Original Research Article

Toxic Activity of *Tinospora bakis* (Irg al-hagar) roots in Wistar Albino Rats

ABSTRACT

Aim: This study aims to evaluate the toxic effect of *Tinospora bakis* roots on body weight, haematology, biochemistry and histopathology on rats.

Methodology: Twenty four male Wistar albino rats were divided into four groups, each of 6. For sub chronic toxicity, the aqueous extract was administered orally at a dose of 50 and 500 and 2000 mg/ kg - for four weeks- to group 2, 3 and 4, respectively whereas Group 1 was kept as a control. Clinical signs and mortality were observed daily. The weights of the animals were recorded weekly. Blood samples were collected for hematology and biochemistry analysis. Specimens of Liver and kidney were kept in 10% formalin for histopathology.

Results: The results revealed that all animals in the four groups survived, and no mortality was recorded. The body weights of the animals increased in group 2 and 3, decreased in group 4. The extract had no adverse effects on haematology, biochemistry and histology of rats at doses of 50 and 500 mg/ kg, but caused significant alteration at dose 2000 mg/kg White blood cells (WBCs) were significantly ($P<0.05$) increased; Red blood cells (RBCs), Haemoglobin (Hb) and Packed cell volume (PCV) were significantly ($P<0.05$) decreased. Total protein and albumin were significantly ($P< 0.05$) decreased whereas Urea, creatinine, Alanin Transaminase (ALT), Asparate Transaminase (AST) and Alkaline phosphatase were significantly elevated. These findings correlated with histopathological changes on liver and kidney.

Conclusion: The low doses of *T. bakis* aqueous extract were safe, but the high dose caused hepato-renal toxicity.

Key words: Tinospora bakis, aqueous extract, toxicity, rats.

1. INTRODUCTION

Many plants contain chemical constituents which are used for different medical purposes. However, over dosage of plant products containing medical compounds may cause toxic effects when introduced into the body [1]. The toxic phytochemicals produced by plants include alkaloids, sulpher, phenol, tannin, proteins and enzyme inhibitors [2]. Toxins have direct and indirect mechanisms of actions on the most frequently induced organs (liver, kidney, brain, lung, intestine and others). The mechanisms of actions include direct and indirect damage of tissue, effect on function and genetic defect [3].

In view of the various medical uses and pharmaceutical results of *Tinospora bakis*, Preliminary screening of the plant for *in vivo* toxicity is needed

Tinospora bakis (A.Rich) Miers in Hook. Niger. Fl.: 215 (1849) belongs to the family Menisperaceae. It is known in Sudan as Irg al - Hagar. It is found at low plains and distributed in West and Central Africa [4].

Palamtine isoquioine alkaloid was isolated from the plant [5]. The woody part of the root of *Tnospora bakis* (A. Rich) Miers has a high character in West Africa as a diuretic and febrifuge [6]. In addition, the root is used against jaundice, hematuria, bilious fever and yellow fever, malaria and schistosomiasis. Externally, the decoction is applied against various skin problems. The leaves are similarly used as diuretic and tonic. The aqueous extract of the root containing the alkaloid fraction showed moderate activity against *Plasmodium falciparum* [7]. Syrup of the aqueous root extract induced a significant increase of the billiary secretion in rats [8]. Columbine, in small doses, was found to increase the bile, stomach and intestine secretion; in high doses it produced greasy degeneration of the liver. Palmatine showed a stronger antipyretic effect in rabbits [9]. A lyophilized aqueous extract of *T.bakis* at concentration of 1-4 mg/ml showed *in vitro* hepato-protective activity after treatment with CCl_4 [10]. In

mice, the aqueous extract of the root administered either intra peritoneal or subcutaneously showed LD_{50} of 360 and 425 mg/ kg, respectively.

On the other hand, intra peritoneal administration of *T.bakis* methanolic extract to rats at a dose of 100 mg/ kg was toxic [11]. *In vitro* study of the plant caused cytotoxicity on lymphoblast cells at concentration of 10 mg/ ml [12].

Other species of *Tinospora*, such as *T.tomentosa* aqueous and methanolic extracts were found to be non toxic in mice and rats at doses up to 3.5 g/ kg [13]. *Tinospora rumphii* is a folkloric medicinal plant that is widely distributed in Asia and Africa. It has been used to treat many diseases including jaundice which is a manifestation of liver damage [14].

