

SUBCHRONIC ORAL TOXICITY OF RABELLA POWDER IN EXPERIMENTAL RATS

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It has recently become obvious that the prevalence of obesity has been rapidly increasing in Vietnam, as well as other countries, over the past two decades. There has been a current trend for researchers to discover new natural ingredients which are safe and effective in the treatment of obesity. RABELLA powder was a herbal-derived product which contained Amorphophalus konjac (K. Koch starch). This plant was used as an oral medication for controlling body weight in the past as well as in the present. So far, the safety of this product, has not been reported yet in Vietnam. Thus, this study was designed to assess the subchronic toxicity of RABELLA powder in Wistar rats. The method used in this study followed the guidance of the World Health Organization and Organization for Economic Co-operation and Development; 2 oral doses of 1.2 g/kg b.w/day and 3.6 g/kg b.w/day was administered to rats for 12 consecutive weeks. The results show that RABELLA powder caused no significant change in the general condition, hematological indexes, functions and microscopic images of livers and kidneys. We conclude that RABELLA powder did not cause subchronic toxicity in experimental rats. Moreover, this also revealed partly the safety of RABELLA powder in clinical practice.

Keywords: RABELLA powder, Amorphophalus konjac K. Koch, subchronic toxicity, rats.

I. INTRODUCTION

Obesity is a major health and economic crisis facing the modern world. It is associated with excess mortality and morbidity and is directly linked to common conditions such as type 2 diabetes mellitus, coronary heart disease and sleep apnea. According to WHO, obesity has reached epidemic proportions worldwide, with approximately 1.9 billion overweight and 650 million obese adults.¹ NICE currently recommends pharmacological treatment for weight loss maintenance such as orlistat, liraglutide, naltrexone/bupropion in addition to a reduced-caloric diet and optimal physical

exercise. These synthetic drugs, however, caused undesirable effects such as nausea, headaches, and constipation.² Therefore, one of the most urgent mission of researchs was to find the novel drugs derived from herbs which not only exhibit anti-obesity effect but also with limited side effects.

Toxicity refers to unwanted effects on biological systems. In order to evaluate biological toxicity, it is very important to choose the correct system, since no effect may otherwise be seen.^{3,4} Toxicity of a substance can be impacted by many factors, such as the route of exposure (skin absorption, ingestion, inhalation, or injection), the time of exposure (a brief, acute, subchronic, or chronic exposure), the number of exposures (a single dose or multiple doses over a period of time), the physical form of the toxin (solid, liquid, or gas), the organ system involved

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(cardiovascular, nephro-, hemo-, nervous-, or hematopoietic-system) and even the genetic makeup and robustness of the target cells or organisms.⁵ Subchronic systemic toxicity is defined as adverse effects occurring after the repeated or continuous administration of a test sample for up to 90 days or not exceeding 10% of the animal's lifespan.⁶

Amorphophalus konjac K. Koch was the main component of RABELLA powder. This plant has long been used in South East Asia as a food source and as a traditional medicine. Glucomannan (GM), a soluble fiber derived from *Amorphophallus konjac*, is marketed as being helpful in reducing body weight.⁷ However, so far, there has been no reports available on the toxicity of RABELLA powder in Vietnam. Therefore, in this study, we aimed to assess the subchronic toxicity of RABELLA powder in experimental animals.

II. METHODS

1. The preparation of RABELLA powder

RABELLA was manufactured by Ngoc Thien Trading and Pharmaceutical Joint Stock Company. This product was prepared and offered in form of sachets. Each sachet contained 5 g *Amorphophalus konjac* K. Koch starch. The recommended dosage in patients was 2 sachets per day.

2. Experimental animals

Healthy *Wistar* rats (180 ± 20 g) were used in this study. The animals were housed in cages (groups of ten rats/cage) under the standard conditions (temperature $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and relative humidity $80\% \pm 10\%$), 12 hours dark/light time. We fed the rats with standard animal feed and allowed free access to water. They were acclimated to housing for at least 1 week prior to investigation at the Department of Pharmacology, Hanoi Medical University.

