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Acute Toxicity Evaluation of Temulawak *(Curcuma xanthorrhiza* Roxb) Hepatoprotective Supplement.

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ABSTRACT

This study was carried out to evaluate the acute toxicity of Temulawak (*Curcuma xanthorrhiza* Roxb) hepatoprotective supplement. Temulawak is a native Indonesian plant which known to have many pharmacological benefits. In vivo study was conducted in Wistar Rats by oral administration. Temulawak (*Curcuma xanthorrhiza* Roxb) hepatoprotective supplement was given at doses of 2000 mg/kg body weight (BW). Animals were observed for any toxic sign and symptoms during the first 24 hours especially in the first 4 hours. In the absence of animal mortality, the observation is continued for up to 14 days with periodic observations every day. At completion of study animals were sacrificed and macroscopic observation was done. No mortality and clinical signs of toxicity were found in Temulawak (*Curcuma xanthorrhiza* Roxb) hepatoprotective supplement administered groups. The Lethal Dose 50 (LD 50) of this hepatoprotective supplement in female and male rats belongs to the unclassified category according to Organization of Economic Cooperation and Development (OECD) guideline or at least practically non-toxic (LD50> 5000 mg/kg BW) which is equivalent to a dose of 56 000 mg (56 g) in humans (70 kg). From macroscopic observation, there were no significant abnormalities on important organs (lung, heart, liver, kidney, stomach) after the administration of this supplement at 2000 mg/kg BW. The oral LD50 of Temulawak (*Curcuma xanthorrhiza* Roxb) hepatoprotective supplement was greater than 5000 mg/kg BW thus it could regarded as non-toxic. Based on the results can be concluded the safety of Temulawak (*Curcuma xanthorrhiza* Roxb) hepatoprotective supplement in the animal tested.

Keywords: acute toxicity, *Curcuma xanthorrhiza* Roxb, *in vivo*, LD50, supplement

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INTRODUCTION

The liver is an organ that plays a vital role in maintaining homeostasis, and responsible for various metabolic functions of lipids, proteins, and carbohydrates. After the perfect absorption process, the blood is rich in nutrients and xenobiotics and transferred to the liver through the portal vein. Various compounds including ethanol, drugs, toxins in the blood will eventually enter the liver and can cause liver damage. Damages to the liver such as fatty liver, non-alcoholic steatohepatitis, hepatitis A, B, or C, cirrhosis and liver carcinoma could be exacerbated by unhealthy lifestyles, obesity, excessive alcohol consumption, and because of the effects of certain drugs [1].

The use of medicinal plants for health benefits increases throughout the world. These medicinal plants have a significant contribution to promotive, curative and rehabilitative of human health, as well as in disease prevention [2]. Many medicinal plants have been reported as the source of hepatoprotective supplement. In Indonesia, there are many types of medicinal plants traditionally used for liver treatment.

Temulawak(*Curcuma xanthorrhiza* Roxb.) is a native Indonesian herbal medicine that belongs to the ginger family (Zingiberaceae) and produces rhizomes which can be used to various health problems. Temulawak has been used as a traditional remedy to treat various diseases as antiinflammation [3], antioxidant[4], anticancer [5], antimicrobial [6], and hepatoprotective [7]. Curcumin contained in Temulawak has effect hepatoprotective [8].

The use of herbal medicines as a hepatoprotective supplement must synergize the strength of traditional medicine systems with modern concepts based on evidence, standardization and clinical trials to support clinical benefits.

Toxicity evaluation is a crucial step in developing and producing herbal medicines to ensure the safety of these plants. An oral acute toxicity test is generally a preliminary step to provide information on the harmful effects of test compounds on health that may arise from oral or dermal administration. This test is carried out on two types of animals (rodent and non-rodent). Products tested given to experimental animals at doses different, then observing for 14 days.

The Lethal Dose 50 (LD50) is a quantitative parameter from acute toxicity test is while the qualitative parameter is visual observations for mortality and signs of toxicity (salivation, lethargy, diarrhoea, and coma). LD50 data can be used to determine the potential for acute toxicity compounds relative to other compounds. Besides that, it can also be used for estimate the dose of other toxicological test doses [9].

This study was carried out to evaluate acute toxicity Temulawak (Curcuma xanthorrhiza Roxb) hepatoprotective supplement according to Organization of Economic Co-operation and Development (OECD) guideline 420 and 423 for evaluation of chemical compounds [10].

MATERIALS AND METHODS

Experiment Animals

Male and female Wistar rats aged between 8-12 weeks were obtained from the Laboratory of Pharmacology and Toxicology, Faculty of Pharmacy, Gadjah Mada University. Experiment animals were quarantined and acclimatized for one week and kept in animal rooms which rearing conditions as follow: temperature of 25 ± 2^{o}C, relative humidity of 65 ± 10% and a 12 h-light/dark regime. Animals were feed in plastic cages and allowed free access to food and water. All research protocols in this research have been approved by the ethics commission for Preclinical Research in LPPT, UGM, Yogyakarta, with number 00140/04 / LPPT / XI / 2017.

