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Hematological and histological Changes in mice Fed Aqueous Extract of *Senna alexandrina*

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Abstract

This study aims to evaluate the effect of aqueous extract stem of dried of *Senna Alexandria* using male mice once daily at varying doses for 2 and weeks on hematological and histological changer in liver and kidney. In the acute toxicity aqueous extracted of dried stem of *Senna Alexandria* were administrated orally to mice up to 20-25 gr of body weight, in sub-acute study mice was daily administrated orally with same extraction at dose 500,1000,2000 mg/kg for 14 days . Hematological findings have shown in male group increase of RBC, HGB, HCT in increase of PIT in group treated with 500mg/ kg of body weight.

Body weight was increase after application of extract at the dose of 1000 and 2000 mg/ kg . The histology in liver includes congestion of central vein with inflammatory cell in their lamens, vascular dilation ,fatty infiltrations cytoplasmic vacillation necrotic hepatocyte . Whereas in the kidney the changer includes shrunken glomeruli increasing of renal space, swelling and cytoplasmic vaculation of epithelial cells lining the renal tubule. Increase in body weight was with a dose 1000,2000mg/kg week after 2 weeks.

Keywords: Toxicity, Hematology, Aqueous Extract, Mice.

التغيرات الدموية و النسجية في الفئران المعرضة للمستخلص المائي لنبات *Senna alexandrina*

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الخلاصة

هدفت هذه الدراسة للتحري عن تأثير الجرعة المائية لمحلول السيقان الجافة لنبات السنا اسكندراني (السنامكي) على ذكور الفئران بجرعة واحدة يوميا وبتراكيز متخلفة ولمدة 14-20 يوم و تاثيرها على الفحص النسيجي للدم و التغيرات النسجية لكبد و الكلية للفئران المعاملة .ان تاثير معرفة السمي الحاد للمحلول المائي للسيقان الجافة لنبات السنامكي حيث جرعت الفئران (20-25) يوميا اما التاثير السمي تحت الحاد حيث تم تجريب الفئران فمويا بالمحلول السابق وبالجرع 500 , 1000 و 2000 ملغم / كغم و لمدة اربعة عشر يوما و اظهرت نتائج الفحص النسيجي للدم زيادة في كريات الدم الحمراء و الهيموكلوبين و الهيماتوكريت (مكناس الدم نسبة خلايا الدم الاحمر في الدم) و زيادة في قيمة الصفائح الدموية وفي المجاميع المعاملة ل 50 ملغم / كغم من وزن الجسم . ظهرت زيادة في الوهدة . كما لوحظ زيادة في وزن الجسم بعد التجريب بالمستخلص المائي و بالتراكيز 1000, 2000 ملغم /كغم لكل جرعة , كما اظهرت نتائج الفحص النسيجي للكبد و الكلية تغيرات ملحوظة اختلفت باختلاف تركيز الجرعة للمحلول المائي لسيقان نبات السنا , و اظهر نسيج الكبد

توسع الاوعية الدموية و اترشاح خلايا وحيدة النواة و توسع الحبيبات و تفجي السايوتوبلازم للخلايا الكبدية
 و ظهور خلايا متخثره متغلطة الانوية وتحللها بينما في الكلى نلاحظ حدوث انكماش الكبيبات وتتكس و انتفاخ
 و تنخر الخلايا المبطنه للنيبيات الكلوية

1. Introduction

Plants are used since ancient for medicine assumed to be safe. This safety is based on their longer treatment of diseases according to knowledge published over centuries [1]. Although limited evidence suggests that side effects with the use of herbal drugs are less occurrence than conventional drugs, they are usually mild and only affecting a small number of people. evidence suggests that some of the herbs considered to be safe over the last many decades have proven to be associated with health hazards [2].

S. alexandrina Miller. (Syn. *Cassia senna*, Family Caesalpiniaceae), in Iraq the plant founds in south east [3] and was formerly exported through Alexandria is mentioned in all famous herbals of the 15th and 16th century and is described in the last editions of the pharmacopoeias of countries all over the world [4]. The pods and leaves are considered as one of the most used laxatives [5, 6]. The laxative value of senna is because of the presence of sennosides A and B in its leaves and pods, which were isolated in pure form by [7]. Senna, has adverse effects on the heart because regular consumption is reported to deplete the body of potassium causing fatalities. Other adverse reactions consist of grand mal seizures, circulatory failure, high blood pressured and anaphylactic reaction [8]. As the use of senna as laxative drug is wide worldwide, experimental screening of the toxicity of this plant is crucial to assure the safety and effectiveness of this natural source. The aim of this study was to evaluate the toxicity of the aqueous extract of *S. alexandrina* in order to found a safe use of this plant as a medicine.

2. Method and Method

2.1 Plant Material

The leaf of plant obtained from local market in Iraq.

2.2. Preparation of Plant Extract

The aqueous extract was prepared according to the method described by Mbiantcha *et al.*, (2011)[9], with some modifications. Briefly, 150 g of powdered leaf in 1500ml. of distilled water, maintained at room temperature for 4 h. Extract was first filtered on filter paper and then freeze – dried to yield 13 g of water extract.

2.3 Laboratory Animals and Experimental Designs

59 adult mice between (20 - 25 g) were used for acute and sub-acute toxicity test kept in animal house of Alrazi center under laboratory condition of 25-28°C and supplied food and water for the period of research. 59 mice were used for acute and sub-chronic toxicity study. The study has been done as a joint collaboration between a Master's student and an internship team of Veterinary Drug Center, Baghdad, Iraq; were produced and approved the experimental protocol of the study. All animals used once for aqueous reasons. Then all animals were dissected at the end of the study for histological changes and blood parameter test.

2.4 Acute Toxicity Test

The mice were randomly divided into six groups of five animals per each group. Feeding oral doses of plant extracts at (500, 1000, 2000, 3000, 4000, 5000 mg/kg per oral) were separately administered orally to the mice in each group. All the mice were allowed free access to food and water and were seen for over a period of 24 h for signs of acute toxicity. The number of death, within this period was noted.

