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# Assessment of Acute and Sub-Acute Toxicity of Muskmelon Pectin on Wistar Rats Model

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ABSTRACT

Pectin is an ideal film-forming ingredient for fast-dissolving pharmaceutical films because of its enhanced hydrophilicity and biocompatibility. Using the Organization for Economic Co-operation and Development (OECD) protocol, the current study assesses the acute and subacute toxicological profiles of pectin derived from the muskmelon rind of Cucumis melo L. in Wistar rats. The Wistar rats were administered a single dose containing 100, 300, 500, 1000, and 2000 mg/kg of muskmelon pectin for the acute toxicity research. They were then monitored every week for changes in body weight, food intake, and mortality. The other group of rats received daily oral doses of 100, 300, 500, 1000, and 2000 mg/kg body weight as part of a 28-day sub-acute toxicity research to assess the extract's impact on histopathological and biochemical parameters. According to acute research, the minimum fatal dosage of pectin was around 2000 mg/kg per body weight. The outcomes obtained from the subacute toxicity investigations did not show any discernible differences from the control group, biochemical tests, or haematological analyses. The weight of the brain, lungs, heart, liver, and kidneys was the same. The findings showed that consuming 2000 mg/kg of muskmelon pectin daily by oral means was typically safe. The study also showed that pectin was not detrimental to development or reproduction, nor did it have immunostimulating or sensitizing effects.

***Keywords***: Lethal dose, Mortality, Toxicity, Muskmelon pectin, Wistar rat.

## Introduction

Natural polymer pectin has been explored for usage as a film-forming element in fast-dissolving delivery formulations because of its hydrophilicity, affordability, and biodegradability. Studies have shown that natural polymers, such as pectin, can increase the solubility of medications, shorten the time it takes to dissolve, enhance the properties of films, and serve as agents that produce films. Patients of various ages can take advantage of these benefits. Researchers have focused on employing natural polymers to generate safe and efficient medications that dissolve rapidly for fast drug release; pectin represents one naturally occurring polymer investigated in this context[1]. Natural film-forming materials, such as pectin, are a viable choice for pharmaceutical formulations because they supply nutritional supplements and facilitate quick release of drugs in fast-dissolving films[2].

Pectin extraction from various plant sources, including sugar beet, citrus, banana, mango, grapefruit, and passion fruit peels, has been described in the literature. Finding naturally occurring polymers in new varieties and fruit wastes has become increasingly important to satisfy industrial needs. The Cucurbitaceae family includes the yearly climber muskmelon, which can reach a height of 1.5 meters[3]. Its leaves are grooved and round to kidney-shaped. Its skin is warty and scaly. Its flesh is thick. The fruit is grown all summer long and traded abroad. *Cucumis melo* L. has 64 g of linoleic acid for every 100 grams of total fatty acids.

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Thirty-two milligrams of the fruit contain 3.5% carbs, 0.6% of the total protein, and 0.2% fat[4]. The pectin from muskmelon is believed to enhance formulations' stability and resistance to high temperatures.

Toxicants can affect tissues, cells, the extracellular matrix, and the surface of cells. Consequently, determining a molecule's toxicity is crucial before employing it as a practical excipient. This study evaluated muskmelon rind pectin's acute and subacute cytotoxic properties. The term "acute toxicity" describes adverse reactions that happen quickly after either one oral dose or multiple doses given over a day. Sub-acute toxicity is the phrase used to describe the emergence of side effects following repeated or continuous medication during a 24 to 28-day period. When adverse side effects arise from repeated or continuous treatments, usually over weeks or months, it is referred to as "chronic toxicity." Whether a film-forming polymer is derived from a plant or manufactured in a lab, it is always important to double-check its toxicity profile. Toxicity tests on Wister rats helped determine how much muskmelon pectin could be incorporated into dosage[5].

## Materials and Methods

### *Chemicals*

Analytical grades of anhydrous Citric acid, sodium citrate, and methanol were purchased from Finar Limited (India).

