Original Research Article

Assessment of Sub chronic Toxicity of *Sonchus cornutus* in Rats

ABSTRACT

Aim: To assess the toxicity of the aqueous extract of *Sonchus cornutus* in Wistar albino rats.

Methodology: The aqueous extract of *Sonchus cornutus* aerial part was administered orally to rats in group 2, 3 and 4 at a dose of 50, 500 and 2000 mg/ kg, respectively for four weeks whereas, group 1 was kept as a control. The animals were observed daily for clinical signs and mortality. Weekly, the weights of the animals were recorded, and blood samples were collected for haematological and biochemical analysis. Specimens of Liver and kidney were kept in 10% formalin for histopathology

Results: The results revealed that all the animals in the four groups survived. The extract had no adverse effects on haematology, biochemistry and histology of rats at doses of 50 and 500 mg/ kg. But dose 2000 mg/kg proved to have significant alteration in White blood cells (WBCs), Red blood cells (RBCs), Haemoglobin (Hb) and Packed cell volume (PCV). In addition, total protein, albumin, urea, creatinine, Alanin Transaminase (ALT), Asparate Transaminase (AST) were significantly ($P<0.05$) changed. These findings correlated with the histopathological changes on liver and kidney.

Conclusion: The highest dose of *S.cornutus* aqueous extract (2000 mg/kg) was not fatal, but may have some toxic effects on liver and kidney.

Key words: Sonchus cornutus, aqueous extract, toxicity, rats.

1. INTRODUCTION

Medicinal plants are often assumed to be efficient and safe; however there are some reports on poisoning consecutive to plant based-medicine administration [1]. Thus, interest is accorded to toxic effects of plant extracts.

Many plants contain phytochemicals 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 [2]. The toxic phytochemicals include alkaloids, sulpher, phenol, tannin, proteins and enzyme inhibitors [3]. Toxins have direct and indirect mechanisms of actions on the most frequently induced organs (liver, kidney, brain, lung and intestine). The mechanisms of actions include direct and indirect damage of tissue, effect on function and genetic defect [4].

Sonchus cornutus has been used traditionally as a remedy to treat many diseases beside the biological activities. Sonchus was used as herbicide [5]. *Sonchus asper* methanolic extract had protective effect against CCl_4 induced kidney damage in rat [6]. Additionally, it possessed antioxidant activity and was used for treatment of liver and kidney disorders [7]. The plant was used in folk medicine for treatment of hormonal disturbance and oxidative stress [8]. The aqueous methanolic extract of *Sonchus asper* administered to rats at doses of 250, 500 and 1000 mg/kg exerted considerable antihypertensive activity [9].

Although extensive works have been conducted *on S.cornutus*, no conspicuous information on toxicity is available so far. Therefore, attention has been directed towards toxicity of the plant. *Sonchus cornutus* aqueous extract showed partial cytotoxicity at concentrations of 5000 and 10000 µg/ ml [10]. However, little was made available for other species such as *S.arvensis*, *S.oleraceus* and S.*transcaspicus.* Two eudesmanolides isolated from *S.transcaspicus* whole plant showed *in vitro* cytotoxicity against cultured human cell lines [11]. Likewise, *S.oleraceus* was mildy toxic as it may contain large quantities of nitrates [12].

In Sudan, *S. cornutus* was assessed for antitheilerial activity [10]. Traditionally, it was used as a remedy for treatment of malaria, diabetes mellitus and hypertension and [13].

The objective of this study is to elucidate the toxic effects of *S.cornutus* since this has not been previously done in depth.

2. MATERIALS AND METHODS

2.1 THE PLANT

Sonchus cornutus Hochst. ex. oliv. and Hiern, is locally known as Moleita or Malot. The aerial part of the plant was collected in August from the banks of the Blue Nile River, South of Khartoum state. The plant was identified and authenticated by a botanist at the Medicinal and Aromatic Plants Research Institute, Khartoum, Sudan. The voucher specimen has been deposited in the herbarium museum of the Institute. The voucher includes the vernacular name, description and distribution of the plant [5]. The plant air-dried in the shade, was 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 (113- 118 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 prior to 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*. All principles involving the animals were conducted with strict adherence to standard guidelines of laboratory procedures.