2.5 Sub-chronic Toxicity Study

The mice were randomly divided into four groups of 6 mice of per group. They were fed orally with different concentrations of aqueous extract of *Senna Alexandrian* daily for 14 days. It is shown as flows for 14 days; Group I: The control was given water 0.25 ml of water Group II: They were given 500 mg/kg of aqueous leaf extract of plant. Group III: This group was administered with 1000 mg/kg of the leaf extracts of *Senna Alexandrian* Group IV: They were administered 2000 mg/kg of the aqueous leaf extract of *Senna Alexandrian*.

2.6 Collection of Blood and Organ Samples

Fourteen (14) days after feeding the mice with the aqueous extracts of *Senna Alexandrian*, they were fasted overnight, anaesthetized with chloroform and sacrificed. Blood samples were collected by

cardiac puncture using syringe and needle. Blood samples from each animal were collected into dry sample bottles for clinical chemistry analysis and EDTA (Ethylenediaminetetraacetic acid) container for hematological examination. The sample bottle with the whole blood was allowed to stand for 20 minutes to clot and further spun at 12,000 rpm for 5 minutes using the centrifuge. The liver and kidney were carefully removed and placed into 10% formalin saline for histological analysis.

2.7 Procedures Used for Haematological

Packed Cell Volume (PCV), Haemoglobin level (Hb), White Blood Cells count (WBC), platelets and red blood cell indices (MCV, MCHC, and MCH) were analyzed using the methods outlined[9]. All other reagents used were of analytical grade.

2.8 Histopathological Studies

The treated and control rats organs were taken out. They were weighed and examined for the evidence of gross lesions. Similar samples were fixed in 10% formalin solution, dehydrated in graded (70-90%) alcohol, cleared in xylene, and placed and embedded in paraffin wax. To perform histology of tissues, 5-6 μm sections were prepared using Microtome. These sections then deparaffinated in xylene, passed through 70% to 90% alcohol, and stained with hematoxylin and eosin (H&E). The slides prepared by this process were observed under light microscopy.

2.9 Statistical Analysis

One-way analysis of variance (ANOVA) with the RTM Statistic software package, version 3.0.3 and excel package were used for numerical analysis. The normal distribution of the data and the homogeneity of variance were tested by Bartlett homogeneity test. One-way ANOVA with a Tukey test post-hoc was used to identify statistical changes among groups. A p-value of ≤ 0.05 was considered statistically significant.

3. Results

3.1 Effect on Body Weight

Body weight tests have shown that the extract application for two weeks, have affected body weight mainly with large doses as shown in Figure-(3.1), where the weight has increased by about 50% of its original value with the dose of 1000 mg/kg and reached an increase of about 60% at the dose of 2000 mg/kg.

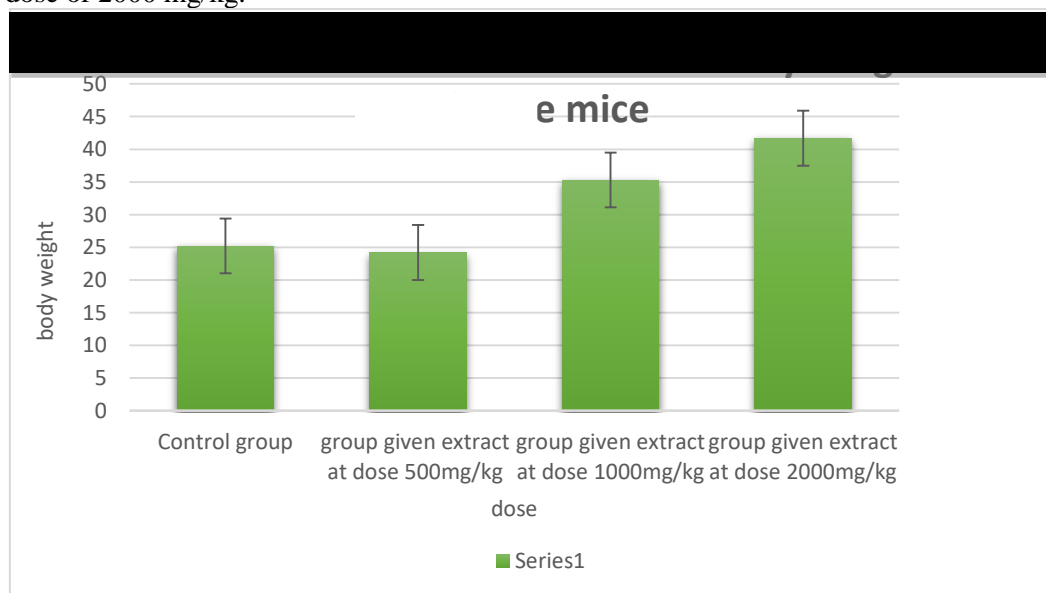


Figure 3.1-Effect of Senna Alexandria extract on body weight of mice for two weeks.

3.2 Hematological Findings

Results have shown that with mice, the *Senna Alexandria* extract increase RBC,HGB, HCT and PLT mainly in high doses. While there is high increase in PLT value in group treated with 500mg/kg b.w Table-(3.3). As while RBC,HGB,HCT increase with doses of 500, 1000 and 2000 mg/kg, as compared to the control group, they show slight drop with the dose of 1000 mg/kg relative to the 500 and 2000 mg/kg doses, but PLT follows the same trend as with group. That represent an agreement with[10,11] as in Table-(3.3)

Table 3.3-effect of Senna Alexandria on blood parameter of mice

Hematology	Unit	Control	Group given extract at dose 500mg/ kg b.w	Group given extract at dose 1000mg/ kg b.w	Group given extract at dose 2000mg / kg b.w
WBC	X10 ³ /μl	3.67±0.1	0.61±0.1*	2.44±0.1	1.35±0.1
RBC	X10 ⁶ /μl	7.19±0.2	7.12±0.2	3.02±0.2	8.14±0.2*
HGB	g/dl	12.68±0.1	10.88±0.1	8.40±0.1	14.26±0.1*
HCT	%	36.5±0.1	32.9±0.1	25.2±0.1*	43.3±0.1**
MCV	fl	50.8±0.2	46.2±0.2	86.75±0.2***	53.2±0.2
MCH	pg	17.6±0.1	15.3±0.1*	27.81±0.1	17.5±0.1
MCHC	g/dl	34.7±0.1	33.1±0.1	33.28±0.1	32.9±0.1
PLT	X10 ³ /μl	722±0.2	911±0.2**	881±0.2*	812±0.2*

