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Original Article

ACUTE AND SUBCHRONIC ORAL TOXICITY STUDIES OF ETHYL ACETATE EXTRACT OF SONCHUS ARVENSIS L. LEAVES

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ABSTRACT

Objectives: To evaluate acute and subchronic oral toxicity of ethyl acetate extract of *Sonchus arvensis* L. leaves.

Methods: Extract was prepared by maceration in ethyl acetate. Acute oral toxicity test was conducted in ddY mice using the conventional test. Sixty mice were divided into 6 groups, namely control group and five test groups, each given the extract at 0.19 g/kg body weight (b.w), 0.56 g/kg b.w, 1.67 g/kg b.w, 5 g/kg b.w and 15 g/kg b.w, respectively. Subchronic toxicity test was carried out in *Wistar* rat after daily administration of ethyl acetate extract of *Sonchus arvensis* L. leaves for 90 days at 100 mg/kg b.w, 400 mg/kg b.w, and 1000 mg/kg b.w. The rats in all groups were observed for behaviour, body weight development, haematological, clinical biochemistry, organ to body weight ratio, and organ histology.

Results: No mortality was observed both in acute and subchronic toxicity test in male and female animals. Observed behaviour, body weight profile and organ histology among experimental groups were comparable. Haematological, clinical biochemistry parameters, organ to body weight ratio were not significantly different ($p>0.05$).

Conclusion: There were no toxic effects after the use of single dose and repeated dose of ethyl acetate extract of *Sonchus arvensis* L. leaves in animal tested. Results of the present study suggest that ethyl acetate extract of *Sonchus arvensis* L. leaves is safe after single administration at high dose and repeated administration during 90 days.

Keywords : Acute, Subchronic, Oral toxicity, Ethyl acetate extract, *Sonchus arvensis* L.

INTRODUCTION

The plant *Sonchus arvensis* L., known in Indonesia as tempuyung, has been used widely as traditional drug. The most well-known activity of this plant is anti-kidney stone[1]. Two active compounds in tempuyung, Apigenin 7-glucoside and Luteolin 7-glucoside, were suggested to have important role for such activity[1]. In other countries, another species of this plant have been reported to have various efficacies. Anti-depresant like-effect of *Sonchus oleraceus* extract was shown in mouse models in immobility test that was comparable to amitriptyline (10 mg/kg, p.o.)[2]. Meanwhile, *Sonchus oleraceus* infusions given to diabetic rats resulted in a marked amelioration of the impaired glucose tolerance and lowered insulin as well as C-peptide level[3]. Furthermore, *Sonchus asper* methanolic extract showed protective effect against CCl_4-induced kidney damage in rat[4]. In addition, ethanolic extract of *Sonchus arvensis* L. leaves showed diuretic effect which was comparable to furosemide causing lowered sodium dan pottasium blood level[5].

Previous study showed that the ethyl acetate fraction of ethanol extract of *Sonchus arvensis* L. gave the highest activity in xanthine oxidase inhibition (unpublished data).

Besides efficacy, ethyl acetate extract of *Sonchus arvensis* L. leaves should meet the safety requirement for its development as an alternative medicine. In this study acute and subchronic toxicity test was carried out on the extract to explore its safety feature.

MATERIALS AND METHODS

Sonchus arvensis L. leaves was collected during December 2012 – March 2013 from Manoko Botanical Garden in Lembang, West Java, Indonesia. This plant material has been determination as *Sonchus arvensis* L. at School of Life Sciences and Technology, Bandung Institute of Technology.ddY mice aged 5-6 weeks, weighing 20 – 35 g, was supplied by PT. Bio Farma, Indonesia. Wistar rat aged 8-12 weeks, weighing 120 – 150 g, was supplied by D` Wistar, West Java, Indonesia. They were housed under standard laboratory condition at room temperature of $22^o \pm 3^o$ C and relative humidity of 50-60%, with 12 hours light and dark cycle. Food and water were provided *ad libitum* according to laboratory standard. This study was performed following *Guide for The Care and Use of Laboratory Animals Eight Edition*, 2011 Published by The National Academies Press, Washington, D.C[6].