2.2.1 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 of this study for the use of laboratory animals was approved by the Ethical Approval No. EA/0019/ 2018, The Sudan Veterinary Council, Ministry of Cabinet, Republic of The Sudan.

2.3 PREPARATION OF PLANT EXTRACT

The plant extract was prepared as described previously [14]. Hot distilled water (500 ml) was added to 100 g of the coarsely powdered plant and left to cool down with continuous stirring at room temperature. The extract was filtered through Whatman No. 1 filter paper and then transferred to the freeze- drier (Trivac, U.S.A.). The yield percentage of the aqueous extract of *S. cornutus* (w/w) was 15.4%.

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 was based on the body weight.

2.4 SCREENING of THE AQUEOUS EXTRACT OF *SONCHUS CORNUTUS* FORTOXICITY

Twenty four male Wistar albino rats were divided into four groups, each of 6 rats. Group 2, 3 and 4 were used for evaluation 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) as low, intermediate and high level.

The aqueous extract of the plant was administered orally to rats in group 2, 3 and 4 at doses of 50, 500 and 2000 mg/ kg/ day, respectively for four weeks whereas, group 1 was kept as a control.

Clinical signs of toxicity and 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 sacrifice.

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 differences observed at $P<0.05$ were considered significant.

3. RESULTS

3.1 EFFECT OF THE EXTRACT ON MORTALITY AN BODY WEIGHT

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

The effect of the extract on body weights of rats was summarized (Table 1). The weights of rats were significantly ($P< 0.05$) increased in group 1, 2 and 3. However, the lowest weight gain was recorded at dose 2000 mg/kg.

3.2 EFFECT OF THE EXTRACT ON HAEMATOLOGICAL AND BIOCHEMICAL PARAMETERS

The toxicological effects of *S.corntus* aqueous extract on haematological and biochemical parameters were summarized in Table 2 and 3, respectively. The plant extract altered the haematology and biochemistry of rats in group 4 only.

3.6 HISTOPATHOLOGICAL CHANGES

Necropsy of rats in group 1, 2 and 3 showed normal livers and kidneys. Histopathological changes in livers and kidneys of rats occurred in group 4. The liver characterized by necrosis of liver cells, dissociation of hepatocytes with degeneration of cytoplasm, dilatation of sinusoid, and inflammation of cells (mononuclear cells) (Fig. 1 B) compared with the control (Fig. 1 A). The kidney revealed dilated and segmented glomerular tuft, necrosis of tubular epithelial cells (Fig. 2 B) using (Fig. 2 A) as a control.

4. DISCUSSION

The aqueous extract of *S.cornutus* at the dose of 2000 mg/kg caused haematological, biochemical and histopathological changes. The literature concerning *S.cornutus* is rare and scanty. Due to the bio- active properties of plants from Asteraceae family, *S.oleraceus* was mild toxic as it may contain large quantities of nitrates [15]. Hence, toxicity of *S.cornutus* could be due to nitrates or other compounds

In the current study, the increase in the percentage of body weight gain indicated that the extract of *S. cornutus* did not have general toxic effects at doses of 50 and 500 mg/ kg. However, the lowest body weight gain at the dose of 2000 mg/kg confirmed the toxic effect of the extract.

On the other hand, changes on haematological as well as biochemical parameters are indicators of abnormalities and/or toxicities in the body. This means that the doses of 50 and 500 mg/kg had no toxic effects on both parameters as well as the histological findings. The combined effects of physiological and chemical factors in the metabolism system of animals could lead to increase in WBCs [16]. This information support the present result exhibited increase in number of WBCs in group 4, associated with inflammation seen in histopathological investigation. The alteration in RBCs and Hb may be due to defective haematopoiesis inhibited erythopoiesis or increase in destruction of red blood cells [17, 18].

Decrease of serum total protein and albumin could be indicative of impaired liver excretory and synthetic function. Primary and secondary hepatic disease can cause elevation of both ALT and AST [19]. Elevated transaminases are suggestive of liver necrosis [20]. On the other hand, urea and creatinine were determined to diagnose the function of kidney [21]. The elevation in the level of serum renal function parameters in rats was associated to renal dysfunction and metabolic disturbances [22, 23].

