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Safety Assessment for Mannuronic and Guluronic Acids, as Alginate Residues in Toxicology Studies

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ABSTRACT

Objective: The present study aims to search the safety assessment for Mannuronic and Guluronic acids as Alginate residues in toxicology examinations. Concerning the fact that Alginate is a safe agent and is vastly used in the food industry, many investigations were conducted to evaluate the safety property of Mannuronic(M) and Guluronic (G) acids as the natural uronic acids and Alginate residues.

Material and Method: In the major studies, for the acute toxicity assessment, the BALB/c mice and Wistar rats received orally five different single doses of (M) in one study and (G) in another study and were then kept under observation for 14 days. In the chronic study, the animals were divided into four groups and treated orally once daily with (M) and/or (G) at dose levels of 0, 50, 250, and 1250 mg/kg body weight for at least 63 and 90 days. The mortality, clinical signs, biochemical and hematological parameters, body weight changes, gross findings, organ weights, and histopathological determinations were monitored during this evaluation. In the minor studies, the safety property of (M) and (G) was assessed indirectly using *in vitro* and *in vivo* examinations.

Results: In these studies, the results of acute toxicity revealed that the LD50 for Mannuronic and Guluronic acids are 4600 and 4800 mg/kg, respectively. The findings of these investigations showed no mortality, morbidity, or abnormality in the chronic study of any animal's body weight, organ weight, or necropsy. Moreover, the accumulated data showed no significant difference in these animals' biochemical, hematological, and histopathological determinants.

Conclusions: The results of toxicology studies show that Mannuronic acid and Guluronic acid have high safety when administered orally in animals.

Keywords: Mannuronic Acid; Guluronic Acid; Safety; Acute Toxicity; Chronic Toxicity; Toxicology; Alginate Residues

Abbreviations: RBC: Red Blood Cell Count; WBC: White Blood Cell; Hb: Hemoglobin Concentration; HCT: Hematocrit; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration; PLT: Platelet Count; ALP: Alkaline Phosphatase

Introduction

Alginate is a linear polysaccharide discovered in 1880 [1]. Alginate is considered both a biopolymer and a polyelectrolyte, comprising 40% of the dry weight of algae. It is essentially produced by brown algae [mainly sargassum algae and kelp] cell walls [2]. Alginate is prepared from the algae cell wall by sodium carbonate solution and pH value adjusting with hydrochloric acid [HCl] at pH 2.85 [2,3]. Some of the main species of Algae used for Alginate extraction are Laminaria hyperborean, Macrocystis pyrifera, Laminaria digitata, and Ascophyllum nodosum [4]. Alginates are copolymers composed of Mannuronic acid [M] and Guluronic acid (G) covalently linked together by 1,4-glycosidic bonds in different blocks, including G blocks (G residues), M blocks (M residues), or alternating M and G residues called MG/ GM blocks (Figure 1) [2,5,6].Various proportions of GG, MG, and MM blocks in an irregular pattern have arranged their structure. The ratio of the M/G block and then its length determine the physicochemical properties of Alginate [7], for instance, their molecular weight and physical properties [5].

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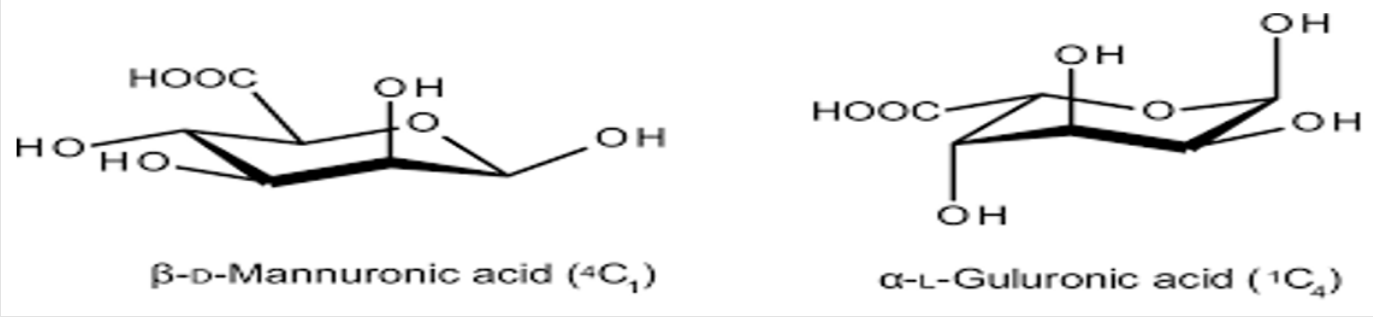

