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Acute and Sub-Acute Toxicity Studies of Starch Glutamate: A Novel Superdisintegrant

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ABSTRACT

Starch glutamate was a novel superdisintegrant which was synthesized from the native potato starch and glutamic acid by esterification process. The prepared starch glutamate toxicity was evaluated by performing acute and sub-acute toxicity studies on the Wister rats and Swiss albino mice. Acute toxicity studies were conducted on the Swiss albino mice by dividing them randomly into 10 groups (6 rats in each group) and treated with starch glutamate up to the concentration of 2000mg/Kg body weight, and then they were closely observed for 12 hrs for any toxic symptoms. Sub –acute toxicity studies were performed on the Wister rats by dividing them into 5 groups (6 rats in each group) and treating them with starch glutamate up to the concentration of 2000mg/Kg body weight once daily for 28 days and on 29th day animals were sacrificed and blood samples were analysed for change in haematological parameters and biochemical parameters. The prepared starch glutamate was safe up to the concentration of 2000mg/Kg body weight and it did not exhibit any change in the haematological parameters and bio-chemical parameters.

Keywords: Superdisintegrants, Starch glutamate, Acute toxicity, Sub-acute toxicity.

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INTRODUCTION

Superdisintegrants are the substances which were added to the fast dissolving tablets to improve the disintegration process of the tablet. Superdisintegrants were added to the formulation at a lower concentration typically 1-10% by weight relative to the total weight of the dosage form to fast up the disintegration process. Superdisintegrants can be divided into natural superdisintegrants and synthetic superdisintegrants. Whether the superdisintegrnat obtained from the plant origin or synthesized in laboratory it is necessary to screen its toxicity data. Toxicity studies[1] will be conducted to know the adverse effects of a substance on human or animal health or on environment. Toxicity can be divided into acute toxicity, sub-acute toxicity and chronic toxicity. Acute toxicity defined as adverse effects occurred within a short period of time after administration of a single oral dose of a substance or multiple doses given within 24 hours. Sub-acute toxicity can be defined as an occurrence of adverse effects due to continuous or multiple exposures between 24h and 28days.

In the present study, a new superdisintegrant starch glutamate was synthesized from the native potato starch by esterification process. Toxicity studies have been performed for the starch glutamate on Wister rats and Swiss albino mice to identify the safe concentration of it in the formulation of dosage forms.

MATERIALS AND METHODS:

Materials:

Glutamic acid, potato starch, acetone, hydrochloric acid, potassium di hydrogen phosphate, dimethyl sulfoxide procured from the SD Fine chemicals. Mercuric chloride, Sodium chloride, Glacial acetic acid, Ammonium oxalate, Sodium hydrogen phosphate, Potassium dihydrogen phosphate purchaged from Merck, Mumbai. Crystalline sodium sulphate, Ethanol, Gentian violet, Wrights stain, Methanol from Qualigens Fine Chemicals, Mumbai. Merck analytical kit.

Preparation of Starch Glutamate:

10 parts of potato starch and 10 parts of glutamic acid were accurately weighed. 10 parts of previously weighed potato starch was dispersed into 25 parts of distilled water to make starch slurry.10parts of glutamic acid was dissolved in

distilled water and then it was added to the starch slurry. pH of the dispersion (Glutamic acid and starch slurry) was adjusted to 3.5 by adding 10ml of sodium hydroxide, then it was conditioned for 16hr to complete the reaction between potato starch and glutamic acid. After conditioning, unreacted glutamic acid was removed by washing this dispersion with distilled water, and then this solid mass was dried at 60°C temperature to form starch glutamate. The dried starch glutamate was passed through #120 sieve to obtain uniform sized particle and stored in a desiccator.

Toxicity Studies of Starch Glutamate :

Toxicity of prepared starch glutamate was evaluated by performing acute, sub-acute and chronic toxicity studies. Toxicity studies of starch glutamate on animals were approved by the Institutional Animal Ethical Committee, GITAM Institute of Pharmacy, GITAM (Deemed to be University), and Visakhapatnam (Approval No: 1287/PO/Re/S/09/CPCSEA).

Animal Housing [2]:

The animals (Wister rats and Swiss albino mice) were housed in a stainless steel cages which containing automatic watering system. Six Wister rats were placed in one cage, where as one Swiss albino mice were placed in separate cages. All these animals were maintained in a clean room, with temperature as 68-76°F and humidity as 42%-72% respectively. These animals were allowed to expose for 12hr complete light and dark cycles in a day.

Acute Toxicity Studies:

Swiss albino rats were selected as an animal model to conduct acute toxicity studies. Swiss albino rats weighing 20-25g of body weight of either sex were taken and divided into 10 groups, each group containing 6rats. These rats were fastened overnight before conducting experiment. These Swiss albino rats were treated with starch glutamate through oral route with distilled water at four levels i.e. 50, 300, 1000 & 2000mg/Kg of body weight[3] and these were closely observed for 12hr for any toxicity symptoms[4] like increased motor activity, anesthesia, tremors, arching and rolling, chronic convulsions, ptosis, tonic extension, lacrimation, straub reactions, exophthalmos, pilo-erection, salivation, muscle spasm, opisthotonus, writhing, hyperthesia, loss on righting reflex, depression, ataxia, stimulation, sedation, blanching, hypnosis, cyanosis, analgesia and for 12h mortality rate.

