Acute Oral Toxicity of Pea Protein Isolate (Nutralys) in *Wistar Rats* and *Cd1 Mouse*

Chentouf Aouatif\(^a\), Philippe Looten\(^a\), Srinivasan M\(^b\), Srinivas A\(^b\)

\(^a\) Biology and Nutrition Department, Toxicology Service, Roquette Freres, 62136 Lestrem, France  
\(^b\) International Institute of Biotechnology and Toxicology (IIBAT), Padappai-601 301, Tamil Nadu, India

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**Abbreviations:**  
OECD: Organization for Economic Co-operation and Development  
LD\(_{50}\): Lethal dose inducing 50% mortality

**Corresponding Author:**  
Srinivasan M.*  
Senior scientist  
Email: sini256@sify.com  
Phone: +914427174246

Aouatif C.  
Senior Toxicologist  
Email: aouatif.chentouf@roquette.com  
Phone: +91321635488

Looten P.  
Toxicologist  
Email: philippe@roquette.com  
Phone: +91321635488

Srinivas A.

**Abstract**  
Pea Protein Isolate (Nutralys), a human food product, derived from animals and vegetables is generally considered to be non-toxic to the consumer. However, the question of whether oral administration might exert a negative influence has, hitherto, remained unclear. The present study was therefore conducted to determine the acute toxicity effect and establish oral LD\(_{50}\) in Wistar rats and CD1 Mice by as per OECD 423 “Acute Oral Toxicity -Acute Toxic Class method”. Split dose oral administration of Pea Protein Isolate in rat and mice did not show any systemic toxicity for rats and mice. The body weight gain in both rats and mice was normal. Therefore, we conclude that Pea Protein Isolate exhibited no toxicity at high dose and it’s LD\(_{50}\) of was found to be > 2000 mg/kg b.w. under present experimental conditions.

**Citation:**  
intakes have generally been shown to have a positive effect on muscle protein synthesis and size gains.

To ensure the safety of medicines there are international regulations establishing that wide pharmacotoxicological research must be conducted on experimentation animals before initiating its application in human beings (United States Food and Drug Administration, 2006a). In human food there are essential nutrients that constitute the basic engine of our metabolism and without which it’s not possible to receive minimum nutrition. However, it’s important to add many other ingredients that provide for a better health. For that reason, several formulations have been created or several natural sources have been added to nutritional supplements in different forms to make up for the shortage of some nutrients. The use of nutritional supplements has remarkably increased in recent years (Ervin, 2004 and; Timbo, 2006). They have started to grow important and to be frequently taken by consumers at their own choice as they seek to achieve an adequate diet. A great number of dietary supplements with different health benefits have been illegally adulterated with discontinued pharmaceutical ingredients which are potentially harmful to those who take them (Clewell et al, 2010 and; United States Food and; Drug Administration, 2006b).

Considering the fact that Pea Protein Isolate (Nutralys), is derived from animals and vegetables for human consumption, it is generally considered to be non-toxic to humans. However, the question of whether oral administration might exert a negative influence has, hitherto, remained unclear. The present study was therefore conducted to determine any acute toxicity effect and establish oral LD₅₀ in Wistar rats and CD1 mouse according to the OECD Test Guideline 423 for ‘acute oral toxicity study in rodents. All procedures using animals were reviewed and approved by the institutional animal ethics committee (IAEC), International Institute of Biotechnology and Toxicology (IIBAT), Chennai, India.

2. Materials and Methods

2.1 Test items

Pea Protein Isolate (Nutralys) manufactured and supplied by Roquette Freres, France. Is a high quality white powder source food grade with 85% Pea Protein content. Test substance identity was confirmed by Department of Analytical Chemistry, IIBAT.

2.2. Housing and feeding conditions

The current acute oral toxicity study of Pea Protein Isolate (Nutralys) in Wistar rat and CD1 mice, as per the Organization for Economic Co-operation and Development (OECD) 423 (OECD, 2001) & OLIS – 1998 guidelines were conducted in the Animal house facility of IIBAT during the year 2012. Healthy female Wistar rats of age 8 - 12 weeks corresponding to the body weights of 180–220 g were used for the current study. Similarly, the healthy female mice of age 8 and 12 weeks and body weights of 22–26 g were used. All the females were nulliparous and non pregnant. The animals were procured from the breeding facilities of IIBAT. Animals were housed in polypropylene cages with stainless steel grills and gamma-irradiated corn cobs were used as bedding. Bedding material, cages, grills and water bottles were changed on alternate days. Animals were housed individually. Animals were acclimated for a minimum period of 5 days in the controlled environment (temperature: 22 ± 3_C; relative humidity: 50 + 20% and light: 12-h light/dark cycle) and were provided with ad libitum supply of reverse osmosis water and a standard rodent pellet feed (supplier: M/s. Tetragon Chemie Pvt. Ltd, Bangalore, India). Feed alone was withdrawn overnight prior to the dosing and following dosing, for a period of 3 hours. For mice, Feed alone was withdrawn four hours prior to the dosing and following dosing, for a period of 1 hour.

2.3. Experimental Design

In Step1 of the experiment, three female rats and three female mice were administered the test substance by oral intubation at 2000 mg/kg b.w. None of the animals exhibited either clinical signs of toxicity or mortality. In order to confirm the above dose level, in step-2 48 h after step-1 dosing, three female rats and three female mice were administered with the test substance at 2000 mg/kg b.w. None of the animals exhibited any clinical sign of toxicity or mortality.