The leaf was extracted with ethyl acetate using maceration. Leaf extract was concentrated by rotary evaporator under reduced pressure. Extract was dissolved in 10% acacia gum to prepare doses of acute and subchronic toxicity test. The dosage form was administered by oral gavage.

Acute toxicity test

There were 6 groups for each sex (5 mice per group), consisting control and five test groups. Control group was given only vehicle without extract. Test groups were given the above extract doses. The drugs were given in a volume of 0.5 ml/20 g b.w. Behaviour was observed at 0 and 30 min and 1, 2, 4 and 24 hours. All groups were observed for toxicity signs for 14 days. Body weight was measured every day. On the 14^{th} day all animals were sacrificed. Macroscopic presentation of organ was observed for changes in size and color.

Subchronic toxicity test

Wistar rats were divided in 6 groups (10 rats per group) consisting test, control and satellite group which given repeated dose during 90 days. Three test group were administered 3 doses (100, 400 and 1000 mg/kg b.w.) while control group was only given vehicle. Two satellite group were given extract at dose 1000 mg/kg b.w and only vehicle for other group. Behaviour was observed at 0 and 30 min and 1, 2 hours, 91 days, and 121 days for satellite group. Animal in test and control group were sacrificed on the 91^{th} day while satellite group on the 121^{th} day. At the end of experiment animals were put in metabolic cages, urine were collected for 16 – 24 hours to determine pH, density, volume and urine colour. Five microliters blood was taken from animal tail to determine haematological parameters. There were white blood cell (WBC), red blood cell (RBC), haemoglobin (Hb), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), haematocrite (Ht) and platelet (PLT). Blood samples

for clinical biochemstry parameters was collected in Eppendorf then centrifuged on 12000 rpm for 10 minutes and blood serum was kept in freezer (-20°C) before measurement. The parameters were SGPT (serum glutamic-pyruvic transaminase), SGOT (serum glutamic-oxaloacetic transaminase), total cholesterol, triglyceride, glucose, creatinine and blood urea nitrogen (BUN). Organ to body weight ratio was measured for heart, lung, liver, spleen, kidney, adrenal gland, testis, ovaries and uterus. Liver and kidney were fixed in 10% formalin for microscopic examination using hematoxylin-eosin staining.

RESULTS AND DISCUSSION

Following administration of ethyl acetate extract of *Sonchus arvensis* L. leavesin single dose up to 15 g/kg b.w no death was observed in both male dan female animals. Therefore LD50 of extract was higher than 15 g/kg b.w which is categorized practically non-toxic. Body weight development profile between control and test group were comparable as shown in Fig. 1 (acute oral toxicity) and Fig. 2 (subchronic oral toxicity). Body weight increase in both female and male indicates ethyl acetate extract of *Sonchus arvensis* L. leaves did not have general toxic effect and influence animal appetite[7].

