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Research Article

SUBCHRONIC TOXICITY STUDY OF CORN SILK (*ZEA MAYS* L.) IN COMBINATION WITH BINAHONG (*ANREDERA CORDIFOLIA* (TEN.)) STEENIS LEAVES ON WISTAR RATS

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ABSTRACT

Objective: Nowadays, modern medication has been shifting into natural-based medication. These phenomena lead to the elevation of the use of traditional medicine. Similar to modern medicine, nature-based medicine should meet the standard which is including safety, efficacy, and quality aspects. The medicine must be safe during the consumption period and scientifically proven through preclinical toxicity test. It can be obtained through acute and subchronic toxicity test. Besides, it should have abilities to treat certain diseases and ease of accessing such medicines should be guaranteed particularly for patients. From our previous study, a combination of ethanolic extract of corn silk (CS) and binahong leaves (BL) showed activity in ameliorating kidneys function on renal failure rat's model. Therefore, to obtain standardized traditional medicine, a study on safety must be accomplished.

Methods: A 90-day oral toxicity study using Wistar rats was conducted to evaluate subchronic toxicology. The combination of extracts were given in three different levels daily for 90 days and a 120 days toxicity test was carried out for satellite groups.

Results: Administration of extracts in combination to rats for 90 days did not alter behavioral, motoric activities, urine, hematological, chemical, and histopathological parameters, and it was suggested a no-observed adverse-effect level of CS 450 mg/kg and BL 600 mg/kg in combination. Even though the number of white blood cells in all treated groups arose compared to the control group, but the values were still in the normal range.

Conclusion: These results indicate that the combination of ethanolic extract of CS and BL can be generally meet safety requirement as traditional medicine.

Keywords: Corn silk, *Zea mays* L., Binahong leaves, *Anredera cordifolia* (Ten.) Steenis, Subchronic toxicity test.

INTRODUCTION

The trend of the use traditional medicine has been increasing recently and people's paradigm to go back to nature gains the popularity. There are a lot of herbs that are used traditionally, and almost all of them have not scientifically proven. Ensuring the efficacy, safety, and quality of medicine is a crucially important step. Thus, natural-based treatments should also meet these requirements before it is used as therapeutic agents.

Toxicity test is a common type of test to detect any toxic effects on biological systems and to obtain specific dose-response character from tested substances. Acquired data are then occupied in predicting the level of toxicity in human; thus, the acceptance safe dose can be determined [1].

Corn silk (CS) is made from stigmas, the yellowish thread like strands from the female flower of maize. *Zea mays* belong to Poaceae family. It is a waste material from corn cultivation and available in abundance [2,3]. Its appearance is shown as reddish, pink, yellowish brown, brown to purplish red thread with a slight odor and metallic taste [4]. Empirically, it has been used as a treatment for inflammation on urinary bladder and prostatic disorders, as well as urinary tract irritation [5]. It is beneficial for treating frequent urination which is caused by irritation of the bladder and urethral walls as well as for difficulty in passing urine (e.g. prostate disorders). It soothes and relaxes the lining of the urinary tubules and bladder relieving irritation and improving urine excretion [6,7]. According to ancient literature, CS also has been employed to relieve prostate related problems, bed-wetting, carpel tunnel syndrome, edema, obesity, and post-menstrual syndrome. In addition, it shows activity as an anti-lithiatic agent, uricosuric, and antiseptic.

CS also possess antibacterial activity against corn earworm due to its content of maysin flavone glycoside [8]. In another study, flavonoid compounds isolated from CS were beneficial as anti-fatigue and anti-diabetes [9], diuretic and anti-hypertension [10]. Its antioxidant properties come because of phenolic compounds and flavonoid in CS [11]).