Following dosing, step -1, and step - 2, each animal was observed for mortality and clinical signs of toxicity for 14 days. Body weights were recorded weekly. Animals were sacrificed by CO₂ exposure at the end of the
14-day of observation period for gross pathological examination.

Prior to the dosing of each step, rats were fasted overnight and the mice for 4 h. The test solution was prepared shortly prior to the administration. The dose volume maintained for all the groups were 10 ml/kg b.w with distilled used as the vehicle.

3. Objective of Research

To find the acute oral lethal dose (LD50) of the Pea Protein Isolate (Nutralys) in Wistar rats and CD1 mouse according to the OECD Test Guideline 423 and understand the mechanisms of toxicity if any, induced by Pea Protein Isolate (Nutralys).

4. Results and Discussion

Since Pea Protein is more often used as food supplement, possible toxic effects of this product needs to evaluate before its use commercially. Assessment of skin and eye irritation and skin sensitization potential of chemicals along with the acute oral, dermal, and inhalation toxicities, (the commonly termed 'six pack' of acute toxicity studies) is required by regulatory authorities around the world for the purposes of classification and labeling, risk assessment, and risk management of substances, in support of public health protection. These acute in vivo studies generate preliminary toxicity information and are used as a basic screening tool by many regulatory agencies around the globe to understand the toxic potential of the respective product. Hence we assessed the acute oral toxicity of pea protein in rats and mice as animal model. These studies were conducted by well established OECD guidelines.

In rats and mice oral toxicity study, the results showed that a dose concentration of 2000 mg/kg b.w. of Pea protein isolate when administered, did not produce oral toxicity signs like dullness, abnormal body posture, Tremors, seizures; Restlessness etc in any of treated animals. It has been documented that, body weight, the most sensitive parameter to show an adverse effect, is an important indicator of the physiological alterations that could occur in the organism after being exposed to a certain substance (Morbery and; Hayes, 1989). The rapid loss of body weight (approximately 15 to 29% in a period from five to seven days) is something to be taken into account when leading toxicological studies (United States Food and Drug Administration, 2006a). However, in the Pea protein administered animals, normal body weight gain was observed throughout the observation periods suggesting that no adverse effect is induced by the test compound (Table- 1&2). It was proved in our previous studies that skin irritation, eye irritation and skin sensitization potential study showed no toxicity to rabbits and mice (data not published). Future, gross pathology examination conducted at the end of the experimental period did not reveal any gross lesions confirming the non toxic nature of this protein.

Table 1: Weekly Individual Animal Bodyweight and Percentage Body Weight Gain-Rats (Step - 1 and Step - 2)

<table>
<thead>
<tr>
<th>Group/ Dose (mg/kg b.w.)</th>
<th>Animal No.</th>
<th>Sex</th>
<th>Body Weight (in grams)</th>
<th>% body weight gain Day 0-7</th>
<th>% body weight gain Day 7-14</th>
<th>% body weight gain Day 0-14</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Day 0</td>
<td>Day 7</td>
<td>Day 14</td>
<td>Day 7</td>
</tr>
<tr>
<td>G1/step-1 2000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>177 31</td>
<td>F</td>
<td>219</td>
<td>240</td>
<td>9.59</td>
<td>261</td>
<td>8.75</td>
</tr>
<tr>
<td>177 32</td>
<td>F</td>
<td>210</td>
<td>230</td>
<td>9.52</td>
<td>250</td>
<td>8.70</td>
</tr>
<tr>
<td>177 33</td>
<td>F</td>
<td>215</td>
<td>233</td>
<td>8.37</td>
<td>252</td>
<td>8.15</td>
</tr>
<tr>
<td>Mean ± SD (n=3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>214.67 ± 4.51</td>
<td>F</td>
<td>215</td>
<td>233</td>
<td>8.37</td>
<td>263</td>
<td>12.88</td>
</tr>
<tr>
<td>G1/step-2 179 52</td>
<td>F</td>
<td>215</td>
<td>233</td>
<td>8.37</td>
<td>263</td>
<td>12.88</td>
</tr>
</tbody>
</table>
Proteins perform a nutritive function, exert colloidal osmotic pressure, and aid in maintenance of acid-base balance. They are also essential for synthesis of enzymes, antibodies, coagulation factors, hormones, and transport substances. Hence their exits an extensive demand for protein to maintain the normal function of human body. Consumption of Pea protein serves as exogenous source and compensates the extensive demand of protein in human beings. However, extensive toxicological evaluations need to perform before labelling the Pea Protein safe for human consumption. It has been reported that Pea protein stimulates the formation and excretion of bile acids that leads to reduction of VLDL cholesterol in liver thus preventing the formation of blocks in blood vessels (Spielmann et al., 2008). Further the genotoxic potential of Pea protein assessed with the aid of battery of genotoxic assays (AMES, in vitro chromosomal aberration test and in vivo micro nuclease assay) in our recent publication confirms that this protein as a non-mutagenic and non-genotoxic (Chentouf et al., 2013).

In summary, the results of the acute oral toxicity study acquiesce that a 2000 mg/ kg b.w. dose of Pea Protein Isolate taken orally doesn’t produce signs of toxicity or death in rats and mice, suggesting a LD$_{50}$ higher than 2000 mg/ kg b.w. According to OECD, the substances that have a LD$_{50}$ higher than 2000 mg/ kg b.w. orally can be considered as non-toxic (OECD 423). However, these acute studies may yield preliminary toxicological information, but further conduct of repeated dose studies by various applicable routes are essential to demonstrate Pea Protein Isolate as a safe compound and to recommend its use as a dietary supplement.

**Conflict of interests**

The authors declared no conflicts of interest.

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References