Tinospora bakis, T.cordifolia, T.crispa, T.sinensis, T.smilacina, and *T.sagittata* have been reported to possess significant immunomodulatory effect [15].

In Sudan, the macerated roots of *T.bakis* are used for headache in folk medicine [4]. The plant extract investigated against *Plasmodium falciparum* [16], *Madurella mycetomatis* [17]. Furthermore, *Tbakis* was evaluated for trypanocidal [11], antitheilerial [12] and antidiabetic activity [18].

The objective of plant toxicity test is to clarify the toxic effects of the plant. The toxicity of *T. bakis* roots extract is necessary since this has not been previously done in depth.

2. MATERIALS AND METHODS

2.1 PLANT COLLECTION

The roots of *T.bakis* were collected from Ingassana hill in East- south of The Sudan. The plant was identified and authenticated by a at the Medicinal and Aromatic Plants Research Institute, Khartoum, Sudan. The voucher specimen has been deposited in the herbarium museum of the Institute. The plant air- dried in the shade, coarsely powdered and kept in polythene bags at room temperature.

2.2 ANIMALS

Clinically normal, twenty four male Wistar albino rats, 4-5 weeks of age, weighing (114-117 g) were brought from the Medicinal and Aromatic Plants Research Institute, Khartoum, Sudan. The animals were kept in metal cages to adapt for one week before the start of the experiment. The rats were fed with a standard diet which is manufactured commercially for poultry (Layers), and vegetables. Feed and water were provided *ad libitum*. This work was carried out according to the international regulations for the use of laboratory animals.

2.3 PREPARATION OF PLANT EXTRACT

The plant extract was prepared as described previously [19]. Hot distilled water (500 ml) was added to100 g of the coarsely powdered plant roots and left to cool down with continuous stirring at room temperature. The extract was then filtered through Whatman No. 1 filter paper, and then transferred to the freeze-drier (Trivac, U.S.A.). The yield percentage of the extract was 7.89 %.

The required weight of the extract for each group was calculated according to the dose, dissolved in 6 ml of distilled water. The volume of the extract administered orally to each animal based on the body weight.

2.4 EXPERIMENTAL DESIGNS

Twenty four male Wistar albino rats weighing were divided into four groups, each of 6 rats. Group 2, 3 and 4 were used for testing of sub chronic toxicity, and group 1 was kept as a control. The extract was given at one of the fixed dose level (50, 500 and 2000 mg/kg).

2.4.1 Screening of the aqueous extract of *T.bakis* roots for toxicity

The aqueous extract of the plant was administered orally to the rats in group 1, 2 and 3 at doses of 50, 500 and 2000 mg/ kg/ day, respectively for four weeks; keeping group 1 as a control.

Clinical signs of toxicity and/or mortality were observed daily. The weights of the rats were recorded at the day of dosing, at weekly intervals thereafter, and at the time of death or when the animals were sacrificed.

2.5 BLOOD COLLECTION FOR HAEMATOLOGICAL AND BIOCHEMICAL ANALYSIS

Blood samples were collected weekly-starting from week zero (Control) - from the orbital sinus of rat's eye - in Ethylene Diamine Tetra acetic acid (EDTA) and plain vacutainers, for hematological and biochemical tests, respectively. Sysmex Haematology System KN-21N/Germany and Sysmex Biochemistry System / Germany) instrument were used for analysis. The procedures were carried out as described in the manual of the automated machines.

2.6 PATHOLOGICAL EXAMINATION

Rats in group 1, 2, 3 and 4 were sacrificed at the end of the experiment. Specimens of normal and abnormal liver and kidney were fixed in 10% neutral buffered formalin and processed for histopathological examination.

2.7 STATISTICAL ANALYSIS

The data collected during the study were analyzed using the computer program SPSS version 20. The statistical analysis was done using One Way ANOVA, followed by Duncan multiple comparison test. The data are expressed as mean ±SD. The results with $P<0.05$ were considered significant.

3. RESULTS

3.1 EFFECT OF THE EXTRACT ON MORTALITY AND BODY WEIGHT

There was no mortality recorded even at the highest dose (2000 mg/kg) after oral administration of the extract.

The aqueous extract of *T.bakis* roots was well tolerated by the animals after oral administration of the doses 50, 500 and 2000 mg/ kg to group 2, 3 and 4, respectively.