Binahong (*Anredera cordifolia*) originally comes from Tiongkok, and it can be found in tropical regions in South America. It also widely spread to several tropical countries such as Vietnam and Indonesia. Indonesian especially those in Java Island cultivates this plant. Its leaves contain several secondary metabolites such as saponin, flavonoid, quinon, steroids, monoterpenoid, and sesquiterpenoid. Whereas its rhizome well-known of its contents of flavonoid, polyphenol, saponin, tannin, and steroids. With regards to the study of Lemmens [12], there was a method to isolate the triterpenoid saponin from binahong leaves (BL) which are known as bousingosida A1 [12]. Several triterpenoids have been found from binahong, including larreagenin A, oleanolic derivatives, and ursolic acid. Empirically, BL are used to normalize blood pressure, prevent stroke attack, relieve peptic ulcer, and treat gout. Moreover, its potency to improve vitality and immune system response, to treat urinary incontinence, ulcer, and hemorrhoids has been reported through several studies. According to the traditional use of binahong in Columbia and Taiwan, it is pharmacologically active to relieve pain and treat diabetes. There was a study reporting that ethanolic extract of BL inhibits activity on isolated gastric fundus from rats induced by spasmogen. Water extract of BL also possessed anti-hepatotoxic activity at a dose of 30 mg/kg bw [12].

On our previous studies, administration of ethanolic extract CS and BL in combination improved kidneys function and showed attenuation on kidneys oxidative stress on renal failure model in rats [13-15].

However, there was no toxilogical studies conducted on the safety of this combination. This study was conducted to observe toxicological properties that may appear through repeated oral administration for 90 days.

METHODS

Extract preparation

CS and BL were purchased from Manoko, Lembang and then were extracted by reflux using ethanol 96%. Each liquid extract was evaporated until obtaining concentrated extracts. These extracts were then combined in three different concentrations according to groups in subchronic toxicity test.

Animals

Male and female Wistar rats with approximately 3-4 months in age were used in this study. Rats were provided by the Animal Laboratory of School Pharmacy of ITB and kept under usual management condition in this institution. All methods were conducted according to OECD instruction for document number 408.

Experimental design

There were six groups (n=20, 10 male and 10 female) in this study including control, combination of CS 37.5 mg/kg and BL 50 mg/kg which was referred as dose 1 group, CS 150 mg/kg and BL 200 mg/kg which was referred as dose 2 group, CS 450 mg/kg and BL 600 mg/kg as dose 3 group, satellite control, and satellite dose 3 group.

The extracts were prepared by dispersing it in 0.5% CMC Na solution. It was given by oral route with the volume of 1 ml/100 g bw. All rats received extracts according to its group, whereas control and satellite control received the vehicle only (CMC-Na 0.5%). The experiment was conducted for 90 days in which all groups were given the either extracts or vehicle once daily. While all groups were sacrificed on day 91, satellite groups (control and dose 3) were sacrificed 30 days after.

Evaluation

There were several parameters observed including behavioral aspects, motoric activity, body weight, the number of mortality, urine, hematology, clinical biochemistry, organ index, gastric mucousal observation, and histological studies. In terms of behavioral aspects, parameters were including straub, piloerection, ptosis, pineal reflexes, corneal reflexes, lacrmation, cathalepsy, posture, hanging ability, retablishment, flexion, Haffner response, mortality, grooming, respiration, salivation, vocalization, tremor, seizure, defecation, urination, and writhing.

RESULTS

Throughout the 90 days toxicity study, there were no clinical signs or adverse effects observed on all rats that may be attributed to administration of combined extracts compared to control. The same figure was found in satellite group of the highest dose (dose 3) from day 91 to day 120 compared to control satellite group.

Body weight changes

The group mean weekly body weights versus time are presented in Fig. 1. There were no significant differences in body weight in both male and female rats between all treated groups and control group ($p>0.05$). The body weight increased throughout the periods. However, these findings were in the normal range, and the differences were therefore attributed to normal biological variation.

Organ index

Overall, there were no significant differences between all treated groups and control in male group. The results of organ index were shown in Table 1. However, weight of spleen in satellite dose 3 group increased significantly as compared to control. It needs to be observed further whether it may provide immunostimulant effect.

