



The Safety Properties of Sodium Alginate and its Derivatives

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ABSTRACT

Alginate is a biomaterial with a vast use in biomedicine, food and pharmaceutical industry due to its advantageous properties such as abundance, biocompatibility and easiness of gelation. The Food and Drug Administration (FDA) has confirmed some alginates salts and related derivative to “generally regarded as safe” components for oral use. According to the vast use of alginate as a harmless agent in food and pharmaceutical industries, logically its hydrolysate and ultimately its residues including Mannuronic and Guluronic acids, as alginate hydrolysate components are safe and non-toxic ingredients. In this regard, a number of studies have been conducted to reveal the safety of these agents. Hear, we aimed to review the safety of sodium alginate and its hydrolysate in the literature. In conclusion, the outcomes of acute and chronic toxicity studies indicate that the sodium alginate and its hydrolysate have a high safety profile when administered orally to animals.

INTRODUCTION

Alginate is a biomaterial with a vast use in biomedicine, as well as industry due to its advantageous properties such as biocompatibility and easiness of gelation. Structurally, alginate is a hydrophilic polysaccharide composed of linear copolymers comprising blocks of α -L-guluronate (G) and β -D-mannuronate (M) blocks covalently connected by 1,4-glycosidic bonds (1). The quantity and arrangement of these blocks as well as the source and extraction

method of alginate lead to an exclusive physicochemical nature to the alginates (2). X-ray diffraction studies showed that alginate contains all four possible glycosidic bonds. This property leads to the flexibility of alginate chains according to M and G arrangement and the structure of the chain (3-5).

One of the remarkable peculiarities of alginates is their capability to make gels through binding multivalent cations or acid precipitation. Interestingly, in spite of other polysaccharides such as agar and gelatin, the sol/gel transition of alginates is not be affected by altering the temperature (6).

However, alginates are primarily extracted from brown seaweeds including *Laminaria*, *Lessonia*, *Macrocystis*, *Ascophyllum*, *Saccharina*, *Ecklonia* seaweed, and *Durvillaea* (7-10) but, these polymers can be also pulled out from bacterial sources such as mucoid strains of *Pseudomonas aeruginosa* and *Azotobacter vinelandii* (11).

The extraction procedure of alginate is a simple but multistage process, normally initiates with treating the dried algae with a weak mineral acid. After more refinement and in the presence of calcium, achieved alginic acid will be transformed into soluble sodium salt (12, 13). However, microbial fermentation is an alternative production method to reach extra physicochemical properties (14).

The type of algae, place and season of harvesting as well as the extraction conditions including temperature, time, and PH significantly alter the chemical composition such as purity, M and G rearrangement and ratio, molecular weight, and rheological characteristics of the extracted alginate (15, 16).

Alginate can be easily transformed into hydrogels with amended physicochemical and biological properties by physical or chemical cross-linking. There are various methods to form alginate cross-linking, such as free radical polymerization, thermal gelation, ionic cross-linking, and covalent cross-linking (17).

Alginates are recognized as nonimmunogenic, nontoxic, and biocompatible ingredients (12); therefore, these natural multifunctional polymers have been used in the biomedicine, food, pharmaceutical, and cosmetic industries as thickening or viscosity-enhancing agents (18-22).

Application of Alginate in the Pharmaceutical Industry

In the pharmaceutical context, alginate and its most known derivatives, sodium alginate, are well-accepted in the production of solid oral dosage forms. However they have many benefits as abundance, relatively low cost, safety, and biodegradability, but their high variability may limit their use as excipients for tablet formulation (23). In tablet-making industries sodium alginate has been applied as a suspending substance, tablet binder, taste improver, and controlled-release matrix. Also in soft tableting, for enhancing the performance of pressure-sensitive drugs, sodium alginate is used (22, 24-26).

The investigation of bio-sourced direct compression excipients is an interesting issue in the pharmaceutical industry. Proper pharmaceutical excipients should be directly compressible

and capable to being mixed with drugs without any influence on the quality of the tablet (27-29).

