

ACUTE TOXICITY STUDY OF TOMATO POMACE EXTRACT IN RODENT

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ABSTRACT

Tomato and tomato products are considered to be healthy food for the human diet. Although tomatoes have been widely studied for their phenolic content, less emphasis has been laid on toxicological effect of this plant. Thus, the purpose of the present study is to determine the acute toxicity effect of *Lycopersicon esculentum*, or commonly known as tomato, was administered orally in the form of dried tomato pomace extract *in vivo*. Adult male rats were orally administered single dose of 1000 and 5000 mg kg⁻¹ dried tomato pomace extract. There were 10 rats in each group. All animals were sacrificed after 2 weeks of treatment. Seven parameters were tested: cage side observation, body weight gain measurement, food and water consumption, absolute organ weight, hematology, biochemical analysis and histopathology, to look for evidence of acute toxicity. No mortality was observed when varying doses of the extracts were administered per day for a period of 2 weeks. There were no significant differences in body weight, behavior, food consumption, absolute organ weights between controls and treated animals. Hematological analysis showed no differences in most parameters examined. In the biochemistry parameter measurement, no significant change occurred. Pathologically, neither gross abnormalities nor histopathological changes were observed. These findings suggest that none of the organs appeared to be target and the data could provide satisfactory preclinical evidence of safety to launch clinical trial on standardized formulation of tomato pomace extracts to be the dietary supplement.

Keywords: *Lycopersicon Esculentum*, Acute Toxicity, Tomato Pomace, Dietary Supplement

1. INTRODUCTION

Several studies have suggested a strong link between dietary phytochemical intakes and a reduced risk for chronic diseases, including cancer and coronary artery disease. Dietary antioxidants from fruits and vegetables, which are rich sources of antioxidants and many studies have shown that an important food sector promoting health benefits and reduce the risk of chronic disease via

functional ingredients in these products (Verschuren, 2002; Phachonpai *et al.*, 2012).

Tomato or *Lycopersicon Esculentum* (*L. esculentum*) consumption worldwide has greatly increased over the past 2 decades. It had been reported that consumption of tomato or tomato products has been associated with decreased risk of some cancers and prevented illness, promoting growth (Shi and Maguer, 2000) and proper immune system functioning (Sandhu *et al.*, 2000). In

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addition, tomato also contains valuable phytochemicals and antioxidants including carotenoids such as lycopene and beta-carotene (Canene-Adams *et al.*, 2005).

Recently, previous studies reported that the waste product of tomato or tomato pomace obtained from food industry processing contained skin, pulp and crushed seeds of tomato were still contains lycopene and beta-carotene (Sabio *et al.*, 2003). It had been reported that dried tomato pomace exhibited cognitive enhancing effect in normal and cognitive impairment conditions (Thukhammee *et al.*, 2012). Therefore, the development of tomato pomace extract as functional food or dietary supplementation is very much interesting. However, less emphasis has been laid on toxicological effect of this extract. Information about toxicity of dried tomato pomace extract is very important as a baseline before exploring further to develop this extract as a new herbal dietary supplement. Therefore, the purpose of the present study is to determine the acute toxicity effect of dried tomato pomace extract in healthy male rats.

2. MATERIALS AND METHODS

Preparation of dried tomato pomace powder: Tomato pomace is consisting of tomato skins, pulp and crushed seeds that remain after the processing of tomatoes for juice. Wet tomato pomace was dried with oven at 50°C for 2 h. The %yield of tomato pomace is 12.75. The dried tomato pomace was grinded as powder and determined both lycopene level and total phenolic compound and used as markers for quality control. It was found that the dried tomato pomace contained lycopene 160.94 mg 100 g⁻¹ sample while contained total phenolic compound 413.07±34.45 mg of gallic acid equivalent/mg. The dried tomato pomace was kept in airtight container at room temperature until use.

2.1. Animals

Adult male Wistar rats (180-200 g, 8 weeks old) were obtained from Animal Unit, Faculty of Medicine, Khon Kaen University and were housed in group of 5 per cage in standard metal cages at 22±2°C on 12:12 h light-dark cycle. All animals were given access to food and water *ad libitum*. Experiments were performed to minimize animal suffering in accordance with the internationally accepted principles for laboratory use and care of European Community (EEC directive of 1986; 86/609/EEC) and approved by the Ethical Committee of the Khon Kaen University.