Gastric mucousal damage and urine parameters

There were no damages on gastric mucousal found through macroscopic observation in all groups. With regards to observation on urine, there were no significant differences on parameters including urine specific gravity, volume, and pH. The result on urine was shown in Table 2.

Clinical biochemistry values

In general, clinical biochemistry data, such as serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), creatinine, urea, total cholesterol, triglycerides, and glucose, were not significantly affected by extract administration, although using the highest dose of the combination.

According to the measurement of SGOT and SGPT (Table 3), there were no statistically significant changes between groups that were given by extracts and control group (>0.05). SGOT and SGPT are common types of biochemical parameters to evaluate liver function. In terms of serum creatinine and urea (Table 3), there were reductions on both parameters, but the values were statistically the same compared to control group.

According to the Table 3, the concentration of triglyceride and glucose went down in all treated groups. However, it did not show significance comparing to the control group. Despite elevation on cholesterol concentration, when it compared to the control group, it was similar statistically.

Hematological observation

With regards to Table 4, parameters related to hematology measurement: Red blood cells (RBC), mean platelet volume (MPV), hematocrite (HCT), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), and hemoglobin (Hb), there were no statistical differences between female rats and control. The number of white blood cells (WBC), granulocytes, monocytes, lymphocytes on satellite dose 3 group showed the significant difference as oppose to control, but similar results happened in satellite control as illustrated in Table 4.

After 90 days treatment, the number of RBC, MPV, HCT, MCH, platelet, MCHC, MCV, and Hb on male rats were not affected by the administration of extract. Despite elevation on WBC in satellite control and satellite dose 3 group, but its value remained in the normal range. There were statistically difference on the number of monocytes and granulocytes between satellite dose group and control (Table 4).

Histopathological studies

Administration of extract did not affect the histological appearance of organs: Kidneys, liver, and lungs as compared to the control groups. By contrast, white pulp dilatation in the spleen was found in all treated groups (Fig. 2). It needs to be observed further through immunological response and measurement of lymphocytes in the spleen.

DISCUSSION

All results showed that there was no significant alteration. In our previous study, combination of binahong and CS extract showed better activity to improve kidney function on renal failure model [14]. In the former study, administration of binahong extract alone was considerably safe in 90 days oral toxicity test [15]. Indeed, through this study, the safety aspect of CS and binahong extract in combination has been proven scientifically.

Previous studies relating to CS revealed that this crude plant contained flavonoid, phenolic compound [11,16,17]. Because of that CS extract is potential to be utilized for treating diseases associated with oxidative stress [14]. There is only a few publication mentioning antioxidant activity of binahong extract even though some studies revealed binahong activity against bacterial infection and as wound healing agent [18].