### *Collection and Preparation of the muskmelon rind*

Muskmelon rind (*Cucumis melo* L) was collected at a local fruit juice shop (https://maps.app.goo.gl/r4fHJZ33jPxrck7w6 ) in March 2023. The rinds were thinly sliced and air-dried until the remaining liquid evaporated. The dried rinds were pulverized into a powder.

### *Pectin Extraction*

The powdered rind (5 g) was weighed using an analytical balance (Shimadzu-ATX 224, Shimadzu scientific instruments, Japan). Then, 150 mL of distilled water was added, and the mixture was adjusted to a

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pH of 2 by adding powdered anhydrous citric acid. The mixture was heated to 80 °C with occasional swirls for an hour. Then, the mixture was filtered using a muslin cloth, and the filtrate was combined with an equivalent amount of ethanol and placed into an incubator set at 4°C for three hours to separate the pectin. The precipitate was then collected and subjected to multiple ethanol purifications. The cleaned precipitate was dried using an oven at 40°C (Remi RDHO-50, Remi electrotechnik Limited, India). The dried pectin was stored in a desiccator after screening with a #120 sieve to create uniformly sized particles[6].

*Animals used in experiments*

Mature and young albino rats of the Wistar breed (body weight:150-200rgms) between 8 and 12 weeks were used in the acute and sub-acute toxicity experiments[7]. Before testing animals were kept in clean, stainless-steel top-grill polypropylene cages inside a laboratory animal room for at least a week. The standard parameters in the cage were 25 ± 2 °C in temperature, 65% relative humidity, and a 12-hour light-dark cycle. Fresh paddy husk was used to make the bedding. The rats were given clean water and pellets as a usual diet. SS sipper tubes and polypropylene water bottles were frequently on hand. The water bottles and bedding in the cages were cleaned every day[8]. All experimental techniques were approved by Vaageswari College of Pharmacy's Institutional Animal Ethical Committee (IAEC) in Karimnagar, Telangana, India (CPCSEA Number VCP/IAEC/2024/05). The OECD protocols for the care and use of animals were followed in this study.

*Acute toxicity study*

The acute toxicity tests were carried out on Wister rats according to OECD recommendation 423, with a few minor modifications. The investigation was conducted using male, single-sex rats. Twenty-four rats divided into six groups (5-treatment and control groups) of 4 rats each were used for this experiment. The sample to be tested was administered orally to the rats in the test group at doses of 100, 300, 500, 1000, & 2000 mg/kg BW each day[9]. The animals from the control group received food and water. Every animal was weighed, registered, and given unlimited access to water for an entire night before the experiment began. The rats were starved for an extra four hours following the dosing. Every animal in each group was observed continuously for the initial four hours and the following twenty-four hours to detect any abnormalities or fatalities. Subsequently, they were monitored twice a week to observe any adverse consequences, such as changes in body weight, exhaustion, lacrimation, nasal bleeding, paralysis, piloerection, skin, food, and water intake, and mortality[10].

*Sub-acute toxicity study*

The OECD recommendation 407 was followed in the sub-acute toxicity study using Wistar albino rats. The same acute toxicity study procedure was followed for this assay, except the animals were given the pectin extract for 28 days. The test animals were administered oral doses of the sample at 100, 300, 500, 1000, & 2000 mg/kg BW, while the control group rats received food and water[11]. The weights of the animals were observed once a week and for 28 days, and changes in morphology or behaviour were also noted. The animals were sacrificed by euthanasia on day 29 post-treatment, and their organs and tissues were carefully removed and examined for any morphological changes. Before this, blood samples were collected from the tail vein puncture into EDTA and heparinised tubes. They were sent for haematological and biochemical examination[12].

*Biochemical Analysis*

Blood samples were tested using CBC equipment. The parameters examined include WBCs, lymphocytes, basophils, eosinophils, neutrophils, RBCs, PLTs, and haemoglobin count. Additional parameters include serum creatinine, bilirubin, liver glycogen, blood urea, SGPT, and SGOT[13].

