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Original Research Article

Acute and Sub-Acute Toxicity Studies of Starch Hyaluronate in Wistar Rats

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ABSTRACT

Starch hyaluronate is used as a new superdisintegrating agent in the development of fast-dissolving tablets of poorly soluble drugs because of its improved biocompatibility, and hydrophilicity. The purpose of this study is to assess the acute and subacute toxicity profiles of starch hyaluronate in Wistar rats. The starch hyaluronate was administered to Wistar rats in a single-dose acute study and monitored for seven days. Wistar male and female rats were used in a 28-day sub-acute study that examined the effects of oral doses of 100, 200, and 600 mg/kg body weight/day on body weight, food intake, mortality, biochemical analysis, and histopathological evaluation. An acute study revealed that the synthesized starch hyaluronate minimal oral fatal dose for rats was larger than 2000 mg/kg body weight. The subacute toxicity evaluation found no significant changes when compared to the control group, and there was no change in hematological or biochemical parameters. The weights of the liver, kidneys, and pancreas remained constant. Based on the findings, it was concluded that starch hyaluronate at 600mg/kg body weight/day was neither immunogenic nor sensitizing, and was not a reproductive or developmental toxicant, implying that it is relatively safe when taken orally.

***Keywords*:** Starch hyaluronate, Wistar rats, Toxicity, Mortality

Introduction

Superdisintegrants are compounds that were added to fast-dissolving tablets to enhance the tablet's disintegration process.[1] A variety of excipients have been frequently used as tablet disintegrants for some years, and only a few recognized disintegrants are already available to pharmaceutical industry formulators. Simple starches are among the most commonly used disintegrants. However, simple starch cannot always provide an acceptable, sufficient, and rapid disintegration time for a solid dosage form. So, to enhance the disintegration of immediate-release tablets, "superdisintegrants" have been developed.[2] Toxicants have qualities that can affect the extracellular material, cell surface, and space beneath the cells and tissues. As a result, determining the toxicity of any molecule before it is employed as a pharmaceutical excipient has become a must. In the current investigation, the acute and subacute toxicity profile of starch hyaluronate was evaluated.[3,4] Acute toxicity is defined as undesirable effects that occur within a short period of time after even a single oral intake of a product or repeated doses given within 24 hours. Sub-acute toxicity is defined as the occurrence of harmful effects caused by continuous or multiple treatments between 24 hours and 28 days.[5] Chronic toxicity is defined as the occurrence of harmful effects caused by continuous or multiple treatments usually weeks or months. [6]Esterification was used to synthesize starch hyaluronate as a new superdisintegrant from native potato starch and hyaluronic acid.[7] It is important to check the toxicity data of every superdisintegrant whether it is acquired from plants or synthesized in a laboratory. Researchers are now concentrating on the development of superdisintegrants using a starch modification, to boost hydrophilicity and cross-linking to prevent gel formation and solubility upon contact with water.

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This modified starch showed enhanced stability and extreme temperature resistance. Toxicity experiments on Wister rats were conducted to determine the safe concentration of starch hyaluronate in dosage form formulation.[8]

Materials and Methods

Materials

Sodium hydroxide, as well as potato starch, were obtained from SD Fine Chemicals Ltd in Mumbai, India in 2022. We bought hyaluronic acid from Rass Bio solution Pvt. Ltd. In a lab starch hyaluronate was synthesized, analytical kit by Merck.

Methods

Processing of Starch Hyaluronate

Potato starch was purchased from SD Fine Chemicals Ltd. Potato starch of 10 grams was suspended in a beaker containing distilled water to a volume of 15 mL. 10 grams of hyaluronic acid was added to the starch slurry, and the pH of the slurry was then adjusted to 3.5 with 10 mL sodium hydroxide and kept idle for esterification reaction. The mixture was then treated using distilled water to remove any remaining amounts of hyaluronic acid before being dried at 60^0C to form a dry mass. The dried starch hyaluronate was screened with a #120 sieve to uniform-sized particles and preserved in the desiccator.[9,10]

Experimental animals

In order to evaluate acute and sub-acute toxicity experiments, adult healthy male and female Wistar albino rats (age, 8-12 weeks; body weight (males), 150-200 g; body weight (females), 120-150 g) were employed. Cages made of polypropylene held three rats. The chosen female rats were Nulliparous and not pregnant, while the male and female rats were housed separately. The animals were kept in sanitary polypropylene cages with stainless steel top grills for at least a week in the laboratory animal room before the tests. Standard cage conditions were used (temperature of 25 ± 2 °C, relative humidity of 65%, and a cycle of light and dark lasting 12/12 hours). The bedding was made out of brand-new paddy husk. The animals were given standard rat pellet food (supplied by M/s Hindustan Lever Ltd., Mumbai), and clean

polypropylene water bottles with stainless steel sipper tubes were available at all times. Daily cleanings were performed on the water bottles and cage bedding. The Institutional Animal Ethics Committee (IAEC) of the Balaji Institute of Pharmaceutical Sciences, Narsampet, Warangal, Telangana, India, gave its approval to all experimental procedures (CPCSEA Number 01/BIPS/IAEC/2022). The guide for the care and use of laboratory animals was followed when doing experiments on animals