Figure 1: The chemical structure of Mannuronic and Guluronic acids.

In the primary structure, the position of carboxyl group on the C5 site is different in M and G molecules, and this tiny difference causes a significant difference in their design and physical properties. The MM blocks with a β-1,4-glycosidic bond cause a linear flexible structure. In contrast, GG blocks with α-1,4-glycosidic bonds lead to a folded and rigid structural conformation, which plays an essential role in the stiffness of the molecular chain [7,8]. Thus, the higher G residues, creates a stronger the Alginate gels [6]. The commercial alginates are widely used in different fields of industry, including food processing, environmental contaminants treatments, pharmaceuticals, and agricultural industries, and that is because of their ability to gel with calcium ions and also its non-toxic, biocompatible, biodegradable, and non-immunogenic properties [2,9,10].

In the presence of divalent cations, mostly Ca2+, Alginate can form a gel matrix, which is a hydrogel and could be used in various sorts of compounds, such as food ingredients (without any effect on their flavor) [11], bioactive compounds, and chemical/ drugs [5]. Given the fact that Alginate gel particles are non-toxic, biodegradable, and low price [since it is widely found in seaweeds], and its easy production, therefore they can be used as a safe agent in gel formation in food industry [5]. Besides, they have non-immunogenic properties and can encapsulate and protect nutrients, cells, and their components [2,12]. Because of the Alginate's ability in ionic gel formation in the presence of multivalent cations, it is widely used in encapsulation substances in the food industry. This ability mostly depends on G content in the Alginate [13], as there is mainly G that binds to divalent cations [5]. Alginate with high G content can format strong and brittle gels with good thermal stability, while Alginate gels with high M content are weak and more elastic, with good freeze-thaw properties [5,8]. Alginate has unique colloidal properties, such as thickening, stabilizing in gel formation process which are used in food industry [14,15].

In food applications, Ca-alginate gel particles are well known for encapsulating plant polyphenols causing more functionality and stability of polyphenols in food products. For instance, Ca-alginate gel particles, used for encapsulating lemon balm extracts showed its ability to save the antioxidant activity of lemon balm extracts without any chemical interaction between lemon balm extracts and Alginate [16]. Interestingly, probiotics could be encapsulated and protected in high acid and bile environments by Ca-alginate gel [17]. In the dairy industry, Ca-alginate gel particles of less than 30μm, as a safe agent are used to minimize the powdery or grainy sensation of yogurt and ice cream without affecting their taste [18]. The Ca-alginate gel particles can also be used to prepare low-fat mayonnaise and other similar emulsions, which may contribute to the decrease in overconsumption of fatty foods in humans and thus reduce the incidence of some diseases in humans [19,20].

The added Alginate to products related to meat, fish, dairy, and Cereal-based causes to improve their quality [21]. For example, in bakery applications, it plays a role in fruit or cream fillings, as the thickening and structuring agent in low-fat margarine and also in controlling the ice cream melting behavior [6,22]. Besides, they can be used as dietary fibers. Currently, Alginate, as a natural dietary fiber and a restricted-energy food supplement, is used by obese people. It has a high viscosity and extraordinary shear-thinning effect. It can improve weight loss by enhancing the activity of digestive enzymes such as pancreatic lipase [23,24], and reducing food intake, apparent protein digestibility, and blood glucose [10,25].Recently, calcium alginate has been considered a probable salt-adsorbing material in health foods or supplements, which can prevent salt-sensitive hypertension and kidney dysfunction [26].Studies report that the sodium alginates, as food additives are biocompatible, biodegradable, and 'Generally Recognized as Safe (GRAS) compounds [27].