The safety of the extract at doses of 50 and 500 mg/kg was confirmed by previous findings which exhibited that in sub chronic toxicity test of ethyl acetate extract of *Sonchus arvensis* leaves at doses of 100, 400 and 1000 mg/ kg had no toxic effects on body weight, haematological and biochemical parameters, and histological changes [24].

5. CONCLUSION

The results revealed that the aqueous extract of *Sonchus cornutus* at doses of 50 and 500 mg/ kg was safe, but dose 2000 mg/ kg may have hepato-renal toxicity. Phytochemical analysis and mechanism of actions are recommended to define the toxic compounds that may exist.

CONSENT

It is not applicable.

REFERENCES

1. Fennel CW, Lindsey KL, McGaw LJ, Sprag SG, Staffort GI, et al. Assessing African medicinal plants for efficacy and toxicology. J Ethnopharm. 2004; 94: 205- 217.

2. Farah HF, Elamin TH, El Hussein AM, Khalid HE. Assessment of antitheilerial activity of *Kigelia africana* fruits against *Theileria lestoquardi.* Eur J Med Plants. 2015; 5 (1): 101-108. ISSN: 2231-2927.

3. Maiga A, Diallo D, Fane S, Sango K, Paulsen BS, Cisse B. A survey of toxic plants on the market in the district of Bamako, Mali: Traditional knowledge compared with literature search of modern pharmacology and toxicology. J Ethnopharm. 2005; 96: 183-193.

4. Chandra SJ, Sandhya S, Vinod KR, David B, Sudhakar K, chaitanga R, Plant toxins harmful effects. Hygeia J Drugs Med. 2012; 4 (1): 70—90. www.hygeiajournal.com

5. Braun M, Burgstaller H, Hamdoun AM, Walter H. *Sonchany.us CGermornutus*, Asteraceae, Common Weeds of Central Sudan. Vier-Türme-verlag, Benedict Press, Münster schwarzach, Germany, Weinketsheim: Mergrraph; 1991.

6. Khan RA, Khan MR, Sahreen S, Bokhari J. Prevention of CCl_4 induced nephrotoxicity with *Sonchus asper* in rat. Food Chem Toxicol Suppl 8-9. 2010; 48: 2469- 2476

7. Khan MR, Badar I, Siddiquah A. Prevention of hepato-renal toxicity with *Sonchus asper* in gentamicin treated rats. Complement Altern Med. 2011; 11: 113. http://www.biomedcentral.com/1472-6882/11/113.

8. Khan RA. Protective effects of *Sonchus asper* (L.) Hill, (Asteraceae) against CCl_4 induced oxidative stress in the thyroid tissue of rats. BMC Complement Altern Med. 2012; 12: 181-188. http://www.biomedcentral.com/1972-6882/12/181

9. Mustaq MN, Akhtar MS, Alamgeer, Ahmad T, Khan HU, Maheen S, Tabassum N et al. Evaluation of antihypertensive activity of *Sonchus asper* L. in rats. Acta pol phar. 2016; 73 (2): 425- 431. PMID: 27180435 [Indexed for MEDLINE].

10. Farah HM, Elamin TH, Khalid HE, Elhussein AM. Evaluation of *in vitro* antitheilerial activity of *Sonchus cornutus* aqueous extract. Topcls J Herb Med. 2013; 2 (5): 90- 94.

11. Han Y, Zhang G, Gav K, Jia Z. Natural product chemistry new sesquiterpens from *S.transpicus.* Planta Med. 2005; 71: 543-547

12. Leonard DB. Plants and food. *Sonchus oleraceus.* Med at your Feet. 2007; 1- 4.

13. Fashir GA, Abdalla NI, Fangama IM. Assessment the consumption of *Sonchus cornutus* (Hochst) in Khartoum State, Sudan. Intern J Curr Microbiol Appl Sci. 2015; 4 (6): 833-839. ISSN: 2319-7706. http://www.ijcmas

14. Harborne JB.editor. Phytochemical Methods. 2nd ed, Chapman and Hall Ltd., London.1984; 4-7.

15. Wegiera M, Smolarz HD, Jeduch M, korczak M, Kopron K. Cytotoxic effect of some medicinal plants from Asteraceae family on J-45.01 leukemic cell lines. Pilot study. Acta Pol pharm. Drug Res. 69 (2): 263-268.

