Estimation of the Cytotoxic Effects of *Yucca Gloriosa* Variegata Leaves Extract on Albino Mice

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Abstract

Yucca gloriosa Variegata L. is a stemless. The whole plant of *Y. gloriosa* L. has vast medicinal uses. The Native Americans and North New Mexico used a tea from the leaves and roots to treat asthma, headache, wound healing. As well as it was being consumed as daily dietary. All part of *Y. gloriosa* L. is rich in saponin steroidal glycosides. Saponin extracts are well-known to be highly toxic. Hence, present study was carried out to investigate the toxicity of saponin and estimate the LD_{50} value which helps in determining the safe dose range for the drug that be used, as well as to determine hematological aspects and examine histological effect. Different concentrations of saponin extract were injected into male mice (10,000, 8000, 6000, 4000, 2000, 1600, 1200, 600, 300) mg/kg. The LD_{50} value of the saponin extract was estimated to be (3100mg/kg). Hematological study was assessed by using various hematological parameters like RBC, WBC, LYM, MID. Complete blood pictures of the treated mice indicated an evident leukocytosis and erythrocytopenia effect of the drug. Treated-mice kidney tissues specimens were examined microscopically indicated clear signs of heavy inflammation, necrosis and swelling of renal tubes, degeneration of renal tubes as well as hyaline and hemorrhagic cast at high concentrations of leaves extract.

Key words: *Yucca gloriosa, Saponin, Hematological parameters, Histological effect, Necrosis, Hyaline cast, Hemorrhagic cast.*

Introduction

Yucca, of the family Agavaceae, is perennial shrubs [1]. It has forty to fifty species that are remarkable for their rosettes of evergreen leaves and produced white flowers on the terminal position of large panicles. This plant is native to the arid parts of the Americas and the Caribbean. *Y. gloriosa* L. is commonly known as variegated Spanish Dagger [2]. The leaves are very stiff, spine-tipped, edged creamy yellow that changed to red in the cold months of the year [3]. This plant has been described as one of the promising plants that can contribute in agricultural, pharmacochemical as well as textile industry and reputed in folk medicine. The whole plant *Y. gloriosa* L. is used for ulcer, jaundice, asthma and bronchitis [4]. The leaves are used for dandruff, hair loss, skin sores and phthisis. The roots are crushed to make pack for wound healing. The roots are used to cure rheumatism. The fruits are eaten raw as a laxative, blood purifier. Now, Yucca sp. gains scientific validation and acceptance of consumer. Capsules, beverages and herbal tea obtained from vast group of yucca extracts are marketed. Virtually, every part of yucca possesses bioactive compounds: leaves, shoots, rhizomes, seed pods, flowers and bark [5]. Many studies evaluated *in vitro* on the antimicrobial effect of *Y. gloriosa* L. extract. The whole plant extract was investigated to determine the antimicrobial activity against different Gram negative organisms and different Gram positive organisms as well as against fungal strains. Anthelmintic activity of *Y. gloriosa* L. extract on Indian adult earthworms, *Pheretima posthuma,* was also assayed due to its anatomical and physiological similarity with the intestinal roundworm parasites of humans [6,7]. The antimycotic activity of steroidal glycoside crude extract of *Y. gloriosa* flowers was examined *in vitro* against a group of human pathogenic organisms including yeasts, fungi [8]. It is used as antiallergric drug [9], gastro-ulcer therapy [10] and has

hepatoprotective activity [11]. Saponins are essential components of *Yucca* with broad-spectrum biological properties. They play key roles in food, cosmetics and pharmaceuticals [12]. Saponins are bioactive compounds produced mainly by plants. Chemically, they occur as glycosides of steroids or polycyclic triterpenes [13]. Because of their properties as lyobipolar, they are affected on the cell membranes and decreased the surface tension of an aqueous solution. This activity is the reason for the name "saponin", derived from the Latin word "sapo", which refers to the formation of stable soap-like foam in aqueous solution [14]. They are commonly known by their sweeter to bitter taste, acid hydrolyzed, high toxicity towards coldblooded species and hemolytic properties due to saponins interaction with membrane sterols of the erythrocyte causing an increase in permeability, bursting erythrocyte membrane and a loss of haemoglobin, thus they are highly toxic when injected into the blood stream [15]. A vast range of herbs are known for their therapeutic potential with a wide range of pharmacologically important saponins that exhibited a broad spectrum of biological activities attributed to their structural diversity and therapeutic capabilities [16]. Saponins are known to their toxicity towards cold-blooded species, but have weak toxicity if taken orally by warm-blooded species and lethal dose 50% (LD_{50}) values extent from 50-1000 mg/kg that are perhaps due to low absorption rates and acid hydrolization leading cleavage the ester-linked sugars to produce aglycones and carbohydrates. However, they are very toxic when taken intravenously [17]. Thus, the present study aims to determination of $\mathbf{LD_{50}}$ value of *Y. gloriosa* Variegata saponins extracted from leaves which help in determining the safe dose range for the drug to be used and to estimate the hematological effects of saponins extract and to investigate the histological state of body organ (kidney) after the use of *Yucca* saponins as therapeutic agents.