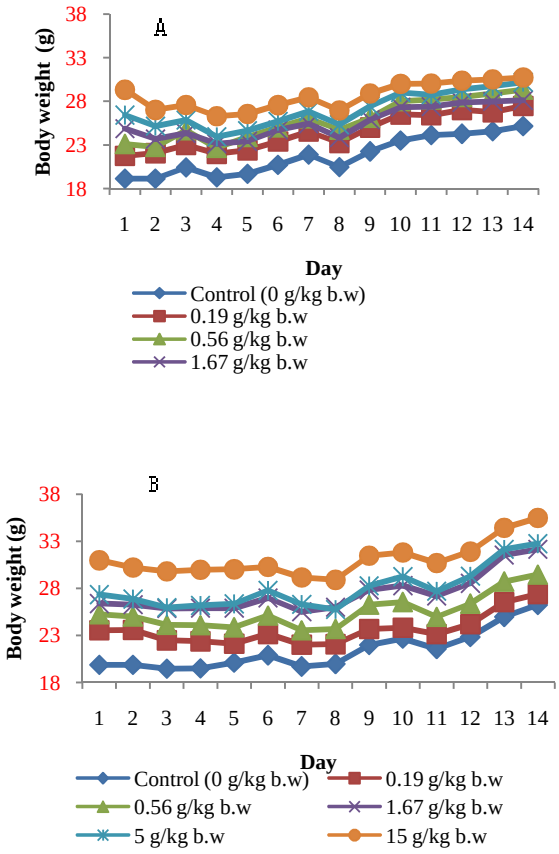

Fig. 1: Profile body weight development in male (A) dan female mice (B) for 14 days after administration ethyl acetate extract of *Sonchus arvensis* L.

Graphic information Fig. 1 part A must be include 6 groups as well as part B. (control (0 kg/b.w, 0.19 g/kg b.w, 0.56 g/kg b.w, 1.67 g/kg b.w, 5 g/kg b.w and 15 g/kg b.w)

No signs of straub, piloerection, ptosis, catalepsy, lacrimation, vocalisation, salivation, tremor, convulsions, and writhing were observed until 24 hours after drug administration and 90 days for subchronic toxicity test. It confirmed that ethyl acetate extract of *Sonchus arvensis* L. leaves did not cause toxic effect on central nervous system. Motor activity in control and test group were comparable. Body posture, respiration, urination and defecation were normal. Furthermore, mice in control and test groups showed normal reflexes, which indicates that ethyl acetate extract of *Sonchus arvensis* L. leaves did not affect spinal integrity in central nervous system.

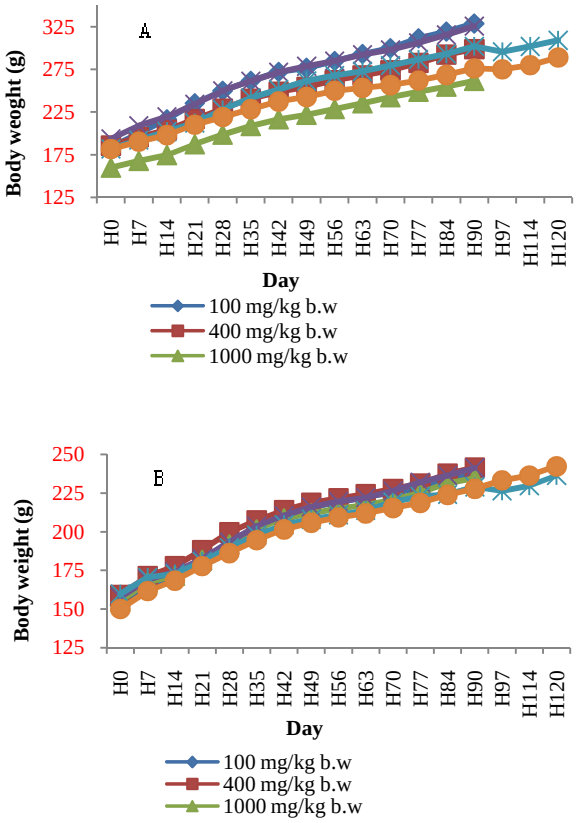

Fig. 2: Profile body weight development in male (A) dan female rats (B) for 90 days after administration ethyl acetate extract of *Sonchus arvensis* L

Graphic information Fig. 2 part A dan part B must be include 6 groups. (control, 100 mg/kg b.w, 400 mg/kg b.w, and 1000 mg/kg, control satellite and satellite 1000 mg/kg b.w)

