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Carboxymethyl cellulose synthesis from wheat straw and physiological effects as food additive on some haematological and biochemical parameters of male mice

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Abstract: High purity carboxymethyl cellulose (CMC) was prepared by using α-cellulose extracted from wheat straw by etherification process. Presence of toxic metals in the prepared CMC was tested by Atomic Absorption Spectroscopy which was within the WHO/FAO recommended value. Purity of the CMC was 99.98 % that was higher than proposed limit. Microorganisms were tested by pour plate method. No colonies were observed after incubation of different media indicating absence of microorganisms. Repeated-dose sub-chronic oral toxicity was studied in Swiss albino mice following OECD guideline 408. 24 mice were divided into 4 groups fed diets with 0 (control), 2, 4 and 8% CMC for a period of 3 months. No abnormalities showed and no animals died during the administration period. Haematological and biochemical parameters were determined at every month of the experiment. No significant differences were observed between control and treated mice with different percentages of CMC. Therefore, the present study found no toxic effect of CMC that support the safety use of CMC as additive for foods and pharmaceuticals.

Keywords: Wheat straw, Waste, CMC, Food additive, Toxicity, Mice.

INTRODUCTION

Wheat straw is an important ligno-cellulosic agro-waste mainly consists of cellulose, hemicellulose and lignin. Every year in the world, tons of unused wheat straw are generated as agro waste. Only few percentages are used as feedstock and energy production, mostly dumped on field which causes environmental pollution. Production of useful component from agro residue is quite environmental friendly. Many researchers have tried to transform this waste into value-added products. Wheat straw has already been used for making composites, panel boards and anion exchangers[1,2]. A limited number of studies have reported the use of wheat straw cellulose for its derivatives production[3-5]. Today CMC has got ample scientific attention, especially due to its polyelectrolyte character and it is the most widely used cellulose ether with applications in many areas of industry and human life[6] as food additives. The continuously increasing demand of instant and defatted food makes the CMC market grow dramatically in recent years[7].

It is reported that more than 2, 5000 types of additives are used to maintain quality and consistency of food [8,9]. Toxicity studies for assessing the safety of food and food additives have developed over the past years in the field of toxicology and has expanded. Evaluation of toxic properties of a substance is crucial when considering for public health protection because exposure to chemicals can be hazardous and results to adverse effects on human being [10]. Recent years in Bangladesh, the use of CMC as additive in foods, pharmaceuticals and cosmetics has extensively increased. The present study carried out to determine the purity, biochemical and haematological toxicity of synthesized CMC, with the aim to obtain information on the safety use of CMC as additive.

MATERIALS AND METHODS

Materials: Wheat straw were collected from Regional Wheat Research Institute, Rajshahi, Bangladesh. Healthy Swiss albino mice and pellet diet for mice were purchased from International Center for Diarrheal Diseases Research, Bangladesh (ICDDR'B). Chemicals used during the study were sodium hydroxide (Merck, India), monochloroacetic acid (BDH, England), ethanol (Merck, Germany), methanol (Merck, Germany), glacial acetic acid (BDH, England), silver nitrate (BDH, England), n-hexane (Merck, Germany), sodium chlorite (Merck, Germany), etc. All chemicals were of reagent grade and used without further purification.

Methods

Preparation of Sample*:* Wheat straw were cut manually into small pieces and dried in the sun light to remove moisture. The dried samples were milled into powder using a grinding disk mill (model: FFC-15, Taian City Up International Trade Co.,Ltd , Shandong, China). The powdered straw samples were then passed through 230 mesh (64 µm) screen (Sieve type: OHIO 44060, USA) and stored in a desiccator for further use.

Synthesis of carboxymethyl cellulose: α-Cellulose was extracted from straw sample following by the method of Mondal and Haque [11]. Then CMC was prepared from α-cellulose by the method of Mondal et al. [12] with some modification. At first certain amount of cellulose was treated with 30% aqueous NaOH using 95% ethanol as solvent for 30 min at room temperature. Then 12.6 mol/L aqueous mono-chloroacetic acid ($ClCH_2COOH$) was added drop-by-drop to the mixture and heated at 60°C for 3 hrs. The mixture was separated into two phases. The liquid phase was removed and the solid phase was suspended in methanol, and neutralized

by acetic acid. Then, it was filtered and washed for five times with ethanol. The residue from filtration was oven dried at 60°C and CMC was obtained.