Materials and Methods

Plant materials

The leaves of *Yucca gloriosa* Variegata were collected from plants obtained with labels showed the scientific name of the studied plant and its photograph from local nursery in Baghdad.

Preparation of saponin extract

Steroidal saponins extraction from *Y. gloriosa* Variegata leaves was carried out following the method of Kaur *et al*. [18]. Different concentrations of saponin extract was prepared.

Experimental animals

Albino mice (25-30g) of male sex only were maintained at room temperature (25°C) with free access to feed. Male mice were injected intraperitonealy with different concentrations of the saponin extract (10,000 mg/kg, 8000 mg/kg, 6000 mg/kg, 4000 mg/kg, 2000 mg/kg, 1600 mg/kg, 1200 mg/kg, 600 mg/kg, 300 mg/kg.) to determine of $LD_{50.}$

Determination of $\mathbf{LD_{50}}$

LD_{50} of saponins extract of *Yucca gloriosa* leaves was determined by up-down method [19]. Albino mice were injected intraperitonealy with one dose of (0.3 ml) of different concentrations of the extract. Mortality was recorded after 24 hours. The LD_{50} was determined using the following formula:

$\mathbf{LD_{50} = xf + kd}$

LD_{50}: Intermediate Lethal Dose
xf : Last dose that was used
k : Value from the table.
d : Decrease and increase in the dose.

Hematological parameters

Blood was collected from control and treated mice using insulin syringe, and stored in labeled EDTA tubes. The total and differential count of blood cells was done for the collected blood sample by using an auto-hematology analyzer.

Preparations of histological sections

The tested organ (Kidney) was taken from control and mice treated with the saponin extract of *Y. gloriosa* Variegata leaves. The organ was kept in formalin 10%

and then they were dissected. The method described by Bancroft and Gamble [20] that was employed for dissecting. The specimens were fixed, embedded in paraffin, sectioned using microtome and then stained with Harris haematoxyline and Eosin (H&E) and microscopically examined.

Results and Discussion

Acute toxicity study

When male mice were injected peritonealy, the LD_{50} of the saponin extract was estimated to be (3100 mg/kg). In toxicology, the median lethal dose, LD_{50} or LC_{50} (lethal concentration, 50%) is a measure of the lethal dose of a toxin, radiation, or pathogen. LD_{50} is frequently used as a general indicator of a substance's acute toxicity. A lower LD_{50} is indicative of increased toxicity. Saponins are known to their toxicity towards cold-blooded species, but have weak toxicity if taken orally by warm-blooded species and lethal dose 50% (LD_{50}) values extent from 50-1000 mg/kg that are perhaps due to low absorption rates and acid hydrolization leading cleavage the ester-linked sugars to produce aglycones and carbohydrates. However, they are very toxic when taken intravenously [21].

Hematological examination

A complete blood picture for the samples exhibited the following results as shown in table (4-1). The table describes the effect of crude saponins on blood parameters.

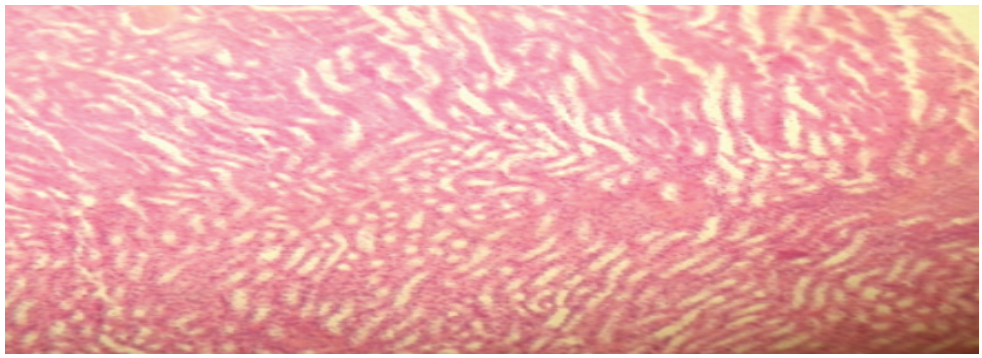
Table (4-1) Blood parameters of mice injected with different concentration of saponins after 24 hours.