With respect to the fact that, Alginate has been introduced as a safe agent in food and pharmaceutical industries, therefore, logically seems that the Mannuronic and Guluronic acids, as the residues and components of Alginate might be the safe and non-toxic agents. In this connection, the various investigations have shown that the Mannuronic and Guluronic acids, as the natural uronic acids are the safe agents. In the present study, our aim is to show the safety of Mannuronic ($C_6H_{10}O_7$) and Guluronic acids ($C_6H_{10}O_7$), based on the results of various research which have been made on these two natural uronic acids.

Toxicology Assessment of Guluronic Acid

There are some scientific studies in connection with Guluronic acid and its safety, tolerability, and efficacy in experimental model examinations. During an investigational study, Nazeri, et al. [28]

analyzed acute and sub-chronic toxicity in BALB/c mice after Guluronic acid oral administration. This study explained Guluronic acid as a safe substance for oral administration. In the acute toxicity tests, six groups of mice (one control group and five treated groups) and eight animals in each group [four male and four female] were used. Guluronic-treated-group was single-gavaged orally with water-soluble doses of 2000, 3000, 4000, 5000, and 6000 mg/kg body weight, and the control group received the same volume of deionized water. Mice were monitored carefully within 14 days after the treatment and assessed based on the general behavior, signs of toxicity, and mortality. In the acute toxicity examination in BALB/c mice, the 4800 mg/kg, as the Guluronic acid LD50 (lethal dose 50) value was assigned.

During this experiment, neither mortalities nor obvious clinical signs were observed in the animals administered with Guluronic acid. The mean weekly body weight for treated groups (Group 2 to 5) was comparable with the control group (group 1). Besides, the weekly water and food consumption changes in treated mice were insignificant in comparison with control group [28]. In this research, four groups of six BALB/c mice [three male and three female] were used to analyze the sub-chronic toxicity. Drinking Guluronic-deionized water was prepared daily at dose levels of 0 (group I – control), 50 (group II – low dose), 250 (group III – mid dose), and 1250 (group IV – high dose) mg/kg body weight for 90 consecutive days. The control group received only the same volume of deionized water. Each day, the doses were given at a similar time, adjusted with the animal's body weight. In this experiment, the highest dose administered to animals was 50 times higher than the optimum-recommended dosage of 25 mg/ kg BW. The other selected dose levels were approximately 2 and 10 times more than the optimum-proposed dosage of 25 mg/ kg which had been determined in other investigations [29,30]. The health conditions of the animals, including morbidity and mortality, were recorded daily. An overnight fastening before blood collection was compiled and calculating organ-to-body ratio before euthanasia was performed.

Table 1: Effect of Guluronic acid on hematological determinants in male and female mice.