*Significantly difference from the control, $p < 0.05$

3.3 Histopathological Changes

3.3.1 Kidney

Histological readings showed no abnormalities in kidney in treated mice Figure-(3.3), in compare to control Figure-(3.2), the sections of kidneys at dose of 500 mg/kg, had revealed sub capsular tubular vascular degeneration and the cortical region revealed marked interstitial nephritis with marked of lymphocytes associated with tubular vascular degeneration Figure-(3.4 and vascular degeneration Figure-(3.6 and 3.7), while the sections of dose 2000 mg/kg, were similar to these in concentration of 1000mg/kg, but at this concentration the degenerative changes were extended to involve some of the glomeruli, the changes revealed tubular vascular degeneration, congestion of inter tubular blood vessels, tubular necrosis and marked metaplasia of parietal epithelium of bowman capsule with marked dilation of bowman space (Fig.3.8 and 3.9).

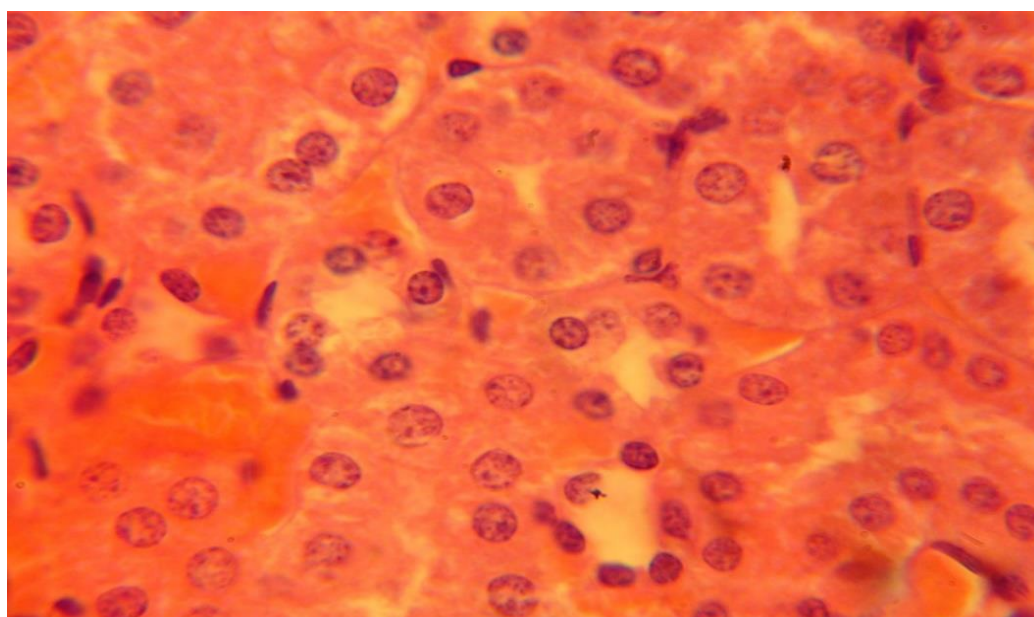


Figure 3.2-Microscopic section of kidney (control)(H&E stain, 100x.)

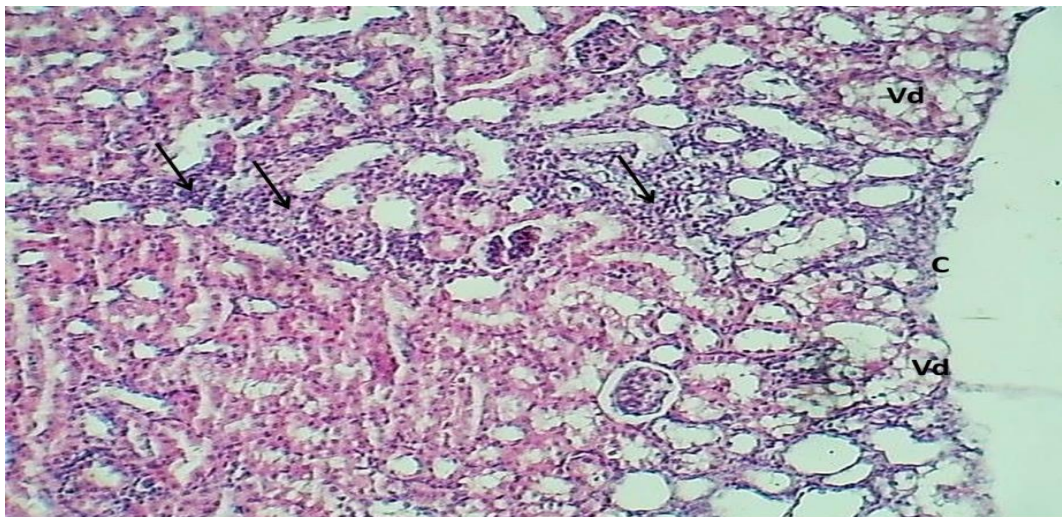


Figure 3.3-Microscopic section of the kidney with dose (500mg/kg) Senna Alexandria extract shows: capsule (C), vascular degeneration (Vd) & infiltration of lymphocytes (arrows). (H&E stain, 40X)