Macroscopic observation on heart, liver, lung, stomach mucosa, ovaries, testis, spleen, kidney revealed no abnormalities. The colour-changing and organ hypertrophy indicate toxic effect of the test material[7]. Organ to body weight ratio after 90 days administration is shown in Table 1. Heart, liver, lung, spleen, kidney for male and female test group were not significantly different with control group. Testis in male animals, ovaries and uterus in female animals were not significant changed compared to those in control groups. Adrenal gland for female animals at dose 100 mg/kg b.w and 1000 mg/kg b.w were significantly increased compared to control group. It could possibly be because female animals were more sensitive to the chemical substance compared to male animals[7]. But in satellite groups, after 30 days of extract-free period, the condition returned to normal. There were no significant difference observed between satellite 1000 mg/kg b.w with control satellite.

As shown in Table 2 WBC (White Blood Cell), Hb (Haemoglobin) and PLT (Platelet) counts for all test group were not significantly different with control group. It indicates that extract was not toxic for the circulating WBC as well as Hb and did not interfere with the platelet function. In male animals, after extract administration at dose 100 mg/kg b.w, RBC (Red Blood Cell) countswere significantly decreased compared to those in control group, but this was not the case for the higher dose, and female animals. However the extract might not be considered toxic because after 90 days administration there were no changes in animal behaviour, and body weight increased in all treatment groups.

Table 1 : Organ to body weight ratio after 90 days ethyl acetate extract of *sonchus arvensis* L. leaves administration

Group	Control	100 mg/kg	400 mg/kg	1000 mg/kg	Satellite (Control)	Satellite (1000 mg/kg)
Male						
Heart	2.88±0.21	2.71±0.28	2.77±0.18	2.88±0.21	3.04±0.24	2.94±0.30
Liver	28.4±2.70	27.78±3.08	28.46±2.01	29.36±2.68	25.88±1.67	25.11±1.84
Lung	4.53±0.80	4.48±0.85	4.79±0.67	5.11±0.85	4.93±0.61	4.91±0.49
Kidney	4.53±0.80	6.52±0.79	6.46±0.46	6.37±0.57	6.26±0.43	6.30±0.48
Adrenal gland	0.16±0.03	0.16±0.02	0.17±0.02	0.18±0.04	0.18±0.03	0.16±0.01
Spleen	2.43±0.51	2.55±1.17	2.36±0.75	2.33±0.53	2.12±0.34	2.05±0.83
Testis	6.40±0.53	6.52±0.79	6.46±0.46	6.37±0.57	6.26±0.43	6.30±0.48
Female						
Heart	3.13±0.43	2.93±0.16	2.93±0.25	2.96±0.21	3.03±0.22	3.03±0.22
Liver	28.40±8.75	28.15±2.54	28.75±2.89	27.82±7.88	30.99±2.92	29.00±2.82
Lung	5.65±1.62	5.02±0.77	5.41±0.93	5.65±1.62	5.22±1.66	5.55±0.79
Kidney	6.35±0.73	5.76±0.39	6.05±0.47	6.03±0.42	6.12±0.35	6.41±0.50
Adrenal gland	0.30±0.07	0.24±0.03	0.26±0.03	0.24±0.05	0.27±0.04	0.28±0.04
Spleen	2.75±0.49	2.52±0.56	2.77±0.83	2.55±0.47	2.42±0.41	2.26±0.28
Uterus	2.45±0.67	2.31±1.07	2.36±1.14	2.28±0.84	3.06±1.08	2.99±1.40
Ovaries	0.40±0.07	0.36±0.06	0.36±0.09	0.37±0.09	0.38±0.07	0.36±0.06

Table 2 : Profile of haematological parameters after 90 days of ethyl acetate extract of *Sonchus arvensis* L. leaves treatment

