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Toxicity Evaluation of Na-CMC synthesized from Cellulose of Water Hyacinth (Eichhornia crassipes (Mart.) Solms).

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ABSTRACT

Carboxymethylcellulose sodium (Na-CMC) has been synthesized from water hyacinth (*Eichhornia crassipes* (Mart.) Solms) cellulose in previous research either without or with epichlorohydrin as crosslinker. This study is aimed to evaluate the toxicity of uncrosslinked and crosslinked Na-CMC synthesized from water hyacinth cellulose. Research was conducted through acute oral toxicity study in female mice, pharmacological screening, and subchronic oral toxicity study in rats, haematological test, biochemical analysis, organ index and histopathological examination on liver, kidney, and lung. Results showed that LD_{50} of uncrosslinked and crosslinked Na-CMC for female mice were 14 g/kg body weight of mice, equivalent to 9.8 g/kg body weight of rat, categorized as practically non-toxic according to Loomis criteria (LD_{50} 5-15g/kg body weight of rat). Pharmacological screening results showed that uncrosslinked and crosslinked Na-CMC only gave effects in grasping ability and catalepsy. Subchronic toxicity study showed that there were no significant difference on the haematological and biochemical analysis in male rats group. However, there were significant difference in female rats positive control and experimental group on the haematological and biochemical analysis towards the control group. Histopathological examination showed that crosslinked Na-CMC synthesized from water hyacinth cellulose caused liver, kidney, and lung abnormality.

Key words: toxicity, acute, subchronic, carboxymethylcellulose sodium, crosslink, epichlorohydrin.

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INTRODUCTION

Water hyacinth (*Eichhornia crassipes* (Mart.) Solms), an easy-to-grow aquatic weed has 72,63% of cellulose [1]. This cellulose contain was high enough to be used as a raw material for carboxymethylcellulose sodium (Na-CMC). Previous research has produced Na-CMC from water hyacinth with shortcoming on water holding capacity (WHC) and oil holding capacity (OHC) compared to the standard Na-CMC [2]. Research conducted by Hasanah et al [3] has produced Na-CMC from water hyacinth cellulose by crosslinking with epichlorohydrin to overcome that. This co-processed pharmaceutical excipient should be evaluated for its safety [4]. The first step of pharmacological safety evaluation is acute toxicity study. Acute toxicity study is one of the pre-clinical study to determine the toxicity degree of a single dose of a substance, or multiple doses given within 24 hours. Its quantitative measurement is the median lethal dose (LD_{50}) [5]. The observed signs of toxicity are important to ensure the safety of this pharmaceutical excipient. The next safety evaluation is the subchronic toxicity study to investigate the substance safety from the repeated exposure over a relatively limited period of time [6]. This research was done to evaluate acute toxicity of crosslinked Na-CMC compare to uncrosslinked and measuring its pharmacological safety as an excipients.

MATERIALS AND METHODS

Instrumentation

Mortar and pestle, graduated cylinder, 1 mL syringe (OneMed), oral gavage needle, analytical balance (Mettler Toledo Dragon 204), and animal balance (Ohaus Triple Beam).

Materials

Experiment materials

Aquadest, standard Na-CMC, Na-CMC synthesized from water hyacinth cellulose and its synthesis with epichlorohydrin as crosslinker using the previously published method [2, 3].

Experiment animals

Experiment animals were albino mice of Swiss Webster strain weighing 20-30 gram (ethical approval No. 21/UN6.C1.3.2/KEPK/PN/2015) and albino rats of Wistar strain weighing 150-200 gram (ethical approval No. 398/UN6.C1.3.2/KEPK/PN/2016). These animals were acclimated for one week prior to the experiment. Animals were categorized as healthy if the body weight changes were not more than 10 % and their activities were normal [6, 7].

Methods

Acute Toxicity Study

Mice were divided into ten groups of negative control (aquadest), positive control (standard Na-CMC), each four dose variances of uncrosslinked and crosslinked Na-CMC (1,75; 3,5; 7; and 14 g/kg body weight). Three female mice were used for each group. All mice were fasted approximately 18 hours prior to dosing, and water was provided continuously. Pharmacological screening was conducted prior to the oral administration. After the administration, the mice mortality was observed for 14 days. Then, the LD50 value was determined by probit analysis. Besides, all mice were observed for the sign of toxicity at 0.5; 1; 2; 4; and 24 hours after administration (effects on central nervous system: motor activity, forelimb grip strength, grasping, tremor, convulsion, catalepsy, sedative, straub tail, tail pinch, toe pinch, pineal response, respiration, and autonomic nervous system: piloerection, salivation, lacrimation, abnormal urination, diarrhea). The body weight was also observed daily for 14 days. Resulted data was analysed statistically [7].

