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Original Research Article

Acute and Sub-Acute Oral Toxicity Profile of Purified Tomato Extract on the Liver and Kidney Functions of Male Wistar Rats

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ABSTRACT

The pharmacological properties of lycopene-containing extracts are numerous. Purified tomato extract (PTE) is a lycopene-rich extract with a wide range of pharmacological properties; however, toxicity testing is required to examine the potential risks associated with the constituent chemicals. This study was conducted to evaluate the acute and sub-chronic toxicities of PTE on the liver and kidney functions of Wistar rats. PTE was obtained by extracting tomatoes using a solvent mixture of n-hexane: acetone: ethanol (1:1:2) and the solvent was then evaporated. For the acute toxicity testing, the rats were divided into two groups: normal control and PTE (750 mg/kg *b.w.*) treatment groups. The rats were observed for seven days for bodyweight and physiological changes (stool, activity, tremor, and coma), as well as death. In the sub-chronic toxicity testing, the rats were divided into five groups: normal control, PTE (50, 100, or 150 mg/kg *b.w.*) treatment, and negative control groups. The rats were observed for 28 days for bodyweight changes. Also, the serum urea, creatinine, aminotransferase, aspartate aminotransferase levels were determined. There were no significant differences in the body weight between the normal control and PTE treatment groups, which were 182.85 ± 6.36 g and 184.28 ± 6.72 g, respectively. A similar result was obtained for the physiological conditions which included normal stool, principal activity, no tremor, no coma, as well as no deaths in both groups. The sub-chronic study results showed that PTE was safe. The administration of a single dose of PTE for 28 days did not cause any damage.

Keywords: Acute toxicity, Lycopene, Purified tomato extract (PTE), Sub-chronic toxicity, Tomato.

Introduction

An acute toxicity testing is required to evaluate the adverse effects that might occur following the oral administration of a substance in single dose or multiple doses given within 24 hours. It is preliminary testing that has a highly important pharmacological and toxicological significance. In oral acute toxicity testing, the preparations are made in various dosage levels, and toxic effects and death are observed.[1] Sub-chronic oral toxicity with repeated doses can be performed after information on acute toxicity is obtained. The assay evaluates the risk of side effects after administering single or multiple doses of the test sample for 14-28 days.[1]

Tomatoes are commonly used to make juice or pasta. They provide a wide range of pharmacological benefits; including antioxidant, antihypercholesterol, and antidiabetic properties.[2] These pharmacological effects exist due to the presence of some phytochemical compounds in them. The chemical compounds that are present in tomatoes include lycopene, vitamin C, zinc, and beta carotene.[2] Lycopene is a natural compound from tomato that regulates the inhibition of HMG-CoA reductase to reduce cholesterol levels.[3] Vitamin C is found in many fruits and vegetables, including tomatoes. It acts as an antioxidant and can neutralize free radicals.[4]

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Tomato lycopene extract (TLE) has been reported to reduce blood glucose levels.[5] Tomato extracts have also been reported to reduce TC (total cholesterol), TG (triglyceride), LDL-C (low density lipoprotein cholesterol), and increase HDL-C (high density lipoprotein cholesterol) in the blood.[6] The extract contains lycopene and beta-carotene with anti-atherosclerosis.[7]

Lycopene-containing extract has abundant pharmacological properties. PTE is an extract containing lycopene that has abundant pharmacological properties. Toxicity testing is needed to assess the risks that may arise from the constituent compounds. To recognize a chemical substance, it is necessary to identify the hazards that may arise. The purpose of the acute toxicity test is to evaluate the toxic symptoms that appear and determine the occurrence of death in 50% of the test animals. The sub-acute toxicity test is used to detect the toxic effect of a chemical that is given orally and repeatedly, for 28 days.[1] Sub-acute toxicity test provides information on the toxic effect on the affected target organs. Both of these toxicity tests are required to observe the death of the test animals after being given pure tomato extract (PTE). The study was therefore aimed at evaluating the acute and sub-acute toxicity effects of PTE on Wistar male rats.

Materials and Methods

Source of chemical materials and equipment used

Analytical grades of acetone (Bratacho, Bali), ethanol (Bratacho, Bali), hexane (Bratacho, Bali). ALAT (GPT) Diasys, ASAT (GOT) Diasys, creatinine FS Diasys, urea FS Diasys were purchased from PT, Kurniajaya Sentosa, Surabaya, Indonesia. The equipment used consisted of a rotary evaporator (Heidolph) and UV-Visible spectrophotometer (Shimadzu).

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Test animals

Wistar male rats (2 months old, 150-200 g) were acclimated at an ambient temperature of 27-30^{o}C for one week. The animals were kept in a cage where humidity and hygiene were maintained. These rats were placed in cages and fed with B511 and water *ad libidum*. The ethical clearance for the animal handling was provided by the Faculty of Veterinary Medicine, Udayana University (Clearance No.: 445/UN14.2.9/PD/2019).