Group	Control	100 mg/kg	400 mg/kg	1000 mg/kg	Satellite (Control)	Satellite (1000 mg/kg)
Male						
WBC $x10^6$/µL	4.42±1.23	4.05±0.62	4.61±0.85	4.86±1.24	5.10±1.51	4.51±1.55
RBC $x10^6$/µL	1.28±0.29	0.99±0.34	1.07±0.28	1.21±0.40	1.11±0.16	1.10±0.12
Hb g/dL	4.90±1.07	4.61±1.34	4.70±1.29	5.32±1.48	4.44±0.99	4.59±0.31
MCH pg/cell	38.42±3.53	44.07±7.93	40.54±3.56	42.28±7.49	40.45±8.84	42.14±3.40
MCHC g/dL	102.46±10.84	119.43±22.88	109.33±10.54	114.51±19.80	108.94±23.76	114.12±9.66
MCV fl	37.56±0.79	36.97±0.47	37.10±0.41	37.33±0.39	37.10±0.33	36.93±0.37
HCT %	5.03±1.29	3.64±1.32	3.93±1.08	4.08±1.08	4.11±0.64	4.06±0.47
PLT $x10^5$/µL	1.31±0.21	1.43±0.25	1.44±0.29	1.51±0.28	1.56±0.28	1.54±0.07
Female						
WBC $x10^6$/µL	5.12±2.43	4.00±1.35	4.35±1.42	4.17±1.95	5.12±0.95	4.98±1.41
RBC $x10^6$/µL	1.41±0.36	1.23±0.32	1.33±0.42	1.44±0.37	1.00±0.13	1.04±0.17
Hb g/dL	4.77±0.56	4.37±0.73	4.73±0.87	4.66±0.94	4.36±0.36	4.46±0.69
MCH pg/cell	33.92±6.93	35.38±6.16	36.47±6.72	39.73±8.54	43.83±3.42	43.73±3.62
MCHC g/dL	96.71±25.98	100.41±24.62	97.97±19.70	106.77±25.16	117.36±11.33	116.98±11.92
MCV fl	37.38±0.69	37.23±0.82	37.19±0.66	37.38±1.04	37.44±1.92	37.47±1.60
HCT %	5.27±1.44	4.58±1.29	5.31±1.54	5.45±1.51	3.74±0.45	3.84±0.66
PLT $x10^5$/µL	1.25±0.26	1.26±0.32	1.39±0.25	1.30±0.26	1.44±0.16	1.50±0.28

Table 3: Profile of clinical biochemistry parameters after 90 days of ethyl acetate extract of *Sonchus arvensis* L. leaves treatment

Group	Control	100 mg/kg	400 mg/kg	1000 mg/kg	Satellite (Control)	Satellite (1000 mg/kg)
Male						
SGOT U/L	148.64±16.80	144.13±22.54	150.21±31.50	174.01±30.07	155.84±48.21	171.86±38.08
SGPT U/L	42.28±12.09	40.88±12.14	43.10±7.63	47.16±14.10	41.00±18.76	42.96±13.00
Glucose mg/dL	155.87±46.39	133.16±44.47	148.65±45.03	140.57±36.33	114.37±25.09	118.12±26.77
Cholesterol mg/dL	58.23±7.56	53.00±12.20	58.09±12.32	60.41±12.86	60.46±19.42	65.16±20.57
Triglyceride mg/dL	108.69±40.00	90.93±35.31	107.08±35.89	102.80±36.55	79.66±47.79	66.83±30.54
Creatinine mg/dL	0.58±0.19	0.60±0.12	0.59±0.13	0.33±0.23	0.47±0.25	0.52±0.17
BUN mg/dL	40.35±6.44	43.28±15.87	37.44±6.98	41.12±8.69	50.16±6.79	51.31±5.83
Female						
SGOT U/L	171.70±97.98	142.61±53.98	217.91±110.11	172.47±52.40	151.15±61.63	165.37±52.05
SGPT U/L	39.72±13.22	30.82±9.37	34.47±12.80	30.17±8.74	34.41±9.26	34.82±7.03
Glucose mg/dL	124.54±44.82	155.79±56.91	177.93±62.64	150.64±37.49	129.59±31.15	136.45±45.17
Cholesterol mg/dL	124.54±44.82	69.51±9.46	70.18±16.76	65.41±10.00	69.84±19.35	65.97±17.78
Triglyceride mg/dL	90.53±22.60	95.52±23.71	97.38±14.02	104.47±27.17	101.40±30.33	94.16±33.65
Creatinine mg/dL	0.51±0.14	0.52±0.17	0.47±0.27	0.49±0.12	0.60±0.32	0.63±0.19
BUN mg/dL	50.76±12.16	41.49±6.32	44.38±6.16	48.11±8.46	74.09±12.19	73.21±9.51