Test Substance

The herbal hepatoprotective supplement contains Temulawak (*Curcuma xanthorrhiza*. Roxb.) was provided by PT SOHO Industri Pharmasi. All other chemicals and solvents used in the study were of analytical grade.

Sample preparation

Temulawak (*Curcuma xanthorrhiza* Roxb) hepatoprotective supplement tablet was made into powder and prepared in 0.5% Na CMC carrier as suspension.

Observation

This study was carried out in accordance with OECD guidelines for no. 420 and 423 [10] for acute oral toxicity. Four doses:5,50,300 and 2000 mg/kg body weight (BW) were or 14 days. Individual animal body weight was recorded on day one and at the end of the experiment. administered in the two different rats groups. Signs of toxicity, food and water consumption of each animal was observed f

RESULTS

Lethal Dose (LD) 50

From the experiment, the result shows that Temulawak (*Curcuma xanthorrhiza* Roxb) hepatoprotective supplement has not been found to be toxic even at limit test of 5000 mg/kg BW in experimental animals as shown in table 1.

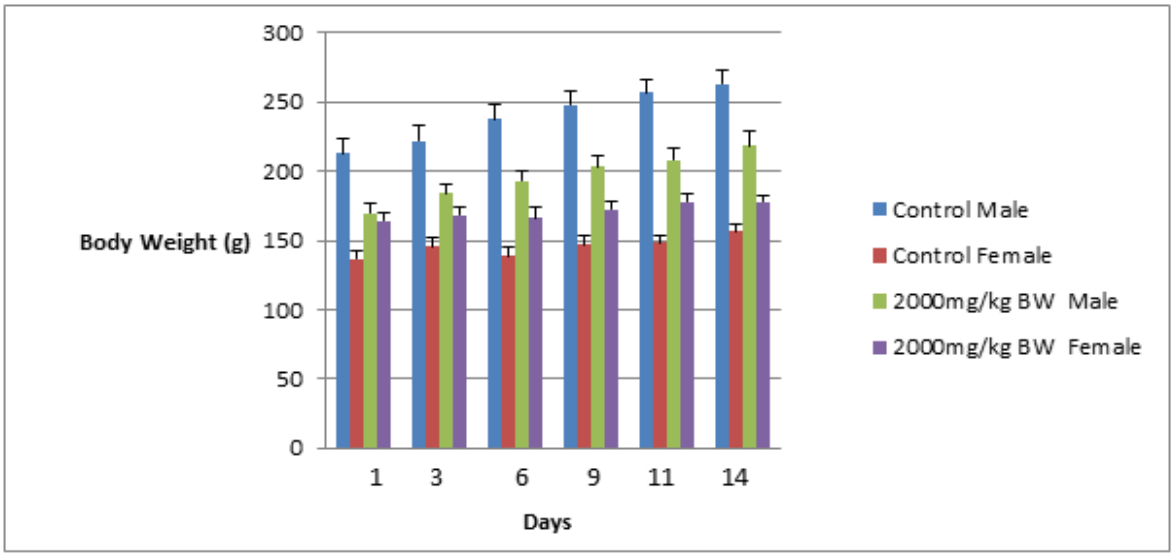
Table 1: Lethal Dose 50 (LD50)

Group	Sex	N	Mortality	LD50 (mg/kg)
Control	Male	5	0	>5000mg/kg BW
Control	Female	5	0	
Limit test (2000mg/kg BW)	Male	5	0	
Limit test (2000mg/kg BW)	Female	5	0	

Body Weight

The administration of Temulawak (*Curcuma xanthorrhiza* Roxb) hepatoprotective supplement with a limit of 2000 mg/kg BW did not statistically affect the body weight of male and female rats as shown in figure 1.

Figure 1:. Effect for body weight for 14 days



Toxic Symptoms

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Administration of Temulawak (*Curcuma xanthorrhiza* Roxb) hepatoprotective supplement for 14 days at 2000 mg/kg BW/day did not show any toxic effect nor any mortality on male and female rats (Table 2).

Tabel 2: Toxic symptoms with dose of 2000 mg/kg/day on male and female rats for 14 days

Group	Sex	N	Toxic Symptoms
Control	Male	5	No
Control	Female	5	No
Limit test (2000mg/kgBW)	Male	5	No
Limit test (2000mg/kgBW)	Female	5	No