Analysis	Injection Dose Concentration mg/kg			
	Control	**300**	**600**	**1200**
RBC	5.93x10*12	1.80x10*12	1.50x10*12	1.00x10*12
WBC	6.4x10*9	5.7x10*9	8.2x10*9	20.2x10*9
LYM	4.9	4.0	5.9	13.50
MID	0.8	0.9	2.0	3.1
GRAN	0.7	0.8	0.30	3.6
RDWa	24.1	21.2	22.3	22.8
MCH	17.6	15.6	16.6	16.2
PLT	116x10*9	110x10*9	126x10*9	103x10*9
MPV	6.5	6.5	7.0	6.6
PDW	9.1	8.8	9.6	9.1

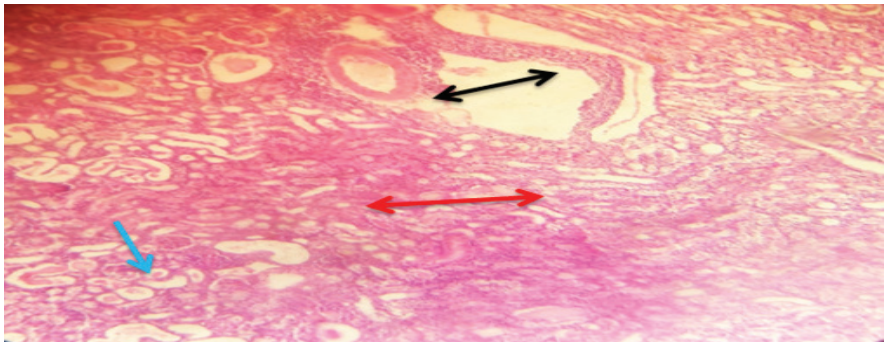
From data recorded in the table, it was observed that there is a clear decrease in the number of erythrocytes count, which indicates an evident hemolysis of the red blood cells. There's a clear increase in the number of leukocytes and lymphocytes count, which indicates an evident leukocytosis due to heavy inflammation. The elevate rates of GRAN (Granulocytes White Blood Cells) tests indicate infection. MID (Minimum Inhibitory Dilution) exhibited a slight increase which too indicates an inflammatory status. The saponins extract did not affect the number of platelets levels nor their shape (width). The mean measurement of RDW (Red Cell Distribution Width) determines the morphological shape of a red blood cell, which showed no differences. So it is safe to say the saponins extract did not affect the shape of RBCs. MPV (Mean Platelets Count) and PDW (Platelet Distribution Width) results were very close to each other with very tiny and slight difference. The results of the corpuscular hemoglobin (MCH), or "mean cell hemoglobin" (MCH), which is the average mass of hemoglobin per red blood cell in a sample of blood, were almost fixed with very slight differences. Thus, the saponins extract didn't affect the hemoglobin mass in each red blood cell. The results in agreement with Lee *et al.* [22] which reported that the use of *Pueraria niruri* had no adversely effect on the blood cells. The main biological activity ascribed to saponins and their cell membrane permeabilizing properties. Saponins have a specified capability to form pores in membranes; and have a lytic effect on erythrocyte membranes. These actions are believed to be the result of the affinity of the aglycone moiety for the phospholipids existent in the cell membrane that leads to alteration of the molecular structure of cell membrane, reduction of surface tension between the aqueous and lipid phases of erythrocyte membrane, causing emulsion of the lipids and subsequently erythrocyte membrane rupture [23].