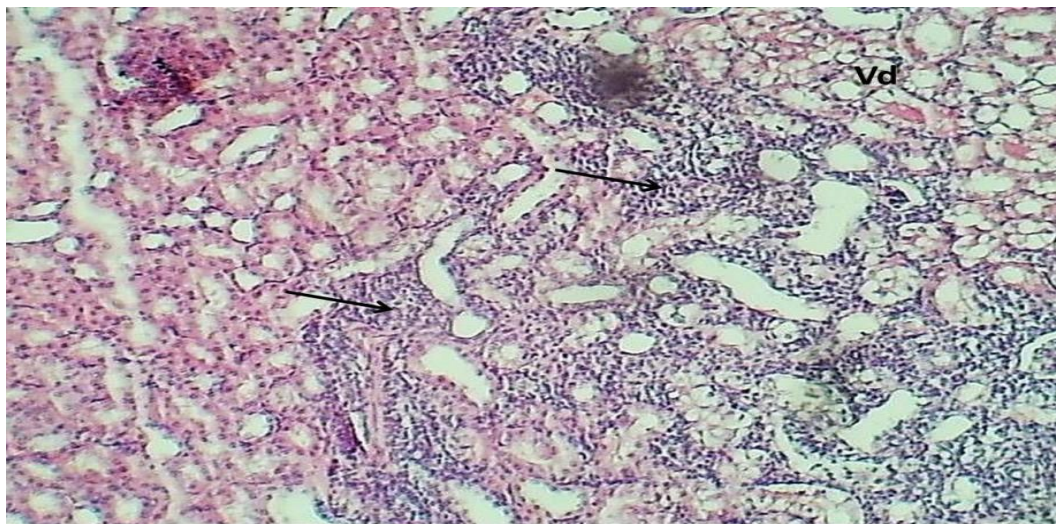


Figure 3.4-Microscopic section of the kidney with dose (500mg/kg) Senna Alexandria extract shows: Vascular degeneration (Vd) & infiltration of lymphocytes (arrows).. (H&E stain 100X)

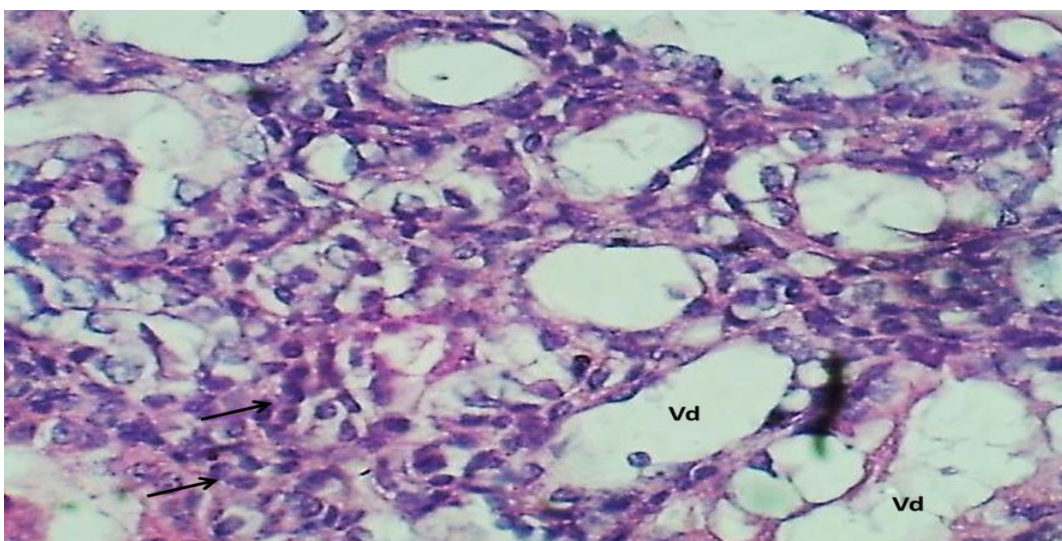


Figure 3.5-Microscopic section of the kidney with dose (500mg/kg) Senna Alexandria extract Shows: vascular degeneration(Vd)&infiltration of lymphocyte(arrows) (H&E stain 40X)

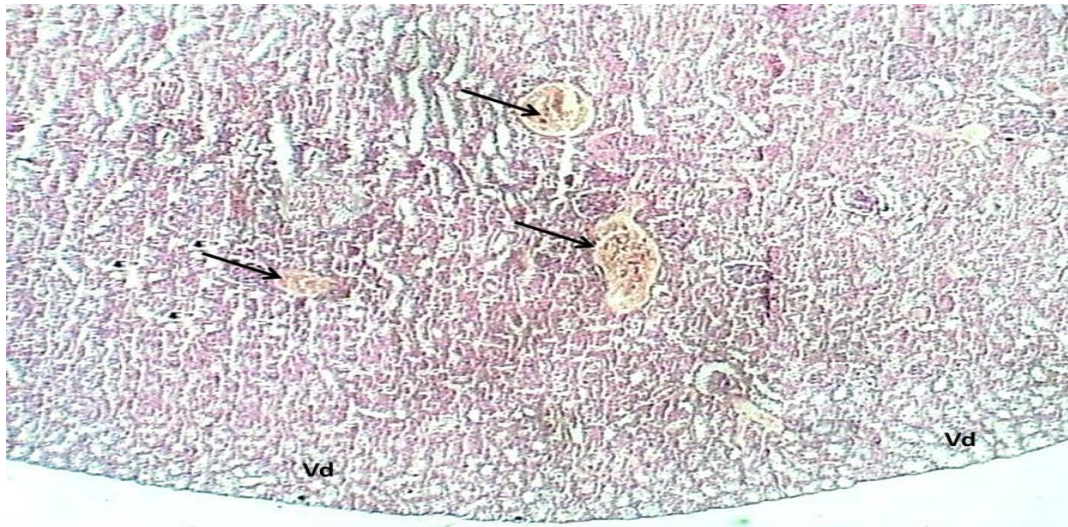


Figure 3.6-Microscopic section of the kidney with dose (1000mg/kg) Senna Alexandria extract shows: Vascular degeneration (Vd)& multiple focal hemorrhage (arrows).. (H&E stain 40X)