*Histopathological study*

Histopathological changes in the liver, kidneys, and heart were assessed. Following euthanasia, the liver, kidneys, as well as heart were surgically removed. The organs were then cleansed with filter paper, weighed with a Shimadzu-224 ATX analytical scale, and their respective weights were recorded as absolute values[i4]. The organs were then preserved in 10% formalin in normal saline. Using a rotating microtome (Bexco-EP-AD5S-440T, India), 5 mm sections were cut, and hematoxylin-eosin (HE) 40 was applied to the sections. Subsequently, a microscopic (BOECO-BIB100, Germany) examination of the sections was performed to check for pathological abnormalities[15].

*Statistical analysis*

The data were examined using Dunnett's multiple comparisons "t" test and one-way ANOVA. The results were reported as the mean ± SD or SEM. Graph Pad Prism version 8.4.3, 2020, was used to compute the data for statistical analysis.[15]

## Results and Discussion

The acute toxicity study found that muskmelon pectin was safe at all the doses examined (100 - 2000 mg/kg BW). In the treated populations at the recorded levels, there were no deaths or changes in behaviours such as fatigue, lacrimation, nasal haemorrhage, paralysis, piloerection, skin colour, and food and water intake. Muskmelon pectin may have $LD_{50}$ above 2000 mg/kg body weight. Rat weight variations after acute muskmelon pectin poisoning testing are shown in Figure 1. The sub-acute toxicity study showed no discernible pathological alterations to the experimental or control groups' hearts, livers, or kidneys at the administered doses (100 - 2000 mg/kg BW). All test animals showed no signs of toxicity or death during the subacute investigation. As shown in Figure 2, rats' mean organ weights in both the control and experimental groups did not differ significantly ($p \geq 0.05$).

The sub-acute toxicity study's experimental groups' haematological parameters, including red blood cells, white blood cells, PLT, and HB all remained within the reference range for rats. The haematological outcomes of the treatment and control groups indicated in Table 1 did not differ noticeably. The biochemical markers related to blood urea, creatinine levels, liver glycogen, SGPT, SGOT, ALP, & bilirubin for each group of animals during the sub-acute toxicity examination were all found to be under the range of reference for rats. Biochemical parameters (Table 2) showed no discernible difference between those receiving musk melon pectin and control groups. In the histological analyses, sections of the treated rats' kidneys, liver, and hearts showed normal overall structure when stained with hematoxylin and eosin, showing no significant distinctions from the control group. A 10-magnification lens was used to capture these photos from histopathology slides, resulting in 100 scale bars per image (Figure 3). This study evaluates the toxicity assessment in rats in two steps. The first phase determines acute toxicity, while the second assesses sub-acute toxicity. Body weight variations function as an index for toxicity assessment. Acute exposure to musk melon pectin did not result in negative consequences or changes to the rats' body weights; therefore, it could be considered relatively safe. A sub-acute toxicity study looks at the damage caused by repeatedly feeding rats oral dosages of medication over 28 days. This study illustrates changes in the organism's haematological state, organ structure, or biochemical characteristics. The results of this study may provide the basis for identifying the negative consequences of the plant extract. Harmful compounds could be found investigating haematological parameters. Parameters including creatinine, urea, uric acid levels, ALT, SGPT, SGOT, and liver glycogen are among those for which biochemical markers are important therapeutic indicators. An assessment of the kidney and liver's functions is necessary to determine the potentially harmful consequences of muskmelon pectin. Urea levels can be used as a measure of kidney function. Many short-term and long-term kidney illnesses may cause it to rise. Normal levels for rat serum urea are 15–45 mg/dl. Creatinine is a marker of the glomerular filtration rate. Creatinine in rats typically ranges from 0.2 to 0.8 mg/d. In contrast, ALT is an accurate test that can detect damage to any liver cells. Haematological and biochemical markers did not show any adverse effects from pectin from muskmelon.

