Preclinical Safety Assessment of Chemically Cross-Linked Modified Mandua Starch: Acute and Sub-Acute Oral Toxicity Studies in Swiss Albino Mice

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ABSTRACT: In the present era, 28 days of repeated-dose-toxicity study following the Organization for Economic Cooperation and Development (OECD) guidelines 407 is compulsory for every drug to go through phase 1 clinical trials. The increasing demand for high-resistant starch containing nutraceuticals and the applicability of modified starch in development of targeted drug delivery inspired us to investigate the toxic profile of mandua starch chemically cross-linked by epichlorohydrin and compare it with alkali-isolated starch in healthy adult Swiss albino mice, which can be the first step for exploring the use of epichlorohydrin-cross-linked mandua starch (ECC-MS) as a pharmaceutical excipient. Histopathological examinations of the kidney and liver did not expose noteworthy abnormalities in the treated mice. There were no clinical and mortality symptoms of toxicity observed during the repeated-dose-toxicity study. The oral consumption of ECC-MS did not pose any harm as it was neither lethal nor had any harmful hematological, biochemical, psychological, anatomical, and behavioral effects. The use of ECC-MS and alkali-isolated mandua starch (AMS) was found safe at a dose of 2000 mg/kg body weight in the acute toxicity study and at doses of 2000, 1500, and 1000 mg/kg body weight in the sub-acute toxicity study as no detrimental effects were observed after oral administration in mice for 14 and 28 days, respectively.

1. INTRODUCTION

Toxicity denotes the degree of adversity caused by the interaction between the cells and the toxicant. This relationship varies according to the cell membrane and chemical properties of the toxicants, which can influence the extracellular matrix, cell surface, and area underneath the cells and the tissues. Hence, it has become a primary necessity to evaluate the toxic nature of any compound before accepting it as a pharmaceutical or nutraceutical excipient. In research, the toxic profile may be confirmed by several practices like acute, chronic, sub-acute, reproductive, and carcinogenic consequences.

Starch is a readily usable, reusable, low-cost, biodegradable, and biocompatible polymer. These characteristics contribute to starch’s growing acceptance and applicability in various medicinal, agricultural, and non-food and food applications. However, due to certain limitations as a raw material for industrial usage, it has been upgraded using various alteration techniques. Starch is modified to satisfy the specific needs of the end customers, resulting in a variety of speciality items. Cross-linking, on the other hand, is regarded as an effective route of starch modification. It can incorporate covalent bonds and augment the naturally occurring intermolecular hydrogen bonds, enhancing the industrial applicability and versatility of starch by enhancing its mechanical properties. The main cross-linkers that stabilize food starches are acetic anhydride, monosodium phosphate, vinyl chloride, epichlorohydrin (ECHD), sodium trimetaphosphate, phosphoryl chloride, and sodium tripolyphosphate. However, some of the chemicals used for alteration have been seen to have a detrimental impact on human health when used in an unregulated amount, i.e., in excess or beyond the limit. Therefore, the United States Food and Drug Administration (FDA) framed 21 CFR 172.892 to ensure the use of chemically modified starch in foods in a controlled manner. The overall permitted starch substitutions in the form of acetate, phosphate, and hydroxypropyl levels are 2.5, 0.4, and 10%, respectively. In cross-linked foods, starches...
having the mono-substituent cross-linking group per 1000 anhydroglucose units are considered safe for industrial use.\(^2,5\)

Numerous researchers worldwide have identified ECHD as a commonly used cross-linker for starch modification. It has been reported that ECHD reacts bifunctionally with starch. Notably, cross-linking starch with ECHD reduces its digestibility, allowing it to be used in control release formulations.\(^6\) It is stated that after cross-linking cassava starch with ECHD, the \textit{in vitro} \(\alpha\)-amylase digestibility of the modified starch decreased gradually as the degree of cross-linking increased.\(^4,5\) Despite being rich in carbohydrate content (~60%), the potentials of mandua starch are still undervalued and unexplored in comparison to other cereals such as rice, wheat, barley, and maize. Hitherto, mandua starch was cross-linked with ECHD in the current research.