Gross Macroscopic Examination and Organ Weight

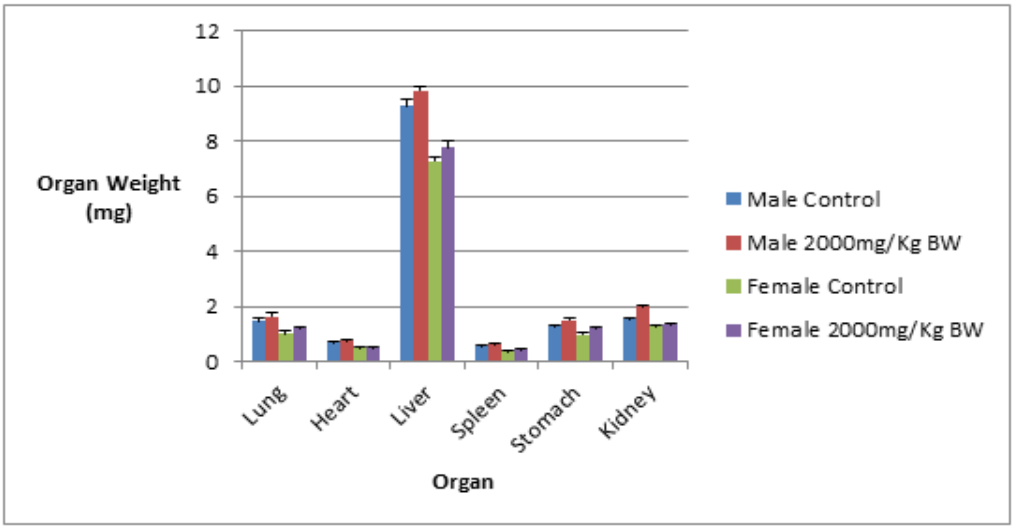
From macroscopic observation, there were no significant abnormalities in vital organs (lung, heart, liver, kidney, stomach) after the administration of Temulawak (*Curcuma xanthorrhiza* Roxb) hepatoprotective supplement at 2000 mg/kg BW/ (Table 3).

Table 3: Gross Macroscopic Examination

Group	Sex	N	Lung	Heart	Liver	Kidney	Stomach	Intestine	Spleen
Control	Male	5	No change	No change	No change	No change	No change	No change	No change
Control	Female	5	No change	No change	No change	No change	No change	No change	No change
Limit test (2000mg/kg BW)	Male	5	No change	No change	No change	No change	No change	No change	No change
Limit test (2000mg/kg BW)	Female	5	No change	No change	No change	No change	No change	No change	No change

The administration of Temulawak (*Curcuma xanthorrhiza* Roxb) hepatoprotective supplement with a limit of 2000 mg/kg BW also did not statistically affect the organ weight of male and female rats (Fig. 2).

Figure 2: Effect for Organ Weight on 14th day after the administration of supplement



DISCUSSION

To ensure the safety of a substance, lethal dose 50 (LD_{50}) toxicity test is often performed in rodents. The LD_{50} is a statistically derived amount of a substance that can be expected to cause death in 50% of the animals when given as a single dose and observed for a specified period [11].

The result shows that Temulawak *(Curcuma xanthorrhiza* Roxb) hepatoprotective supplement has not been found to be toxic even at limit test 5000 mg/kg BW in experimental animals. Based on the classification of the OECD 420 global harmonization system and classification by Loomis (1978) [12], the LD50 of Temulawak *(Curcuma xanthorrhiza* Roxb) hepatoprotective supplement (LD50>5000 mg/kg BW) is classified as unclassified or minimally practically non-toxic [10,12]. A dosage of 5000 mg/kg BW in test animals is equivalent to 56 000 mg (56 g) in humans (70 kg). So a single administration of more than 56 g is not expected to cause real toxic symptoms in humans.

In this study, the administration of this supplement did not affect food intake and water consumption or suppress the appetite. The administration of Temulawak (*Curcuma xanthorrhiza* Roxb) hepatoprotective supplement with a limit of 2000 mg/kg did not statistically affect the development of body weight of male and female rats. This result indicates there was no disturbance in food metabolism. The body weight change is one indicator of adverse effects of drugs and chemicals and, it will be significant if it occurs more than 10% of the initial weight [13].

A single administration of temulawak based hepatoprotective supplement at 2000 mg/kg of also does not affect the central and somatomotor nervous system, autonomic, respiratory, gastrointestinal, genitourinary, mucous membranes and eyes.

The administration of 2000 mg/kg BW Temulawak (*Curcuma xanthorrhiza* Roxb) hepatoprotective supplement also did not statistically affect the development of organ weight of male and female rats. Organ weight is an important marker of physiological and pathological status in animals. Toxic substances can affect the heart, liver, kidney, spleen and lungs organs caused the metabolic reaction was changed [14]. The relative organ weight is necessary to diagnose whether the organ was exposed.

CONCLUSION

No death or signs of toxicity were observed in rats treated with extract at dose 2000 mg/kg thus establishing its safety in use. Based on these results, can be concluded that the potential of acute oral toxicity (LD50) of the Temulawak (*Curcuma xanthorrhiza* Roxb) hepatoprotective supplement in male and female rats belongs to the unclassified category or at least practically non-toxic (LD50> 5000 mg/kg BW) which is equivalent to a dose of 56000 mg (56 g) in humans (70 kg). From macroscopic observation, there were no significant abnormalities on important organs (lung, heart, liver, kidney, stomach) after the administration of Temulawak (*Curcuma xanthorrhiza* Roxb) hepatoprotective supplement at 2000 mg/kg BW.

CONFLICTS OF INTERESTS

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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