Observation on clinical biochemistry parameters (the results are shown in Table 3) was performed to evaluate any toxic effect in kidney and liver function. Elevation in SGOT and SGPT is an indicator of liver and heart damage[8]. After 90 days ofextract administration there were no significantly different in the values of SGOT, SGPT, cholesterol, and triglyceride in female and male animals test groups compared to control groups. It indicates the extract was not toxic for liver and did not affected lipid metabolism. A significant increase was only observed in glucose level in female test group at dose 400 mg/kg b.w, which was absent in groups treated with higher and lower dose. The levels of urea & creatinine are indicators used to diagnose functioning of the kidney[9], and changes in serum

creatinine concentration are more reliably reflect changes in glomerular filtration rate[9]. In renal failure serum creatinin and urea are higher than normal values[9]. Serum urea and creatinin levels in satellite groups given 1000 mg/kg b.w significantly increased compared to those in test groups receiving the same dose, but there were no significant difference when comparison was made to satellite control groups. Urine volume and urine density can be valuable in assesing renal function because these parameters demonstrate the concentrating capacity of kidneys[10]. Animals with impaired ability to concentrate urine due to renal disease have decreased urine spesific gravity and increased urine volume[10]. All urine parameters (Table 4) pH, density, volume were not significantly different between test groups and control groups. It indicates the exctract did not have toxic effect to kidney function.

Table 4: Profile of urine parameters after 90 days of ethyl acetate extract of *Sonchus arvensis* L. leaves treatment

Group	Control	100 mg/kg	400 mg/kg	1000 mg/kg	Satellite (Control)	Satellite (1000 mg/kg)
Male						
pH	7.50±1.08	7.60±0.84	7.70±0.82	7.33±0.86	7.10±0.87	7.20±0.91
Density g/mL	1.09±0.03	1.08±0.05	1.07±0.03	1.09±0.02	1.10±0.05	1.11±0.07
Volume mL	10.37±10.74	14.93±10.56	12.48±12.12	4.23±4.27	8.81±5.53	6.61±5.56
Colour	++	+	++	++	++	++
Female						
pH	6.90±0.56	7.10±0.56	7.00±0.66	7.30±0.67	7.40±0.96	7.20±1.03
Density g/mL	1.08±0.04	1.07±0.03	1.09±0.02	1.08±0.04	1.08±0.02	1.07±0.02
Volume mL	9.66±8.11	10.74±5.53	8.32±4.75	8.57±7.07	5.53±3.52	7.42±8.09
Colour	++	+	++	++	++	++

+ (very light yellow). ++(light yellow)

There were no changes observed in microscopic presentation of liver and kidney in all groups. Results from control groups were comparable with test groups at three doses (100, 400 and 1000 mg/kg b.w). Liver microscopic examination was shown in Fig. 3. Microscopic appearance of central vein and hepatocytes were normal. There were no Kupffer cell in sinusoidal spaces which might indicate the absence of pathological changes to the liver. Histopathological condition of the liver is characterized by vacuolations, varying shapes and sizes of nuclei in hepatocytes, loss of sinusoidal spaces, inflammatory cells scattered all over hepatic tissue and there will be dilated of central vein filled with blood[11]. Fig. 4 shows microscopic examination of the kidney. Bowman's capsule and nuclei in all test groups were not different with control group. In pathological condition. contraction of the glomerus made capsular space in the Bowman's capsule increase. There are also contraction of proximal and distal convulated tubules and the renal cell nuclei will have varying shapes and sizes[11].