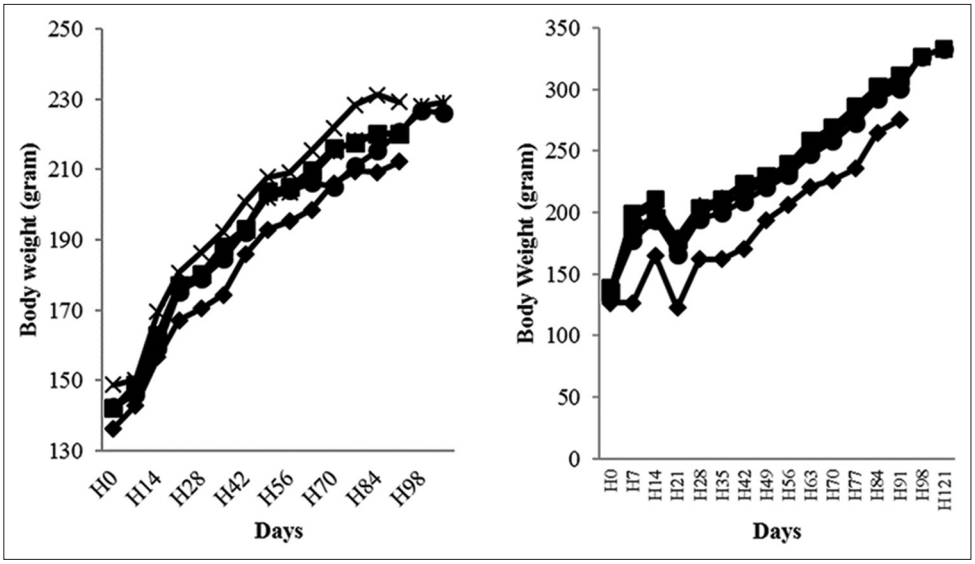

Fig. 1: Body weight changes on female group (left) and male group (right). (⬥) Control; (■) Dose 1; (▲) Dose 2; (×) Dose 3; (✱) Satellite control; (●) Satellite dose 3

Table 1: Organ index

Group	Control	Dose 1	Dose 2	Dose 3	Satellite control	Satellite dose 3
Male						
Liver	2.5119±0.26	2.5620±0.51	2.5944±0.26	2.6108±0.58	2.4392±0.33	2.5857±0.16
Heart	0.3056±0.03	0.2985±0.05	0.2866±0.01	0.3000±0.04	0.3067±0.03	0.2971±0.05
Lungs	0.6276±0.35	0.5073±0.14	0.5757±0.12	0.6201±0.17	0.8006±0.20	0.7893±0.22
Spleen	0.1826±0.02	0.1659±0.03	0.1609±0.02	0.1696±0.03	0.1917±0.04	0.1741±0.02
Kidneys	0.6208±0.06	0.6464±0.13	0.6577±0.10	0.6110±0.13	0.6443±0.89	0.6415±0.09
Adrenal glands	0.0156±0.005	0.0156±0.003	0.0129±0.004	0.0373±0.071	0.0169±0.002	0.0206±0.003
Testis	1.0024±0.11	1.0197±0.19	1.0070±0.11	0.9437±0.21	0.9363±0.09	0.9572±0.12
Vesica seminalis	0.4671±0.08	0.4985±0.12	0.5252±0.16	0.4443±0.13	0.3988±0.09	0.4143±0.06
Female						
Liver	2.6994±0.303	2.6875±0.313	2.5501±0.209	2.8164±0.173	2.5460±0.166	2.8009±0.262
Heart	0.3293±0.034	0.3203±0.030	0.3381±0.074	0.3319±0.035	0.3040±0.028	0.3438±0.052
Lungs	0.7215±0.217	0.6891±0.327	0.6583±0.197	0.6849±0.130	0.9257±0.213	0.8873±0.253
Spleen	0.1899±0.035	0.2053±0.023	0.1980±0.021	0.1983±0.024	0.1932±0.023	0.2284±0.020*
Kidneys	0.5850±0.069	0.5759±0.075	0.5816±0.063	0.6354±0.050	0.5890±0.034	0.6170±0.090
Adrenal glands	0.0244±0.0012	0.0247±0.0012	0.0296±0.0030	0.0260±0.0020	0.0270±0.0025	0.0279±0.0023
Ovarium	0.0498±0.014	0.0394±0.006	0.0439±0.009	0.0536±0.013	0.0483±0.0145	0.0920±0.1258

Values are expressed as mean±SD. *Significantly different from control group ($p<0.05$). SD: Standard deviation

Table 2: Urine parameters: Specific gravity, volume, pH

Group	Specific gravity (g/mL)		Volume (ml)		pH	
	Male	Female	Male	Female	Male	Female
Control	1.0755±0.097	1.0093±0.022	7.90±3.48	9.10±5.00	7.90±0.99	7.30±0.82
Dose 1	1.0177±0.025	1.0241±0.032	7.80±2.90	10.60±5.58	7.70±1.06	7.50±0.71
Dose 2	1.1320±0.170	1.0704±0.138	8.20±7.21	8.65±6.47	7.20±0.79	7.40±1.17
Dose 3	1.1500±0.127	1.0330±0.104	8.10±8.35	5.75±3.07	7.20±1.14	7.30±1.06
Satellite control	1.1100±0.074	1.1270±0.196	8.15±5.36	8.55±8.43	7.30±0.95	7.20±1.03
Satellite dose 3	1.0660±0.139	1.0900±0.218	10.07±4.10	8.10±7.54	7.10±0.88	6.70±0.67

Values are expressed as mean±SD. *Significantly different from control group ($p<0.05$). SD: Standard deviation

Body weight change can be used to evaluate individual response towards particular drugs or compounds [19] and to indicate side effects of drugs [20]. In this study, body weight in all groups increased as time went by. It may be concluded that administration of extracts was not likely to decrease rat's appetite.