2.2. Dosage of Extract on Animals

Single dose toxicity studies were carried out by adapting OECD guideline nos. 423 (OECD, 2003). In order to study any possible toxic effect or changes in normal behavior, 2 groups of rat (all males) were used in this experiment. The acute toxicity of the extract was studied by preparing two different concentrations of the standardized extract (1000 and 5000 mg kg⁻¹) and administered orally using a feeding needle to two groups of animals. The third group was taken as a control and given as vehicle (distilled water). The maximum volume of distilled water administered was not greater than 2mL 100⁻¹ g BW. Animals were fasted prior to conducting the experiment (only food but not water was withheld overnight). Following the 24 h of fasting, the animals were weighed and the test substance was administered. After the substance has been administered, food may be withheld for a further 3-4 h. The acute toxic effects of the extract were assessed on the basis of mortality, which was expressed as LD50. Lethal Dose (LD 50) was calculated using the following equation (Turner, 1965):

$$LD50 = \frac{\text{the apparent least dose lethal to all in a group} \times \Sigma (a \times b)}{N}$$

2.3. Cage Side Observation and Body Weight Measurement

All animals were observed individually after substances administration and special attention was given during the first 4 h and every 12 h daily thereafter, for a total of 2 weeks. All observations were systematically recorded, with individual records being maintained for each animal. Cage side observation included evaluation of fur and skin, eyes, respiratory effects, autonomic effects such as salivation, diarrhea, urination and the central nerve effects including tremors and convulsions, changes in the level of activity, gait and posture, reactivity to handling or sensory stimuli and altered strength (Kumarnsit *et al.*, 2006; Demma *et al.*, 2006; Mukinda and Syce, 2007; Obici *et al.*, 2008). Individual body weight was measured and recorded prior to the oral administration of extracts for 2 weeks. Weight changes were also calculated and recorded.

2.4. Food Consumption

Amount of food consumed was measured daily from the quantity of food supplied and the amount remaining after 24 h for 2 weeks of the study period (Kumarnsit *et al.*, 2006; Demma *et al.*, 2006; Obici *et al.*, 2008).

2.5. Hematology

At the end of the acute toxicity study (2 weeks), all animals were sacrificed and bled via inferior vena cava puncture. The samples were collected in plastic test tubes containing EDTA anti-clotting. Hematological examinations included the following parameters: White Blood cell Count (WBC), Red Blood cell Count (RBC), Hemoglobin concentration (Hb), Hematocrit (Ht), Platelet count (PLT), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) (Demma *et al.*, 2006; Obici *et al.*, 2008; Tan *et al.*, 2008).

2.6. Biochemical Analysis

Serum biochemistry was performed to examine Aspartate aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), Creatinine (CRN), Triglycerides (TG), Cholesterol (Cho), Total Bilirubin (T-Bil), Blood Urea Nitrogen (BUN) and Blood Sugar (BS) (Demma *et al.*, 2006; Obici *et al.*, 2008; Tan *et al.*, 2008).

Analyses for hematology and serum biochemistry were conducted at the laboratory of Srinagarind Hospital, Faculty of Medicine, Khon Kaen University, Thailand.

2.7. Absolute and Relative Organ Weight

At the end of the acute toxicity study (2 weeks), all organs were collected using standardized surgical procedures. The abdominal cavity of each animal was dissected and organs namely the heart, liver, lungs, spleen, kidneys, adrenals, thymus, salivary gland and brain were quickly removed, cleaned with saline, weighed and preserved in 10% formalin for histopathology analyses (Demma *et al.*, 2006; Obici *et al.*, 2008; Tan *et al.*, 2008).

2.8. Histopathological Observation

The four organs of all rats (heart, lung, liver and kidney) were examined macroscopically. The morphology of the internal organs was visually observed for signs of toxicity. These organs have undergone Hematoxylin and Eosin (H and E) staining. Abnormalities of the slides were graded as mild, moderate and severe (Jain *et al.*, 2008; Tan *et al.*, 2008).

2.9. Statistical Analysis

Nonparametric one way statistical analysis of variance, Kruskal-Wallis test and posthoc test (Mann-

Whitney test) were used in order to see any significant changes between Tomato pomace extract groups and vehicle treated group. Differences between groups were considered significant at $p < 0.05$. All data points show the mean of Standard Error of Means (SEM).