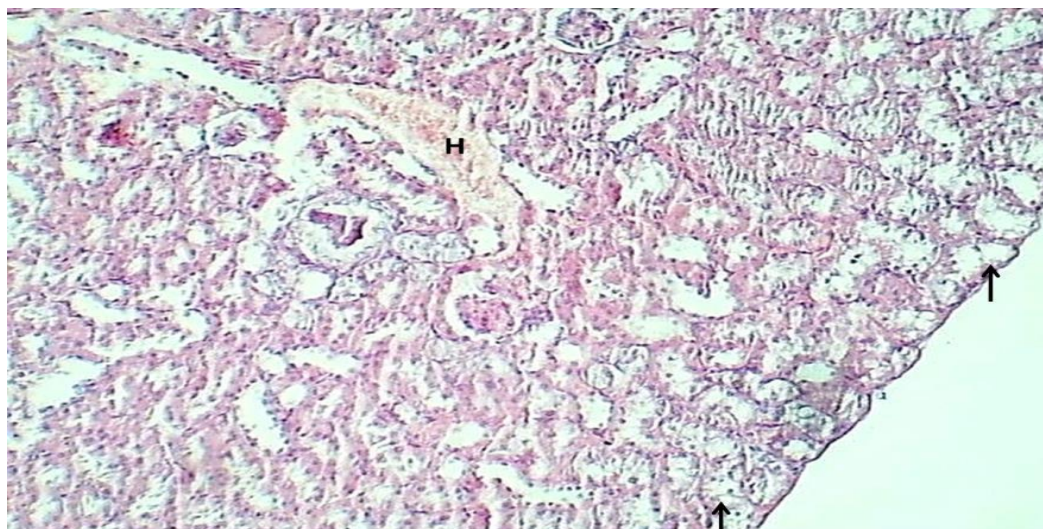


Figure 3.7-Microscopic section of the kidney with dose (1000mg/kg) Senna Alexandria extract shows: Vascular degeneration (arrows) & focal hemorrhage (H).. (H&E stain 100X)

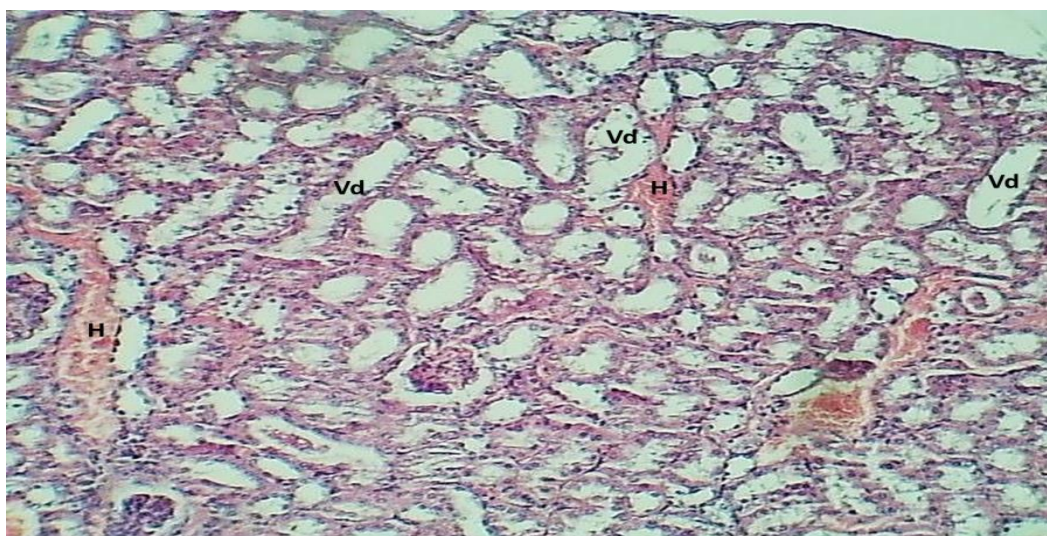


Figure 3.8-Microscopic section of the kidney with dose (2000mg/kg) Senna Alexandria extract shows: Vascular degeneration (arrows) & focal hemorrhage (H). (H&E stain 40X)

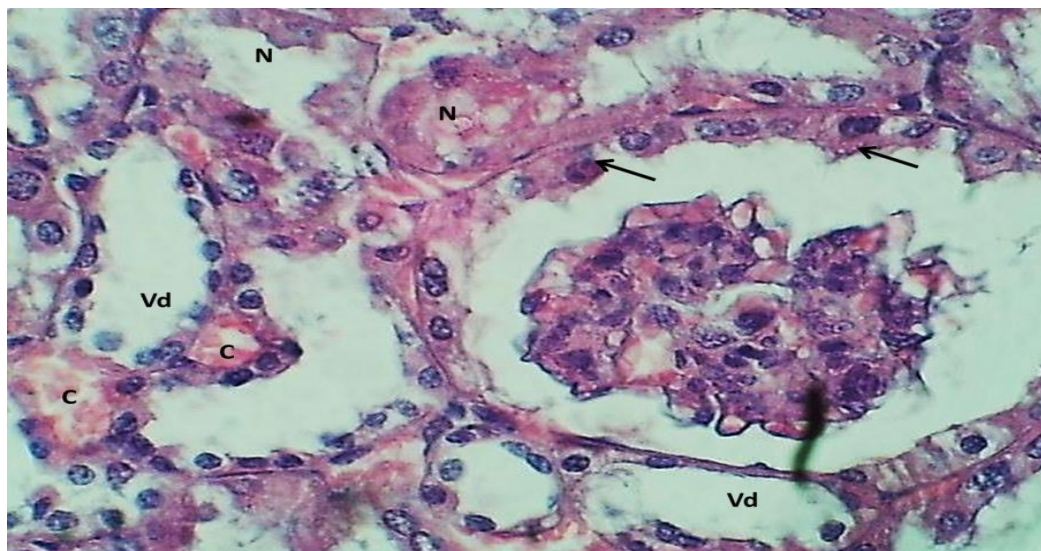


Figure 3.9-Microscopic section of the kidney with dose (2000mg/kg) Senna Alexandria extract shows: tubular vascular degeneration (Vd), congestion of inter tubular blood vessels (C), tubular necrosis (N) & marked metaplasia of parietal epithelium of Bowman capsule (arrows). (H&E stain 40X).

3.3.2 Liver

Histological studies revealed no abnormalities in liver in treated mice. In compare to control group (Fig.3.10), the sections of liver showed only in 500 mg/kg moderate central venous congestion, periportal steatosis with marked biliary hyperplasia and moderate hepatitis which characterized by multifocal hepatic necrosis infiltrated with mononuclear leukocytes mainly lymphocytes other section showed finding of vascular amyloidosis Figure-(3.11, 3.12, 3.13 & 3.14). At 1000 mg/kg, the sections of liver showed moderate central venous congestion and vascular amyloidosis Figure-(3.15). The liver parenchyma showed necrosis and atrophy of hepatocytes, other hepatocytes revealed variable stages of necrosis characterized by nuclear hypertrophy and karyorrhexis while other revealed hypnosis (Fig.16), while at a dose of 2000mg/kg, the sections of liver parenchyma had showed mild variable stages of nuclear changes (hypertrophy and karyorrhexis) Figure-(3.17).