Characterization of the Synthesized CMC

***Purity test*:** Purity or the percentage of CMC in the sample was calculated by deducting 100 % of the sum of percentages of sodium chloride and sodium glycolate (free glycolate), determined separately by the procedures described by Mondal *et al*.[12].

Carboxymethyl cellulose content (%) = 100 – (% NaCl + % sodium glycolate)

***Toxic metal test*:** Toxic metals such as arsenic (As), lead (Pb), cadmium (Cd) and mercury (Hg) in the prepared CMC were determined by Atomic Absorption Spectroscopy (Model: AA-68000, Shimadzu, Japan) coupled with an auto-sampler (ASC-6100). Metal ion of a sample was quantitatively determined by measuring the radiation absorbed by atoms of the sample solution with respect to a known concentration.

***Microbiological test*:** Food products and its ingredients in which microorganisms can survive and grow, routine microbiological analysis is important to confirm the safety of foods. The presence of microorganisms such as Yeasts and Molds, *E. coli*, *Coliform* and *Salmonella* in the prepared CMC were determined following by the method described in the literature[13].

Toxicity test

Experimental Animals and Housing Conditions: Healthy male Swiss albino mice collected from International Center for Diarrheal Diseases Research, Bangladesh (ICDDR'B) of body weight 28 ± 2 g were used in the study. Mice were divided into four groups named A, B, C (treated groups) and D (control group). Each group contains 6 mice per cage with cutting straw bedding. A 12 h light-dark cycle was maintained while the temperature and relative humidity of the animal rooms were maintained at 24°C ±3°C and 50–60%, respectively. Animals care and handling conformed to accepted guidelines[14,15]. All animals were allowed to acclimatization for 7 days to the laboratory conditions before the experiment. The experiment was performed in accordance with the guidelines established by the European Community for the Care Use of Laboratory Animals and were approved by Institutional Animal Ethical Committee[16] , and the Organization for Economic Cooperation and Development guidelines [17] .

Sub-chronic Oral Toxicity Study: Commercially available food ingredients, pellet diet were used as normal food for experimental mice (control and treated groups). Test sample such as CMC powder was administered orally with normal food. The diet supplied to each mouse was about 4-5 g per day, which was approximately isocaloric. Group-A received diet with 2% CMC, group- B received diet with 4% CMC, and group-C received diet with 8% CMC and group-D received food with only water. During the period of CMC administration, the animals were observed closely for signs of any toxicity. Repeated dose 90-days oral toxicity studies were performed on male Swiss albino mice with CMC as per the OECD guidelines no. 408.[17]

Laboratory Investigations: A Blood samples were collected from the tail veins of each group of mice by conventional way on days 30, 60 and 90 and collected in a clean and dry test tube for haematological and biochemical examinations. Haematological investigations were performed for the measurement of hemoglobin (Hb) levels, platelet, total count of red blood cells (RBC), white blood cells (WBC) and its differentials (neutrophil, lymphocyte and monocyte). Biochemical parameters measured were serum glucose, cholesterol and triglyceride, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase

(ALP), serum urea and creatinine. All parameters were determined at room temperature (27±0.5) °C following standard laboratory procedures[18].

RESULTS AND DISCUSSION

Purity test: CMC was purified by absolute alcohol washing that removes sodium chloride and sodium glycolate as by-products. Where the CMC product is intended for human consumption, CMC must be purified to a level of minimum 99.5 percent [19]. The percentages of CMC content and sodium salts as impurities in the prepared CMC were determined and presented in **Table 1**. It can be observed from the **Table 1** that percentages of sodium chloride and sodium glycolate in the prepared CMC are very low, 0.011% and 0.009% respectively. Lower salts content means higher purity. Sodium glycolate is toxic in nature. It is reported from the literature [20,21] that sodium chloride and sodium glycolate contents in the food additives should not more than 0.5% each or in combination. The content of CMC increased suggests that, during carboymethylation, more MCA molecules are substituted to the cellulose polymer, thus decreasingthe possibility of MCA to react with NaOH to form by-products[22].