Parameters	GI (control) Male	GI (control) Female	GIV (high dose) Male	GIV (high dose) Female	Reference ranges Male	Reference ranges Female
RBC (106/ll)	9.2 ± 1.02	9.9 ± 0.22	9.7 ± 0.47	9.8 ± 0.37	6.93–12.24	8.16–11.69
HB (g/dl)	13.4 ± 1.1	14.6 ± 0.37	14.2 ± 0.5	14.4 ± 0.24	12.6–20.5	12.4–18.9
HCT (%)	41.9 ± 3.1	45.3 ± 1.2	43.2 ± 1.6	45.1 ± 0.98	42.1–68.3	43.5–67
MCV (fL)	45.2 ± 1.6	45.3 ± 0.24	44.2 ± 0.49	46 ± 2.4	50.7–64.4	50.8–64.1
MCH (pg)	14.5 ± 0.4	14.6 ± 0.12	14.5 ± 0.23	14.7 ± 0.67	13.2–17.6	13–17.6
MCHC (g/dl)	31.9 ± 0.25	32.3 ± 0.15	32.8 ± 0.32	32 ± 0.23	23.3–32.7	23.9–33.1
WBC (103/ll)	7.09+0.19	7.2 ± 0.4	6.5 ± 2.7	6.4 ± 2.7	3.48–14.03	5.69–14.84
Neutrophils (%)	10 ± 1	9.7 ± 1.7	7.1 ± 1.4	8.1 ± 0.55	9.86–39.11	10.39–27.88
Lymphocytes (%)	84.5 ± 2.5	83.7 ± 1.7	81.5 ± 2.4	84.4 ± 0.46	48.81–83.19	55.06–83.82
Monocytes (%)	4 ± 2	2.7 ± 0.28	4.4 ± 2.7	2.9 ± 0.57	3.29–12.48	3.75–14.33
Eosinophils (%)	0	0	0.6 ± 0.6	0	0–4.9	0–4
Basophils (%)	2.5 ± 0.5	3.7 ± 0.28	6.1 ± 2.4	4.5 ± 0.55	0–1.8	0–1.5
Platelets (103/ll)	86 ± 4	111.3 ± 37.3	131.3 ± 22.5	89 ± 32.3	420–1698	476–1611

Note: Although this experiment was carried out using all three low to high doses (50, 250, and 1250 mg/kg) for toxicity assessment, but this table just compares the amounts between the control and the highest group to emphasize more on the safety of the highest one.

Hematology and Biochemistry Assessment

After treating period, the collected blood samples were used for hematology assessment, including red blood cell count (RBC), total white blood cell (WBC), hemoglobin concentration (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin [MCH], mean corpuscular hemoglobin concentration [MCHC], platelet count (PLT), and differential leukocyte count (Table 1). Furthermore, the blood collected was used for clinical chemistry tests, such as glucose, serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin, blood urea nitrogen [BUN], blood creatinine, uric acid, total cholesterol, triglycerides, total serum protein, albumin, calcium, inorganic phosphorus, sodium (Na), and potassium (K). After statistical analysis, the results showed that, there were no significant differences in the above mentioned hematological and serum biochemical factors between male and female mice in the treated and control groups (Table 2).

Table 2: Effect of Guluronic acid on biochemical determinants in male and female mice.

Parameters	GI (control) Male	GI (control) Female	GIV (high dose) Male	GIV (high dose) Female	Reference ranges	Reference ranges Female
Glucose (mg/dl)	191.5 ± 14.5	146.6 ±23.4	201 ± 7.8	114 ± 47.8	129–329	85–281
Creatinine (mg/dl)	0.4 ± 0	0.46 ± 0.03	0.43 ± 0.03	0.43 ± 0.03	0.2–0.4	0.2–0.5
BUN (mg/dl)	23.8 ± 3	20 ± 5.5	20.5 ± 2.6	21.4.6 ± 3.3	7–26	7–31
Triglycerides(mg/dl)	62.5 ± 12.5	83 ± 19	106 ± 20.8	109 ± 2.3	107–535	101–595
Cholesterol (mg/dl)	109.5 ± 9.5	111.3 ± 9.3	146 ± 21.9	133 ± 10.6	111–246	81–208
Calcium (mg/dl)	10.4 ± 0.4	9.5 ± 0.3	10.3 ± 0.87	10.2 ± 0.66	9.4–12.5	8.4–12.7
Phosphorous (mg/dl)	11.3 ± 0.71	10.1 ± 0.7	12.8 ± 1.4	10.1 ± 0.8	9.4–12.5	7.8–13.5
Potassium (mmol/l)	9.7 ± 0.6	9.1 ± 1.05	8.6 ± 0.98	9.8 ± 1.1	7.3–12.24	7.17–12.07
Sodium (mmol/l)	163 ± 5.8	160 ± 10.6	169 ± 9.8	155 ± 3.6	125.3–187.4	129.8–171.5
Total bilirubin (mg/dl)	0.3 ± 0.03	0.4 ± 0.05	0.3 ± 0.05	0.4 ± 0.05	0.2–0.5	0.2–0.7
Total protein (g/dl)	6.8 ± 0.43	6.3 ± 0.56	7 ± 0.95	5.6 ± 0.66	4.8–8.7	4.9–7.3
Albumin (g/dl)	3.5 ± 0.05	3.2 ± 0.08	3.1 ± 15.3	3.3 ± 0.08	2.7–4.6	3.1–5.3
ALT (U/l)	122.5 ± 82.5	69.3 ± 7.6	76.6 ± 20.2	110 ± 62.5	41–131	40–170
AST (U/l)	261 ± 53.5	153 ± 35	138 ± 29.5	159.6 ± 36.5	55–352	67–381
ALP (U/l)	240 ± 30	233.6 ± 20.9	205 ± 10	257 ± 15.5	118–433	108–367