Application of Alginates in the Food Industry

Alginate primarily has been used in food manufacturing as a gelling, thickening, stabilizing, emulsifying (30), coating (31, 32), and pathogen-banning agent (33). Alginate-based films are completely eatable and biodegradable which have been applied in packing products like coffee, instant teas, powdered milk, as well as some medicines in powder form (34). These environmentally friendly products lessen the non-recyclable wastes, by this means decreasing the environmental pollution (35, 36). Ca-alginate gel is well recognized for encapsulating plant polyphenols leading to the preservation of properties of polyphenols in food products. Hydrogel coating of lemon balm extracts has been endorsed to protect its antioxidant function without any interaction between its ingredients and alginate (37). Interestingly, probiotics could be encapsulated in Ca-alginate gel microbeads and stay alive in the gastrointestinal environment (38). Ca-alginate gel particles are used in dairy products like yogurt and ice cream to decrease the grainy feeling (39). Also, Ca-alginate gel is used to produce low-calorie mayonnaise and other similar products to limit fat intake which may be helpful in keeping a healthy lifestyle (40, 41). Moreover, they are considered in weight loss diets by several functions such as their role as a natural dietary fiber and decreasing food consumption, improving pancreatic lipase function (42, 43), as well as improving protein digestion, and blood glucose (44, 45).

Safety Assessment of Alginates

Biocompatibility of alginates has been validated in vivo after oral (46), topical (47), local (48, 49), ocular (50), and nasal (51) administration. Currently, the Food and Drug Administration (FDA) has confirmed propylene glycol alginates derivative and some alginates salts of sodium, potassium, calcium, and ammonium as GRAS (generally regarded as safe) components for oral use (52). There are some data show that the chemical composition of alginates can affect the immunogenicity of polymers. For example, results of a study by Otterlei et al. revealed that alginates which are rich in M constituents are more effective in stimulating cytokine production compared to alginates with high G blocks (53). Furthermore, several impurities are present in alginates including endotoxins, proteins, heavy metals, and polyphenol compounds could lead to immunogenic reactions (54, 55). Consequently, appropriate refinement methods should be applied alongside the extraction process to guarantee the pureness of alginates (55). According to the vast use of alginate as a harmless agent in food and pharmaceutical industries, logically its hydrolysate and finally its residues including Mannuronic acid and Guluronic acids, as alginate hydrolysate components are safe and non-toxic ingredients. In this regard, a number of studies have been conducted to reveal the safety of Mannuronic (C₆H₁₀O₇) and Guluronic acid (C₆H₁₀O₇), as alginate hydrolysate components. In the present study, we aimed to review the safety of sodium alginate and its hydrolysate in the literature.

Toxicity Evaluation of Alginate Hydrolysate Components

Alginate hydrolysate is prepared following the hydrolysis of alginate and its derivatives, such as sodium alginate which is ultimately led to the separation of its components, as the safe and biocompatible molecules (Mannuronic and Guluronic acids).

The several experimental model have been conducted on Guluronic acid and its safety, tolerability, and efficacy. Nazeri et al. in 2017 ((56) analyzed acute and subchronic toxicity in BALB/c mice after oral use of Guluronic acid. This study concluded that Guluronic acid is a safe element for oral use. Six groups of mice (one control group and five treatment groups) with eight animals in each group (four males and four females) were used for acute toxicity testing. Guluronic treatment groups received a single oral gavage at water-soluble doses of 2000, 3000, 4000, 5000, and 6000 mg/kg body weight, and the control group received an equal volume of deionized water. Mice were closely monitored and evaluated for general behavior, signs of toxicity, and mortality within 14 days after the treatment.

In the acute toxicity phase of the study, a guluronic acid (lethal dose) LD50 value of 4800 mg/kg was apportioned. During this experiment, no deaths or obvious clinical symptoms were observed in animals receiving guluronic acid. The average weekly weights of the treatment groups (groups 2 to 5) were similar to those of the control group. Additionally, weekly changes in water and food intake in treated mice were not significantly different compared to the control group (56).

In this study, four groups of six BALB/c mice (three males and three females) were used to analyze subchronic toxicity. Drinking Guluronic-deionized water was prepared daily at dose levels of 0 (group I – control), 50 (Group II - low dose), 250 (Group III - medium dose), and 1250 (Group IV - high dose) mg/kg body weight administered for 90 consecutive days. The control group only gave the same amount of deionized water. Doses were administered at similar times each day, adjusted to animal weight. In this experiment, the highest dose administered to the animals was 50 times the optimal recommended dose of 25 mg/kg body weight. Other selected doses were approximately 2-fold and 10-fold higher than the optimal dose of 25 mg/kg which was determined in other studies (57, 58).

After the treatment period, the blood samples were analyzed for hematological evaluation, such as red blood cell (RBC), total white blood cell (WBC) count, hemoglobin concentration (Hb), hematocrit (HCT), mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), and white blood cell differential count. Additionally, the collected blood was tested for glucose, serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin, blood urea nitrogen (BUN), blood creatinine, total cholesterol, triglycerides, calcium, sodium, potassium, inorganic phosphorus, uric acid, total serum protein, and albumin. Statistical analysis revealed that there were no significant differences in the above hematological and serum biochemical factors between male and female mice in the treatment and control groups.