Table 1: Determination of CMC, NaCl and sodium glycolate contents.

Parameters	**Test results**
Content of CMC or purity (%)	99.98±0.002
Content of NaCl (%)	0.011±0.06
Content of sodium glycolate (%)	0.009±0.06

Results are expressed as mean ± standard deviation; n=3

Toxic metal test: Mankind today is exposed to the highest levels of toxic metals in recorded history [http://drlwilson.com/articles/toxicmetals.htm, 18.12.2015]. Toxic metal content in the prepared CMC was determined and compared with the recommended values shown in **Table 2**. From the results in **Table 2**, it can be seen that the concentrations of Pb, Cd, Hg and As are 0.0004, 0.001, 0.0002 and 0.0003 ppm respectively. These values were within the World Health Organization (WHO) permissible limits for food additives and emulsifier. Both the *Food Chemicals Codex* and the Food and Agriculture Organization of the United Nations World Health Organization (FAO/WHO) have established specifications for identity and purity of CMC which are also met by prepared CMC.

Table 2: Concentration of toxic metals in the synthesized CMC.

Heavy metals	**Studied values (Concentration in ppm)**	**Proposed general limit [JECFA, 1967; JECFA, 2000]**
As	0.0003	0.05 mg/kg body weight/day
Pb	0.0004	0.005 mg/kg body weight/day
Cd	0.001	1 mg/kg body weight/day
Hg	0.0002	0.001-0.0003 mg/kg body weight/day

Microbiological test: It is essential to detect microorganisms, in particular pathogenic microorganisms in the areas of the food and pharmaceutical industries to confirm the authenticity of foods. The presence of microorganisms such as Yeasts and Molds, *E.coli*, *Coliform* and *Salmonella* in the prepared CMC were tested and the cultural responses of tested organisms are given in **Table 3**.

Table 3: Microbiological test of the prepared CMC.

Tested organisms	**Cultural response**
Total plate count, cfu/g	<100
Mold, cfu/g	<100
Yeast, cfu/g	<100
Colliform/g	Negative
Salmonella/ g	Negative
E. coli/ g	Negative

The *total plate count* is intended to indicate the level of microorganisms in a product. No colonies were observed in sample containing cultured media after 48±2 h incubation at 28±1°C. Where no colonies are visible, the result is expressed[23] as less than 100 cfu/g .Yeasts grow as creamy to white colonies and Molds grow as filamentous colonies of various colours on Potato Dextrose Agar (PDA) media. When 1 g sample aliquot was poured in a plate containing PDA, no colonies were visible after 5 days incubation of plate at 20-25°C that confirms the absence of Yeasts and Molds in the sample. *E.coli* grows as dark blue to violet colonies, *coliform as* salmon to red colonies and *Salmonella* as colourless colony. Results in **Table 4** also show that *Coliform, Salmonella* and *E.coli* either failed to grow or produced negative results with their respective culture media.

Toxicity test: The mortality and behavioral patterns of all animals were observed visually which lived upto 90 days after the administration diet with CMC. The behavioural patterns of animals were observed first 6 h and followed by 14 h after the administration. No significant changes were observed in wellness parameters, and the animals in both control and treated groups were normal. Haematological and biochemical parameters of mice were determined at every month of the experiment.

Haematological parameters: Haematological parameters are very important tool for assessing the injures that caused by certain substances[24]. The results of haematological data of mice are shown in **Table 4**.

Table 4: Effect of haematological parameters on Swiss albino mice diet with CMC for 90 day.