3. Subchronic toxicity study

Subchronic toxicity study were carried out according to WHO Guidance and OECD guidelines.^{8,9}

The study was carried out in a continuous 12-week period. *Wistar* rats were divided into three groups of ten animals:

- Group 1 (control) was served as the distilled water control group. Each rat was applied 1 ml distilled water/100 g b.w/day by oral route of administration;
- Group 2 was given orally RABELLA powder at the dose of 1.2 g/kg b.w/day (equivalent to human recommended dose, conversion ratio 6);
- Group 3 was given RABELLA powder at the dose of 3.6 g/kg b.w/day (3 times as high as the dose at group 2).

Animals were treated daily by oral route of administration one a day in the morning for 12 consecutive weeks and observed once daily to detect signs of toxicity.

The signs and indexes were checked during the study including:

- General condition consisting of mortality and clinical signs.
- Body weight changes.
- Hematopoietic function test: red blood cells (RBC), hemoglobin (HGB), hematocrit, total white blood cells (WBC), WBC differentials, platelet count (PLT).
- Serum biochemistry: aspartate amino transferase (AST), alanine amino transferase (ALT), total bilirubin, albumin, total cholesterol and creatinine levels.

The parameters were checked at the times: before treatment, 4 weeks after treatment, 8 weeks after treatment and 12 weeks after treatment. At the end of the experiment, all animals were subjected to a full gross necropsy. Liver and kidney of 30% rats of each group

were removed for histopathology examinations. The micro-histological examination was carried out at Center for Research and Early Detection of Cancer (CREDCA). Assoc.Prof. Le Dinh Roanh, Director of CREDCA gave results of pathological image analysis.

4. Statistical analysis

Data were analysed using Microsoft Excel software version 2010. The levels of significance between the experimental groups and the control group were made using student's t-test. Data was shown as mean ± standard deviation. All data were considered significantly at $p < 0.05$.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with the control group.

$\Delta p < 0.05$, $\Delta\Delta p < 0.01$, $\Delta\Delta\Delta p < 0.001$ compared

with the time point “before treatment”.

III. RESULTS

1. General condition

Animals had normal locomotor activities and good feedings. None of the animals in all treated groups showed any macroscopic or gross pathological changes compared with the control group.

2. Body weight changes

Table 1 showed that after 4 weeks, 8 weeks and 12 weeks of treatment, body weight in all rats increased substantially compared with body weight “before treatment”. No significant differences were observed between groups treated RABELLA powder and control group (group 1) ($p > 0.05$).

Table 1. The effect of RABELLA powder on body weight

Time	Body weight (g)		
	Group 1	Group 2	Group 3
Before treatment	196.0 ± 50.4	197.0 ± 29.8	204.0 ± 31.0
4 weeks after treatment	239.0 ± 58.6	245.0 ± 30.3 $\Delta\Delta$	221.0 ± 22.3
8 weeks after treatment	262.0 ± 48.9 $\Delta\Delta$	258.0 ± 32.9 $\Delta\Delta\Delta$	234.0 ± 20.7 Δ
12 weeks after treatment	265.0 ± 50.2 $\Delta\Delta$	249.0 ± 21.3 $\Delta\Delta\Delta$	242.0 ± 18.1 $\Delta\Delta$

$\Delta p < 0.05$, $\Delta\Delta p < 0.01$, $\Delta\Delta\Delta p < 0.001$ compared with the time point “before treatment”

3. Effect on hematological examination

Table 2. Effect of RABELLA powder on hematopoietic function

Parameters	Group	Before treatment	4 weeks after treatment	8 weeks after treatment	12 weeks after treatment
Red blood cells count (T/L)	Group 1	10.58 ± 1.19	9.56 ± 1.11	9.89 ± 1.02	10.34 ± 1.12
	Group 2	10.92 ± 0.83	10.65 ± 1.32	10.81 ± 1.07	10.19 ± 1.24
	Group 3	10.37 ± 0.97	10.74 ± 1.41	10.63 ± 0.86	10.90 ± 0.62
Hemoglobin level (g/dL)	Group 1	14.58 ± 1.56	12.73 ± 2.38	13.20 ± 1.44	13.28 ± 1.24
	Group 2	14.91 ± 1.86	14.63 ± 2.28	14.38 ± 1.42	14.11 ± 2.28
	Group 3	14.29 ± 1.63	14.38 ± 1.84	13.60 ± 0.77	13.58 ± 0.45