With regards to organ weight, the highest dose of extracts significantly elevated the spleen weight compared to the control group. However, the similar pattern was not found in the remaining groups. It seems that the highest dose of extract administration may attribute to stimulate immune response as the immune organs such as spleen became heavier. The spleen is classified as a major secondary lymphoid organ involved in elicitation of the immune response [21]. The spleen is responsible to filtering blood and trapping blood-borne antigens in which contribute to systemic infection responses [21].

The function of vital organs, such as kidneys and liver, were represented from biochemical values. SGOT and SGPT can be used to determine liver function [22]. Meanwhile, creatinine and urea levels were utilized in evaluating the function of kidneys [23,24]. In all biochemical parameters,

Table 3: Clinical biochemistry measurement

Group	SGPT (U/l)	SGOT (U/l)	Creatinine (mg/dl)	Urea (mg/dl)	Glucose (mg/dl)	Cholesterol (mg/dl)	Triglyceride (mg/dl)
Male							
Control	31.01±11.65	51.73±18.29	0.85±0.42	33.94±13.13	130.88±20.05	53.45±15.08	66.37±20.77
Dose 1	22.47±5.72	46.14±10.61	0.75±0.35	24.51±16.38	120.34±41.40	54.83±15.77	47.43±16.68
Dose 2	23.53±6.99	45.84±8.09	0.80±0.54	22.97±12.22	103.26±27.04	57.49±19.65	66.62±41.03
Dose 3	31.84±19.25	44.10±7.53	0.90±0.46	31.67±11.16	119.30±61.51	56.67±16.06	50.35±14.06
Satellite control	23.53±10.48	40.92±14.25	0.95±0.20	36.33±12.53	110.72±40.52	55.61±13.08	53.94±24.68
Satellite dose 3	23.15±9.59	39.38±11.65	1.01±0.68	42.80±7.30	136.24±39.67	51.57±11.37	55.56±17.20
Female							
Control	17.34±4.98	45.10±8.46	0.73±0.18	32.25±16.07	131.39±44.61	50.19±19.79	46.30±18.16
Dose 1	15.65±5.56	40.16±7.83	0.87±0.30	28.61±4.81	125.90±29.47	52.52±19.73	61.04±11.23
Dose 2	16.15±5.84	38.66±9.59	0.83±0.29	42.70±12.88	119.16±32.48	53.73±20.88	53.09±13.07
Dose 3	26.45±22.29	43.45±11.57	1.09±1.33	41.97±18.81	124.14±31.37	56.42±16.41	45.43±15.78
Satellite control	23.62±8.04	46.98±7.39	0.89±0.44	40.43±7.14	102.05±31.28	50.93±18.83	50.54±12.11
Satellite dose 3	22.29±9.04	42.42±10.49	0.80±0.33	43.44±10.72	109.69±23.85	53.39±15.65	52.88±23.51

Values are expressed as mean±SD. *Significantly different from control group ($p<0.05$). SD: Standard deviation, SGOT: Serum glutamic oxaloacetic transaminase, SGPT: Serum glutamic pyruvic transaminase