16. Nyarko AK, Okine LKN, Wedzi RK, Addo PA, Ofosuhne M. Sub chronic toxicity of the antidiabetic herbal preparation ADD 199 in the rat: Absence of organ toxicity and modulation of cytochrome P450. J Ethnopharm. 2015; 97 (2): 319-325. http://hdl.handle.net/123456789/3231.

17. Keinänen M, Kunutila S, Bloomfield CD, Elonen E, de la Chapelle A. The proportion of mitosis in different cell line changes during short-term culture of normal human bone marrow. Blood. 1986; 67; 1243-1247.

18. Selmanoglu G, Barlas N, Songür S, Kockaya EA. Carbendazim induced haematological , biochemical and histopathological changes to the liver and kidney of male rats. Hum Exp Toxicol. 2001; 20: 625-630.

19. Cheesbrough M. Medical laboratory manual for tropical countries. Microbiology Tropical Health Technology/ Butterworth Scientific Publications, Boston; 1991 http://www.isorjournals.org

20. Wuruchekke, AU, Anthony AG, Obiolah W. Biochemical effects on liver and kidney of rats administered aqueous extract of *Xemenia Americana.* African J Biotech. 2008; 7: 2777-2780.

21. Sharma A, Hirluukar N B, Wadel P, Das P. Influence of hyperglycemia on renal function parameter in patients with Diabetus Mellitus. Int J Pharmaceut Biol Arch Suppl 2. 2011; 2: 734- 739.

22. Bayramoglu G, Senturk H, Bayramonglu A, Uyanoglu M, Colka S, Ozmen A, et al. Carvacrol partially reserves symptoms of diabetes in STZ- induced diabetic rats. Cytotech. 2014; 66: 215- 217.

23. Madinov IV, Balabadkin MI, Markov DS, Markov TN, Main causes of hyperureamia in diabetes mellitus. Terk Arkh. 2000; 55-58

24. Nurianti Y, Henandriani R, Sukandar EY, Anggadiredja K. Acute and subchronic oral toxicity studies of ethylacetate extract of *Sonchus arvensis* L. leaves. Int J Pharm and Pharmaceut Sci. 2014; 65 (5): 343-347.

.

Table 1. Percentage of weight gain of rats given aqueous extract of *Sonchus cornutus*

Group No.	Dose (mg/kg)	Mean weight of rats per week (g)					Weight gain (g)	Weight gain (%)
		W0	W1	W2	W3	W4		
1	0	114.52±1.05	124.00±0.63*	133.17±1.47*	142.17±1.72*	153.67 ±2.16*	39.17	34.22
2	50	115.17±0.75	124.17±0.75*	133.20±1.37 *	142.67±0.82*	152.33 ±0.82*	37.17	32.32
3	500	116.83±0.98	125.67±0.52*	134.87±0.52*	144.33±0.52*	154.00±0.00*	37.16	31.82
4	2000	115.33±0.52	122.83±0.75*	129.80±0.84*	136.50±1.05*	136.50±0.00*	21.17	18.36

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

219

220 **Table 2. Hematological changes on the blood of rats given aqueous extract of *Sonchus cornutus***

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	6.78 ± 0.12	6.72 ± 0.10	11.80 ± 0.10	37.65 ± 0.19
	1		6.78 ± 0.12	6.72 ± 0.10	11.82 ± 0.10	37.65 ± 0.19
	2		6.82 ± 0.08	6.70 ± 0.10	11.82 ± 0.10	37.68 ± 0.17
	3		6.78 ± 0.12	6.65 ± 0.10	11.80 ± 0.10	37.73 ± 0.16
	4		6.78 ± 0.12	6.70 ± 0.13	11.80 ± 0.10	37.73 ± 0.16
3	0	500	6.32 ± 0.75	6.90 ± 0.06	11.93 ± 0.12	37.72 ± 0.15
	1		6.35± 0.10	6.90 ± 0.06	11.93 ± 0.12	37.72 ± 0.15
	2		6.35 ± 0.08	6.90 ± 0.06	11.90 ± 0.09	37.73 ± 0.14
	3		6.38 ±0.08	6.83 ± 0.08	11.88 ± 0.10	37.75 ±0.10
	4		6.37 ± 0.08	6.82 ± 0.10	11.90 ± 0.09	37.72 ± 0.15
4	0	2000	6.68 ± 0.10	6.97 ± 0.12	11.98 ± 0.08	37.97 ± 0.16
	1		8.62 ± 0.08*	5.45 ± 0.10*	9.73 ± 0.31*	37.78 ± 0.28*
	2		8.93 ± 0.08*	5.00 ± 0.14*	9.62 ± 0.23*	35.70 ± 0.33*
	3		8.97 ± 0.04*	4.02 ± 0.17*	8.82 ± 0.23*	35.57 ± 0.37*
	4		8.92±0.00	4.00± 0.10*	8.82± 0.34*	35.54 ± 0.38