Figure 3.10-Microscopic section of liver (control) H&E stain, 100x



Figure 3.11-Microscopic section of liver with dose (500mg/kg) of Senna Alexandria extract shows: amyloid deposit (Am) & multi focal necrosis (arrows) (H&E stain 100x.)

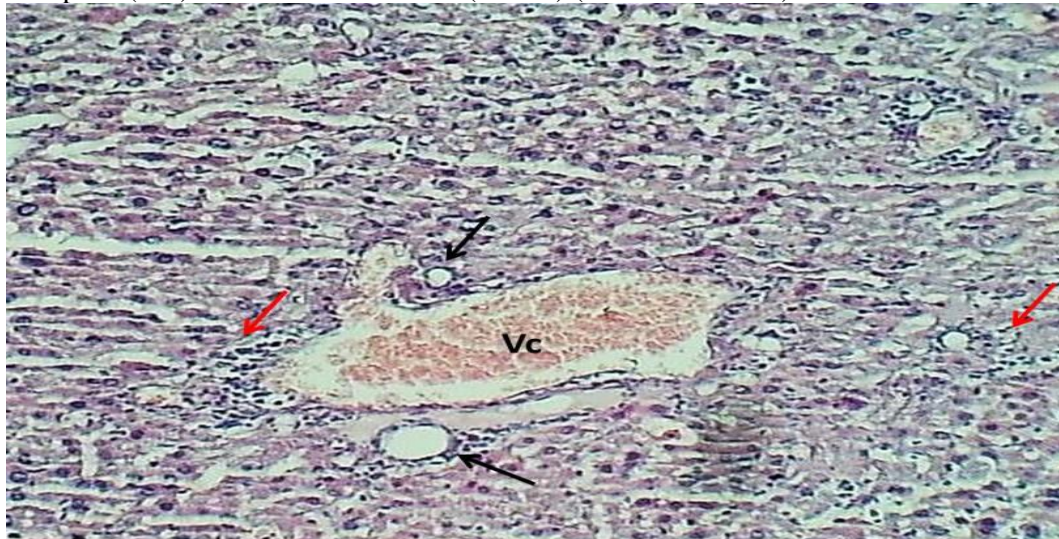


Figure 3.12-Microscopic section of liver with dose (500mg/kg) of Senna Alexandria extract shows: venous congestion (Vc), biliary hyperplasia (Black arrows) & multi focal necrosis (arrows) . (H&E stain 100x.)

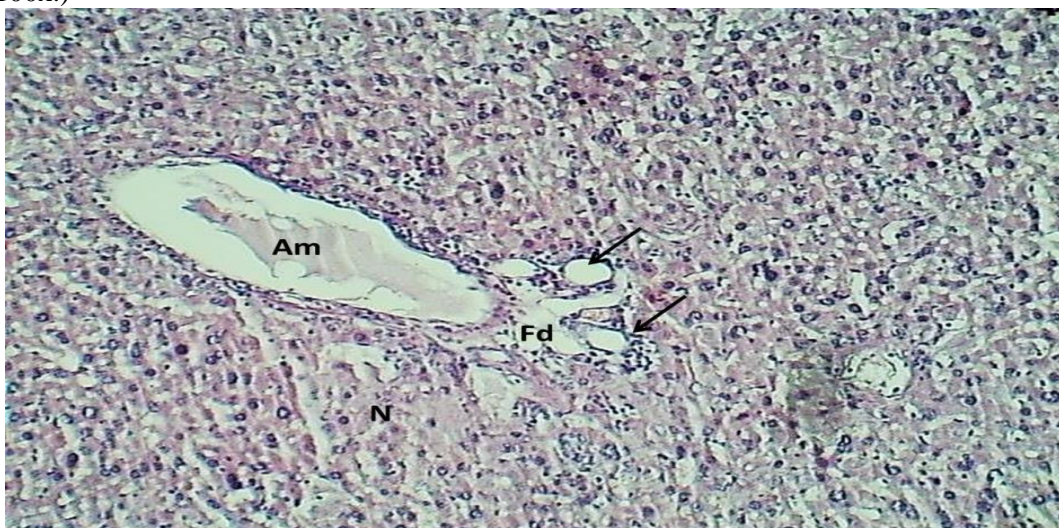


Figure 3.13-Microscopic section of liver with dose (500mg/kg) of Senna Alexandria extract shows: fat droplet (Fd), amyloidosis (Am), biliary hyperplasia (Black arrows) & necrotic hepatocytes (N). (H&E stain 100x.)

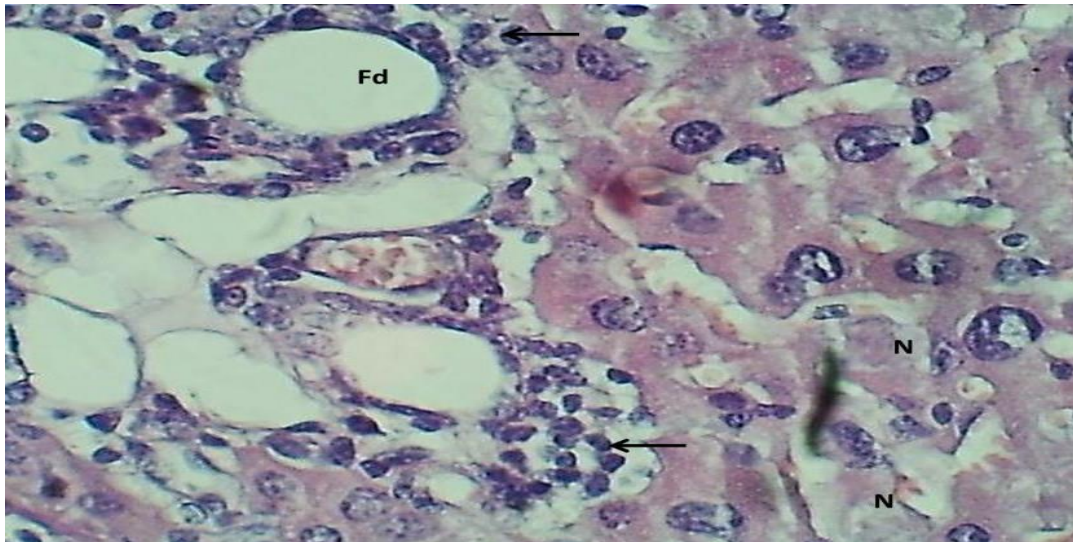


Figure 3.14-Microscopic section of liver with dose (500mg/kg) of Senna Alexandria extract shows: fat droplet (Fd), inflammatory cells (Black arrows) & necrotic hepatocytes (N) . (H&E stain 40x.)