Days	**Parameters**	**Control group**	**CMC treated group**		
		D	**A (2% CMC)**	**B (4% CMC)**	**C (8% CMC)**
30	White blood cell ($\times 10^9$/L)	9.38±3.07	10.80± 1.70	11.11±3.33	9.77±2.97
	Red blood cell ($\times 10^{12}$/L)	6.78±0.53	7.00±0.20	6.98±0.45	7.10±0.35

	Platelet ($\times10^{12}$/L)	1015.70±108.20	1009.10±122.80	1005.08±247.80	999.87±141.90
	Hemoglobin(g/dL)	10.96±0.90	11.00±1.30	11.05±1.00	11.20±1.40
	Lymphocytes (%)	77.11±5.90	79.30±4.80	74.00±3.50	76.70±4.30
	Monocyte (%)	5.12±1.20	4.79±0.60	4.67±0.90	5.09±1.00
	Neutrophils (%)	23.78±1.90	23.99±1.60	33.30±0.90	32.80±1.10
60	White blood cell ($\times10^{9}$/L)	9.99±2.17	10.17± 1.50	10.11±3.03	11.08±2.90
	Red blood cell ($\times10^{12}$/L)	6.80±0.59	7.02±0.10	6.86±0.75	7.00±0.55
	Platelet ($\times10^{12}$/L)	1015.80±108.20	1012.10±122.80	1006.70±247.80	998.30±141.90
	Hemoglobin(g/dL)	11.65±0.91	10.55±1.03	11.19±1.01	10.80±1.40
	Lymphocytes (%)	79.71±4.90	86.30±3.80	77.80±3.50	80.70±3.30
	Monocyte (%)	4.68±1.20	4.07±0.65	4.51±0.70	5.11±1.09
	Neutrophils (%)	24.20±1.80	23.70±1.60	32.30±0.90	30.80±1.11
90	White blood cell ($\times10^{9}$/L)	9.79±2.77	10.03± 1.71	11.00±2.33	10.00±1.97
	Red blood cell ($\times10^{12}$/L)	6.99±0.55	7.13±0.22	7.01±0.80	7.14±0.30
	Platelet ($\times10^{12}$/L)	1020.80±108.20	1016. ±122.80	1016.80±247.80	999.30±141.90
	Hemoglobin(g/dL)	10.61±0.90	10.93±1.30	11.29±1.00	10.95±1.40
	Lymphocytes (%)	88.59±5.80	92.30±4.80	79.80±3.10	87.10±3.30
	Monocyte (%)	5.16±1.20	4.93±0.60	5.01±0.60	5.21±1.11
	Neutrophils (%)	25.20±0.90	26.70±1.61	33.29±0.70	34.70±1.09

Value presented as mean ± standard deviation (n = 6 mice/group), at 5% level of significant ($p<0.05$). Statistical tests: ANOVA followed by Dunnett's tests.

It can be seen from the Table 4 that there was increase in WBC and RBC concentration in the test groups compared to the control group. Platelets were decreased in mice of all treated groups compared to control groups. But, the values were statistically not significant. Therefore, it is plausible to assume that the prepared CMC is not hematotoxic. This observation was in agreement with [25]Kumar and Sastry and [26]Attanayaka et al. [2015]. There were no significant differences in hemoglobin concentration at all dose levels of treated groups compared to control level. Lymphocyte was increased in the mice of CMC treated A group and monocytes were decreased in the A and B groups. It can also be seen from the Table that neutrophils showed significant difference at the groups of B and C compared to the values at the groups of A and control. All differences of these parameters were considered to be fortuitous since they were not dose related, or were numerically very small.

Biochemical parameters: Liver, kidney and heart are selected to conduct a detailed assessment of biochemical parameters. Liver cell damage is characterized by increase in hepatic enzymes like aspartate amino transferase

(AST), alanin amino transferase (ALT) and alkaline phosphatase (ALP) [27-29]. AST and ALT are released in the heart and an elevation in their plasma concentrations are indicators of cardiac damage[30,31].

The effects of CMC at different concentrations on biological parameter of mice are shown in **Table 5**. It can be seen from the Table that the activity of AST showed no significant changes in all concentration levels of CMC. At CMC concentrations of 2% and 4% compared to the control group, there was also no significant change in the activity of ALT. However, a significant increase was observed at concentration of 8% CMC than in control. The activity of plasma ALP increased at 8% CMC concentration, but no significant difference was observed at lower concentrations such as 2% and 4% CMC compared to the control. Since in this study the enzymes showed no appreciable increase or decrease in the treated animals, it implies that the product has no hepato-toxic effect.