Epichlorohydrin is considered as a cross-linking agent for polymers. However, a large amount of ECHD or its residue/or unreacted fraction may cause toxic side effects. It has been reported that ECHD induced morphological defects and neurotoxicity in the early embryos of \textit{Rhinella arenarum} as well as extreme teratogenic consequences.\(^7\) Sub-acute and sub-chronic inhalation sensitivity to ECHD vapors caused hepatic and renal abnormalities.\(^8\) It has been documented that a single 4-h inhalation of ECHD at a concentration of 100 parts per million caused specific, temporary decrement in rat sperm velocity. ECHD is classified as a health hazard in the CLP Regulation (Classification, Labelling and Packaging, 2008).\(^28\)

When ECHD was administered by gastric intubation to Weanling Wistar rats of both sexes for 2 years, 5 days a week, mean body weight in survivors.\(^2,5\)

2. MATERIALS AND METHODS

2.1. Collection of Plant Material. Mandua Grains were procured from Vivekananda Parvartiya Krishi Anushandhan Sansthan, Almora (Uttarakhand). All reagents used were reagent grade.

2.2. Isolation and Modification of Starch. Starch was isolated by the alkali stepping method with a slight modification.\(^11\) Alkali-isolated mandua starch (AMS) was further cross-linked by applying ECHD as a cross-linking agent.\(^1\)

2.3. Fourier Transform Infrared Spectroscopy (FTIR) Characterization. The AMS and cross-linked mandua starch (ECC-MS) were ground in a glass mortar and dried. To confirm the modification, the samples were scanned using a Fourier transform infrared spectrometer at a resolution of 8 cm\(^{-1}\) in the scanning range of 400–4000 cm\(^{-1}\).

2.4. Morphological Analysis. A starch sample was sprinkled on a double-sided adhesive tape attached to a circular specimen stub, and then coated with gold using a sputter coater. The sample was viewed using a scanning electron microscope (Zeiss Gemini FE-SEM) at different magnifications.

2.5. Experimental Animals. The healthy Swiss albino mice of both sexes (weighing ~24 to 35 g) aged 12–24 weeks from the vivarium of the Bilwal Medchem and Research Laboratory Pvt. Ltd. (Jaipur, India) were used in the acute and sub-acute toxicity studies. All of the animals were acclimatized for 7 days to the laboratory conditions before experimentation. Clinical investigations of all animals were performed before starting the study to evaluate any parasite infestation and overall health. The non-suited animals were eliminated in the initial stage after seven days of acclimatization. The animals were kept in standard suspended polycarbonate cages (300 × 190 × 130 mm\(^3\)) with a stainless-steel wire cover and paddy husk as the bedding. The paddy husk was autoclaved and replaced on a weekly basis. The mice were exposed to a 12 h dark/light cycle at 25 °C temperature and a relative humidity of 60 ± 5%. The animals were fed a standard diet (Lipton, Mumbai, India) with free accessible water before and during the experimentation. They were euthanized under the effects of overdose anesthesia (150 mg/kg of ketamine 4%) and were handled as per the CPCSEA guidelines for the care and use of laboratory animals. The ethical consent was acquired from the Institutional Animal Ethics Committee (reg. no. BMRL/AD/CPCSEA/IAEC/2019/10/2-I).

2.6. Acute and Sub-Acute Toxicity Studies of ECC-MS and AMS. 2.6.1. Acute Toxicity Study. Acute toxicity testing of mandua starch samples was conducted in Swiss albino mice according to OECD (Organization for Economic Cooperation and Development) guideline no. 423.\(^13\) Before and after the experimentation, the animals were served a regular diet with free access to water. Individual mice were weighed and categorized into three groups: A, B, and C. Each group included mice of both sexes (male and female) \((n = 3)\). Animals of group A were treated as control and received normal saline. Afterwards, regular food supply was provided to all animals of the three groups. Animals of group B were considered as the test sample and ECC-MS was administered orally at a dose of 2000 mg/kg body weight. Group C animals were treated as the standard and AMS was administered orally at a dose of 2000 mg/kg body weight.

In order to conduct the acute toxicity study, a dose of 2000 mg/kg body weight of the test and standard samples was administered orally after overnight fasting. During this study, no food was given to the animals for the whole night before AMS and ECC-MS administration; following the overnight fasting, the experimental dose was administered orally. Afterwards, normal food and water supply to the animals was continued. The animals were observed constantly for 3 h after oral dose administration of the sample for behavioral, autonomic, and neurological profiles and then every 30 min for the consecutive 4 h and lastly for mortality after 24 h, 48 h, 7 days, and 14 days (2 weeks) for any change in behavior or mortality. The mice were analyzed for signs of toxicity on their skin, hair, pupils, mucous membrane, salivation, lethargy, sleep, coma, convulsions, tremors, diarrhea, oral activity, abdominal, and external genitalia. The mice were separated from their cages during the study to assess the survival, morbidity, and general health.