Subchronic Toxicity Study

Experiment rats of both male and female Wistar rats were divided into 4 groups, each group consisted of 6 rats. Group-I was the normal group. Group-II negative control group received aquadest. Group-III positive

control group received standard Na-CMC suspension (1.25 g/kg body weight). Group IV experimental group received crosslinked Na-CMC (1.25g/kg body weight). The animals were dosed daily for 28 days. Finishing the 28 days period, haematological test (erythrocyte, leukocyte, hemoglobin, hematocrit), blood biochemical analysis (SGOT, SGPT, creatinine, BUN), organ index determination (liver, kidney, lung, brain, heart, and stomach), histopathological examination of liver, kidney, and lung were done for each group. The organs were taken and fixed in Bouin for 48 hours. Specimens were prepared by dehydration, washing, infiltration, embedding, slicing with microtome, and staining with hematoxylin and eosin. Then, the specimens were placed on the glass slides and examined under a microscope with 100 times magnification [6].

RESULTS AND DISCUSSION

Acute Toxicity Study Result

Acute toxicity study was a preliminary study about substance safety of Na-CMC synthesized from water hyacinth cellulose and its synthesis with epichlorohydrin as crosslinking agent. This toxicity study included the mortality and body weight observation for 14 days, and the mice behaviour observation for 24 hours subsequent to the administration of the substance.

The mortality observation for 14 days is presented in Table 1. No mortality was observed in this experiment, which indicated the LD_{50} of the substance was 14 g/kg body weight of mice or equivalent to 9.8 g/kg body weight of rat. According to Loomis criteria, Na-CMC synthesized from water hyacinth cellulose and crosslinked Na-CMC were categorized as practically non-toxic (5-15 g/kg body weight of rat) [8]. Besides, the mice body weight observation was done to investigate the effect of substance administration to the body weight for 14 days. The body weight development is shown in Figure 1 The body weight measurement was analysed statistically using Kruskal Wallis test and resulted that there were difference of mice body weight among the groups.

Within 14 days, body weight fluctuation was observed. Significant body weight decrease by more than 20% compared with control animals, or by more than 25% over a period of 7 days or more may indicate the animal's condition deterioration, which is usually accompanied by a change in food and water consumption [9]. In this research, no animal was detected in this condition.

Pharmacological behaviour observation of a substance was done to give the description of a substance effect on the body and to direct the further study. Pharmacological screening result was statistically analysed using Kruskal Wallis test. The result showed that the substance administration gives significant difference effects in grasping ability and catalepsy.

Subchronic Toxicity Study Result

Haematological parameter of male and female rats were examined as shown in Table 2 and 3 respectively. Based on statistical analysis using t-test, each parameter (erythrocyte, leukocyte, hemoglobin, hematocrit) on all groups (except leukocyte value in female positive control and experimental group) showed the probability value is greater than α (0.05), so there is no significant difference compared to normal control group. Examination of the biochemical parameters in male and female rats are presented in Table 4 and 5 respectively. According to the statistical analysis using t-test, SGOT, SGPT, creatinine, BUN in all groups (except BUN value in female positive control and experimental group) showed the probability value is greater than α (0.05), so there is no significant difference compared to normal control group. The male and female rat organ index are shown in Table 6 and 7 respectively. The statistical t-test showed no significant difference in organ index (liver, kidney, lung, brain, heart, and stomach) in both experimental and control group (probability value > α (0.05). This showed that organ indexes in experimental group were in normal range.

The histopathological features in this study were displayed in Figure 2. The liver tissue in male and female experimental groups showed dilatation of sinusoid, inflammatory cell infiltration, and apoptosis. Liver sinusoid is the capillary bed carrying portal venule and hepatic arteriole blood [10]. Apoptosis usually occurs as a response of cell renewal or regeneration from the old or damaged cells [11]. Apoptosis in the group indicated the test substance had caused damage on the organ. Histopathological condition of the male and female kidney is characterized by bleeding, inflammatory cell infiltration, and undetected Bowman's Capsule. This condition

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indicates the inflammation of the kidney [12]. Fibrosis was occurred in female lung. Fibrosis is the irreversible phenomenon of excessive formation of fibrous connective tissue in an organ that used to describe a pathological state [13].