Note: Although this experiment was carried out using all three low to high doses (50, 250, and 1250 mg/kg) for toxicity assessment, but this table just compares the amounts between the control and the highest group to emphasize more on the safety of the highest one.

Histopathology Assessment

At the end of the experimental period, all groups of mice were euthanized, and the tissues (kidneys, liver, stomach, heart, and testes) were collected, and routine processing was done for histopathological examination. Tissue sections of both treated and control groups showed no pathological alteration after staining and microscopic evaluation. Additionally, there were no toxic or toxico-allergic effects of Guluronic acid in the test groups of mice. The mean relative organ weights of the liver, kidney, stomach, heart, and testes of control and treated mice were insignificant between both groups. The various parts of the kidney (renal corpuscles, tubules, blood vessels, etc.,) heart tissue (endocardium, myocardium, and epicardium), liver, stomach (both glandular and non-glandular sites with mucosal, sub-mucosal, and external muscular layers), testes (seminiferous tubes with spermatogenic and Sertoli cells, Leydig cells, and intermediate layer) were examined and after microscopic evaluation, there was no evidence of pathological effects in any of them.

Collectively, The evaluation of the results of acute and repeated-dose for chronic toxicity studies showed that Guluronic acid is a safe agent after oral administration in BALB/c mice, since there were no adverse events [side effects], as evaluated by the general conditions and appearance of the animals, growth, food consumption, and their clinical observations had no changes. The results of this study on treated mice showed that Guluronic acid has high safety when administered orally, without no mortality or abnormality in clinical signs, body weight, relative organs weight, or necropsy. In addition, findings of this toxicology research showed no significant difference in hematological, biochemical, and histopathological parameters in treated compared to non-treated animals [28]. In another toxicology study, the acute and sub-acute toxicity profiles of Guluronic acid were investigated through the [iv] route by Mahdian, et al. [31] during 28 days [31].

In this research, for the acute toxicity study, 32 healthy BALB/c mice (male and female) were divided into four groups [eight mice in each group]. All three treated groups received single doses of Guluronic acid based on the results of oral toxicity analysis [28]. They were low dose (300 mg/kg), medium dose (600 mg/kg), and high dose (1000 mg/ kg), respectively, whereas the control group, received a single injection of phosphate buffer saline (PBS). All mice were monitored carefully during 14 days, twice per day, for any mortality, toxicity symptoms, or behavioral and clinical changes. Data showed that, there was no apparent change or death due to Guluronic acid toxicity after the fixed period. At the end of the 14 days, all mice were anesthetized and sacrificed for blood and organ collecting. The results of hematological parameters (including WBCs, RBCs, HGB, HCT, MCV, PLTs, and total and differential leucocyte counts) in this research were in the normal range, similar to healthy controls. Moreover, no significant pathological signs were observed in the vital organs of Guluronic acid-treated mice compared to control group. Given that,

the highest received dose (1000 mg/kg) was safe and without evident toxicity, it is revealed that the (iv) LD50 of Guluronic acid is higher than 1000 mg/kg.