2.6.2. Sub-Acute Toxicity Study. This study was conducted as per OECD guideline no. 407.\(^16\) Three groups of Swiss albino mice with either sex were used in the study \((n = 10\) for each group). Animals of group A (control) received 1 mL of normal saline (vehicle) and normal food and water supply for 28 days. Group B (test) and C (standard) received the
chemically modified starch (ECC-MS) and alkali-extracted starch (AMS), respectively, at a dose of 1000, 1500, and 2000 mg/kg body weight per oral dose, respectively, once daily for 28 days. During this study, the mice of all groups were observed for behavioral changes. Afterwards, the animals were fasted for 12 h and anesthetized.

2.6.2.1. Biochemical and Hematological Parameters. At the end of the acute and sub-acute toxicity studies, the mice of all groups were weighed and the blood was withdrawn from the tail vein in two separate tubes: one containing anticoagulant for instant analysis of hematological parameters such as hemoglobin, total red blood corpuscles (RBCs), total leukocyte count (TLC), neutrophils, lymphocytes, eosinophils, monocytes, and basophil count. Another blood sample fraction was centrifuged for 10 min at 1000 rpm to separate the serum for evaluation of biochemical parameters like urea, creatinine, serum glutamate oxaloacetate transaminase (SGOT), and serum glutamate pyruvate transaminase (SGPT).

2.6.2.2. Histopathological Studies. For histopathological examinations, on the 30th day of the study, the vital organs such as kidneys and liver of the animals in the different groups were excised, immediately detached, any non-essential tissues cut off, positioned on a saline-soaked gauze pad to hinder

Figure 1. FTIR spectra for AMS (A) and ECC-MS (B).

Figure 2. SEM Images of alkali-isolated mandua starch (AMS) and epichlorohydrin-cross-linked starch (ECC-MS).
detached organs from each group of mice were fixed in 10% formaldehyde. The sections were cut on glass slides and stained with hematoxylin and eosin. The slides were examined under a light binocular microscope.

2.7. Mortality and Clinical Observations. Each animal of every group was observed during the acute and sub-acute toxicity studies and their responses were recorded. Animals were examined for any sign of alteration in the skin, mucous membrane, and autonomic posture for improvements in the overall response.

2.8. Statistical Analysis. The results obtained in each experiment were compared to the control group by Student’s t-test using an excel program, a significance level of \( p < 0.05 \), and a 95% confidence level. The variables analyzed were expressed as mean ± standard deviation (SD).

3. RESULTS AND DISCUSSION

3.1. Fourier Transform Infrared Spectroscopy (FTIR).

The FTIR spectra of the AMS and ECC-MS displayed all of the typical absorption bands for polysaccharides. The IR spectra of AMS and ECC-MS exhibited the characteristic peaks for the starch backbone. The spectrograms of AMS and ECC-MS indicated that the vibration bands of C-H, O-H, and C-O in native and ECC-MS starch had no distinctive differences. The IR spectra of the ECC-MS exhibited the characteristic peaks for the starch backbone. The strong band at 3401 cm\(^{-1}\) in alkali-isolated mandua starch is assigned to the –OH stretching and the band at 1020 cm\(^{-1}\) is due to the C–O–C in anhydroglucose units. Comparatively, the absorption peak at 3401 cm\(^{-1}\) in AMS was seen to be broadened and less sharp in the ECC-MS spectra due to the hydrogen bond stretching. Additionally, in the IR spectra of the cross-linked starches, the typical absorption bands of starch at 1019 and 1155 cm\(^{-1}\) due to C–O stretching vibrations were highly diminished in intensity. The absorption bands at 1640 cm\(^{-1}\) showed the presence of C=O stretching in alkali-isolated starch. The presence of the band at around 2930 cm\(^{-1}\) in raw and modified starch is assigned to the C–H vibration stretch. The peaks at 709 cm\(^{-1}\) indicate the low content of coordinated water molecules due to the wagging vibrations of the –OH groups.

In all, FTIR confirms the cross-linking reaction between starch and ECHD (Figure 1).