Parameters	Group	Before treatment	4 weeks after treatment	8 weeks after treatment	12 weeks after treatment
Hematocrit (%)	Group 1	58.77 ± 7.09	51.93 ± 7.73	52.10 ± 7.54	53.55 ± 6.18
	Group 2	61.21 ± 2.77	57.09 ± 9.26	57.21 ± 5.54	55.54 ± 8.57
	Group 3	56.41 ± 6.19	56.72 ± 7.60	52.61 ± 3.29	54.17 ± 2.04
MCV (fL)	Group 1	55.50 ± 1.58	52.10 ± 5.15	51.60 ± 5.80	52.50 ± 4.28
	Group 2	56.10 ± 2.08	54.40 ± 2.27	53.50 ± 3.47	52.80 ± 4.66
	Group 3	55.10 ± 2.38	53.40 ± 2.37	51.90 ± 4.63	52.00 ± 4.81
Platelet count (G/L)	Group 1	475.9 ± 136.6	592.7 ± 147.8	603.0 ± 158.6	627.8 ± 184.1
	Group 2	589.5 ± 118.1	730.1 ± 174.4	638.7 ± 123.4	556.1 ± 191.3
	Group 3	550.9 ± 118.7	602.1 ± 58.0	628.3 ± 132.5	619.3 ± 149.8

MCV: Mean corpuscular volume

There was no significant difference in red blood cells count, hematocrit, hemoglobin level, MCV and platelet count between groups treated RABELLA powder and group 1 ($p > 0.05$) (Table 2).

Table 3. Effects of RABELLA powder on total WBC count and WBC differentials

Parameters	Group	Before treatment	4 weeks after treatment	8 weeks after treatment	12 weeks after treatment
Total WBC count (G/L)	Group 1	10.6 ± 3.6	9.5 ± 3.9	10.5 ± 2.4	11.6 ± 3.4
	Group 2	10.8 ± 3.7	10.1 ± 3.2	12.0 ± 4.1	9.4 ± 2.9
	Group 3	9.3 ± 1.7	8.5 ± 1.7	8.9 ± 2.0	9.2 ± 2.2
Lymphocytes (G/L)	Group 1	7.6 ± 2.8	6.6 ± 2.8	7.4 ± 1.8	8.5 ± 2.7
	Group 2	7.2 ± 2.5	6.8 ± 2.2	8.1 ± 3.0	6.6 ± 2.7
	Group 3	7.0 ± 1.9	5.6 ± 1.6	6.2 ± 1.9	6.3 ± 2.2
Neutrophils (G/L)	Group 1	1.6 ± 0.4	1.4 ± 0.6	1.8 ± 0.9	1.4 ± 0.2
	Group 2	1.5 ± 0.6	1.4 ± 0.5	1.7 ± 0.5	1.4 ± 0.6
	Group 3	1.4 ± 0.3	1.5 ± 0.3	1.5 ± 0.5	1.5 ± 0.5

WBC: white blood cells

Table 3 showed that at all time points, there was no significant difference in total WBC count, lymphocytes and neutrophils at groups treated RABELLA powder compared with group 1 and the time point "before treatment" ($p > 0.05$).

4. Effect on liver parameters

There were no significant differences in aspartate amino transferase (AST) level and alanine amino transferase (ALT) level, total bilirubin, albumin concentration and total cholesterol concentration between groups treated RABELLA powder and group 1 ($p > 0.05$). The results were shown in table 4.