The effect of the extract on body weights of rats was shown (Fig 1). The extract significantly ($P<0.05$) increased the bodyweights of rats in group 1, 2 and 3; decreased in group 4.

3.2 EFFECT OF THE EXTRACT ON HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS

The plant extract altered the haematology and biochemistry of rats in group 4 only.

The hematological changes on blood of rats administered orally aqueous extract of *T.bakis* were summarized (Table 1). WBCs, RBCs, Hb and PCV were not affected in group 2 and 3, but significantly ($P<0.05$) changed in group 4.

The toxicological effects of the extract on the biochemical parameters were presented (Table 2). Oral administration of the aqueous extract at doses of 50 mg/ kg (group 2) and 500 mg/ kg (group 3) had no effect. However, a dose of 2000 mg/ kg (group 4) was significantly ($P< 0.05$) altered all the parameters.

3.3 HISTOPATHOLOGICAL CHANGES

Necropsy of rats in group 1, 2, 3 showed normal livers and kidneys. Though, the extract caused histopathological changes in liver and kidney (Group 4). The liver characterized by vesicular nuclei and accumulation of cytoplasm at the boundaries of hepatocytes (Fig. 2 B), compared with the control (Fig. 2 A). The kidney revealed dilated and segmented glomerular tuft (Fig. 3 B) referring to the control (Fig. 3 A).

4. DISCUSSION

There were no changes on the animals' behavior and/or mortality recorded after oral administration of repeated doses 50, 500 and 2000 of the aqueous extract of *T.bakis* roots. Similar findings mentioned that different doses of the extract were safe up to a dose of 2000 mg/ kg [18]. However, the current result disagree with the author [4] who found that the aqueous extract of the root administered either intra peritoneally or subcutaneously to mice showed LD_{50} of 360 and 425 mg/ kg, respectively could be due to the animal spp. or the route of administration. On the other hand, the dose 2000 was toxic but not fatal. Relevant to this finding is the previous study which revealed that the aqueous and methanolic extracs of *T.tomentosa* were found to be non toxic in mice and rats at doses up to 3.5 g/ kg [9].

In the present work, the increase in the body weight confirmed that the extract of *T.bakis* did not have general toxic effects and influence on animal food intake at doses of 50 and 500 mg/ kg. The findings were supported by previous study in which the aqueous extract of the plant improved the body weight of diabetic rats at a dose of 400 mg/kg/day [18]. However, decrease in bodyweight (group 4) indicated abnormality or toxicity which influenced food consumption and metabolism.

On the other hand, changes on haematological as well as biochemical parameters are indicators of toxicities in toxicological studies [20]. This means that the doses of 50 and 500 mg/kg had no toxic effects on both parameters as well as the histological. Though, other author found that intra peritoneal administration of *T.bakis* methanolic extract to rats at a dose of 100 mg/ kg was toxic; causing a variable decrease in haematological values and an increase in serum enzymes [11]. This could be due to the variation in the solvent used, the time of collection and maturity of the plant.

The combined effects of physiological and chemical factors in the metabolism system of animals could lead to increase in WBCs [21]. This could be the case with rats in the present study (group 4). The mechanism of action of WBCs and its component are defensive against foreign substances. The alteration in RBCs and Hb may be due to defective haematopoiesis inhibited erythopoiesis or increase in destruction of red blood cells [22, 23].

Additionally, the alteration in biochemical parameters is indicator of toxicity. Decrease of serum albumin could be indicative of impaired liver excretory and synthetic function. The observed increase ($P< 0.05$) of serum urea and creatinine in group 4 suggest renal malfunction [24]]. Primary and secondary hepatic diseases can cause elevation of both ALT and AST [25]. The increment in the activities of transaminases indicates that the plant may induce hepatic parenchyma injury, hepatic dysfunction and leakage of these enzymes from liver cytosol to the blood stream [26, 27]. Overall, the biochemical changes were correlated with the histopathological findings on the liver and kidney.