On the other hand, for sub-acute toxicity analysis, 48 mice of both sexes were treated for twenty-eight-day experiments. Animals were divided into three treatment groups (six males and six females), which received a low dose (25 mg/kg), medium dose (50 mg/kg), and high dose (100 mg/kg) of Guluronic acid once a day through the (iv) route, while the healthy controls received PBS through the same route. During these 28 days, the body weight of animals was recorded weekly (5 times), in addition to monitoring of morbidity, the water and food consumption, behavior features, and morphological changes. The treated groups showed no death, no clinical or behavioral changes compared with the control group and no significant change in body weight after Guluronic acid administration. On day 28, three males and three females of each group were anesthetized. Blood samples and organs were collected for evaluation, including hematological, biochemical, and histopathological analysis. Concerning the hematological parameters, all related factors were reported in the normal range and without notable statistical changes both in the treated and control groups. The biochemical analysis for serum concentrations of ALT, AST, and ALP was examined, and they were relatively same in both the treated and control group. As a result, receiving tested doses of Guluronic acid through the (iv) route is not toxic to the liver. In addition, the serum concentration of phosphorus, calcium, sodium, potassium, urea, and creatinine were relatively similar between both treated mice and non-treated control, suggesting that Guluronic acid could not lead to renal toxicity and disfunction.

Following the histopathological examinations of vital organs, such as the lung, heart, liver, spleen, and kidneys of treated mice and control ones, there was no obvious abnormality in none of them. Moreover, no lesions, inflammation, necrosis, or pathological changes were observed in the organs of tested mice, and they were too similar to those of the control group. The remaining six mice in each group were left alive and followed for an additional six weeks for survival assays. No remarkable changes were reported in survival rates or the appetite between all groups. This research study on Balb/C mice regarding the acute and sub-acute toxicity of Guluronic acid through intravenous administration showed no adverse effect after the(iv) injection. This component could not cause any clinical signs after its administration, which can be considered as a safe application [31].

In the minor studies, the safety of Guluronic acid was reported indirectly, based on in vitro and in vivo examinations. In this research, not only the safety of Guluronic acid was evaluated, but its other properties (anti-aging based on oxidative stress enzymes, anti-cancer, anti-diabetes, anti-fatty liver and anti-inflammatory effect] was also reported. For this purpose, evaluating the mRNA expression of 6 oxidative stress enzymes, including Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione S-transferase, inducible nitric oxide synthase (iNOS), and myeloperoxidase (MPO) in Guluronic acid-treated PBMCs (peripheral blood mononuclear cells) suggested that the Guluronic acid can modulate the gene expression of these enzymes. As a result, Guluronic acid has immunomodulatory properties, which might be helpful during anti-aging and anti-inflammatory processes [29].

In another study, the gene expression of the abovementioned oxidative stress enzymes was evaluated on Guluronic acid-treated Sprague-Dawley rats. The results of this research showed that the treated rats significantly reduced their MPO level compared with the control group. These two studies explained the safety and efficacy of Guluronic acid even during oxidative stress conditions [32].In a cancer-related inflammation (CRI) study in both in vitro and in vivo conditions was shown that the Guluronic acid could prevent the CRI, tumor cell adhesion, accumulation of immunosuppressive or inflammatory cells, and also tumor-promoting mediators, such as COX-2 (cyclooxygenase 2), MMP2 (matrix metalloproteinase 2), MMP9, VEGF [vascular endothelial growth factor], and pro-inflammatory cytokines, effectively. In addition, this way had no direct toxic effects on the cells [33].