Table 4: Hematology measurement

Measurements	Control	Dose 1	Dose 2	Dose 3	Satellite control	Satellite dose 3
Males						
RBC ($\times10^6$/mm^3)	1.54±0.25	1.42±0.15	1.59±0.12	1.53±0.24	1.49±0.14	1.42±0.12
WBC ($\times10^3$/mm^3)	2.06±0.85	1.48±0.35	1.88±0.36	1.76±0.55	3.30±1.17*	3.02±1.16*
Mid (%)	0.18±0.11	0.18±0.04	0.15±0.10	0.30±0.10	0.15±0.21	0.28±0.092*
MPV (fl)	20.49±4.93	22.10±0.38	22.10±0.23	19.37±6.80	20.29±5.03	21.81±0.28
Granulocytes (%)	0.71±0.41	0.72±0.24	0.72±0.45	0.74±0.24	0.94±0.74	1.61±0.47*
HCT (%)	5.80±0.87	5.38±0.63	6.10±0.48	5.68±0.71	5.65±0.48	5.42±0.48
MCH (pg/cell)	28.32±2.74	29.44±2.45	27.00±0.94	27.00±3.01	27.53±2.53	29.40±2.37
Lymphocytes	0.86±0.16	0.58±0.18	0.64±0.38	0.54±0.15	0.88±0.65	1.13±0.64*
MCHC (g/dl)	74.28±6.76	77.37±7.19	70.36±2.79	71.47±7.35	72.02±6.29	77.09±6.91
MCV (fl)	38.08±0.57	38.06±0.57	38.39±0.23	37.75±0.49	38.18±0.63	38.16±0.46
Platelet ($\times10^2$/mm^3)	882.80±317.63	993.40±273.62	847.50±90.85	676.63±409.19	832.60±323.36	972.60±257.15
Hb (g/dl)	4.27±0.32	4.11±0.35	4.25±0.22	4.05±0.30	4.05±0.32	4.21±0.22
Females						
RBC ($\times10^6$/mm^3)	1.56±0.2	1.67±0.26	1.54±0.23	1.42±0.22	1.75±0.24	1.60±0.20
WBC ($\times10^3$/mm^3)	1.71±0.61	1.80±0.55	1.88±0.47	1.37±0.38	2.62±0.69	4.55±1.83*
Mid (%)	0.18±0.13	0.18±0.07	0.20±0.06	0.19±0.06	0.20±0.00	0.39±0.16*
MPV (fl)	19.03±6.93	18.99±6.75	20.68±5.20	22.06±0.43	17.31±7.94	18.01±9.50
Granulocytes (%)	0.50±0.00	0.76±0.24	0.70±0.26	0.61±0.22	1.07±0.29	2.47±1.41*
HCT (%)	5.94±0.64	6.31±0.92	5.87±0.86	5.39±0.90	6.62±0.77	6.03±0.88
MCH (pg/cell)	25.21±2.31	25.65±2.39	26.48±2.01	27.22±2.25	25.16±2.34	27.41±2.43
Lymphocytes	0.60±0.10	0.73±0.28	0.86±0.11	0.64±0.14	0.88±0.15	1.69±0.77*
MCHC (g/dl)	66.10±5.20	67.14±5.41	69.03±4.78	70.99±6.51	6.45±5.66	71.88±6.57
MCV (fl)	38.10±0.71	38.16±0.77	38.33±0.52	38.14±0.43	38.43±0.76	38.10±0.71
Platelet ($\times10^2$/mm^3)	535.40±257.37	575.80±287.73	620.40±213.64	758.90±165.53	520.80±314.02	535.40±257.37
Hb (g/dl)	4.00±0.24	4.21±0.43	4.02±0.48	3.94±0.53	4.34±0.43	4.00±0.24

Values are expressed as mean±SD. *Significantly different from control group ($p<0.05$). SD: Standard deviation, RBC: Red blood cell, WBC: White blood cell, MPV: Mean platelet volume, HCT: Hematocrite, MCHC: Mean corpuscular hemoglobin concentration, MCV: Mean corpuscular volume, Hb: Hemoglobin