CONCLUSION

The results revealed that the aqueous extract of *T.bakis* roots at low doses was safety, but the high dose may cause hepato renal toxicity. A further work is needed for determination of LD_{50} and LD_{99}. The

phytochemical analysis is recommended to define the toxic compounds that may exist; the mechanism of action is considered necessary.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that “Principles of laboratory animal care” (NIH Publication No. 85-23, revised 1985) were followed, as well as national laws were applicable. The protocol for the use of laboratory animals in this study was approved by the Ethical Approval No. EA /0019/ 2018, The Sudan Veterinary Council, Ministry of Cabinet, Republic of The Sudan.

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Table1. Hematological changes on the blood of rats given aqueous extract of *Tinospora bakis*

Group No.	Week No.	Dose (mg/kg)	WBCs ($\times 10^3/mm^3$)	RBCs ($\times 10^6/mm^3$)	Hb (g/dl)	PCV (%)
1	0	0	5.90 ± 0.14	6.3 2 ± 0.08	11.68 ± 0.19	37.50± 0.40
	1		5.90 ± 0.09	6.35 ± 1,05	11.72 ± 0.21	37.53 ±0.39
	2		5.92 ± 0.80	6.35 ± 0.08	11.72 ± 0.21	37.55 ±0.39
	3		5.93 ± 0.10	6.38 ± 0.08	11.73 ± 0.15	37.57 ±0.42
	4		5.91 ± 0.15	6.37 ± 0.08	11.73 ± 0.15	37.57 ±0.43
2	0	50	6.77 ± 0.10	6.95 ± 0.10	11.93 ± 0.14	38.13 ± 0.26
	1		6.77 ± 0.10	6.97 ± 0.05	11.95 ± 0.12	38.18± 0.26
	2		6.83 ± 0.08	6.93 ± 0.08	11.95 ± 0.12	38.18 ± 0.26
	3		6.85 ± 0.08	6.93 ± 0.05	11.93 ± 0.14	38.12 ± 0.26
	4		6.85 ± 0.08	6.93 ± 0.10	11.93 ± 0.14	38.12 ± 0.26
3	0	500	7.05 ± 0.19	6.80 ± 0.14	11.82 ± 0.17	38.02 ± 0.17
	1		7.45 ± 0.76	6.82 ± 0.15	11.80 ± 0.21	38.00 ± 0.17
	2		7.12 ± 0.15	6.82 ± 0.19	11.77 ± 0.23	38.00 ± 0.17
	3		7.12 ± 0.15	6.77 ± 0.20	11.75 ± 0.26	37.85 ±0.20
	4		7.15 ± 0.14	6.68 ± 0.16	11.58 ± 0.23	37.53 ± 0.22
4	0	2000	6.65 ± 0.19	7.05 ± 0.24	12.02 ± 0.25	38.45 ± 0.33
	1		8.95 ± 0.24*	4.93 ± 0.44*	10.77 ±0.41 *	36.23 ± 0.31*
	2		9.00 ± 0.22*	4.72 ± 0.38*	10.50 ± 0.41*	36.00 ± 0.24*
	3		9.06 ± 0.23*	4.70 ± 0.46*	10.48 ± 0.40*	36.00 ± 0.28*
	4		9.06 ± 0.21*	4.71 ±0.42*	10.48 ± 0.40*	36.00 ± 0.28*

*The data presented as Mean ± SD, *P< 0.05 is significantly different from the control, n=6*

Table 2. Biochemical changes on blood of rats after administration of aqueous extract of *Tinospora bakis*