In an in vitro study on the HepG2 cell line treated with Guluronic acid was revealed that this agent could reduce the viability of cancer cells compared to healthy cells (using the mouse fibroblast cell line L929). Moreover, the Guluronic acid could induce apoptosis in this liver cancer model at a proper time and dose [30].In another investigation, on Guluronic acid-treated-PBMCs of Nonalcoholic Steatohepatitis (NASH) patients was shown the increased levels of TLR4 (Toll-Like Receptor 4), NF-κB (nuclear factor-κB), TNF-α (tumor necrosis factor-α), and IL-6 (interleukin 6) expression and secretion decreased significantly after treatment, compared with the healthy PBMCs [34].In research, treating the PC-3 cells [related to prostate cancer] with Guluronic acid showed that the expression and secretion of COX-2, MMP-2, IL-8, and NF-κB proteins were remarkably reduced, which are involved in the progression and metastasis of prostate cancer. Additionally, MMP-2 activity and cancer cell proliferation were also inhibited after treatment, compared to the control group [35]. In important research, using the experimental model of diabetes and investigating the effects of Guluronic acid on blood sugar and insulin secretion revealed a significant increase in the final weight of the treated group compared with the diabetic control. Moreover, the final fasting blood glucose and insulin level of treated mice showed a statistical decrease and increased, respectively. All these can effectively reduce the severity of inflammation caused by diabetes [36].

Toxicology Assessment of Mannuronic acid

The second vital ingredient of Alginate is mannuronic acid, called M-blocks. Like G-blocks, they play an essential role in the physicochemical properties of the Alginate polymer [37]. Fattahi, et al. [38] designed a standard toxicology study to determine the Mannuronic acid's acute and sub-chronic toxicity. For this purpose, the Mannuronic acid was gavaged orally into healthy mice and rats [38]. To analyze the acute toxicity of Mannuronic acid, six groups of

healthy male NMRI mice (one control group and five treated groups, with five animals in each) were used. The treated groups received 1000, 2000, 3000, 4000, and 5000 mg/kg body weights of the water-soluble Mannuronic acid, while the control group received the same volume of deionized water. During two weeks (14 days), all groups were assessed carefully for their general behavior, signs of toxicity, and mortality, once a day. The obtained LD50 for the acute toxicity study of Mannuronic acid in NMRI mice was 4600 mg/kg.

On the other hand, twenty-four Wistar male rats (10 weeks of age and weighed 240–270 g) in four groups were used for Sub-chronic toxicity examination. Each six-test/control rats were gavaged once daily with the fresh solution of Mannuronic acid in deionized water [vehicle] for 63 consecutive days [9 weeks]. The proposed doses were as below; Group I (control) 0, Group II (low dose) 50, Group III (mid dose) 250, and Group IV (high dose) 1250 mg/kg body weight. The selected doses were considered on the basis of the optimum-recommended dosage of 25 mg/ kg/day, which had been determined in other investigations [29,30]. Therefore, the animals received the Mannuronic acid with 2, 10, and 50 times more than the optimum-proposed dosage of 25 mg/ kg/day. Animals were recorded for their health conditions, weight, food and water consumption, clinical signs of toxicity, and mortality, every day. At the end of the period, heart blood samples, in addition to vital organs were collected from the overnight fasted animals for hematology, clinical chemistry, and histopathological examination.

The results of this investigation showed that there were no significant clinical signs, water and food consumption changes, body weights, and mortalities between the treated and control groups. The hematological parameters, including Hb, RBC, HCT, MCV, MCH, MCHC, and platelet values, did not show any significant differences in treated and non-treated groups. Although the sporadic changes were seen in WBC counts (neutrophil, lymphocyte, and monocyte), but there were no statistically significant differences between the treated and control groups at any time. Moreover, there were no meaningful differences between important biochemical factors, such as glucose, AST, ALT, ALP, total bilirubin, BUN, blood creatinine, uric acid, total cholesterol, triglycerides, total serum protein, albumin, calcium, inorganic phosphorus, Na, and K. The histopathology and macroscopic evaluation explained that all vital organs (liver, kidneys, and stomach) and their related parts were healthy in both the control and tested groups, without any signs of toxic or toxico-allergic effects [38]. Collectively, the results of this experimental toxicology research, as a major study indicate that the Mannuronic acid, similar to Guluronic acid is a safe agent for oral administration.