3. RESULTS

In this study, the oral administrations of the dried tomato pomace at all dosages were given for 2 weeks did not produce any visible sign of acute toxicity or instant death in rats tested during the period of observation.

3.1. Food Consumption and Body Weight Measurement

The amount of food consumed was measured daily from the quantity of food supplied and the amount remaining after 24 h. Values for consumption are based on total intake and average relative body weight of the preceding time interval. These data showed no statistically significant difference between any groups (**Table 1**).

The mean of rats' body weight was measured daily for 14 consecutive days. Again, there is no statistically significant difference between any groups in this parameter.

3.2. Cage side Observation

All the three doses of Tomato pomace extract did not produce significant changes in behavior, skin effect, breathing, defecation, postural abnormalities, yellowing or loss of hair.

3.3. Organ weights

As shown in **Table 2**, the mean of each organ weights did not differ between the extract treated and the control groups.

3.4. Hematological and the Serum Biochemical Parameters

The serum biochemical and blood hematology results for the rats are presented in **Table 3 and 4** respectively. No statistically significant difference was shown among treated groups.

3.5. Histopathology

Our histopathology results were shown in **Fig. 1**. There are no significant morphological changes detected in kidney, heart, lung and liver in all rat samples from all groups of study.

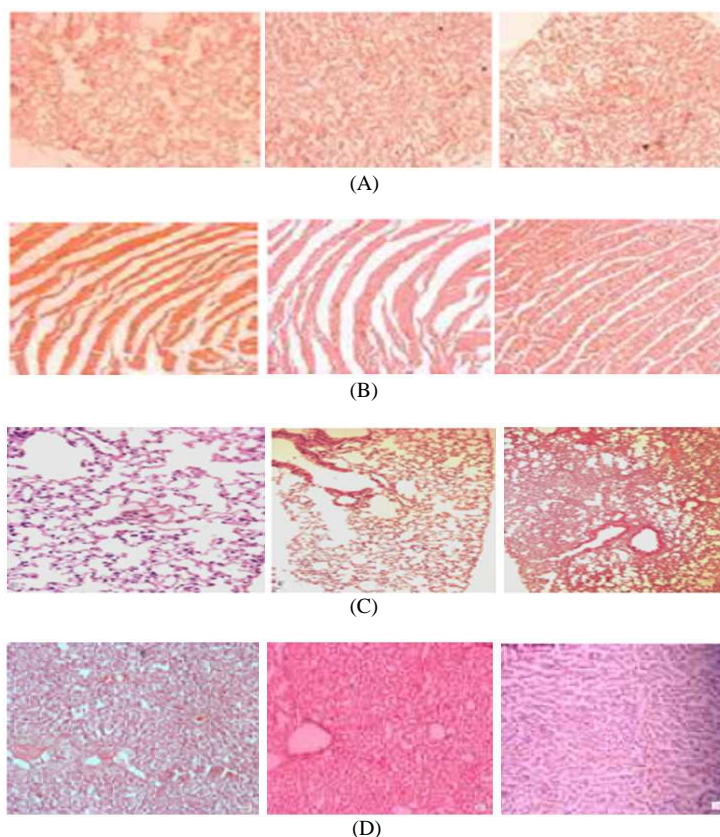


Fig. 1. (A) Kidney sections were stained with hematoxylin and eosin (H and E stained 10×) (B) Heart sections were stained with hematoxylin and eosin (H and E stained 20×) (C) Lung sections were stained with hematoxylin and eosin (H and E stained 40×) (D) Liver sections were stained with hematoxylin and eosin (H and E stained 10×) Vehicle, TP 1000 and TP 5000 treated groups, respectively

Table 1. Effect of the dried tomato pomace on body weight and food consumption in rats

Group	Food consumption (g/day)	Initial weight	Body weight (g) Final weight	Weight gain (%)
Vehicle	18.43±0.52	350.01±1.11	379.13±1.59	6.10 Tomato
Tomato pomace 1000 mg kg ⁻¹	19.40±0.57	349.86±1.07	375.78±1.22	6.16 Tomato
Tomato pomace 5000 mg kg ⁻¹	19.33±0.34	348.82±1.03	375.00±1.83	6.23