Table 1 Cumulative Mice Mortality Percentage for 14 Days After Administration of Na-CMC Synthesized from Water Hyacinth Cellulose and Crosslinked Na-CMC

Group	Cumulative Mortality After Dosage Administration (%) in Days													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Negative Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Positive Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Test Group I	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Test Group II	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Test Group III	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Test Group IV	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Test Group V	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Test Group VI	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Test Group VII	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Test Group VIII	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Description:

Negative Control	: Aquadest 125 ml/kg body weight
Positive Control	: Standard Na-CMC 7 g/kg body weight
Test Group I	: Synthesized Na-CMC 1.75 g/kg body weight
Test Group II	: Synthesized Na-CMC 3.5 g/kg body weight
Test Group III	: Synthesized Na-CMC 7 g/kg body weight
Test Group IV	: Synthesized Na-CMC 14 g/kg body weight
Test Group V	: Crosslinked Na-CMC 1.75g/kg body weight
Test Group VI	: Crosslinked Na-CMC 3.5 g/kg body weight
Test Group VII	: Crosslinked Na-CMC 7 g/kg body weight
Test Group VIII	: Crosslinked Na-CMC 14 g/kg body weight

Table 2 Male Rat Haematological Value

Group	Hemoglobin (g/dL)	P*	Hematocrit (%)	P*	Erythrocyte ($10^6/m^3$)	P*	Leukocyte ($10^3/mm^3$)	P*
Normal	13.54 ± 0.82	-	47.50 ± 1.76	-	4.51 ± 0.42	-	3.25 ± 0.85	-
Negative	14.16 ± 0.67	0.185	46.83 ± 2.14	0.568	4.74 ± 0.24	0.269	3.73 ± 1.04	0.398
Positive	14.46 ± 0.60	0.053	47.33 ± 0.82	0.839	4.790 ± 0.20	0.161	3.87 ± 0.72	0.204
1.25g/kgBW	14.37 ± 0.42	0.053	47.33 ± 1.03	0.845	4.83 ± 0.59	0.134	3.43 ± 0.84	0.713

Description : The number of test animals each group is 6 animals.
: P : Probability; P ≤ 0.05 have significant meaningful differences
: ** : there are significant differences to the normal group

Table 3 Female Rat Haematological Value

Group	Hemoglobin (g/dL)	P*	Hematocrit (%)	P*	Erythrocyte ($10^6/m^3$)	P*	Leukocyte ($10^3/mm^3$)	P*
Normal	12.72 ± 0.55	-	43.67 ± 1.21	-	4.27± 0.23	-	2.59 ± 0.49	-
Negative	13.40 ± 0.68	0.083	43.00 ± 2.28	0.541	4.48 ± 0.09	0.062	3.11 ± 0.79	0.203
Positive	13.51 ± 0.98	0.115	43.67 ± 0.82	1.000	4.48 ± 0.27	0.181	3.63 ± 0.86	0.027**
1.25g/kgBW	13.23 ± 1.19	0.368	45.00 ± 1.27	0.092	4.43 ± 0.43	0.428	3.63 ± 0.77	0.019**

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Description : The number of test animals each group is 6 animals.
: P : Probability; P ≤ 0.05 have significant meaningful differences
: ** : there are significant differences to the normal group

Table 4 Male Rat Biochemical Value

Group	SGOT (UI/L)	P*	SGPT(UI/L)	P*	BUN (mg/dL)	P*	Creatinine (mg/dL)	P*
Normal	159.67 ± 35.94	-	68.17 ± 4.31	-	40.167 ± 2.63	-	0.86 ± 0.072	-
Negative	189.67 ± 35.68	0.177	77.33 ± 13.92	0.154	44.17 ± 3.66	0.055	0.81 ± 0.80	0.239
Positive	201.17 ± 36.48	0.075	65.67 ± 6.41	0.446	38.83 ± 5.42	0.600	0.858 ± 0.11	0.904
1.25g/kgBW	157.83 ± 15.48	0.911	69.83 ± 10.57	0.728	37.00 ± 2.90	0.076	0.847 ± 0.11	0.740

Description : The number of test animals each group is 6 animals.
: P : Probability; P ≤ 0.05 have significant meaningful differences
: ** : there are significant differences to the normal group