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**Table 1:** Hematological parameters of rats during sub-acute toxicity studies

| 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 | 6.20 ± 0.20 | 10.20±0.4 | 6.20±1.1 | 7.10±1.2 | 67.70±1.2 | 28.12±1.4 | 1.0±0.2 | 2.50±1.2 | - |
| II | 6.12 ± 0.54$^{ns}$ | 10.35±0.3$^{ns}$ | 6.30±1.2$^{ns}$ | 7.25±1.5$^{ns}$ | 67.50±1.3$^{ns}$ | 28.34±1.2$^{ns}$ | 1.2±0.3$^{ns}$ | 2.42±1.3$^{ns}$ | - |
| III | 6.22 ± 0.50$^{ns}$ | 10.45±0.4$^{ns}$ | 6.11±1.4$^{ns}$ | 7.30±1.2$^{ns}$ | 66.50±1.1$^{ns}$ | 28.55±1.1$^{ns}$ | 1.1±0.3$^{ns}$ | 2.35±1.2$^{ns}$ | - |
| IV | 6.35 ± 0.22$^{ns}$ | 10.50±0.3$^{ns}$ | 6.40±1.2$^{ns}$ | 7.45±1.4$^{ns}$ | 66.27±1.2$^{ns}$ | 28.40±1.3$^{ns}$ | 1.3±0.2$^{ns}$ | 2.55±1.5$^{ns}$ | - |
| V | 6.40 ± 0.40$^{ns}$ | 10.32±0.2$^{ns}$ | 6.35±1.1$^{ns}$ | 7.50±1.4$^{ns}$ | 68.20±1.2$^{ns}$ | 28.35±1.4$^{ns}$ | 1.2±0.1$^{ns}$ | 2.65±1.4$^{ns}$ | - |
| VI | 6.37± 0.35$^{ns}$ | 10.43±0.3$^{ns}$ | 6.25±1.3$^{ns}$ | 7.55±1.5$^{ns}$ | 68.52±1.1$^{ns}$ | 28.30±1.2$^{ns}$ | 1.2±0.2$^{ns}$ | 2.70±1.3$^{ns}$ | - |

N-Neutrophils; L-Lymphocytes; E-Eosinophils; M-Monocytes; B-Basophils. * n=6, Mean ± S.E.M, ns- Non significance.

**Table 2:** Biochemical parameters of rats during sub-acute toxicity studies

| Group | Liver glycogen (mg%) | SGPT (IU/L) | SGOT (IU/L) | ALP (IU/L) | Bilirubin (mg/dL) | Blood urea (mg%) | Serum creatinine (mg/dL) |
|---|---|---|---|---|---|---|---|
| I | 125.2 ± 1.2 | 28.10 ± 1.2 | 39.11 ± 1.2 | 113.12 ± 1.1 | 0.71±0.12 | 21.22 ± 0.2 | 0.70 ± 0.2 |
| II | 122.5 ± 1.3$^{ns}$ | 30.25 ± 1.1$^{ns}$ | 40.20 ± 1.1$^{ns}$ | 125.25± 1.3$^{ns}$ | 0.72±0.11$^{ns}$ | 22.20 ± 0.1$^{ns}$ | 0.72 ± 0.1$^{ns}$ |
| III | 125.4 ± 1.1$^{ns}$ | 30.20 ± 1.2$^{ns}$ | 40.30 ± 1.2$^{ns}$ | 124.50 ± 1.3$^{ns}$ | 0.72±0.12$^{ns}$ | 21.19 ± 0.3$^{ns}$ | 0.73 ± 0.2$^{ns}$ |
| IV | 128.3 ± 1.0$^{ns}$ | 29.18 ± 1.3$^{ns}$ | 39.42 ± 1.3$^{ns}$ | 120.45± 1.4$^{ns}$ | 0.72±0.14$^{ns}$ | 21.45 ± 0.1$^{ns}$ | 0.75 ± 0.2$^{ns}$ |
| V | 130.4 ± 1.2$^{ns}$ | 28.20 ± 1.1-$^{ns}$ | 40.50 ± 1.4$^{ns}$ | 123.52± 1.2$^{ns}$ | 0.73±0.12$^{ns}$ | 22.50 ± 0.3$^{ns}$ | 0.74 ± 0.3$^{ns}$ |
| VI | 131.6 ± 1.3$^{ns}$ | 30.15 ± 1.1$^{ns}$ | 39.14 ± 1.2$^{ns}$ | 125.65± 1.1$^{ns}$ | 0.72±0.13$^{ns}$ | 21.45± 0.2$^{ns}$ | 0.72 ± 0.3$^{ns}$ |