Table 4. Effects of RABELLA powder on liver parameters

Parameters	Group	Before treatment	4 weeks after treatment	8 weeks after treatment	12 weeks after treatment
AST level (UI/L)	Group 1	79.4 ± 17.0	78.6 ± 18.6	79.2 ± 28.8	76.7 ± 12.9
	Group 2	93.4 ± 15.4	93.6 ± 30.0	106.1 ± 35.4	82.9 ± 10.2
	Group 3	88.2 ± 20.6	92.2 ± 25.7	73.2 ± 10.6	74.4 ± 10.0
ALT level (UI/L)	Group 1	46.5 ± 9.6	36.7 ± 12.4	36.0 ± 13.5	37.1 ± 10.5
	Group 2	48.7 ± 17.5	38.1 ± 5.8	44.1 ± 10.1	35.6 ± 12.4
	Group 3	38.5 ± 8.9	41.0 ± 9.4	31.4 ± 9.3	37.7 ± 12.1
Total bilirubin (mmol/L)	Group 1	13.33 ± 0.55	13.44 ± 0.37	13.45 ± 0.48	13.44 ± 0.29
	Group 2	13.32 ± 0.42	13.45 ± 0.32	13.30 ± 0.51	13.26 ± 0.34
	Group 3	13.46 ± 0.49	13.45 ± 0.26	13.62 ± 0.55	13.34 ± 0.28
Albumin concentration (g/dL)	Group 1	3.22 ± 0.31	3.10 ± 0.23	3.27 ± 0.34	3.14 ± 0.48
	Group 2	3.35 ± 0.32	3.37 ± 0.38	3.36 ± 0.27	3.18 ± 0.25
	Group 3	3.07 ± 0.32	3.24 ± 0.34	3.31 ± 0.18	3.37 ± 0.34
Total cholesterol concentration (mmol/L)	Group 1	1.25 ± 0.25	1.22 ± 0.19	1.12 ± 0.13	1.24 ± 0.25
	Group 2	1.44 ± 0.20	1.41 ± 0.32	1.23 ± 0.26	1.38 ± 0.20
	Group 3	1.48 ± 0.26	1.25 ± 0.28	1.26 ± 0.22	1.46 ± 0.29

5. Effect on kidney function

Table 5 showed that RABELLA powder caused no significant differences in serum creatinine level between groups treated RABELLA powder and group 1 ($p > 0.05$).

Table 5. Effects of RABELLA powder on serum creatinine level

Days	Creatinine level (mg/dl)		
	Group 1	Group 2	Group 3
Before treatment	0.86 ± 0.20	0.79 ± 0.16	0.88 ± 0.16
4 weeks after treatment	0.78 ± 0.11	0.86 ± 0.13	0.83 ± 0.14
8 weeks after treatment	0.79 ± 0.17	0.80 ± 0.14	0.79 ± 0.22
12 weeks after treatment	0.85 ± 0.14	0.78 ± 0.13	0.80 ± 0.15

6. Histopathological examination

No gross lesions or changes in size examined of the hearts, livers, lungs, kidneys and abdominal cavities was observed when subjected all experimental rats to a full gross necropsy.

There was no significant difference in histopathological examination of livers and kidneys between

groups treated RABELLA powder and control group after 12 weeks of treatment (figure 1 and 2).

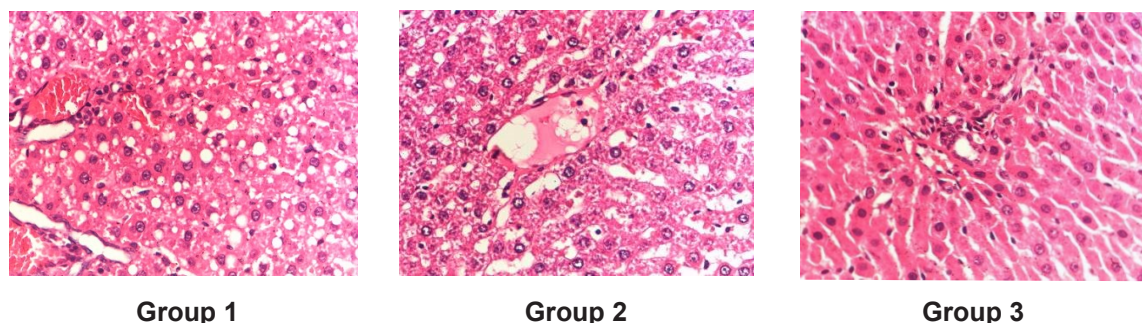


Figure 1. Histopathological images of liver (HE × 400)