3.2. Morphological Investigation. The appearance of mandua starch granules before and after ECHD cross-linking was studied using scanning electron micrographs. SEM investigations revealed that the ECHD-mediated cross-linking of AMS caused slight changes in the granular morphology of ECC-MS as compared to AMS (Figure 2). The granular surfaces of AMS were polygonal with well-defined edges, but the surface of ECHD-cross-linked mandua starch granules became slightly rough with the formation of grooves. The formation of grooves on the surface of the cross-linked starch granules indicates slight fragmentation. These results were consistent with previous reported studies.\(^{29-31}\)

3.3. Clinical Signs of Acute and Sub-Acute Toxicity Studies. 3.3.1. Behavioral Analysis. In chemical cross-linking of mandua starch, different chemicals were used. After processing, there is the possibility of some traces of applied chemicals and reagents being present as residues in the final product and these could result in toxicity of the treated mandua starch. These chemical residues in the materials might be toxic for consumption even when the ECC-MS would be characterized as a “generally regarded as safe (GRAS)” excipient. Several fatalities have been reported due to the usage of widely employed plant and animal-based products due to heart failure, hepatotoxicity etc.\(^{13-16}\) Hence, the toxicity study of treated mandua starch was considered important as it affects public health in different forms, exposure to the chemical substances may be hazardous, and it may cause adverse effects on consumption. In some cases, the presence of behavioral changes in animals after intake of a xenobiotic serves as a sign of toxicity.\(^{12}\)

In the acute toxicity study, no adverse effects were found in animals following oral administration of ECC-MS at a dosage of 2000 mg/kg body weight of mice. Various signs and symptoms related to the animals’ mental, physiological, and autonomic profiles were monitored continuously for 3 h, then every 30 min for the next 4 h, and eventually for mortality after 24 h, 48 h, 7 days, and 14 days (2 weeks); no remarkable changes were observed. The control and test groups reflected the usual fur, skin, mucous membranes, salivation, lethargy, sleep, coma, convulsions, tremors, diarrhea, oral activity, and abdominal and external genitalia.

3.3.2. Gross Histopathological Examination and Macroscopic Findings. Fluctuations in body weight are considered as a responsive indicator of an animal’s general health condition and are described as one of the human end points.\(^{3,14}\) However, a remarkable reduction in body weight specifies the possibility of harmful effects of the test materials and, sometimes, it also relates to the adverse impact on appetite and other related factors.\(^{17}\) In acute toxicity studies of ECC-MS, the changes in body weight of the test and standard group animals were not significant compared to the control group after consumption of a dose of 2000 mg/kg body weight (\( p > 0.05 \)) (Table 1).

Similar results were observed for sub-acute toxicity study (Table 2). These nonsignificant changes in body weight of animals indicated that ECC-MS administration was not harmful following oral administration. Additionally, the weights of body organs such as the liver and kidney were
Table 2. Weight of Mice Treated with Control, Epichlorohydrin-Cross-Linked Mandua Starch (ECC-MS), and Alkali-Isolated Mandua Starch (28-Days Study) (Dose: 1000 mg/kg, Body Weight of the Animal)

| Treatment Duration (days) | Control (normal saline) | ECC-MS | AMS | AMS vs ECC-MS | AMS vs Control |
|---------------------------|-------------------------|--------|-----|--------------|---------------|
| 0                         | 34.56 ± 0.711           | 33.15 ± 1.911 | 34.80 ± 0.511 | 0.439 | 1.013 |
| 7                         | 35.15 ± 1.051           | 34.16 ± 0.653 | 33.67 ± 0.790 | 0.476 | 1.215 |
| 14                        | 35.36 ± 1.103           | 34.51 ± 1.200 | 33.95 ± 1.701 | 0.508 | 1.014 |
| 28                        | 35.52 ± 1.215           | 35.45 ± 1.166 | 34.93 ± 1.434 | 0.540 | 1.009 |

*p* values were obtained by Student's *t*-test, comparing control vs alkali-isolated mandua starch (AMS) and control vs epichlorohydryn-cross-linked mandua starch (ECC-MS).