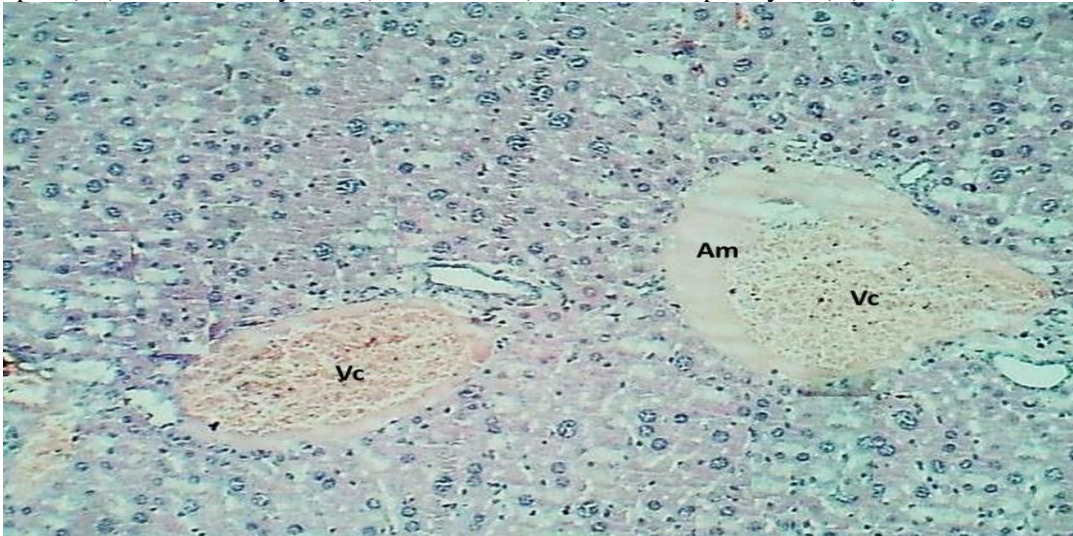


Figure 3.15-Microscopic Section of liver with dose (1000mg/kg) of Senna Alexandria extract shows: vascular amyloidosis (Am) & venous congestion (vc.) (H&E stain. 100X)

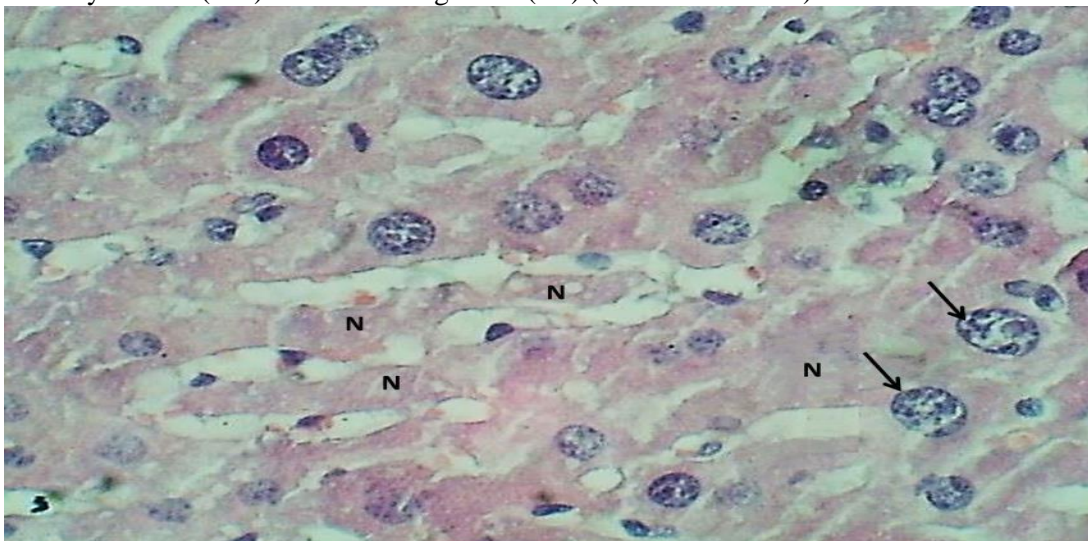


Figure 3.16-Microscopic Section of liver with dose (1000mg/kg) of Senna Alexandria extract (H&E stain. 40X) shows: necrotic and a trophic hepatocytes (N) & hypertrophied karyorrhexis nuclei (arrows).