Acute toxicity study

With a few minor adjustments, the acute toxicity experiments were carried out on Wistar rats in accordance with Organization for Economic Co-operation and Development (OECD) guideline 423. For the investigation, rats of both sexes were employed. The chosen male and female rats were then divided into five individuals of each sex for the normal control and treatment groups. 2000 mg/kg, b.wt., was administered orally to the test group rats as a test sample. The sole vehicle given to the rats in the control group was water. All of the rats were weighed, identified, and fasted the night before the experiment began while being given free access to water. Following dosage, the rats fasted for a further 4 hours, and observations were made constantly for each rat in each group for the first 4 hours and then for the following 24 hours to look for any aberrant changes and deaths. After that, they were monitored twice daily for a week to look for any harmful effects, such as changes in the color of their eyes, hair, or skin, changes in how much they ate or drank, trembling, convulsions, salivation, diarrhea, lethargy, breathing, abnormal behavior, motor activity, sleep, or coma. Acute toxicity was carried out to gather data on the test substance's level of short-term toxicity, which aids in choosing doses for the repeated oral toxicity study.[11]

Sub-chronic toxicity studies

Wistar albino rats were used in a subacute toxicity investigation of starch hyaluronate that followed the procedures outlined in OECD recommendation 407.[12]

Variation in body weight

One day before the start of treatment (Day 0), and then every week after that for the duration of the trial, the body weight of each rat was noted. One day before blood collection, the animals were fasted overnight. The body weight was taken on Day 28.

Variation in weight of the organ

The organs (liver, kidneys, lungs, and thymus). were collected, cleaned with filter paper, and weighed with an analytical balance. (Make: Mettler Toledo) All organ weights were recorded as absolute numbers.

Body and organ weight evaluation

To screen for potential toxicity, the body weights of the control and treatment animals were compared. Three doses of 100, 200, and 400 mg/kg of the target organs underwent a macroscopic investigation to look for any anomalies in size, texture, or shape that could indicate hazardous effects. The principal organs that are targeted are the rat liver, kidneys, lungs, and thymus.

Hematological estimation

Wistar rats under anesthesia had their blood drawn into capillary tubes, where it was analyzed for hematological parameters like hemoglobin (Hb) count, red blood cells (RBC), white blood cells (WBC), and platelet count.[13]

Estimation of Biochemical tests

Blood was centrifuged at 3000 RPM for 10 min at 4 °C for biochemical analysis. After centrifugation, the serum was extracted from the blood and put into storage at -20°C until analysis. Standard methods were used to measure the biochemical markers aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), total protein, serum creatinine, urea, and albumin concentration.[14]

Histology study

The liver, stomach, and kidney were evaluated for histopathological changes. After euthanasia, the stomach, liver, and kidney were surgically removed and preserved in 20 percent formalin in normal saline. On a rotary microtome, 5 m sections were cut, and was subsequently stained with hematoxylin-eosin (HE) 40. The sections were then examined under a microscope for pathological changes.[15]

Statistical analysis

The results were expressed as the mean ± S.E.M and were analyzed by one-way ANOVA followed by Dunnett's multiple comparison "t" test. Data was computed for statistical analysis by using Graph Pad PRISM 5 Software.[16]

Results and Discussions

There was no fatality in the acute toxicity studies. Within 4 hours of continuous observation as well as 24 hours later. Additionally, after the Starch hyaluronate was administered for the seven days of the experiment, no deadly effect was seen. Morphological features are shown normally. None of the Aberrant behaviors, such as salivation, diarrhea, or lethargy, were noticed. Body weight, water, and food consumption and observed common lung activity. Substantial sedation was noted 10–15 minutes after the test dose administration.

This demonstrates that the dose of 600 mg/kg, b.wt. of starch hyaluronate was safe. Because there was no information on fatalities for all doses, the LD_{50} value was thought to be greater than the maximum test dose of 600 mg/kg body weight.

All of the animals were still alive and had a normal appearance on Day 28, the last day of the experiment, throughout the experiment. The body weight increased in every animal. Table 1 shows the results of the body weight

At the end of the treatment time, no differences in organ weight were seen in the males. At the treatment dose of 600 mg/kg/day, the relative liver/body weight of the females was significantly higher than that of the control group. (Table 2).