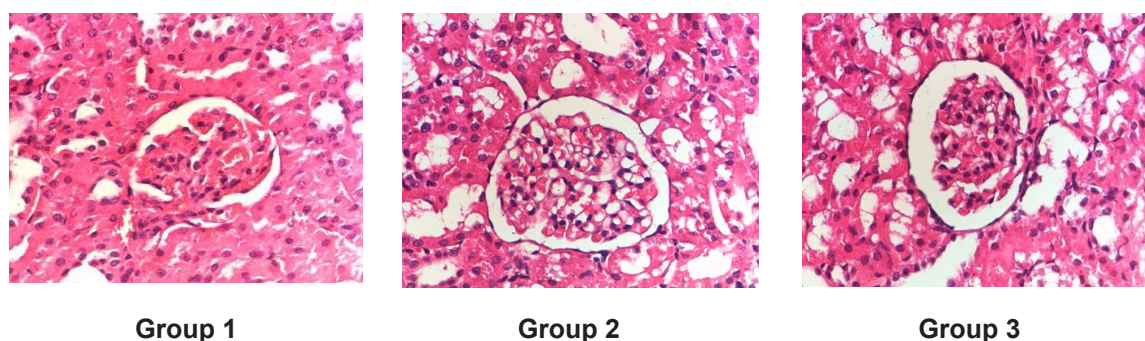


Figure 2. Histopathological images of kidney (HE × 400)

IV. DISCUSSION

Subchronic toxicity of RABELLA powder

Toxicity is the degree to which a substance can harm humans or animals. Toxicity can refer to the effect on a cell (cytotoxicity), an organ (e.g. renal or liver toxicity), or the whole organism. To determine the safety of drugs and plant products for human use, toxicological evaluation is carried out in various experimental animal models to predict toxicity and to provide guidelines for selecting 'safe' therapeutic doses in humans. A subchronic toxicity study provides information on the effects of repeated oral exposure and can indicate the need for further longer term studies.^{8,11} Subchronic studies assess the undesirable effects of continuous or repeated exposure of plant extracts or

compounds over a portion of the average life span of experimental animals, such as rodents. Specifically, they provide information on target organ toxicity.¹²

The body weight changes serve as a sensitive indication of the general health status of animals.¹² Weights were observed in all animals treated with RABELLA powder. It can be stated that RABELLA powder did not interfere with the normal metabolism of animals as corroborated by the nonsignificant difference from animals in the distilled water control group.

The hematopoietic system is one of the most sensitive targets of toxic compounds and is an important index of physiological and pathological status in man and animals.

Furthermore, such analysis is relevant to risk evaluation as changes in the hematological system have higher predictive value for human toxicity when the data are translated from animal studies.^{8,11} After 4 weeks, 8 weeks and 12 weeks of the treatment, there were no significant differences in total red blood cells, hematocrit, hemoglobin level, platelet count, total WBC count and WBC differentials between groups treated RABELLA powder and control group, so it could be concluded that the administration of RABELLA powder did not affect the hematological profile and blood formation process.

Analysis of kidney and liver function is very important in the toxicity evaluation of drugs and plant extracts as they are both necessary for the survival of an organism. The clinical biochemistry analyses were carried out to evaluate the possible alterations in hepatic and renal functions influenced by the plant products.¹³ The liver releases AST, ALT and an elevation in plasma concentration is an indicator of liver damage.⁸ There was no substantial change in AST level and ALT level between the group treated RABELLA powder and the control group. These results indicated that RABELLA powder had no deleterious effect on liver function.

Creatinine level can be used in describing the function of the kidneys.¹¹ No significant differences were observed in blood biochemical parameters between control group and groups treated RABELLA powder at various dose levels ($p > 0.05$). Thus, RABELLA powder did not affect the liver and kidney function.

The histopathological examination revealed the alteration in cell structure when viewed under the light microscope. Further histological study could furnish more information regarding the hepatotoxicity and nephrotoxicity of RABELLA

powder. Our study showed that there was no significant difference in histopathological examination of the liver and kidney between groups treated RABELLA powder and the control group.

Overall, the findings of this study indicated that no significant difference was observed in blood profile, biochemistry parameters and histopathological observations of liver and kidney tissues between groups treated RABELLA powder and the control group.

Previous reports about the toxicity of *Amorphophalus konjac* K. Koch were still limited in the world. A few clinical trials proved the safety of glucomannan (a soluble fiber derived from *Amorphophallus konjac*) in adults.¹⁴

V. CONCLUSION

RABELLA powder at the doses of 1.2 g/kg b.w/day and 3.6 g/kg b.w/day administered orally during continuous 12 weeks did not produce any toxic sign or evident symptoms of subchronic toxicity in experimental rats.

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