Table 2. Effect of the dried tomato pomace on organ weights in rats

Group	Heart (g kg ⁻¹ BW)	Lung (g kg ⁻¹ BW)	Liver (g kg ⁻¹ BW)
Vehicle	3.10±0.02	6.64±0.18	29.70±0.14
Tomato pomace 1000 mg kg ⁻¹	3.18±0.04	6.58±0.18	29.12±0.15
Tomato pomace 5000 mg kg ⁻¹	3.25±0.02	6.39±0.14	28.15±0.18
	Brain (g kg ⁻¹ BW)	Kidneys (g kg ⁻¹ BW)	Thymus (g kg ⁻¹ BW)
Vehicle	15.06±0.02	8.14±0.02	2.35±0.02
Tomato pomace 1000 mg kg ⁻¹	15.13±0.09	8.15±0.03	2.31±0.02
Tomato pomace 5000 mg kg ⁻¹	15.14±0.03	8.16±0.03	2.39±0.02
	Salivary gland (g kg ⁻¹ BW)	Adrenal gland (g kg ⁻¹ BW)	Spleen (g kg ⁻¹ BW)
Vehicle	1.40±0.01	0.72±0.01	3.33±0.02
Tomato pomace 1000 mg kg ⁻¹	1.39±0.01	0.71±0.02	3.32±0.05
Tomato pomace 5000 mg kg ⁻¹	1.40±0.01	0.70±0.02	3.24±0.02

Table 3. Effect of the dried tomato pomace on serum biochemical parameters of rats

Group	BS (mg/dl)	Chol (mg/dl)	TG (mg/dl)	BUN (mg/dl)
Vehicle	97.25±1.99	61.83±0.57	65.25±1.19	10.75±0.39
Tomato pomace 1000 mg kg ⁻¹	97.67±1.70	61.33±0.79	64.44±1.59	11.51±0.43
Tomato pomace 5000 mg kg ⁻¹	96.83±1.07	60.17±0.61	62.42±0.89	11.45±0.52
	CRN (mg/dl)	ALT (U/L)	AST (U/L)	ALP (U/L)
Vehicle	0.38±0.00	32.89±0.61	69.58±1.74	37.00±0.45
Tomato pomace 1000 mg kg ⁻¹	0.37±0.01	33.11±0.57	69.44±1.70	37.67±0.73
Tomato pomace 5000 mg kg ⁻¹	0.37±0.01	33.17±0.44	69.50±1.71	38.42±0.61
	T-Bil (mg/dl)			
Vehicle	0.10±0.00			
Tomato pomace 1000 mg kg ⁻¹	0.10±0.00			
Tomato pomace 5000 mg kg ⁻¹	0.10±0.00			

Table 4. Effect of the dried tomato pomace on blood hematology parameters of rats

Group	WBC (×10 ³)	RBC (×10 ⁶)	Hb (g/dl)	Hct (%)
Vehicle	3.61±0.15	8.58±0.08	12.68±0.21	40.96±0.45
Tomato pomace 1000 mg kg ⁻¹	3.62±0.10	8.94±0.15	12.86±0.19	40.95±0.48
Tomato pomace 5000 mg kg ⁻¹	3.72±0.08	9.06±0.12	13.07±0.16	41.66±0.19
	MCV (fL)	MCH (pg)	MCHC (g/dl)	PLT (×10 ³)
Vehicle	47.46±0.46	15.23±0.12	32.17±0.16	46.00±1.31
Tomato pomace 1000 mg kg ⁻¹	48.42±0.50	15.24±0.15	32.06±0.19	46.15±1.48
Tomato pomace 5000 mg kg ⁻¹	49.78±0.31	15.85±0.14	31.36±0.05	47.02±1.41

4. DISCUSSION

Recently, from the point of view of “self-medication” or “preventive medicine,” several herbal dietary supplements are used in the prevention of life-style related diseases. Our results for the preliminary toxicological screening reveal that the oral administration of dried tomato pomace powder is non toxic substance.

Usually acute (single dose) toxicity study is carried out on laboratory animals by using high dose (sufficient to produce death or morbidity) of the substance in question and/or based on previous report on its toxicity or toxicity of structurally related compounds. Unfortunately, there was no previous report on toxicity of dried tomato pomace extract consumption. Therefore, only two dose levels, the limit dose (usually a dose of 5000 mg kg⁻¹ is considered limit dose in single dose toxicity study) and another low dose (1000 mg kg⁻¹), were selected in single dose toxicity studies of tomato pomace extract powder. In this 2 weeks period of acute toxicity evaluation, rats given dried this extract at dose of 1000 and 5000 mg kg⁻¹ BW, showed no mortality and all rats did not produce any symptom of toxicity. This finding is in accordance with the previous report of dried tomato pomace effect in rats (Thukhammee *et al.*, 2012). However, the dose of the extract, the species of animals and the duration of treatment of the plant extract were different. Moreover, our result on cage side

functional observations such as respiratory, autonomic and nervous system effects in all doses of the extract treated group animals did not produce the toxic effects on these parameters as mention already.