No alterations of toxicological relevance were found in the analysis of the hematological data performed over the course of 28 days of treatment. Some treatment groups showed statistically significant differences in a few metrics when compared to the control group. However, these variations were determined to be of no biological significance because there was no dose-response association and no comparable changes in females. The parameters of the blood biochemistry studies did not show any statistically significant differences. During the necropsy, there were no observable lesions that could be connected to the course of therapy. At the end of the treatment time, no differences in organ weight were seen in the males. At the 600 mg/kg/day dose level, females had a significantly higher relative liver/body weight than the control group. This finding was not deemed to be of biological significance due to the relatively lower liver weights in control females, the absence of a comparable effect in high-dose males, the lack of any histopathological correlates in the liver, and the lack of any effects on indicators of liver function, including the results for serum biochemistry and blood parameters. (Figure 1).

Oral starch hyaluronate administration in Wistar rats for 28 days was assessed with repeated dose administration. The parameters of the urinalysis and blood biochemistry studies did not show any statistically significant differences. All of the groups' biochemical parameter levels were assessed and shown in Figure 2.

The histopathological analysis did not uncover any obvious treatment-related side effects. In high-dose females, there was a tendency for a rise in the incidence of stomach inflammation.

A section of the stomach depicted in figure 3 revealed surface necrosis and erosions of the mucosal epithelium. The serosal surface of the stomach in a small number of animals thickened and deposited fibrinous exudates. Exudates on the serosal surface revealed extensive neutrophil infiltration.

A section of the kidney depicted in figure 4 showed some minor tubular parenchymatous degenerations. While the glomeruli showed no obvious alterations.

Comparing the liver to control animals, no histological alterations were found. However, some animals had fatty liver alterations. (Figure 5)

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Table 1: Effect on the body weight after Starch hyaluronate administered for 28 days

Group	Body weight(g), n=6, Mean ± S.E.M				
	Weight before administration	Weight after 1st week	Weight after 2nd week	Weight after 3rdweek	Weight after 4thweek
1	142 ± 1.1	142 ± 1.3	145 ± 1.3	146 ± 1.6	149 ± 1.2
2	141 ± 1.1^{ns}	142± 1.3^{ns}	144 ± 1.1^{ns}	146 ± 1.3^{ns}	148 ± 1.3^{ns}
3	140 ± 1.3^{ns}	141± 1.2^{ns}	144 ± 1.1^{ns}	147 ± 1.3^{ns}	149± 1.3^{ns}
4	142 ± 1.1^{ns}	144 ± 1.2^{ns}	145± 1.1^{ns}	147± 1.1^{ns}	150 ± 1.2^{ns}

Table 2: Effect on the weight of the isolated organ after Starch hyaluronate administered for 28 days

Dose Starch Hyaluronate (mg/kg)	Liver	Kidneys	Lungs	Thymus
0	2.30 ± 0.25	0.52 ± 0.03	0.38 ± 0.04	0.09 ± 0.02
100	2.47 ± 0.53	0.54 ± 0.06	0.38 ± 0.05	0.1 ± 0.02
200	2.48 ± 0.32	0.55 ± 0.05	0.37 ± 0.04	0.09 ± 0.01
600	2.30 ± 0.23	0.52 ± 0.04	0.37 ± 0.04	0.09 ± 0.01

*n=6, Mean ± S.E.M

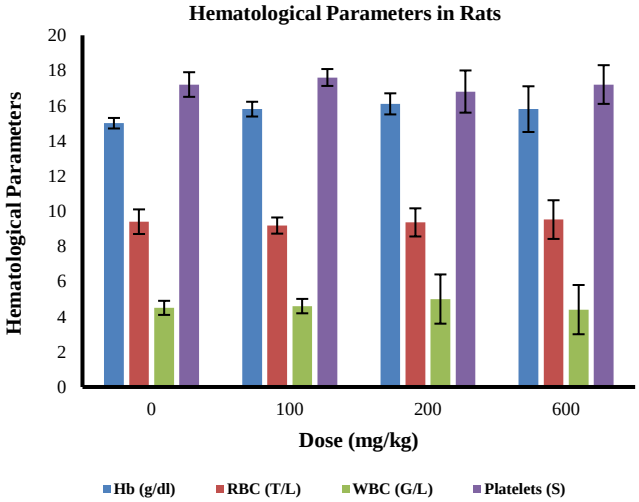

Figure 1: Effects of 28 Days Administration of Starch hyaluronate on Hematological Parameters in Rats (*n=6, Mean ± S.E.M)