*n=6, Mean ± S.E.M, ns- Non significance.

The histopathological analysis did not identify any apparent side effects linked to the pectin treatment. A portion of the kidney with some modest tubular parenchymatous degenerations is shown in Figure 3. The glomeruli, however, did not exhibit any appreciable alterations. No histological differences were seen between the hearts and livers of the experimental animals and the control group.

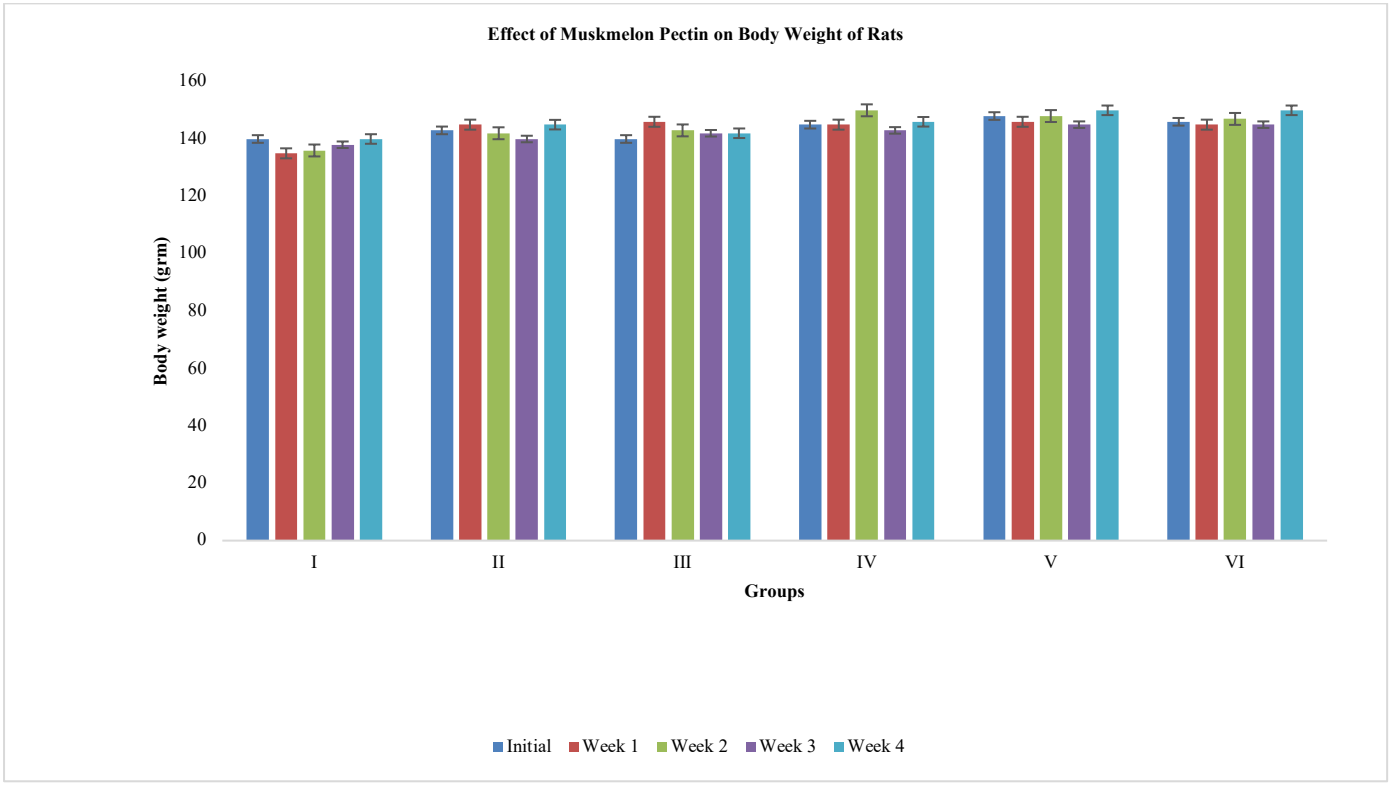


**Figure 1:** Effect of muskmelon pectin on the body (*n=6, Mean ± S.E.M)

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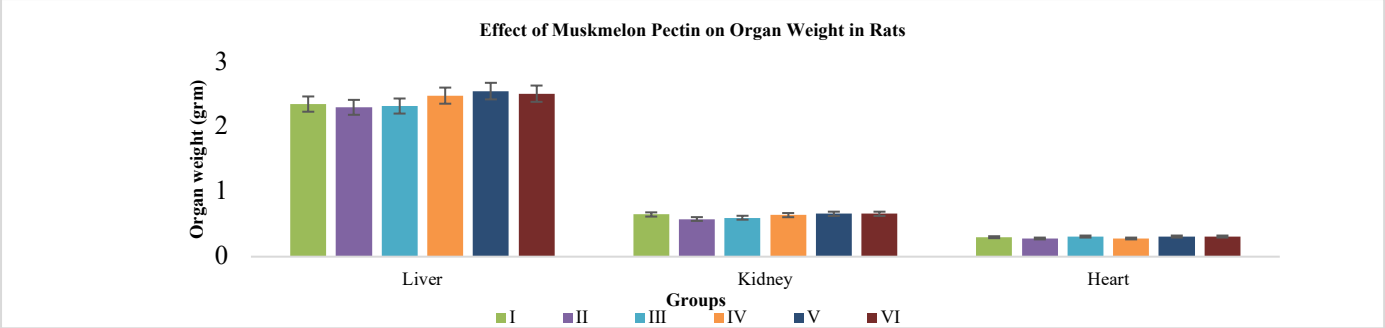


**Figure 2:** Effect of muskmelon pectin on organ (*n=6, Mean ± S.E.M)

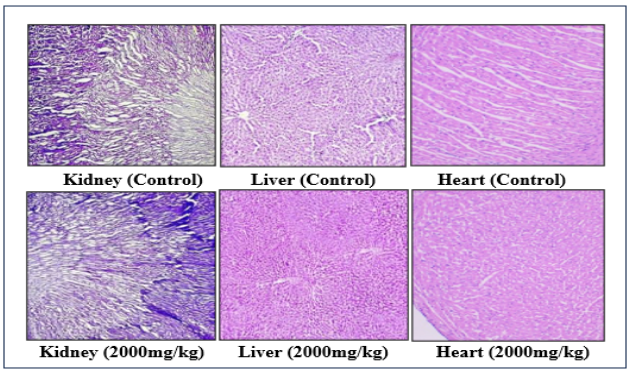


**Figure 3.** Effects of 28 days administration of muskmelon pectin on histopathology study in rats.

## Conclusion

In the acute toxicity test, Muskmelon pectin was safe at doses up to 2000 mg/kg. At the study levels, there was neither morbidity nor death. As a result, oral muskmelon pectin's $LD_{50}$ may be higher than 2000 mg/kg. Regarding the subacute toxicity experiment, no significant differences in test groups from the control group were seen in either haematological or biochemical studies. The heart, kidneys, and liver all had the same weight. The findings show that consuming muskmelon pectin orally at 2000 mg/kg body weight per day is relatively safe.

## 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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