Sub –Acute Toxicity Studies [5]:

In sub-acute toxicity studies, Wister rats of either sex containing a body weight of 130-150g were selected. These Wister rats were randomly selected and divided into 5 groups, each group containing 6 rats. Group-I selected as a control group, which was treated with distilled water for 28 days once in a day. Group-II received 50mg/Kg of starch glutamate once in a day for 28days, Group-III received 300mg/Kg starch glutamate once in a day for 28days, Group-IV received 100mg/Kg starch glutamate once in a day for 28 days, and Group-V received 2000mg/Kg starch glutamate once in a day for 28days. All the animals were monitored for food intake, water intake and body weight for 28days on weekly basis. Animals were sacrificed on 29th day and blood samples were collected and various organs like liver, kidney and heart were isolated and weighed.

Blood samples were analysed to know the changes in the haematological parameter like haemoglobin (Hb), red blood cell (RBC) count , white blood cell count (WBC) , erythrocyte sedimentation rate (ESR), differential count (DC) [neutrophils (N)], lymphocytes (L) , eosinophils (E), monocytes (M), and basophils (B) as well as changes in the biochemical parameters like serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), bilirubin, blood urea nitrogen (BUN) and serum creatinine.

Statistical Analysis [6]:

One way analysis of variance (ANOVA) and dunnetts test was used to calculate the results of acute and sub-acute toxicity studies.

RESULTS AND DISCUSSIONS:

Acute Toxicity Studies:

In acute toxicity studies Swiss albino mice, which were received up to the dose of 2000mg/Kg body weight of starch glutamate did not show any lethal effects and toxicity symptoms like increased motor activity, anaesthesia, tremors, arching and rolling, chronic convulsions, ptosis, tonic extension, lacrimation, straub reactions, exophthalmos, pilo-erection, salivation, muscle spasm, opisthotonus, writhing, hyperthesia, loss on righting reflex, depression, ataxia, stimulation, sedation, blanching, hypnosis, cyanosis, analgesia. From the acute toxicity results it was concluded that the prepared starch glutamate was non-toxic and safe up to the dose of 2000mg/Kg body weight and its LD_{50} could be greater than 2000mg/Kg body weight.

Sub-Acute Toxicity Studies:

In sub-acute toxicity studies Wister rats were received starch glutamate up to the dose of 2000mg/Kg body weight once in a day for 28 days. These rats were monitored for 28 days on weekly basis for change in increment in body weight and results were tabulated in following Table 1.

Table 1: Effect of Starch Glutamate on Body Weight Increment in 28days Treatment

Group	Body Weight in grams, n= 6 Mean ± S.E				
	Initial	1st week	2nd week	3rd week	4th week
I	145 ± 1.4	147 ± 1.3	149 ± 1.1	150 ± 0.8	152 ± 1.2
II.	138 ± 1.1^{ns}	140 ± 0.8^{ns}	142 ± 1.1^{ns}	144 ± 1.2^{ns}	146 ± 1.1^{ns}
III	139 ± 1.2^{ns}	141 ± 1.3^{ns}	143 ± 1.2^{ns}	144 ± 0.3^{ns}	145 ± 0.4^{ns}
IV	142 ± 1.0^{ns}	144 ± 1.1^{ns}	147 ± 1.4^{ns}	148 ± 1.1^{ns}	149 ± 0.5^{ns}
V	144 ± 1.1^{ns}	146 ± 1.2^{ns}	148 ± 1.2^{ns}	149 ± 1.2^{ns}	151 ± 1.2^{ns}

ns: non-significance

In the sub-acute toxicity study, starch glutamate treated groups did not show any significant changes in body increment in weekly intervals compared to that control group.

Organs which were isolated from the sacrificed rats after 28 days were weighed for increment in weight of organs like liver, kidney and heart. The results were tabulated in the Table 2. From the results, it was concluded that the weights of the liver, kidney and heart were unaltered in the experimental groups compared with control group.

Table 2: Effect of 28 Days Oral Administration of Starch Glutamate on Organ Weights in Rats

Group	Organ weight (g/100 g body weight), n=6, mean ± S.E		
	Liver	Kidney	Heart
I	2.35 ± 1.0	0.65 ± 0.5	0.30 ± 0.1
II	2.30 ± 1.1^{ns}	0.58 ± 0.2^{ns}	0.28 ± 0.2^{ns}
III	2.32 ± 1.2^{ns}	0.60 ± 0.1^{ns}	0.31 ± 0.1^{ns}
IV	2.48 ± 1.3^{ns}	0.64 ± 0.4^{ns}	0.28 ± 0.3^{ns}
V	2.55 ± 1.2^{ns}	0.66 ± 0.7^{ns}	0.31 ± 0.2^{ns}

Kidney functions in rats like blood urea and serum creatinine were studied after 28days of administration of starch glutamate. Results were tabulated in Table 3.From the result, it was concluded that starch glutamate treated group rats were did not show any significant changes in the kidney function when compared to the control group.