Preparation of tomato extract

Fresh tomatoes were collected from Kintamani village, Bali, Indonesia, in February 2019. The tomato plant was identified at the Indonesian Institute of Sciences (LIPI) number B-448/IPH.7/AP/V/2019. The tomatoes were steamed for 15 minutes and then crushed using an electric blender. Homogenously crushed tomatoes were macerated with a solvent mixture (acetone: ethanol: n-hexane = 1:2:1). The clear liquid phase (organic phase) was retained and then concentrated using a rotary evaporator.[7]

Acute toxicity testing of purified tomato extract

The Wistar male rats were divided into two groups: a normal group that only received water and a group that was orally given PTE (750 mg/kg *b.w.*) once. The animals were observed consecutively for seven days for physiological changes such as tremor, activities, coma, and body weight as well as death.[1]

Sub-acute toxicity testing of purified tomato extract

Purified tomato extract was dissolved in olive oil and then administered orally to rats. The Wistar male rats were divided into five groups as follows; Group a (Normal group: no treatment), Group b (Negative group: rats given standard feed and olive oil), Group c (PTE 50 mg/kg *b.w.*: rats given standard feed and PTE of 50 mg/kg *b.w.* once daily for 28 days), Group d (PTE 100 mg/kg *b.w.*: rats given standard feed and PTE of 100 mg/kg *b.w.* once daily for 28 days) and Group e (PTE 150 mg/kg *b.w.*: rats given standard feed and PTE of 150 mg/kg *b.w.* once daily for 28 days). In the test animals, liver function was observed by examining the parameters of serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), and liver weight. Meanwhile, the function of the kidney was evaluated by determining the parameters of serum urea (UREA), serum creatinine (CREA), and kidney weight.[1]

Data analysis

ALT, AST, UREA, CREA, kidney weight, liver weight, and bodyweight of the PTE groups were compared with the normal control. The data obtained were analyzed using SPSS software and the significant differences among the groups were determined ($p < 0.05$). The PCA (Principal Component Analysis) Biplot) and PLS-DA was used to assess the kidney and liver profile parameters ($p < 0.05$).

Results and Discussion

The administration of a single oral dose of PTE (750 mg/kg *b.w.*) to male Wistar rats caused no death and no changes in the animal behaviour. The results showed that PTE caused no acute toxicity, and the estimated LD_{50} of the extract is above 750 mg/kg *b.w.* PTE 750 mg/kg *b.w.* did not cause a significant difference in body weight compared with the normal group, which were 182.85 ± 6.36 g and 184.28 ± 6.72 g, respectively (Figure 1). Physiological conditions including normal stools, prevalent activities, no tremors, and no comas of normal control and PTE-treated animals were the same as presented in Table 1. These results showed that PTE administration was safe. Tomatoes containing lycopene are safe for human consumption when prepared with supercritical carbon dioxide.[8] Also, tomato extract (5 mg/kg *b.w.*) has been reported to prevent hepatotoxicity in rats.[9]

The sub-acute toxicity testing was carried out by administering the extract for 28 days. The results indicated that the rats were still healthy, had no deaths and the biochemical parameters of the tested animals were normal. There was no significant difference in the body weights of the animals after 28 days of observation (Figure 2). The results (Figures 3 and 4) showed that there were no significant differences among the groups in the weights of the livers and kidneys on the 28th day. The liver and kidney functions were normal. In the PTE groups and normal controls, the ALT, AST, UREA, and CREA were not significantly different as highlighted in Table 2. PCA biplot and PLS-DA showed that PTE administration did not cause a significant difference ($p > 0.05$) between the normal and negative control group (Figure 5).

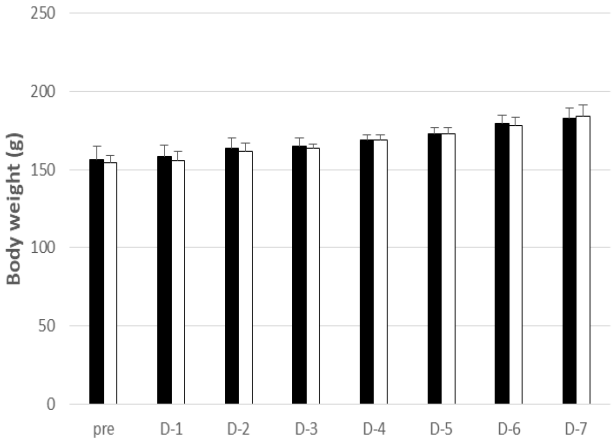

Figure 1: Bodyweights (g) of the experimental rats (n = 10, Average ± SD). ■ : Before acute toxicity of PTE; □ : After acute toxicity of PTE; PTE: Purified tomato extract.