At the histopathological evaluation at the end of the experimental period, mice from all groups were euthanized and tissues including heart, liver, stomach, kidney, and testes were collected and routinely processed for histopathological examination. Tissue sections of both treated and control groups showed no pathological changes after staining and microscopic examination. Furthermore, no toxicity or toxic allergic effects of guluronic acid occurred in the test group. The mean relative organ weights of liver, kidney, stomach, heart, and testis of control and treated mice were not significantly different between both groups. Different parts of heart tissue (endocardium, myocardium, epicardium), liver, the kidney (renal corpuscles, tubules,

blood vessels), stomach (glandular and non-glandular areas including mucosa, submucosa, and external muscularis layer), testes (vas deferens with spermatogonia and Sertoli cells, Leydig cells and stratum intermedium) were examined, and microscopic examination showed no evidence of pathological effects in any of them. Overall, the evaluation of the results of the acute and repeated-dose chronic toxicity studies shows that guluronic acid is a safe drug after oral administration according to not seen adverse events in the general evaluation of the condition. Also, there were no changes in the animals' appearance, growth, feed intake, and clinical observations. The results of this study in treated mice show that guluronic acid is highly safe when administered orally, with no mortality or abnormalities observed in clinical signs, body weight, relative organ weights, or necropsy. Furthermore, the results of this toxicological study showed that there were no significant differences in hematological, biochemical and histopathological parameters in treated animals compared to untreated animals (56).

In another toxicity study, the acute and subacute toxicity profile of guluronic acid via the Intra Venous (IV) rout was explored by Mahdian et al. in 2022 (59). The researchers divided 32 healthy BALB/c mice into four groups (3 treatment groups and one control group) for acute toxicity studies. Based on the results of the oral toxicity analysis (56), a single dose of guluronic acid was administered to all three treatment groups. They received low (300 mg/kg), medium (600 mg/kg), and high (1000 mg/kg) doses, respectively, and the control group received a single injection of phosphate-buffered saline (PBS). All mice were carefully monitored twice a day for 14 days for mortality, toxicity symptoms, behavioral and clinical changes. The data showed that there were no obvious changes or deaths due to guluronic acid toxicity after the specified period. After 14 days, all mice were anesthetized and sacrificed for blood and organ collection. The results of hematological parameters in treatment groups showed that RBC and WBC count, HGB, HCT, MCV, and PLT were within normal limits, similar to healthy controls. Moreover, no significant pathological signs were observed in the vital organs of mice treated with guluronic acid compared to the control group.

Meanwhile, for subacute toxicity exploration, 48 mice of both sexes were treated for 28 days for the experiment. Animals were divided into three treatment groups (6 males, 6 females) and treated with low (25 mg/kg), medium (50 mg/kg), and high (100 mg/kg) doses of guluronic acid. It was administered once a day via IV injection, similarly, healthy controls received IV-injected PBS. During this 28-day period, the animals' body weights were documented weekly (5 times), along with monitoring morbidity, water and food intake, behavioral, and morphological alterations.

The treatment group showed no deaths, clinical or behavioral changes, or significant changes in body weight after guluronic acid administration compared to the control group.

On day 28, every three males and three females from each group received anesthesia. Blood samples and organs were collected for evaluation including hematological, biochemical, and histopathological analyses. Regarding hematological parameters, all related factors were within the normal range in both treatment and control groups, with no significant statistical changes. Biochemical analysis of serum concentrations of ALT, AST and ALP were examined and they were relatively similar in both treatment and control groups. Therefore, receiving the tested doses of guluronic acid by the IV route is not toxic for liver. Furthermore, serum concentrations of phosphorus, calcium, sodium, potassium, urea, and creatinine were relatively

similar in treated mice and untreated controls, indicating that guluronic acid may not cause nephrotoxicity or renal dysfunction.

Histopathological examination of vital organs such as heart, liver, lungs, spleen, and kidneys of treated and control mice showed no recognizable abnormalities in any of them. Furthermore, no inflammation, lesions, pathological changes or necrosis were detected in the organs of the tested mice, which were very similar to the organs of the control group. The remaining six mice in each group were observed for an extra 6 weeks for survival testing. No significant changes in survival or appetite were reported in all groups. This study on the acute and subacute toxicity of intravenously administered guluronic acid in Balb/C mice showed no adverse effects after IV injection. Guluronic acid does not cause any clinical symptoms after administration and is considered safe to use intravenously (59).