In the minor studies, the safety of Mannuronic acid was reported indirectly, based on in vitro and in vivo examinations. In these investigations, not only the safety of Mannuronic acid was evaluated, but its other properties (anti-aging based on oxidative stress enzymes, anti-cancer, anti-diabetes, anti-Alzheimer's disease and anti-inflammatory effect) was also reported. In an in vitro study on the gene expression of six oxidative stress enzymes of healthy PBMCs was shown that the Mannuronic acid with dose of (25 µg/ml) could reduce the mRNA expression of SOD2, GST, iNOS, and MPO enzymes significantly. This research revealed that the Mannuronic acid could modify the oxidative stress process, which may be beneficial in age-related conditions [39]. In another study, using animal model [Sprague-Dawley rats] on the gene expression of the abovementioned enzymes was shown that Mannuronic acid has antioxidant and anti-aging properties [40].

Study on the effect of Mannuronic acid on PC3 cells explained that it had no cytotoxicity effect on this cell line at the concentration of ≤200 µg/ml. Additionally, Mannuronic acid could reduce the gene expression of inflammatory molecules in prostate cancer cells, including MYD-88, NF-kB, IL-8, MMP-9, and COX-2 [41].In a rat model study related to Alzheimer's disease was shown that Mannuronic acid has the potential to change the behavior of these rats, which causes a significant halt in amyloid plaque production pathway. In addition, the amount of Bax/Bcl2, P53, MDA, SOD, and procaspase-3 were normalized in treated experimental rats [42]. During an in vitro study, both treated HEK293 TLR2 cell line and PBMCs with Mannuronic acid indicated that the inflammatory pathway could be positively changed, which it might be due to an increase in SOCS1(Suppressor of Cytokine Signaling-1) and SHIP-1 (Src Homology-2 domain-containing inositol-5′-phosphatase 1), besides a decrease in miR-155 (microRNA-155) levels in treated cells [43]. In a diabetes experimental model, the streptozotocin-induced diabetic rats treated with Mannuronic acid showed significantly lower and higher fasting serum glucose and insulin levels than the diabetic control group, respectively. Besides, it could reduce the severity of diabetes-induced inflammatory symptoms by lowering the serum level of hs-CRP and IL-6 [44].

To study the effect of Mannuronic acid on human dendritic cells, they were treated with this substance. It revealed that Mannuronic acid has no meaningful impact on differentiation, maturation, and function in mature or immature DCs [45]. The anti-inflammatory effect of Mannuronic acid was explained in another in vitro study using the phorbol myristate acetate (PMA)-differentiated THP-1 cells treated with the concentration (25 µg/mL) of Mannuronic acid. It could reduce the expression level of CD147, MMP-2, MMP-9, and TIMP-1 [tissue inhibitor of matrix metalloproteinase], as the inflammatory determinants [46].

Conclusion

Alginate is a natural copolymer comprised of Guluronic acid and Mannuronic acid. It is non-toxic, biocompatible, biodegradable, and non-immunogenic, which is vastly used "to date" in the food industry. In this study, our aim was to search the safety assessment for Mannuronic and Guluronic acids as the natural uronic acids and Alginate residues in toxicology examinations. In these research, the acute and chronic toxicity of Guluronic and Mannuronic acid were evaluated in the BALB/c mice and Wistar rats in various doses for determining the LD50 and their safety assessment. The results of acute toxicity revealed that the LD50 for Mannuronic and Guluronic acids are 4600 and 4800 mg/kg, respectively. In addition, the

chronic animal model studies revealed that the oral administration of Guluronic acid and Mannuronic acid, even with the highest dosage (1250 mg/kg/day) during 63- and 90-days continuous administration are safe and well-tolerated substances. Collectively, the results of acute and chronic toxicology studies show that the Mannuronic acid and Guluronic acid have high safety when administered orally in animals.

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