Table 3: Effect of 28 days administration of starch glutamate on kidney functions in rats

Group	Blood urea (mg%)	Serum creatinine (mg/dL)
I	40.21 ± 0.1	0.72 ± 0.2
II	40.74 ± 0.2^{ns}	0.71 ± 0.3^{ns}
III	40.35 ± 0.3^{ns}	0.72 ± 0.4^{ns}
IV	40.44 ± 0.3^{ns}	0.73 ± 0.6^{ns}
V	40.52 ± 0.3^{ns}	0.74 ± 0.5^{ns}

Blood samples were analysed for changes in the haematological parameter and hepatic functions in rats and results were given in the Table 4 & 5. From these results, it was concluded that the there was no significant changes in the haematological parameters and hepatic parameters in starch glutamate treated groups when compared to controlled groups.

Table 4: Effects of 28days Administration of Starch Glutamate on Haematological Parameters in Rats

Group	ESR(mm/h)	Hb (%)	RBC (X 10^6 mm^3)	WBC (X 10^3 mm^3)	Differential Count Percentage				
					N	L	E	M	B
I	6.51 ± 0.31	10.11±0.2	6.21±1.1	7.11±1.1	67.44±1.2	28.2±1.1	1.2±0.1	2.33±1.1	-
II	6.01 ± 0.37^{ns}	10.54±0.1^{ns}	6.22±1.3^{ns}	7.27±1.2^{ns}	67.57±1.3^{ns}	28.3±1.4^{ns}	1.1±0.2^{ns}	2.41±1.2^{ns}	-
III	6.35 ± 0.78^{ns}	10.22±0.3^{ns}	6.31±1.2^{ns}	7.34±1.4^{ns}	67.26±1.1^{ns}	28.5±1.3^{ns}	1.3±0.2^{ns}	2.37±1.2^{ns}	-
IV	6.55 ± 0.32^{ns}	10.12±0.4^{ns}	6.21±1.4^{ns}	7.40±1.2^{ns}	67.74±1.3^{ns}	28.2±1.2^{ns}	1.2±0.3^{ns}	2.26±1.3^{ns}	-
V	6.66 ± 0.45^{ns}	10.42±0.4^{ns}	6.33±1.1^{ns}	7.25±1.3^{ns}	67.24±1.5^{ns}	28.4±1.2^{ns}	1.1±0.4^{ns}	2.35±1.4^{ns}	-

Data are the mean of 6 animals ± S.E, ESR: Erythrocyte sedimentation rate, Hb: Hemoglobin, RBC: Red blood cells, WBC: White blood cells, N: Neutrophils, L:-Lymphocytes, E: Eosinophils, M: Monocytes, B: Basophils, ns = non-significance

Table 5: Effects of 28days Administration of Starch Glutamate on Hepatic Functions in Rats

Group	Liver glycogen (mg%)	SGPT (IU/L)	SGOT (IU/L)	ALP (IU/L)	Bilirubin (mg/dL)
I	135.3 ± 1.2	42.54 ± 1.2	115.74 ± 1.2	209.74 ± 1.2	0.73±0.12
II	136.2 ± 1.3^{ns}	42.74 ± 1.2^{ns}	115.25 ± 1.3^{ns}	209.58 ± 1.3^{ns}	0.71±0.10^{ns}
III	137.5 ± 1.2^{ns}	42.85 ± 1.1^{ns}	115.22 ± 1.1^{ns}	209.63 ± 1.7^{ns}	0.73±0.12^{ns}
IV	137.4 ± 1.3^{ns}	42.44 ± 1.2^{ns}	115.74 ± 1.5^{ns}	209.71 ± 1.5^{ns}	0.74±0.15^{ns}
V	135.7 ±1.4^{ns}	42.75 ± 1.3^{ns}	115.86 ± 1.6^{ns}	209.28± 1.6^{ns}	0.75±0.14^{ns}

Data are the mean of 6 animals ± S.E, SGPT: Serum glutamate pyruvate transaminase, SGOT: Serum glutamate oxaloacetate transaminase, ALP: Alkaline phosphatase, ns:non-significance.

CONCLUSION:

Starch glutamate which was synthesised from the native potato starch by esterification process did not produce any adverse effects on the behaviour and gross pathology on the rats at treated doses. Therefore the oral LD_{50} of the starch glutamate was greater than the 2000mg/Kg. Sub-acute toxicity studies of starch glutamate also reveals that, it did not produce any adverse effects on the body weight, haematological, and biochemical parameters at test doses. There were no signs of toxicity was observed in the kidney, liver and heart sections of treated animals. From the toxicity studies of starch glutamate, it was concluded that it was safe and non-toxic up to the dose of 2000mg/Kg.Therefore, starch glutamate can be used as a superdisintegrant in the formulation of fast dissolving systems.

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