There are several studies with the main goal of assessing the properties of Guluronic acid including immunomodulation features, anti-inflammatory, anti-aging, anti-diabetes, anti-fatty liver, and anti-cancer effects. These investigations indirectly reported the safety of Guluronic acid, based on in vitro and in vivo examinations. (57, 60-64).

The second important component of alginate is mannuronic acid, called M-block. Similar to G blocks, they play an important role in the physicochemical properties of alginate polymers (65). Fattahi et al. carried out a typical toxicity study to assess the acute and subchronic toxicity of mannuronic acid.

For this purpose, mannuronic acid was orally administered to healthy mice and rats. (66). Six groups of healthy male NMRI mice (one control group and five treatment groups with five animals in each group) were used to analyze the acute toxicity of mannuronic acid. The treatment groups received 1000, 2000, 3000, 4000, and 5000 mg/kg body weight of water-soluble mannuronic acid, and the control group received the same amount of deionized water. For 2 weeks (14 days), all groups were carefully examined once a day for their behavior, symptoms of toxicity, and mortality. The LD50 value of mannuronic acid in NMRI mice was determined at 4600 mg/kg.

Meanwhile, 24 male Wistar rats (10 weeks old, weight 240–270 g) divided into four groups were used for the subchronic toxicity study. Each of the six test/control rats was fed a fresh solution of mannuronic acid in deionized water as a vehicle once daily for 63 consecutive days (9 weeks). Recommended doses are Group I (control) 0, Group II (low dose) 50, Group III (medium dose) 250, and Group IV (high dose) 1250 mg/kg body weight. The selected dose was considered based on the optimal recommended dose of 25 mg/kg/day which was established in the previous studies (57, 58).

Therefore, animals received mannuronic acid at 2, 10, and 50 times the optimal recommended dose of 25 mg/kg/day. Animal health status, body weight, food and water intake, clinical signs of toxicity, and mortality were recorded daily. At the end of the period, vital organs and cardiac blood samples were collected from overnight fasted animals for hematology, clinical chemistry, and histopathology examinations.

The results of this study showed that there were no substantial changes in clinical symptoms, water and food intake, body weight, or mortality between the treatment and control groups.

Hematological parameters such as Hb, RBC, HCT, MCV, MCH, MCHC, and platelet count showed no significant differences between the treated and non-treated groups. Sporadic changes in white blood cell counts (neutrophils, lymphocytes, monocytes) were observed, but there were no statistically significant differences between the treatment and control groups at any time point. Additionally, there was no significant difference was observed in biochemical factors such as glucose, total bilirubin, BUN, blood creatinine, uric acid, total cholesterol, triglycerides, total serum protein, albumin, AST, ALT, ALP, calcium, inorganic phosphorus, and sodium. Histopathological and macroscopic evaluation showed that all vital organs (liver, kidney, stomach) and their related parts in both control and test groups were healthy with no signs of toxicity or toxic allergic effects (66). In general, the results of this experimental toxicology study indicate that mannuronic acid, like guluronic acid, is a safe drug for oral use.

Also, the safety of mannuronic acid has been indirectly reported in other researchers aimed to investigate the properties of mannuronic acid. These in vitro and in vivo studies not only evaluated the immunomodulation, anti-aging, anti-cancer, anti-diabetic, anti-Alzheimer, and anti-inflammatory effects of mannuronic acid, but also revealed the safety of mannuronic acid (66-71).

CONCLUSION

Alginate is a non-toxic, biocompatible, biodegradable, non-immunogenic and is widely used in the food industry and pharmaceuticals products. Alginate is a natural copolymer constructed from guluronic acid and mannuronic acid monomers. Our objective in this study was to review the safety assessment of sodium alginate and its hydrolysate with mannuronic acid and guluronic acid residues as natural uronic acids in alginate hydrolysate construction in toxicity studies. In this study, the acute and chronic toxicity of these components were evaluated at different doses in BALB/c mice and Wistar rats for LD50 determination and safety assessments. The acute toxicity results showed that the LD50 of mannuronic acid and guluronic acid were 4600 mg/kg and 4800 mg/kg, respectively. Furthermore, in chronic animal model studies, oral administration of alginate hydrolysate components was safe and well tolerated, even at the highest dose (1250 mg/kg/day) in the course of continuous administration for 63 and 90 days. In conclusion, the outcomes of acute and chronic toxicity studies indicate that the sodium alginate and its derivative including alginate hydrolysate and its separated components have a high safety profile when administered orally to animals.

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