Table 1: Physiological changes of rats after purified tomato extract treatment

Physiological changes each group		Day of observation						
		D-1	D-2	D-3	D-4	D-5	D-6	D-7
Normal	Stool	N	N	N	N	N	N	N
PTE		N	N	N	N	N	N	N
Normal	Tremor	x	x	x	x	x	x	x
PTE		x	x	x	x	x	x	x
Normal	Coma	x	x	x	x	x	x	x
PTE		x	x	x	x	x	x	x
Normal	Activity	N	N	N	N	N	N	N
PTE		N	N	N	N	N	N	N

D: Day; N: Normal condition; x: Not detected

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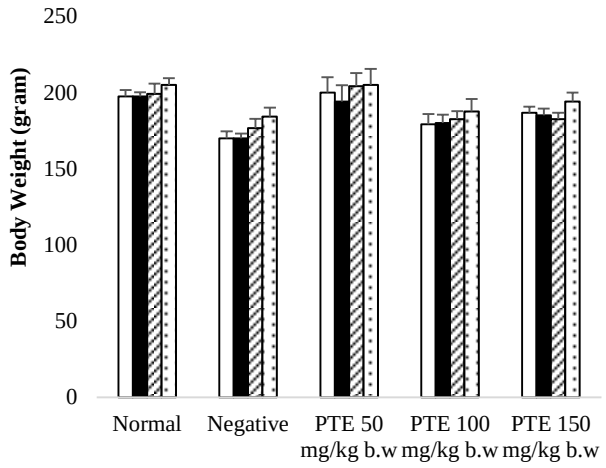


Figure 2: Bodyweights of the experimental rats after 28 days of treatment.
□: Bodyweight on day 7; ■ : Bodyweight on day 14; ▨ : Bodyweight on day 21; ▢ : Body weight on day 28.

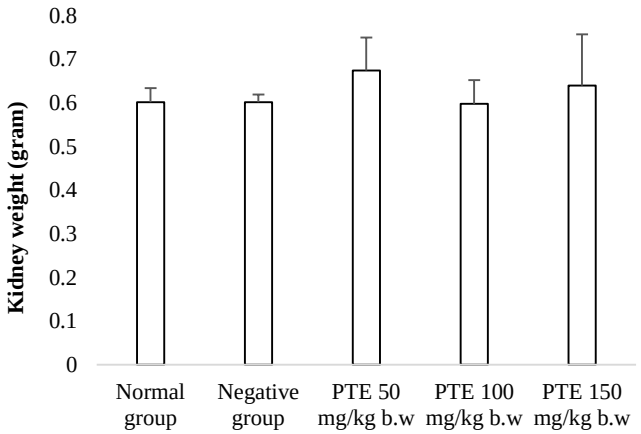


Figure 3: Kidney weights of the experimental rats after 28 days of treatment.

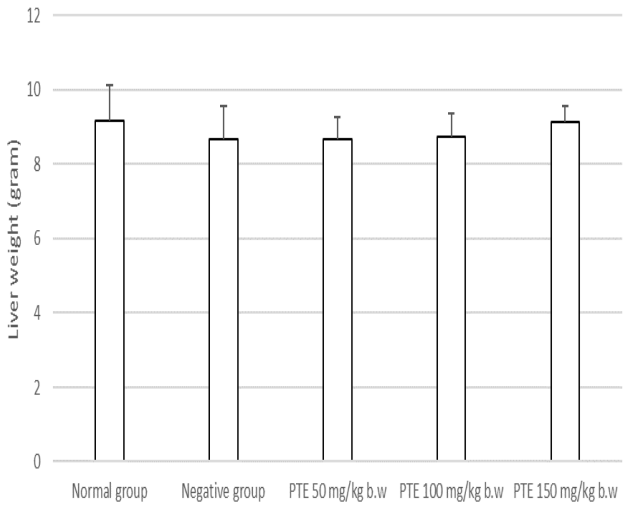
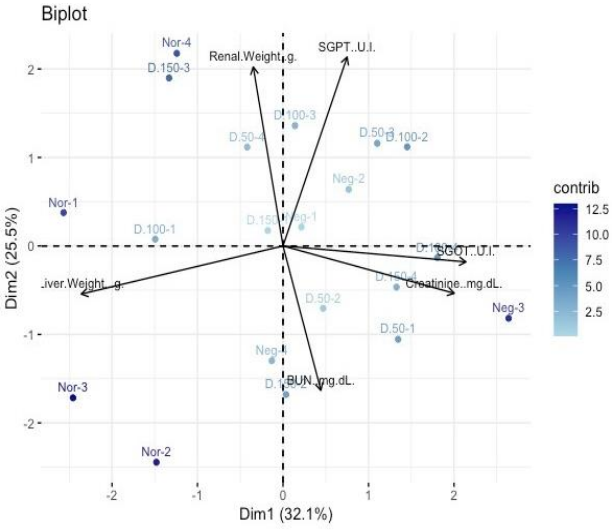


Figure 4: Liver weights of the experimental rats after 28 days of treatment.