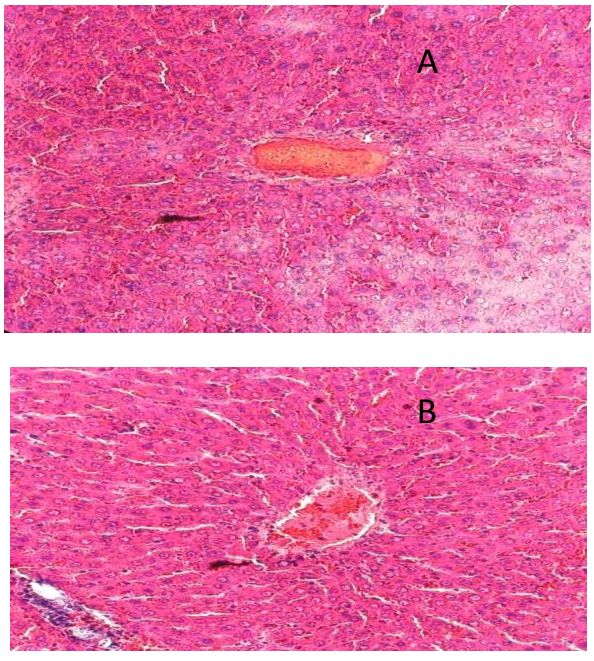

Fig. 3 : Photomicrograph of hepatic tissues in female rats after 90 days of ethyl acetate extract of *Sonchus arvensis* L. leaves treatment. (A) control groups, (B) extract 1000 mg/kg b.w 400x.

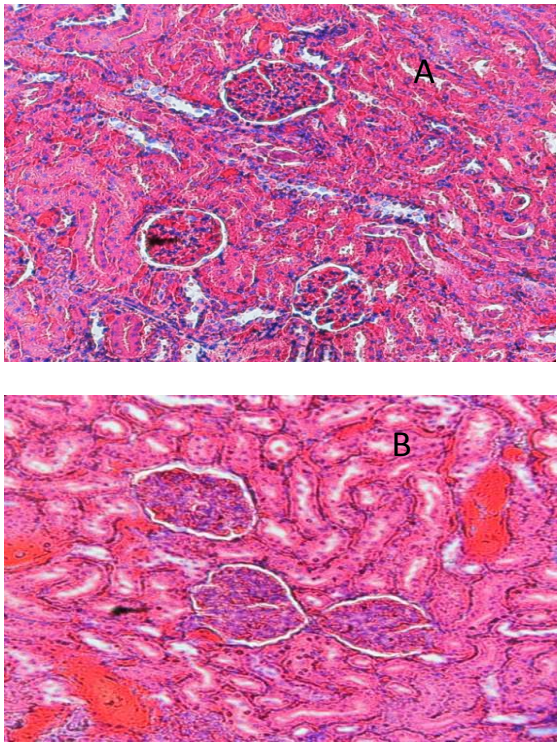

Fig. 4 : Photomicrograph of renal tissues in female rats after 90 days of ethyl acetate extract of *Sonchus arvensis* L. leaves treatment. (A) control groups, (B) extract 1000 mg/kg b.w 400x.

CONCLUSION

Results of the present study showed that ethyl acetate extract of *Sonchus arvensis* L. leaves was safe after single and repeated dose administration based on acute and subchronic oral toxicity studies in mice dan rats. LD50 of the extract was higher than 15 g/kg b.w and classified as practically non-toxic. No abnormalities in behaviour, haematological, clinical biochemistry, and urine parameters. Organ macroscopic and microscopic presentations were not different between control dan test groups.

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