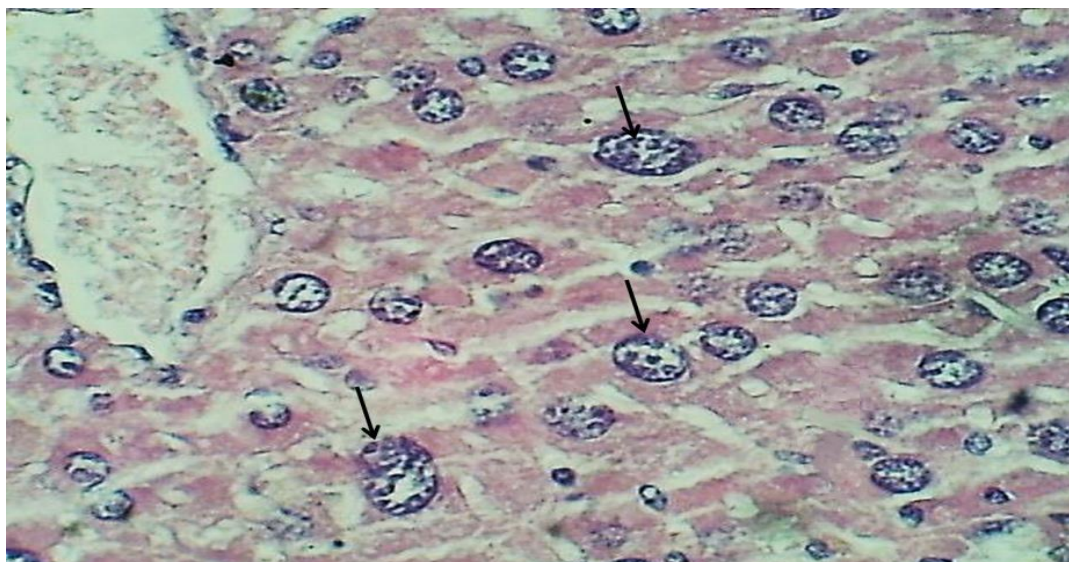


Figure 3.17-Microscopic Section of liver with dose (2000mg/kg) of Senna Alexandria extract shows hypertrophied and karyorrhexis nuclei (arrows) (H&E stain. 40X).

4.2 Discussion

Medicinal plants vary in potency and may contain complex pharmacological phytochemical materials, which may cause toxicity and adverse effects. Various anthranoids obtain from Senna and the most important are sennosides A and B, and aloe-emodin, emodin and chrysophanol [12,13]. The toxicological and mutagenic status of the senna crude extract, however, is less characterized. A study by [14], the laxative effect and acute toxicity of Senna extracts were investigated and could be separated from leaf extract. The most active laxatives and toxic are sennosides A + B the lowest toxicity and fractions with very low laxative activity have the highest acute toxicity. These may be active molecules in the Senna extract that could be responsible for its toxicity. The hydroxyanthraquinones emodin and aloe-emodin gave positive results in genotoxic assays in *Salmonella typhimurium*, V79-HGPRT, rat hepatocytes, and mouse fibroblasts [15]; on the other hand, in another study, such genotoxicity assay in was not observed [16]. In a study by [17], the induction of neoplasms in rat liver, subjected to a diet containing 1% hydroxyanthraquinones for 480 days, was induced. Therefore, standardization of herbal formulations of Senna is essential in order to assess their efficacy and safety.

The acute toxicological investigation (LD50) of the alcoholic extract has showed no mortality even at high doses so that in clarifies the plant extract is nontoxic or slightly toxic according to Hodge and Sterner scale that showed no clinical symptoms at low doses (500 mg/kg), while at (1000 & 2000 mg/kg) tacky cardiac and increase in breathing has appeared also it showed at higher doses (3000,4000,5000 mg/kg) tacky cardiac increase in breathing and tendency to loneliness. The results of the clinical signs are very clear in the high doses considered as a kind of side effects of the extract and are inversely proportional to the dose given may be due to the effectiveness of the active substances contained in the plant extract increased according to dose given or may be depending on the type of solvents used in extraction, concentration, the extraction method and the method of drying after extraction, as all of these things have been shown in the form of side clinical signs.

The study also has demonstrated the hematological parameters variation within the reference range in order to examine the impacts of the alcoholic extract of Senna Alexandria. Results have shown that within the male mice increase in RBC HGB HCT appears mainly in high doses (2000 mg/kg), while high increase in PLT appears in group that was treated with 500mg/kg.

The results have shown that extract causes hematological changes through enhanced levels of HGB, RBC and PLT in all group of mice. That means the plant has effect on hematological parameters through increase defensive mechanisms of body against infection, hence, the plant can be used to treat any fault in hematological system through enhancement of the RBC, PLT counts and PLT which agrees with the results of [18,19].

The animals gained weight after 14 days of administration be due to increase the food intake or induced obesity and fat accumulation by regulation of lipid metabolism in mice [20], or may be the extract controls weight gain by induced lipid accumulation and reduced energy expenditure, and that its action mechanism involves the upregulation of mitochondrial biogenesis in skeletal muscle cells [21].

The sections of kidneys at dose of 500 mg/kg, had revealed sub capsular tubular vascular degeneration and the cortical region revealed marked interstitial nephritis with marked of lymphocytes associated with tubular vascular degeneration, while with a dose of 1000 mg/kg, the sections of kidneys had revealed sub capsular tubular vascular degeneration and the cortical region revealed marked multi focal hemorrhage with mild tubular vascular degeneration in almost similar manner, the sections of dose 2000 mg/kg, the degenerative changes were extended to involve some of the glomeruli. These changes revealed tubular vascular degeneration, congestion of inter tubular blood vessels, tubular necrosis and marked metaplasia of parietal epithelium of bowman capsule with marked dilation of bowman space.

The sections of liver showed only in 500 mg/kg a moderate central venous congestion, peri portal steatosis with marked biliary hyperplasia and moderate hepatitis which characterized by multi focal hepatic necrosis infiltrated with mononuclear leukocytes mainly lymphocytes. Other section showed findings of vascular amyloidosis. At 1000 mg/kg, the sections of liver showed moderate central venous congestion and vascular amyloidosis. The liver parenchyma showed necrosis and atrophy of hepatocytes, other hepatocytes revealed variable stages of necrosis characterized by nuclear hypertrophy and karyorrhexis while other revealed hypnosis, while at a doe of 2000mg/kg, the sections of liver parenchyma had showed mild variable stages of nuclear changes (hypertrophy and karyorrhexis).

Meanwhile the other study by salawa *et al.*[16] using cross apteryx febrifuge saw inflummentary changes histology in liver by infiltration of lymphocyte at portal and central vein of mice treated with at dose level 500 and 1000 mg/kg weight and this shows that the extract exerted deleterious effect on the liver .The liver is capable of regenerating damaged tissue hence the liver function may not be impaired following an insult from toxicant[16]. While Dcgott and poct [17,18] reported that sinusoidal dilatation characterized by widening of hepatic capillaries which may involve entire lobule or media area and can encountered in different situations or infiltrations of sinusoid by various benign of malignant cells[19] .

Based on the result of kidney tissue the observation has seen to suggest that certain medicinal plant might have renal protective ability to prevent kidney dysfunction by accelerating regeneration, harmful effect including polyuria causing dehydration acute renal failure and stone formation [21].

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