Group No.	Week No.	Dose (mg/kg)	Total Protein (g/dl)	Albumin (g/dl)	Billirubin	Urea (mg/dl)	Creatinine (mg/dl)	ALT (IU/L)	AST (IU/L)	ALP (IU/ L)
1	0	0	6.35±0.20	3.53±0.16	0.13±0.05	14.67±0.26	0.53±0.05	13.00±0.89	18.33±0.82	53.00±1.41
	1		6.39±0.18	3.55±0.31	0.13±0.05	14.68±0.23	0.53±0.05	13.05±0.90	18.33±0.82	53.00±1.41
	2		6.46±0.19	3.65±0.26	0.10±0.00	14.68±0.32	0.53±0.05	13.05±0.90	18.42 0.83	53.00±1.67
	3		6.47±0.25	3.73±0.23	0.10±0.00	14.70±0.28	0.58±0.04	13.08±0.94	18.42±0.83	53.17±1.17
	4		6.67±0.16	3.80±0.17	0.10±0.00	14.70±0.26	0.53±0.05	13.10±0.85	18.43±0.80	53.17±1.17
2	0	50	6.35±0.19	3.70±0.09	0.15±0.05	14.5 2±0.15	0.50±0.00	13.60±0.14	18.60±0.26	53.83±1.72
	1		6.47±0.18	3.70±0.09	0.15±0.05	14.5 2±0.15	0.50±0.00	13.60±0.14	18.62±0.24	53.83±1.72
	2		6.47±0.18	3.7 2±0.08	0.15±0.05	14.53±0.16	0.50±0.00	13.62±0.12	18.62±0.24	53.83±1.33
	3		6.43±0.16	3.72±0.08	0.15±0.05	14.53±0.16	0.50±0.00	13.62±0.12	18.65±0.23	53.83±1.17
	4		6.43±0.16	3.70±0.08	0.15±0.05	14.56±0.14	0.50±0.00	13.62±0.12	18.65±0.23	53.83±1.17
3	0	500	6.65±0.19	3.80±0.09	0.12±0.04	14.50±0.66	0.48±0.04	14.00±0.24	18.90±0.23	53.83±1.17
	1		6.65±0.19	3.80±0.06	0.12±0.04	14.50±0.66	0.48±0.04	14.03±0.27	18.95±0.18	53.83±0.89
	2		6.63±0.20	3.83±0.05	0.13±0.05	14.55±0.61	0.50±0.06	14.07±0.28	18.95±0.16	53.83 0.89
	3		6.60± 0.21	3.83±0.05	0.13±0.05	14.58±0.61	0.55±0.08	14.07±0.28	18.95±0.16	54.00±1.26
	4		6.60±0.21	3.80±0.00	0.13±0.05	14.43±0.87	0.55±0.08	14.08±0.21	18.93±0.18	54.00±1.26
4	0	2000	6.72±0.15	3.85±0.08	0.10±0.00	14.30±0.23	0.45 ±0.05	13.90±0.17	18.75±0.10	53.67±0.82
	1		5.45±0.19*	3.05±0.12*	0.57±0.05*	17.45±0.34*	0.68±0.08*	15.90±0.28*	21.47±0.26*	56.50 1.05*
	2		5.00±0.13*	2.00±0.08*	0.60±0.00*	17.97±0.36*	0.75±0.05*	16.13±0.28*	21.48±0.23*	57.00±1.26*
	3		5.00±0.16*	2.00±0.06*	0.60±0.04*	17.99±0.38*	0.76±0.06*	16.15±0.30*	21.50±0.20*	57.20±1.30*
	4		4.90±0.18*	2.00±0.07*	0.61±0.00*	17.98±0.40*	0.77±0.06*	16..20±0.34*	21.87±0.25*	57.50±1.32*

*The data expressed as Mean ± SD, *P < 0.05 is significantly different from the control, n= 6*