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3.3.3. Histopathological Studies. The consumption of poisonous compounds impacts the physiology of organs and tissues. It also influences the changes in microstructure of the organs. In toxicity studies of chemically modified mandua starch, the animals were autopsied after the due course of the study and the internal organs were examined for any structural changes. No significant differences in cellular structures of the liver and kidney were found in histopathological examinations following ingestion of treated mandua starch. The photographs of liver histology revealed that the hepatic parenchyma was regular in architecture and there were no signs of necrosis or hyperemia in animals of the control, test, and standard groups. The centrilobular vein was distinct in its vicinity and the vasculature to all tissues was noticeable and regular. Additionally, the standard cellular architecture and binucleation were also clear and were observed to be unaltered. No symptoms of damage, necrosis, congestion, fatty acid aggregation, or hemorrhagic regions were found in the ECC-MS-treated animals. Neutrality was not found in the blood cells or the tissues and the primary organs were not modified in the sub-acute oral toxicity.

Both the ECC-MS and control groups exhibited no remarkable histological changes on evaluating the renal structure histopathologically. In addition to the test groups, the glomerular structure appeared sensible. In both cases, the distal tubules and the glomeruli appeared healthy, with no interstitial or intrageneric obstructions/atrophies or atrophic-ventricular entric atrophies being detected. Both nephron cells were delicate with distinctly prominent nucleoli, and no signs of degeneration, bleeding, necrosis, or lymphocyte infiltration were observed. Furthermore, histopathological examinations of the liver and kidneys revealed no morphological anomalies consistent with ECC-MS administration.

Serum biochemical and hematological parameters are another essential parameter to identify acute or chronic liver and/or kidney toxicities. In determining the toxicity of medications and plant extracts, evaluation of liver and kidney functions is also an important factor. An elevation in the AST, ALT, and ALP levels in the blood suggests liver toxicity. The cytoplasmic presence of AST and ALT enzymes above the limit also indicates the sign of liver toxicity. On the other hand, creatinine is an excellent predictor of kidney activity and an increase in ALT or AST levels indicates toxicity. As a result, the presence of these indicators in abnormal amounts in the serum may be used to determine any organ injury resulting from toxicity.

In the present study, acute and sub-acute tests revealed no substantial variations in serum AST (SGOT) and ALT (SGPT) levels among mice treated with 1000 mg/kg body weight of ECC-MS (test) and mice treated with the standard (alkali-extracted mandua starch) and control groups (*p* > 0.05). However, the slight variations in serum AST and creatine kinase levels may be due to several other factors such as muscle injury, myocardial infarction, and elevated muscle activity. On the other side, histological portions of the livers of all mice in the acute and sub-acute trials revealed stable structures without lesions. Generally, acute kidney damage determined as the relative organ weight is critical for determining whether the organ is exposed to injury or not. However, no substantial difference in the weight of each organ was found following ECC-MS administration (*p* > 0.05) in the 28-days sub-acute toxicity study (Table 3) and 14-days acute toxicity study (Table 4).
caused by toxicants may result in renal cell failure and is responsible for millions of deaths per year. Concurrent tests of urea, creatinine, and uric acid may be used to determine renal failure and their typical values indicate a decreased risk of renal complications. Creatinine is a noble marker of kidney function, and the changes in ALT, AST, and creatinine levels reflect hepatic and renal toxicity.22 A rise in serum creatinine by \( \geq 0.3 \) mg/dL or to \( \geq 1.5 \) times the baseline is defined as acute kidney injury.23 The glomerular filtration rate is proportional to the number of functioning nephrons in the kidney and it decreases in all types of progressive kidney diseases. Creatinine is a naturally occurring endogenous marker of the glomerular filtration.

Table 3. Organ Weight and Organ Weight Ratio of the Control and Test Groups (Dose: 1000 mg/kg Body Weight of the Animal; 1500 mg/kg Body Weight of the Animal, and 2000 mg/kg Body Weight of the Animal)

| Organ Weight (g or g%) | sub-acute toxicity study (dose: 1000 mg/kg body wt of animal) | sub-acute toxicity study (dose: 1500 mg/kg body wt of animal) | sub-acute toxicity study (dose: 2000 mg/kg body wt of animal) |
|------------------------|---------------------------------------------------------------|---------------------------------------------------------------|---------------------------------------------------------------|
| control (saline water) | AMS (standard)                                                | test (ECC-MS)                                                | AMS (standard)                                                |
| liver (g)              | 2.54 ± 0.512                                                  | 2.67 ± 0.330                                                 | 2.61 ± 0.556                                                 |
| kidney (g)             | 0.47 ± 0.023                                                  | 0.49 ± 0.032                                                 | 0.47 ± 0.016                                                 |
| liver (g%)             | 7.14 ± 1.404                                                  | 7.87 ± 1.312                                                 | 7.20 ± 1.303                                                 |
| kidney(g%)             | 1.33 ± 0.071                                                  | 1.38 ± 0.056                                                 | 1.46 ± 0.167                                                 |