Table 5: Effect of biochemical parameters on Swiss albino mice diet with CMC for 90 days**.**

Days	**Parameters**	Control group	Treated group		
		D	A (2% CMC)	B (4% CMC)	C (8% CMC)
30	AST (U/L)	162.95±0.11	163.01±0.12	163.05±0.13	162.98±0.10
	ALT (U/L)	53.20±1.90	54.90±1.60	55.04±1.90	69.10±3.50
	ALP (U/L)	128.20±1.90	122.70±1.60	130.30±1.90	138.80±1.50
	Serum urea (mg/dL)	34.30±0.90	29.00 ±1.00	30.80±0.80	31.10±1.20
	Serum creatinine (mg/dL)	67.30±0.90	71.00 ±1.00	70.80±0.80	68.10±1.20
	Serum glucose (mg/dL)	125.39±3.07	140.78± 1.70	135.01±3.33	145.17±2.97
	Serum cholesterol (mg/dL)	180.88±1.53	201.06±2.20	198.95±1.45	200.04±2.35
	Serum triglyceride (mg/dL)	120.60±1.70	113.80±1.30	115.00±1.70	99.84±1.40
60	AST (U/L)	164.95±0.17	163.73±0.11	164.11±0.13	163.53±0.17
	ALT (U/L)	55.10±1.90	56.71±1.60	58.34±1.90	71.80±3.50
	ALP (U/L)	132.10±1.20	130.50±1.60	131.30±1.80	143.80±1.00
	Serum urea (mg/dL)	30.10±0.98	29.55 ±1.07	29.80±0.80	28.98±1.22
	Serum creatinine (mg/dL)	66.30±0.90	67.76 ±1.00	70.11.80±0.80	68.12±1.20
	Serum glucose (mg/dL)	126.09±2.07	142.80± 1.90	138.01±2.30	141.08±2.07
	Serum cholesterol (mg/dL)	189.88±1.07	210.06±1.60	195.95±1.45	200.22±2.30
	Serum triglyceride (mg/dL)	110.60±0.99	102.80±1.30	108.00±1.70	101.41±1.34
90	AST (U/L)	161.95±0.11	163.70±0.11	161.10±0.13	162.10±0.10

	ALT (U/L)	52.77±1.90	57.70±1.60	59.31±1.90	71.80±3.50
	ALP (U/L)	131.22±1.41	130.70±1.40	131.33±0.90	140.70±1.05
	Serum urea (mg/dL)	29.40±0.91	28.00 ±1.01	28.90±0.90	27.77±1.22
	Serum creatinine (mg/dL)	68.01±0.90	70.02 ±1.00	69.23±0.80	68.99±1.20
	Serum glucose (mg/dL)	120.39±3.07	141.80± 1.70	139.01±3.03	140.07±2.07
	Serum cholesterol (mg/dL)	180.80±1.60	200.06±2.10	195.85±1.05	201.04±2.30
	Serum tryglyceride(mg/dL)	105.50±1.80	100.80±1.30	102.00±1.70	99.11±1.33

Values are expressed as mean ± S.E.M of 6 animals (one-way ANOVA); p>0.05 vs control group (student t-test). Significantly different from control, p<0.05.

Urea and creatinine are considered as a suitable prognostic indicator of renal dysfunction and kidney failure for any toxic compounds[32]. In this study, it can be seen from the table that serum urea were slightly decreased and serum creatinine were slightly increased by the doses of 2%, 4% and 8% CMC as compared with control. The lack of significant alterations in the levels of creatinine and urea means CMC has no harmful effect on the kidney.

Dietary CMC with different concentrations also significantly increased the serum glucose, serum cholesterol values and significantly decreased the serum triglyceride values compared to control. However, these values remained within the normal range throughout the experimental period indicating there was no CMC toxicity. All results of biochemical parameters were supported by reports in the literature[33-35].

CONCLUSION

Annually the global agricultural sector produces billions of tonnes of agricultural residue as straw, cereals, oil seeds and other plants commodities. Wheat straw is one of the largest biomass generated from wheat processing industries consists higher percentage of cellulose (48%). The produced CMC from straw cellulose was well suited for pharmaceutical and food additives owing to its high purity and no toxicity. The production of food-grade CMC will not only be helpful in the urban agricultural waste management but also be fruitful in saving the country revenue which it expends in importing CMC.

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