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

222

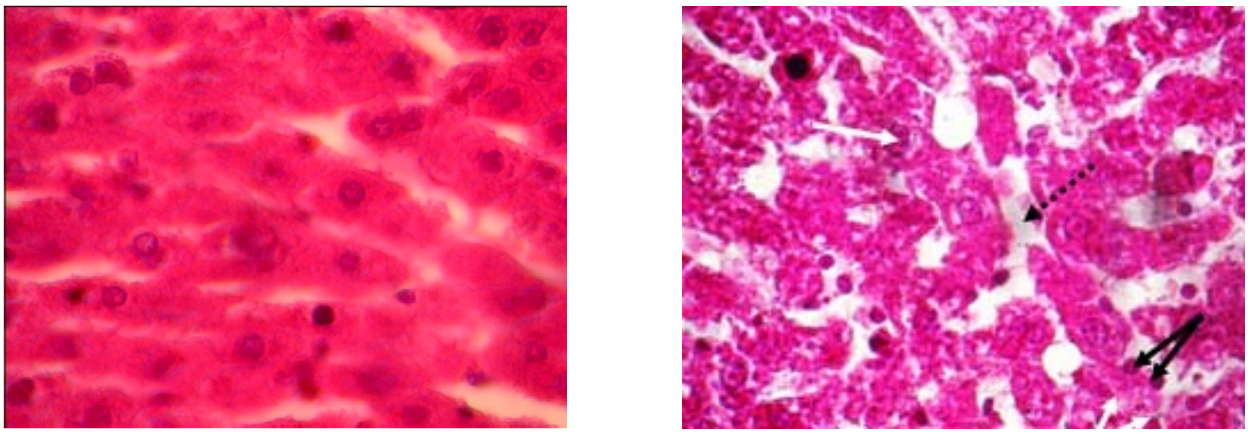
223

224

225 **Table 3. Biochemical changes on blood of rats after administration of aqueous extract of *Sonchus cornutus***