Organ Weight Ratio

| Organ Weight Ratio (g%) | sub-acute toxicity study (dose: 1000 mg/kg body wt of animal) | sub-acute toxicity study (dose: 1500 mg/kg body wt of animal) | sub-acute toxicity study (dose: 2000 mg/kg body wt of animal) |
|-------------------------|---------------------------------------------------------------|---------------------------------------------------------------|---------------------------------------------------------------|
| control (saline water)  | AMS (standard)                                                | test (ECC-MS)                                                | AMS (standard)                                                |
| liver (g%)              | 7.12 ± 0.955                                                  | 7.16 ± 0.859                                                 | 7.15 ± 0.916                                                 |
| kidney (g%)             | 1.35 ± 0.511                                                  | 1.32 ± 1.003                                                 | 1.31 ± 0.948                                                 |

Figure 3. Histopathological analysis of the animal group (dose: 1000 mg/kg) (repeated-dose 28-days toxicity study, sub-acute toxicity study).
filtration rate. The serum or plasma concentration of creatinine is inversely proportional to the glomerular filtration volume. However, plasma creatinine concentration is not a highly responsive measure of renal impairment until the glomerular filtrate flow rate has decreased to less than 50%. Thus, renal activity in the blood can be determined by measuring urea, a byproduct of protein digestion produced in the liver by ammonia and then removed by the kidneys. Hence, urea and creatinine amounts were determined in this study to assess the renal functions of the mice used in the toxicity studies.

The results revealed no statistically difference in serum urea and creatinine levels between groups of animals receiving doses of ECC-MS (test), standard (AMS), and control in both acute and sub-acute trials (\( p > 0.05 \)). The photomicrographs of the histopathological analysis of the test and standard animals are shown in Figures 3 and 4.

3.3.4. Hematological Evaluation. WBC or leukocytes are the first cellular defense lines against foreign materials, infectious organisms, tissue inflammation, and inflammatory processes. Leukocytes are formed in hematopoietic stem cells, more often referred to as the bone marrow. Leukemia is described as an abnormally low count of normal leukocytes or neutropenia with a low RBC count (anemia). Increased white cell count in the blood, on the other hand, is a symptom of infection. Iron deficiency has been associated with hemoglobin-dependent reductions in MCHC values. Considering all of these findings, WBC counts were taken into consideration in all mice. In the biochemical and hematological investigation, no significant differences were observed in hemoglobin, WBCs, lymphocytes, neutrophils, monocytes, eosinophils, and basophils among the test, standard, and control groups of animals (\( p > 0.05 \)). Furthermore, a nonsignificant alteration was noticed in the serum creatinine, urea, AST, and ALT of the treated and control animal groups (\( p > 0.05 \)). The hematological and enzymological parameters after acute and sub-acute toxicity studies of AMS and ECC-MS are shown in Table 5.

3.3.5. Mortality and Clinical Observation. In the 14-days acute and 28-day repeated-dosage sub-acute study, there was no death or clinical symptoms of toxicity. Scientific demonstrations of the treated and concurrent control groups were almost identical.

4. CONCLUSIONS

The current research study demonstrates that ECC-MS, a modified cross-linked mandua starch, may be regarded as safe. It did not produce any lethality or cause any significant behavioral, hematological, anatomical, and biochemical adverse effects after oral administration. Furthermore, ECC-MS showed a no-observed adverse effect level (NOAEL) at doses of 1000, 1500, and 2000 mg/kg body weight in both...
sexes of animals during the 28-days repeated-oral-dose sub-acute toxicity study. This may be because of the proper washing of the cross-linked starch before administration to mice. After cross-linking, proper washing of the cross-linked starch might be the reason for the removal of residual or unreacted epichlorohydrin from the final product. Besides this, the reacted amount of epichlorohydrin in cross-linking of alkali-extracted mandua starch might be within the permissible limit of consumption as it did not produce any signs of toxicity. The reacted amount of epichlorohydrin in cross-linking of alkali-extracted mandua starch might be within the permissible limit of consumption as it did not produce any signs of toxicity.

Moreover, cross-linked modified mandua starch may have the potential as a novel excipient in developing dietary, nutraceutical, or pharmaceutical products.

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**Notes**

The authors declare no competing financial interest.

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