The administration of PTE did not cause changes in the weight of the kidney, liver, and levels of AST, ALT, UREA, and CREA. According to the statistical analysis, there was no significant difference ($p \geq 0.05$) in the values obtained for the PTE's ALT, AST, UREA, and CREA in the normal and negative control groups. Sub-acute toxicity testing in male Wistar rats was conducted by the administration of PTE (50, 100, or 150 mg/kg *b.w.*) for 28 days.

As the main organ of metabolic processes, the liver has to be observed for its function. There were no changes when the transaminase activity (ALT and AST) was measured. Both of the parameters were expected to be elevated due to liver damage.[10] When the liver cell membrane is damaged, these enzymes (ALT and AST) are used as parameters for assessing liver function.[12,13] These results indicate that the 50, 100, and 150 mg/kg *b.w.* PTE did not show any changes in the liver function, where the AST and ALT values were not significantly different ($p \geq 0.05$) from the normal group. In line with Uchendu's research, which showed that tomato extract could protect the liver of rats from severe liver damage, indicating that tomato extract has a hepato-protective effect.[13]



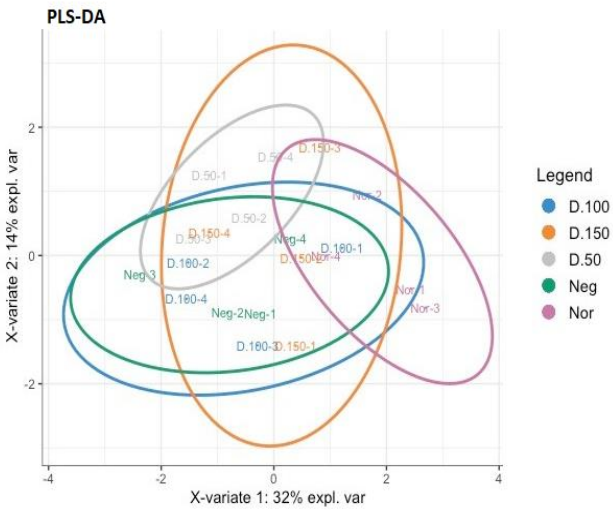


Figure 5: PCA biplot and PLS-DA profile of renal and kidney response-group correlation

Table 2: Serum biochemical parameters of experimental male rats orally treated with purified tomato extract

Groups	AST (U/I)	ALT (U/I)	UREA (mg/dl)	CREA (mg/dl)
Normal group	61.78 ± 1.70	124.64 ± 2.06	17.39 ± 2.15	0.48 ± 0.080
Negative group	65.89 ± 2.05	124.11 ± 2.39	17.62 ± 0.84	0.69 ± 0.056
PTE 50 mg/kg *b.w*	71.79 ± 2.41	126.97 ± 2.69	19.02 ± 2.17	0.68 ± 0.034
PTE 100 mg/kg *b.w*	73.39 ± 2.93	127.50 ± 3.62	16.70 ± 1.36	0.59 ± 0.028
PTE 150 mg/kg *b.w*	73.75 ± 1.35	126.79 ± 2.14	17.62 ± 2.45	0.62 ± 0.056

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; UREA: Urea; CREA: Creatinine

The kidney is the main organ that functions in maintaining the homeostasis of the body. High serum creatinine and blood urea nitrogen levels can be used as a diagnostic marker of acute kidney injury[14]. Administration of 50, 100, or 150 mg/kg *b.w.* of PTE for 28 days revealed no change in the kidney function. Furthermore, there were no significant differences ($p > 0.05$) in the UREA and CREA values among the treated and control groups. Lee *et al*. (2016),[15] also reported that tomato leaf extract has antioxidant abilities to reduce blood creatinine and urea levels. The antioxidant effect of the extract is due to the presence of flavonoid compounds. The development of herbal medicines requires safety tests which include acute, sub-chronic, and chronic toxicity tests. Acute toxicity test results indicated that PTE is safe because it does not cause physiological changes and death in the test Wister male rats. In addition, PTE contains lycopene compounds that have pharmacological properties. Therefore, PTE can be developed as raw material for oral preparations.

Conclusion

The findings of this study revealed that the administration of a single dose of PTE in male Wistar strain rats is safe because it did not cause any physiological changes and death. In addition, the administration of PTE for 28 days did not cause any liver and kidney damage.

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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