assessment of potential environmental toxicants or xenobiotics. The various blood cells (erythrocytes, leucocytes, and platelets) are produced at a turnover rate of about 1 to 3 million per second in a healthy human adult and this value could be altered in certain physiological or pathological states including hemolytic anemia or suppressive inflammation (Guyton, 1991). Certain drugs including alkylating cytotoxic agents could also affect blood formation rate and the normal range of hematological parameters. In the present acute study, treatment of rats with high dose of intraperitoneal acetaminophen did not cause significant ($p>0.05$)

alterations in most of the measured hematological parameters, except for the granulocyte differential and platelet counts which were significantly ($p<0.05$) elevated in dose related fashion (Table 3).

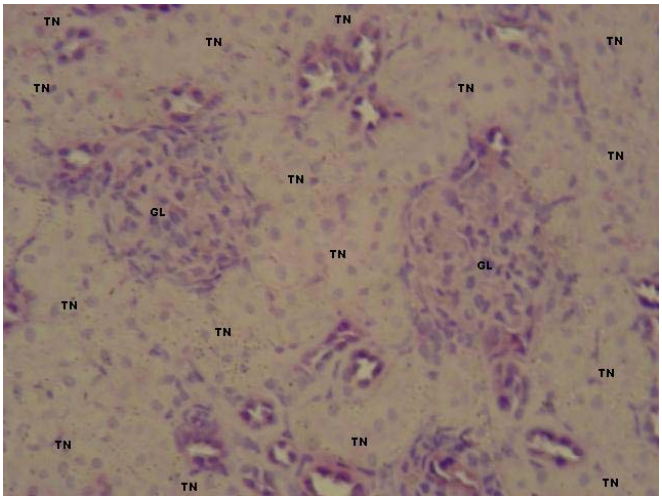

Figure 4. a section of a repeated dose (200 mg/kg) acetaminophen-treated kidney showing glomeruli (**GL**) with complete loss of Bowman's capsule (**BM**) and severe diffuse tubular necrosis (**TN**) (x400 magnification, hematoxylin and eosin staining).

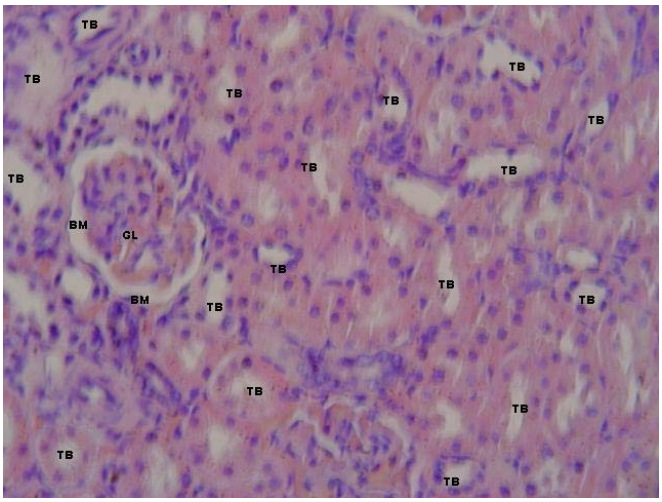

Figure 5. a representative of repeated 500 mg/kg of *Harungana* root extract-pretreated-acetaminophen-treated kidney showing an intact glomerulus (**GL**) and mildly swollen tubules (**TB**) (x400 magnification, hematoxylin and eosin staining)

However, in the repeated dose study, intraperitoneal injection of acetaminophen caused a significant ($p< 0.05$) decrease in the PCV level while causing non-significant ($p>0.05$) alterations in other measured blood indices (Table 4).

The recorded hematotoxicity could be secondary to the deleterious effect of acetaminophen on organs of hematopoeisis in the body which include liver and kidneys. Literature has shown acute or chronic large dose acetaminophen to be associated with overproduction of a highly reactive intermediate, N-acetyl-*p*-benzoquinone-imine (NAPQI), which covalently bound to macromolecules of renal tissues (Prescott, 1989; Fored et al., 2001) resulting in acetaminophen-associated nephropathy (Emeigh Hart et al., 1996). However, oral treatment with graded doses of *Harungana madagascariensis* reversed the significant decrease in the Hb, PCV value recorded for acetaminophen hematotoxicity and also caused a significant ($p<0.05$, $p<0.05$, $p<0.001$) dose related increase in the Hb and TLC, and PCV, respectively. Although, the extract caused non-significant ($p>0.05$) increase in lymphocyte differential, MCV, MCH and MCHC, but had no effect on other measured parameters. Results of this study showed that the extract could contain active biological principle(s) reversing the hematotoxic effect of acetaminophen, with subsequent enhancement of hematopoiesis. The biological principle(s) could also be mediating hematopoietin-like effect or enhancing the release of hematopoietin from hematopoetic organs such as the kidneys or liver. Although the exact hematopoietic mechanism of the extract was not investigated in the present study, this area could constitute an area of future study. Thus, the

overall results of this study suggest that the *Harungana madagascariensis* root extract could be improving the hematological status in rats repeatedly exposed to high dose of acetaminophen.

In conclusion, the overall result suggests that the aqueous root extract of *Harungana madagascariensis* possesses nephroprotective potential and improves hematological derangements associated with repeated dose acetaminophen nephrotoxicity. Although, the active principles were not isolated and their possible mechanisms of actions were not investigated in the present study, these could constitute areas of future studies.

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