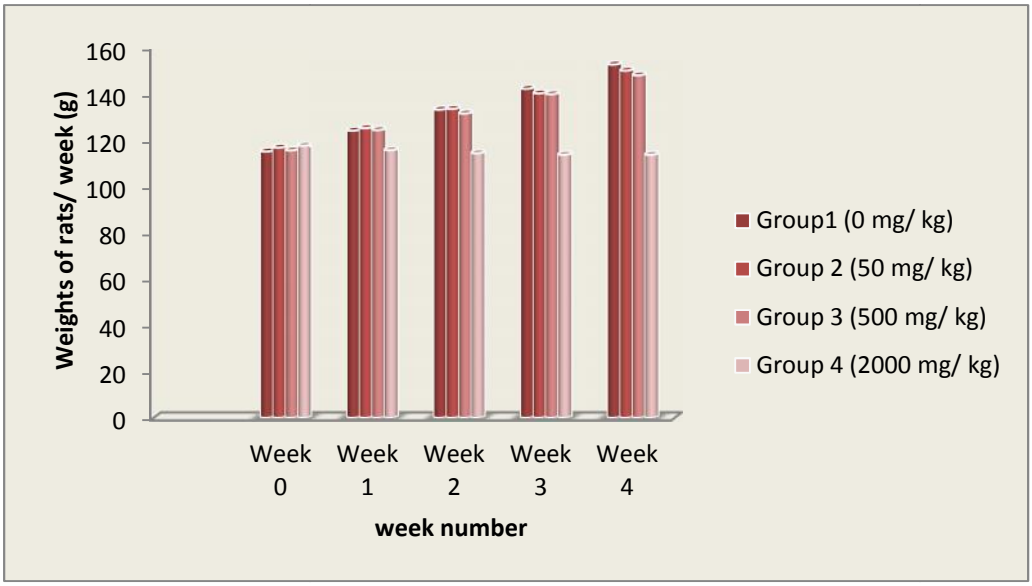
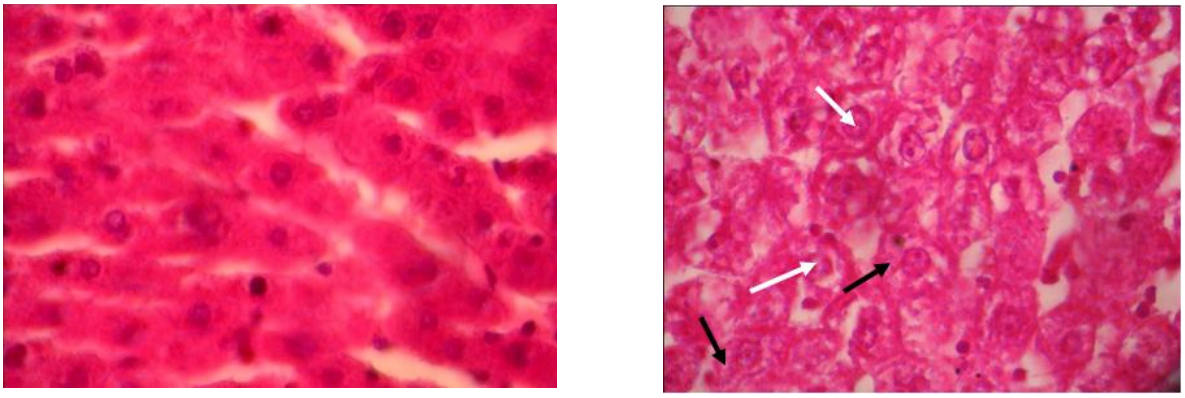
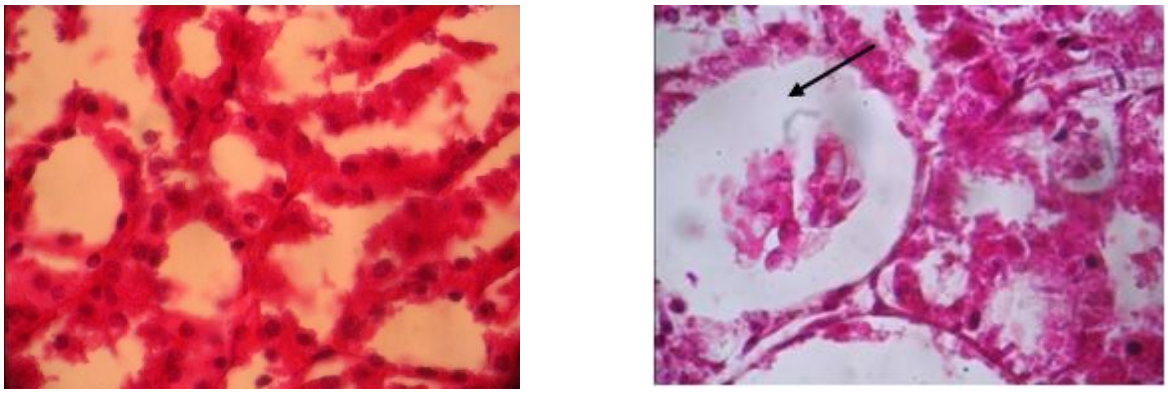


Fig.1 Effect of aqueous extract of *T.bakis* roots at different doses on bodyweight of rats per week



A B

Fig.2. Section of rat liver: (A) Normal control (group 1). (B) After oral administration of aqueous extract of *T.bakis* at a dose of 2000 mg/ kg (group 4) showed vesicular nuclei (white arrows) and accumulation of cytoplasm at the boundaries of hepatocytes (black arrows), H&E (× 10).



A B

Fig.3. Section of rat kidney: (A) Normal control (group 1). (B) Dosing of 2000 mg/ kg aqueous extract of *T.bakis* (group 4) dilated and segmented glomerular tuft (back arrow) were H&E (×40)