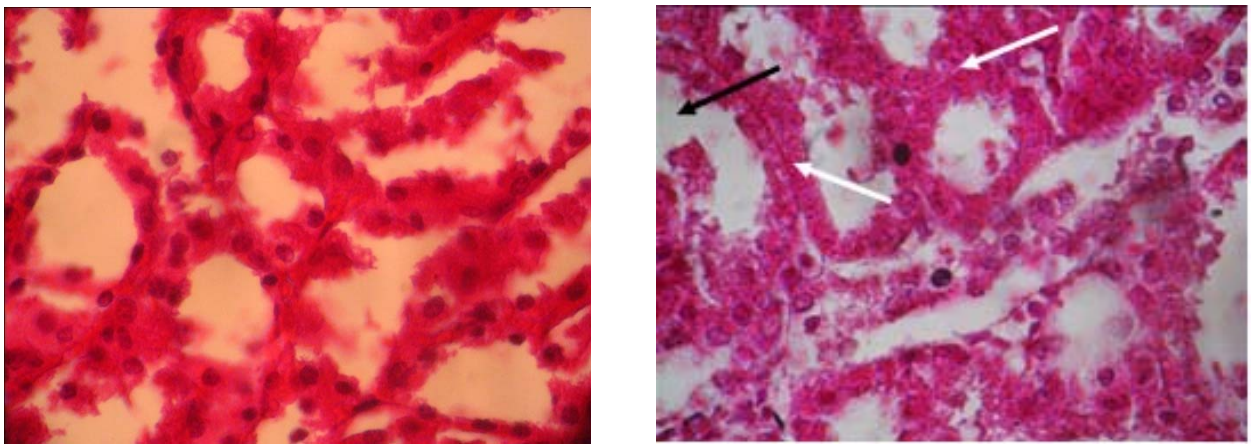
Group No.	Week No.	Dose	Total Protein (g/dl)	Albumin (g/dl)	Urea (mg/dl)	Creatinine (mg/dl)	ALT (IU/L)	AST (IU/L)	ALP (IU/ L)
1	0	0	6.35± 0.20	3.53 ± 0.16	14.67± .26	0.53 ± 0.05	13.00±0 89	18.33±0.82	53.00±1.41
	1		6.39 ± 0.18	3.55 ± 0.31	14.68± 0.23	0.53 ± 0.05	13.05±0.90	18.33±0.82	53.00±1.41
	2		6.46 ± 0.19	3.65 ± 0.26	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	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	14.70± 0.26	0.53 ± 0.05	13.08±0.92	18.43±0.80	53.17±1.17
2	0	50	6.67 ± 0.16	3.67 ± 0.12	14.75± 0.19	0.50 ± 0.09	13.50±0.55	18.95±0.19	54.00±0.63
	1		6.67 ± 0.16	3.67 ± 0.12	14.75 ±0.19	0.50 ± 0.09	13.50±0.55	18.95±0.19	54.00 0.63
	2		6.67 ± 0.16	3.68 ± 0.13	14.75 ±0.19	0.52 ± 0.08	13.53±0.52	18.97 0.19	54.00±0.63
	3		6.69 ± 0.16	3.70 ± 0.13	14.77 ±0.21	0.52 ± 0.08	13.50±0.55	18.97±0.19	54.33±0.52
	4		6.69 ± 0.16	3.70 ± 0.13	14.75 ±0.19	0.50 ± 0.09	13.50±0.55	18.97±0.19	54.33±0.52
3	0	500	6.88 ± 0.10	3.80 ± 0.14	14.87 ±0.05	0.50 ± 0.00	13.75±0.19	18.77±0.21	53.83±0.75
	1		6.88 ± 0.10	3.80 ± 0.14	14.87 ±0.05	0.52 ± 0.04	13.75±0.19	18.77±0.21	53.83±0.75
	2		6.90± 0.11	3.82 ± 0.16	14.87 ±0.05	0.52 ± 0.04	13.80±0.18	18.78±0.17	53.83 0.75
	3		6.90 ± 0.11	3.82 ± 0.16	14.88 ±0.04	0.50 ± 0.00	13.80±0.18	18.78±0.17	54.00±0.63
	4		6.90 ± 0.11	3.83± 0.19	14.88 ±0.04	0.50± 0.00	13.78±0.17	18.80±0.19	54.00±0.63
4	0	2000	6.50 ± 0.14	3.95 ± 0.19	14.90 ±0.13	0.50 ± 0.06	13.83±0.10	18.85±0.19	53.50±0.55
	1		5.10 ± 0.14*	3.07 ± 0.08*	18.48±0.31*	0.70 ± 0.09*	15.87±0.27*	21.10±0.39*	53.50 0.55
	2		4.00 ± 0.06*	2.02 ± 0.04*	19.33±0.38*	0.72 ± 0.08*	16.37±0.28*	21.80±0.43*	53.67±0.52
	3		3.43 ± 0.14*	2.00 ± 0.00*	19.92±0.37*	0.73 ± 0.08*	17.27±0.31*	22.37±0.30*	53.67±0.52
	4		3.43 ± 0.00*	2.01± 0.06	19.90± .35*	0.71± 0.07*	17.27±0.31*	22.36±0.38*	53.67±0.53

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



A B

Fig. 1. Section of rat liver: (A) Normal control (group 1). (B) After given aqueous extract of *Sonchus cornutus* at a dose of 2000 mg/ kg (group 4) showed liver necrosis with vesicular nuclei (white arrow), dilatation of sinusoid, (black dotted arrow), inflammatory cells (mononuclear cells) (black arrows), H&E (× 40)



A B

Fig.2. Section of rat kidney: (A) Normal control (group 1). (B) After dosing of 2000 mg/ kg aqueous extract of *Sonchus cornutus* (group 4) dilated and segmented glomerular tuft (black arrow); necrosis of tubular epithelial cells (white arrows), H&E (×40)