Histological effect of saponin extract

The results of the histological study of mice kidney are shown in the following figures:

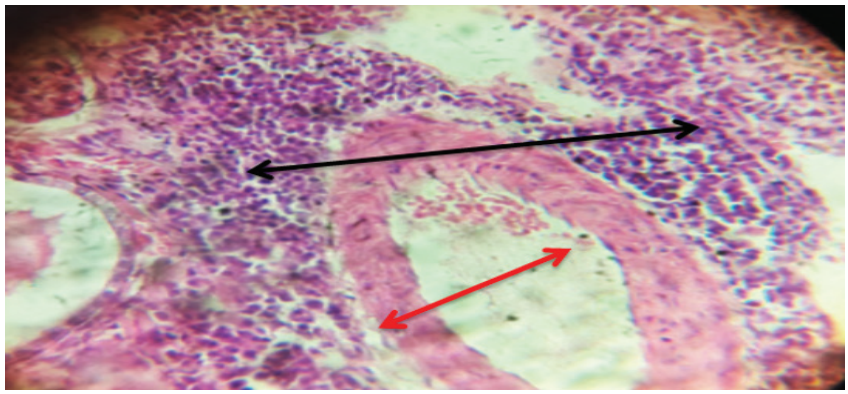


(A)



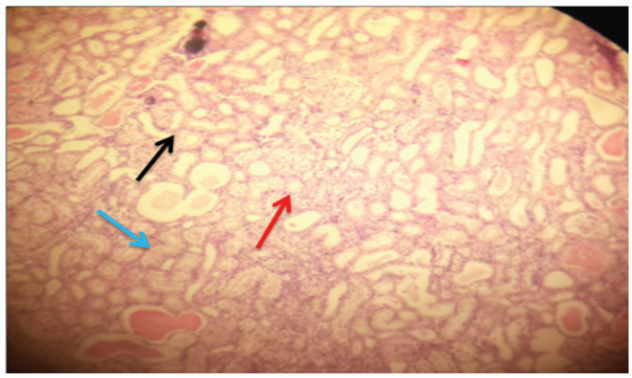
(B)

Cont... **The results of the histological study of mice kidney are shown in the following figures:**

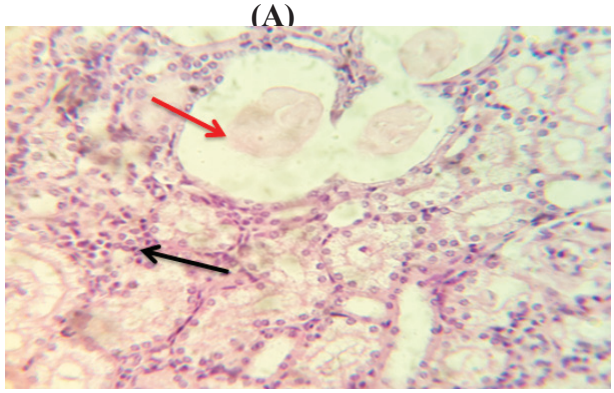


(C)

Figure (3-1) C.S. of kidney from mouse: A, control group showed the normal appearance and normal arrangement of renal tubules. B & C, treated with 300 mg/kg (10X and 40X respectively). B: showed pyelonephritis with inflammatory reaction (black arrow); necrotic and swelling renal tubules (red arrow); atrophic glomeruli (blue arrow). C, showed nephritis with heavy inflammatory reaction tend to be granulomatous reaction (black arrow); congested blood vessel with little RBCs found within thickened lumen due to fibrous connective tissue proliferation (red arrow).



(A)



(B)

Figure (3-2) C. S. of mouse kidney treated with 600 mg/kg: A, (10 X) showed disarrangement and atrophic glomeruli and renal tubules due to dilatation and cloudy swelling and infiltration of inflammatory cells (red arrow); hyaline caste within degenerated renal tubule (black arrow) and hemorrhagic caste (blue arrow); B, 40X (H&E), showed high magnification for hyaline caste within dilated renal tubules (red arrow) and swelling of renal tubules with desquamated epithelial cells (black arrow).