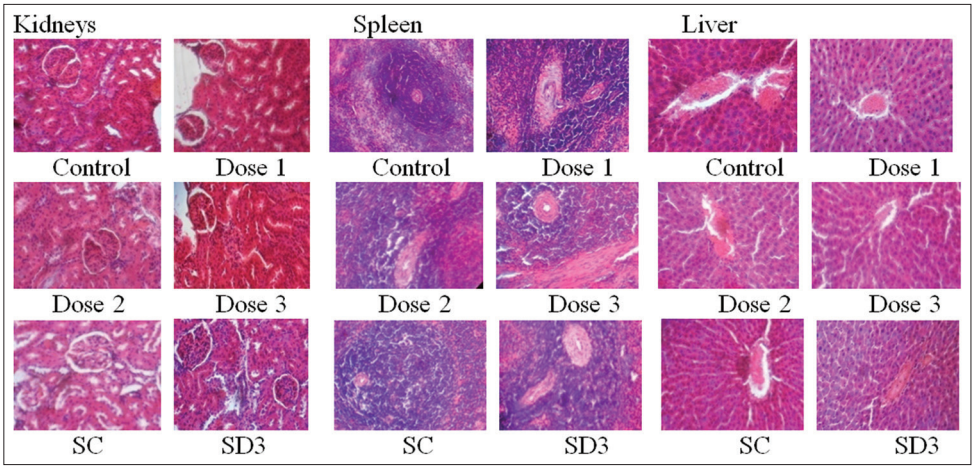

Fig. 2: Histological appearance on kidneys, spleen, and liver. SC is satellite control group; SD3 is satellite of dose 3 group

there were no statistical differences between all treated group compared to the control. Results of biochemical properties showed that administration did not affect kidneys and liver performances.

Metabolic disorder such as hyperlipidemia and hyperglycemia may contribute to fatal diseases such as atherosclerosis and diabetes, which may result in impairment of coronary blood flow [25] and disrupt homeostasis. Lipid profile including total cholesterol, triglyceride, HDL, and glucose serum levels. The results revealed that there were no alteration observed in all parameters similar with that of the control group.

With regards to histopathological findings, there was no significant alteration of histology including heart, liver, kidneys, and spleen. The results were relevant with other parameters and showed a similar pattern with the control group.

CS-BL extract was found to be safe when orally administered to rats for approximately 3 months. It was conducted to evaluate its subchronic toxicology. When it was administered at levels of CS 37.5 mg/kg bw and CL 50 mg/kg, CS 150 mg/kg and BL 200 mg/kg, as well as CS 450 mg/kg and BL 600 mg/kg to rats for 90 days, there were no significant hematological, biochemical, and remarkable histopathological changes in CS-BL treated group. Even though WBC value increased slightly, the number was still in the normal range. By observing vital organs under microscope, there was a pulp dilatation in spleen in all treated groups in which need to be observed more on particular immune response and lymphocytes number in spleen.

CONCLUSION

Overall, administration of CS in combination with BL extract did not alter behavior and physiology in both male and female Wistar rats. There were no differences found in parameters: Behavior, motoric activity, body weight changes, urine, clinical biochemistry, gastric mucousal, and appearance of organs microscopically. However, in terms of immunological aspect, it needs to be observed further to determine the effect of CS and binahong extract in combination.