Table 5 Female Rat Biochemical Value

Group	SGOT (UI/L)	P*	SGPT(UI/L)	P*	BUN (mg/dL)	P*	Creatinine (mg/dL)	P*
Normal	129.67 ± 14.72	-	54.33 ± 3.08	-	38.83 ± 3.43	-	0.73 ± 0.07	-
Negative	118.17 ± 12.66	0.177	49.17 ± 7.11	0.133	44.83 ± 6.27	0.067	0.69 ± 0.10	0.461
Positive	143.67 ± 30.65	0.337	54.50 ± 6.75	0.957	47.33 ± 6.28	0.016**	0.65 ± 0.06	0.071
1.25g/kgBW	152.00 ± 24.35	0.083	52.50 ± 7.01	0.570	44.33 ± 3.45	0.020*	0.81 ± 0.17	0.324

Description : The number of test animals each group is 6 animals.
: P : Probability; P ≤ 0.05 have significant meaningful differences
: ** : there are significant differences to the normal group

Table 6 Male Rat Organ Index

Group	Liver	Stomach	Kidney	Brain	Lung	Liver
Normal	2.85 ± 0.21	1.02 ± 0.11	0.83 ±0.05	0.73 ± 0.10	0.83 ± 0.10	0.34 ± 0.02
Negative	2.68 ± 0.19	0.97 ± 0.09	0.85 ± 0.04	0.70 ± 0.06	0.73 ± 0.11	0.35 ± 0.03
Positive	2.85 ± 0.39	1.00 ± 0.07	0.86 ± 0.08	0.69 ± 0.06	0.81 ± 0.16	0.36 ± 0.02
1.25g/kgBW	2.79 ± 0.20	1.04 ± 0.15	0.87 ± 0.08	0.71 ± 0.08	0.71 ± 0.37	0.37 ± 0.02

Description : The number of test animals each group is 6 animals.
: P : Probability; P ≤ 0.05 have significant meaningful differences
: ** : there are significant differences to the normal group

Table 7 Female Rat Organ Index

Group	Liver	Stomach	Kidney	Brain	Lung	Liver
Normal	2.90 ± 0.21	1.24 ± 0.23	0.85 ± 0.10	0.76 ± 0.08	0.95 ± 0.05	0.38 ± 0.04
Negative	2.68 ± 0.41	1.20 ± 0.41	0.80 ± 0.13	0.70 ± 0.13	0.98 ± 0.26	0.36 ± 0.07
Positive	2.43 ± 1.20	1.23 ± 0.62	0.67 ± 0.35	0.67 ± 0.35	0.81 ± 0.52	0.29 ± 0.14
1.25g/kgBW	2.96 ± 0.30	1.05 ± 0.30	0.82 ± 0.22	0.75 ± 0.220	0.98 ± 0.05	0.38 ± 0.02

Description : The number of test animals each group is 6 animals.
: P : Probability; P ≤ 0.05 have significant meaningful differences
: ** : there are significant differences to the normal group

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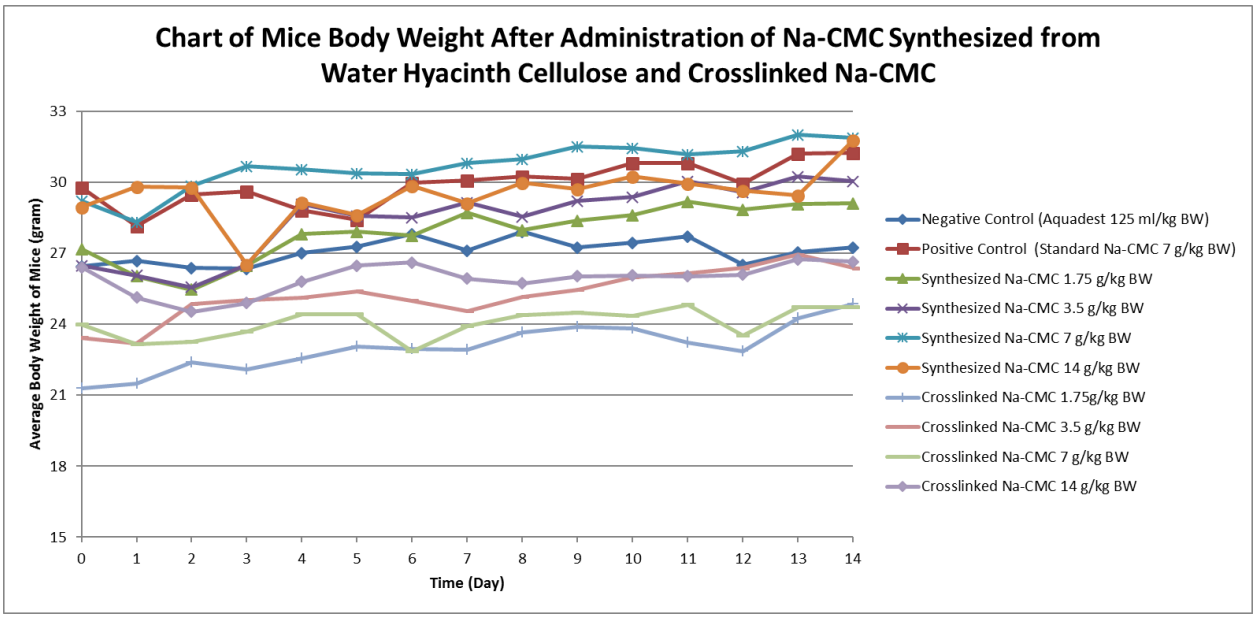