Regardless of the dose used, the tomato pomace extract did not appear to affect the body weight of the rats and caused no significant changes in their food consumption. Utilization of food exhibited normal metabolism in the animals (Mukinda and Syce, 2007) and this suggests that the single oral dose of the extract did not retard the growth of rats. However, the effect of tomato pomace extract on the alteration of body weight gain seems to be in contradiction to the results from (Friedman *et al.*, 2000), who reported that both red and green tomatoes contain glycoalkaloids, is a natural toxin substance could reduce body weight gain in hamsters (Friedman *et al.*, 1994; 2000). One possible explanation for the difference between both phenomenons as mention above that may result from the differences of animal species, doses of the extract and the duration of treatment. However, this study did not investigate about the possible active ingredients and the precise underlying mechanisms of tomato pomace extract on body weight gain; this is planned in future studies.

Our finding indicates that all animals from tomato pomace extract groups showed no significant changes in the absolute organ weights of the heart, liver, lungs, spleen, kidneys, adrenals, thymus, salivary gland and

brain of the rats treated with this extract compared to vehicle control treated group. Similarly, no damage or structural modification was observed during the macroscopic evaluation of important organs such as the liver, kidney, brain and heart of the treated animals when compared with the control group.

The various biochemical and hematological parameters investigated in this study are useful indices of evaluating the toxicity of the extract in animals. Measurement of hematological parameters can be used to investigate the extent of deleterious effect of foreign compounds including plant extracts on the blood constituents of an animal. It can also be used to explain blood relating functions of chemical compounds and plant extracts (Oyedemi *et al.*, 2011). The biochemical parameters were used as indices for assessing organ dysfunction or damage as could arise in toxicity especially the liver plays an important role in many metabolic processes; any disturbance in the liver would affect the normal level of measurable biochemical parameters in this organ. AST, ALT and ALP are marker enzymes present in high concentrations in the liver, when liver cells are inflamed or damaged, these enzymes leak into the blood stream leading to a rise in the plasma level of these enzymes (Nkosi *et al.*, 2005). However, the results of both hematological and biochemical parameters of the present study did not show any worrisome results since all the changes were within the normal expected range for the rat species used in this study.

Taken all data together, the results in this study showed that the acute administration of the dried tomato pomace at all given doses (up to 5000 mg kg⁻¹) did not produce any sign of acute toxicity in rats tested during the period of observation. This, however, suggest that the extract has no acute toxicity when administered orally and the lethal dose if any is above 5000 mg kg⁻¹. Clarke and Clarke (1975) reported that substances with LD50 of 1000 mg kg⁻¹ BW given orally are considered safe or of low toxicity. Similarly, the chemical labeling and classification of acute systemic toxicity based on oral LD50 values recommended by the Organization for Economic Cooperation and Development (OECD, Paris, France) (Walum, 1998) are as follow: very toxic, <5 mg kg⁻¹; toxic, >5<50 mg kg⁻¹; harmful, >50<500 mg kg⁻¹ and no label, >500<2000 mg kg⁻¹. Therefore, the expected high LD50 (>5000 mg kg⁻¹ BW) of the dried extract tomato pomace, is an indication that the extract could be considered relatively safe especially when administered orally where absorption may not be complete due to inherent factors limiting absorption in the gastro intestinal tract. Although, obtaining a lethal dose in

animals may not predict the human lethal dose of a drug or acute poisoning overdose (Chapman *et al.*, 2010). However, these findings could provide satisfactory preclinical evidence of safety to launch clinical trial on standardized formulation of tomato pomace extract to be the herbal dietary supplement.

5. CONCLUSION

In a nutshell, the data of the present study do suggest that oral administration of dried tomato pomace is not toxic. Obviously, this study points to the need for additional studies to better define the roles of tomato consumption that may be responsible for the beneficial effects. No less challenging, but potentially beneficial for human health, would be an assessment whether the cited effects in rats parallel those in humans.

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