REFERENCES

1. The Centre of Drug and Food Analysis. Standardized Procedure of Toxicity Test. Jakarta: The Ministry of Health of Republic of Indonesia; 2009. p. 1-82.
2. Maksimovic Z, Malencic D, Kovacevic N. Polyphenol contents and antioxidant activity of Maydis stigma extracts. Bioresour Technol 2005;96(8):873-7.
3. Hasanudin K, Hashim P, Mustafa S. Corn silk (Stigma maydis) in healthcare: A phytochemical and pharmacological review. Molecules 2012;17(8):9697-715.
4. Ditjen PO, Depkes RI. Materia Medika Indonesia. jilid 6. Jakarta: The Ministry of Healt of Republic of Indonesia, 1995. p. 139-42, 321, 324-5, 336.
5. El-Ghorab A, El-Massry KF, Shibamoto T. Chemical composition of the volatile extract and antioxidant activities of the volatile and nonvolatile extracts of Egyptian corn silk (*Zea mays* L.). J Agric Food Chem 2007;55(5):9124-7.
6. Chevallier A. Encyclopedia of Medicinal Plants. London: Dorling Kindersley; 2001.
7. Steenkamp V. Phytomedicines for the prostate. Fitoterapia 2003;74(6):545-52.
8. Maksimovic ZA, Kovacevic N. Preliminary assay on the antioxidative activity of Maydis stigma extracts. Fitoterapia 2003;74(1-2):144-7.
9. Hu QL, Zhang LJ, Li YN, Ding YJ, Li FL. Purification and antifatigue activity of flavonoids from corn silk. Int J Phys Sci 2010;5(4):321-6.
10. Fadliah F. The effect of water extract of corn silk (Maydis stigma) on gentamicin and piroxicam induced renal failure on rats. Bachelor theses, School of Pharmacy, Bandung Institute of Technology; 2008. p. 35-43.
11. Ebrahimzadeh MA, Pourmorad F, Hafezi S. Antioxidant activities of Iranian corn silk. Turk J Biol 2008;32:43-9.
12. Lemmens RH, Bunyapraphatsara N, editors. Plant Resources of South-East Asia. Vol. 12. Leiden 2003. p. 72-3.
13. Sukandar EY, Fidrianny I, Adiwibowo LF. Efficacy of ethanol extract of *Anredera cordifolia* (Ten.) steenis leaves on improving kidney failure in rats. Int J Pharmacol 2011;7(8):850-5.
14. Sukandar EY, Sigit JI, Adiwibowo LF. Study of kidney repair mechanisms of corn silk (*Zea mays* L.) hair – Binahong (*Anredera cordifolia* (Ten) steenis) leaves combination in rat model of kidney failure. Int J Pharmacol 2013;9(1):12-23.
15. Salasanti CD, Sukandar EY, Fidrianny I. Acute and sub chronic toxicity study of ethanol extract of *Anredera cordifolia* (ten.) V. Steenis leaves. Int J Pharm Pharm Sci 2014;6(5):348-52.
16. Alam EA. Evaluation of antioxidant and antibacterial activities of Egyptian maydis stigma (*Zea mays* hairs) rich in some bioactive constituents. J Am Sci 2011;7:726-9.
17. Bhaigyabati T, Kiritika T, Ramya J, Usha K. Phytochemical constituents and antioxidant activity of various extracts of corn silk (*Ze mays* L). Res J Pharm Biol Chem Sci 2011;2:986-93.
18. Astuti SM, Sakinah MA, Andayani R, Risch A. Determination of saponin compound from *Anredera cordifolia* (Ten) Steenis plant (Binahong) to potential treatment for several diseases. J Agric Sci 2011;3(4):224-32.
19. Winder CV, Lembke LA, Stephens MD. Comparative bioassay of drugs in adjuvant-induced arthritis in rats: Flufenamic acid, mefenamic acid and phenylbutazone. Arthritis Rheum 1969;12 Suppl 5:472-52.
20. Teo S, Stirling D, Thomas S, Hoberman A, Kiorpes A, Khetani V. A 90-day oral gavage toxicity study of D-methylphenidate and D, L-methylphenidate in Sprague-Dawley rats. Toxicology 2002;179(3):183-96.
21. Tiwari U, Rastogi B, Singh P, Saraf DK, Vyas SP. Immunomodulatory effects of aqueous extract of *Tridax procumbens* in experimental animals. J Ethnopharmacol 2004;92(1):113-9.
22. Marshal WJ. Clinical Chemistry. 3rd ed. London: Mosby; 1995. p. 231-2.
23. Wolf PL. Methods and Technique in Clinical Chemistry. New York: John Willey and Sons Inc.; 1972. p. 135-6, 159, 189-90, 377-82.
24. Kanter MW. Clinical Chemistry. Indiana: The Bobbs-Merrill Company Inc.; 1975. p. 65, 74-5, 111-4, 184-6.
25. Shaila HP, Udopa SL, Udopa AL. Hypolipidemic effect of *Terminalia arjuna* in cholesterol fed rabbits. Fitoterapia 1997;68:405-9.