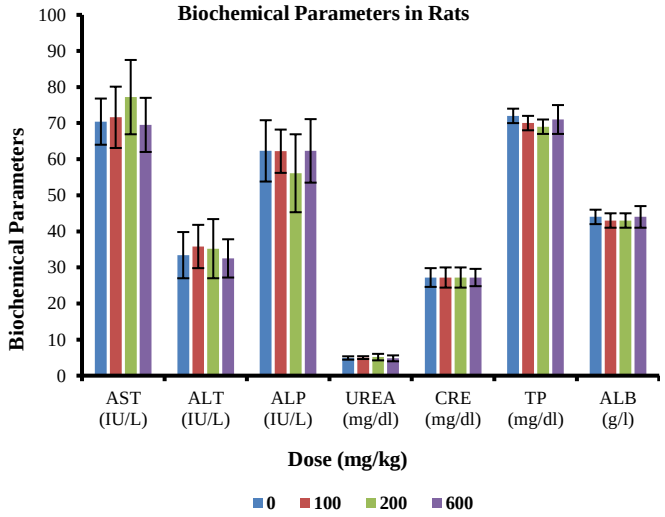

Figure 2: Effects of 28 Days Administration of Starch hyaluronate on biochemical Parameters in Rats (*n=6, Mean ± S.E.M)
AST-aspartate transaminase, ALT-alanine transaminase, ALP-alkaline phosphatase, TP- total protein, CRE- serum creatinine, urea, and ALB- albumin

Conclusion

The oral LD_{50} of starch hyaluronate was higher than 600 mg/Kg. Additionally, research on the sub - acute toxicity of starch hyaluronate has shown that at test levels, it had no negative impacts on body weight, hematological measurements, or biochemical parameters. Starch hyaluronate was shown to be well tolerated by toxicity testing. The results show that starch hyaluronate is safe to use, and daily consumption of 600 mg of this new super disintegrant is advised.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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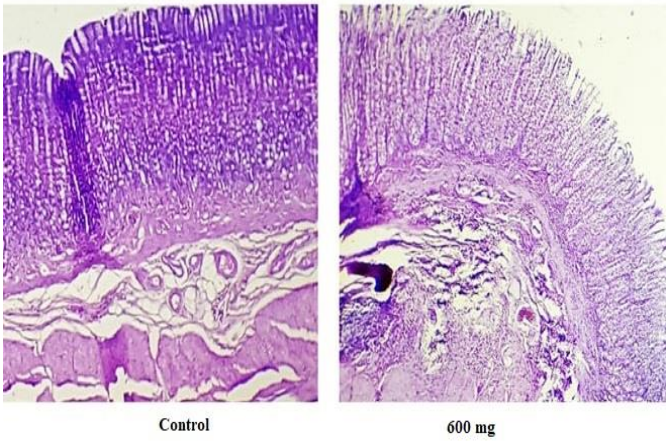

Figure 3: Effect of starch hyaluronate stomach, Control rats and rats treated with the Starch hyaluronate (600 mg/kg),

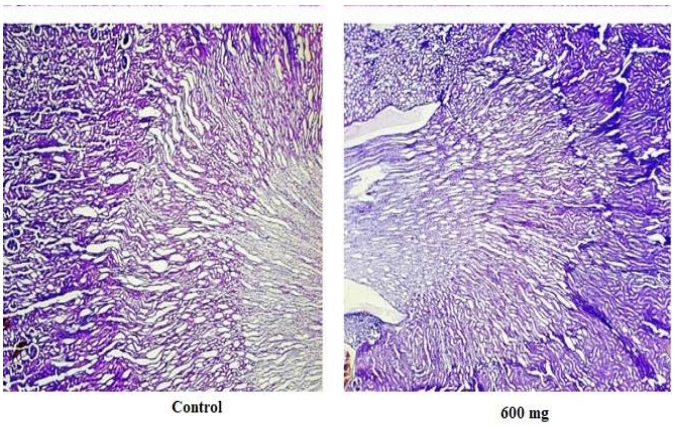

Figure 4: Effect of starch hyaluronate Kidneys, Control rats and rats treated with the Starch hyaluronate (600 mg/kg),

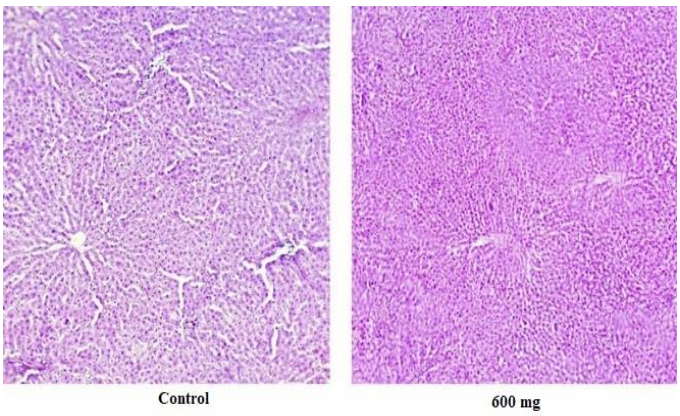

Figure 5: Effect of starch hyaluronate Liver, Control rats and rats treated with the Starch hyaluronate (600 mg/kg),

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