Figure 1 Chart of Mice Body Weight After Administration of Na-CMC Synthesized from Water Hyacinth Cellulose and Crosslinked Na-CMC

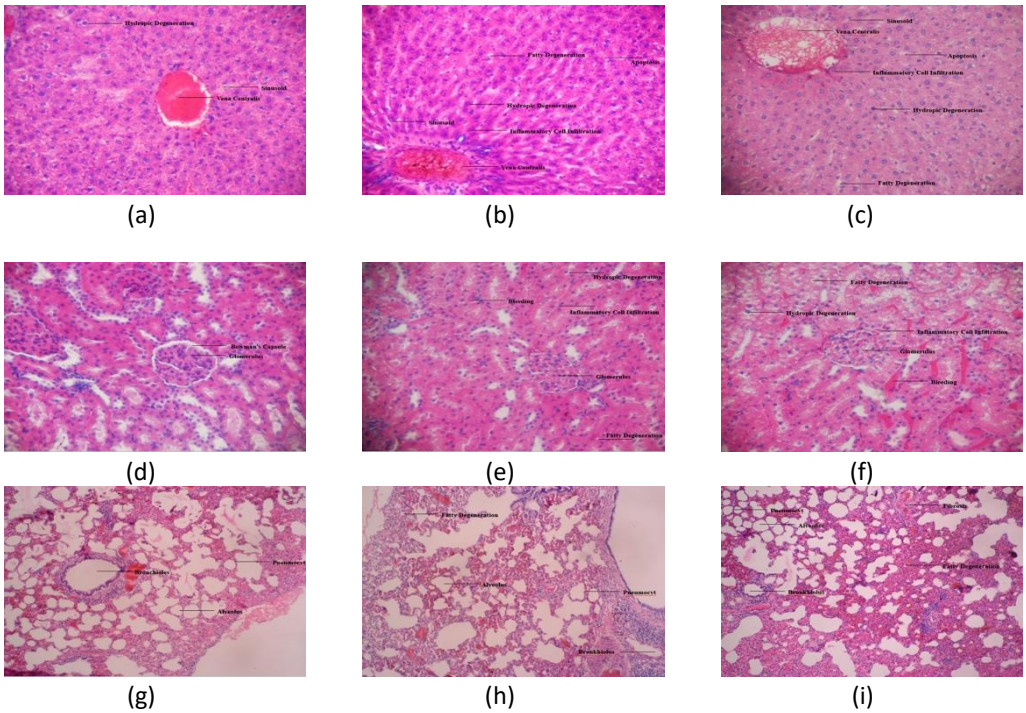

Figure 2 Histopathological examination: rats tissue cross-section (a) normal male liver; (b) experimental male liver; (c) experimental female liver; (d) normal male kidney; (e) experimental male kidney; (f) experimental female kidney; (g) normal male lung; (h) experimental male lung; (i) experimental female lung

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CONCLUSION

Based on the acute toxicity study of uncrosslinked and crosslinked Na-CMC synthesized from water hyacinth cellulose, the LD_{50} value was 14 g/kg body weight of female mice.

The safety level of uncrosslinked and crosslinked Na-CMC synthesized from water hyacinth cellulose were classified in the practically non-toxic category according to Loomis criteria (5-15 g/kg body weight of rat).

Pharmacological screening test revealed that there were no significant difference effect of tested substance in motor activity, forelimb grip strength, tremor, convulsion, sedative, straub tail, tail pinch, toe pinch, pineal response, respiration, piloerection, salivation, lacrimation, abnormal urination, and diarrhea. However, there were significant difference in grasping and catalepsy effect.

The subchronic oral toxicity study of 1.25 g/kg body weight of rat only showed the significant difference on leukocyte and BUN in female positive control and experimental group. The histopathological examination revealed abnormality in liver, kidney, and lung of both male and female positive control and experimental group.

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