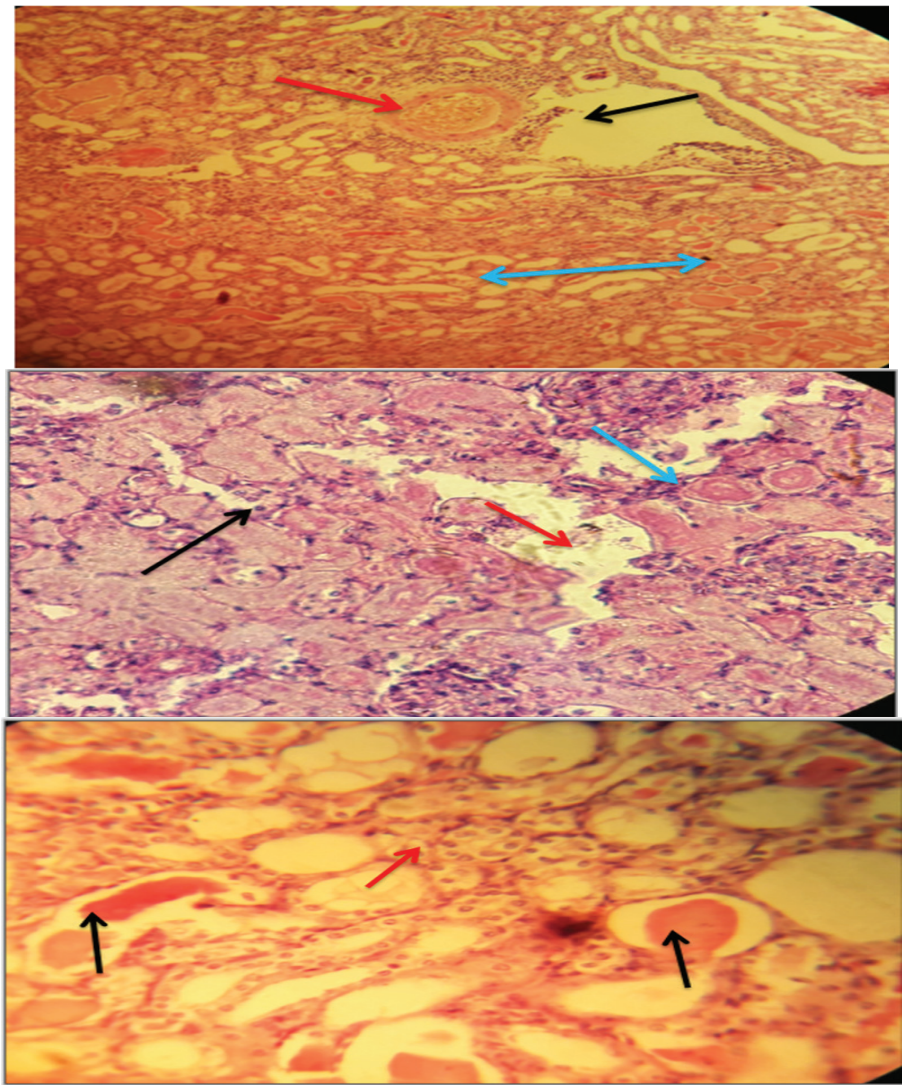


Figure (3-3): C. S. of kidney of mouse treated with 1200 mg/kg; A, showed inflammatory zone within necrotic area (black arrow); congested and inflamed and thickened blood vessels (red arrow); degenerated renal tubules contained hyaline and hemorrhagic castes (blue arrow). 10X (H&E); B, showed destructive changes (Red arrow); infiltration of inflammatory cells (black arrow) and swelling of renal tubules with desquamation and hemorrhagic caste (blue arrow) 40 X; C, showed clear hemorrhagic castes within degenerated renal tubules (black arrow) and swelling of renal tubules with prominent nuclei (red arrow) 40 X.

Results viewed that the kidney of untreated mouse showed no visible lesions and normal arrangement of renal tubules. In treated mice kidney, the general aspects were the appearance of necrotic areas, degenerates renal tubules, contained hyaline cast and hemorrhagic cast with the increase of saponins concentration. The histological results are in agreement with the results of Sung *et al*. [24] which investigated the nephrotoxicity of ICR mice treated with 150, 300 and 600mg/kg of saponin extract of *Asparagus cochinchinensis*. No significant toxicity was recorded based on the organ weight, serum parameters or histological structure and the differences that occurred were attributed to the high saponins concentrations administered to animals. Also, Ajibade *et al.* [25] showed that there were multiple foci of hemorrhages into the interstitium. There were few loci of tubular necrosis and presence of hyaline casts with interstitial cellular infiltration by macrophages. They

considered the presence of hyaline cast in the kidney is normal and has been ascribed to the use of medicines and the appearance of a few loci tubular necrosis in the kidney has been observed to be a reflection of the initial pathogenesis of the infection but not caused by the saponin therapy [26].

Conclusion

Generally, from the results of the present study it was concluded that saponins extracted from *Y. gloriosa* has no toxic effects on kidney tissues of male mice at doses less than 1200 mg/kg.

Conflict of Interest: None

Funding: Self

Ethical Clearance: Not required

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