ACUTE TOXICITY TEST (LETHAL DOSE$_{50}$) OF ETHANOL EXTRACT OF FRUIT AND SEEDS OF BITTER GOURD (*Momordica charantia Linn*) ON MALEMICE *Mus musculus*

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Abstract

The purpose of this study was to show that bitter melon fruit and seeds were safefor consumption by testing the toxicity by determining the LD_{50} value of the ethanol extract of bitter melon fruit and seeds using the Thompson-Weil method and their effect on animal behavior. The test animals used were 23 male white mice (*Mus musculus*) with an average weight of 25 grams and divided into 5 groups.Giving a solution of bitter melon fruit and seed extract orally with various doses: 100 mg/kgbw, 50 mg/kgbw, 25 mg/kgbw and 12.5 mg/kgbw and administration of steriledistilled water as a control group. Mice were observed individually for 1 hour, 2 hours, 4 hours and 24 hours after administration of the extract by looking at the number of dead animals and the visible toxic symptoms. After that, it was observed for 2-14 days after treatment. The results of the research on bitter melon extract found that 1 mice died at a dose of 100 mg/kgbw (20%), while at other doses there were no mice that died. The results of the research on bitter melon seed extract were 1 mice died at a dose of 100 mg/kgbw (20%), at a dose of 50 mg/kgbw, 2 mice died (40%), and at a dose of 12.5 mg/kgbw, mice died much 1 mice (20%), while at other doses no mice died. This indicates that the extract of bitter melon fruit and seeds is included in the non-toxic category.The administration of the extract test material did not cause toxic symptoms in which all rats had normal activities. So that bitter melon and bitter melon seeds are safe for consumption.
Key words : Momordica charantia,_ Extract, Toxicity_ Mus musculus, Thompson-Weil

1. Introduction

Momordica charantia or bitter melon has been widely used by humans as medicinal plants. African people use bitter melon one of them as an antidiabetic. Bitter gourd extracts, especially insulinmimetics and polyphenols, have the potential to lower blood glucose (B Joseph, D Jini. 2013). Toxicity is a term in toxicology which is defined as the ability of a compound to causedamage or injury. The term toxicity is a qualitative term that occurs or does not occur depending on the amount of toxic compound elements that are absorbed. Acute toxicity test is part of a preclinical test designed to measure the toxic effect ofa compound. Acute toxicity refers to the toxic effects that occur after oral administration of a single dose within 24 hours. The median lethal dose or LD50 is a statistical measure after a single dose that is often used to express toxic dose level as quantitative data. While clinical symptoms, physiological symptoms and

toxicmechanisms as qualitative data (Jenova, 2009). The process of destruction only occurs when the target organ has accumulated in sufficient quantities of the toxic part or its metabolites, so this does not mean that the highest accumulation of the toxic agent is in the target organ, but it could also be elsewhere. Furthermore, for most of the toxic compounds at high concentrations in the body will cause more damage. The concentration of toxic compounds in the body is the amount of poison exposed, then related to the rate of absorption, the amount absorbed, and relatedto the distribution, metabolism and excretion of these toxic compounds (Donatus, I.A., 2005). The Thompson-Weil method using the LD_{50} calculation list is a method that is often used in determining the level of toxicity of a compound. This method was chosen because it has a fairly high level of confidence, accurate results, and does not require a large number of experimental animals (Mustapa, 2018).

2. Literature review and hypotheses development

Abundant pre-clinical studies have documented the anti-diabetic and hypoglycemic effects of *M. charantia* through various postulated mechanisms. However, data on clinical trials with human subjects are limited and flawed by poor study design and low statistical power. Most acute toxicity studies are designed to determine the $LD_{50}$of a drug. The LD_{50} of a drug is defined as a single dose of a substance that is statistically estimated to kill 50% of experimental animals (Radji and Harmita, 2008). This test is carried out by giving the chemical being tested once or several times within 24 hours, then observed for 14 days (Hendriani, 2007). shortly after administration with a certain dose. At least four levels of dosage are recommendedin acute toxicity testing, these doses range from a low dose that is not or nearlylethal to all test animals to the highest dose that can be tolerated kill all or almost allof the test animals (Fadli, 2015). The purpose of the acute toxicity test of atraditional drug is to determine the acute toxicity potential (LD_{50}) to assess variousclinical symptoms, the spectrum of toxic effects, and the mechanism of death(Angelina et al, 2008). For the acute toxicity test of traditional medicines, it isnecessary to carry out at least one rodent species, namely mice or rats (Lu, 1995).The initial procedure for determining the acute toxicity of a new compound is toestablish a range of doses to be administered to test animals. The recommendeddose is at least four dose levels, ranging from the lowest dose that has not yet giventhe death effect of all test animals

to the highest dose that can kill all or almost all ofthe test animals (Donatus, 1998). The principle of this toxicity test is that a testsubstance is administered orally using a probe with a 3 inch intubation needle with aball-tipped tip in predetermined doses in several groups of experimental animals.Furthermore, observation for 14 days after administration to see the toxic effectsand death. The final result will be LD_{50} (Barile, 2005: OECD, 1981). Basically, the LD_{50}test value that must be reported in addition to the number of animals that died,must also mention the duration of the observation. If the observations were madewithin 24 hours after treatment, then the result was written "LD_{50} 24 hours". However, as development progresses, this is no longer considered, because ingeneral the LD_{50} test is carried out in the first 24 hours, so writing the test results"LD_{50}" alone is sufficient to represent the LD_{50} test observed in 24 hours. The writingof the results must be accompanied by the duration of the observation (Loomis, 1987). Philippus Aureolus Theophratus Bombast von Hohenheim (1493-1541) stated that everything that is efficacious as medicine is poison, only the dose makes it non- toxic (Wirasuta, 2016). Toxicity tests on *Artemia salina Leach* larvae showed the result that administration of bitter melon extract showed potential acute toxicity (Cahyadi Robby, 2009). The results of other studies related to bitter melon plants show that bitter melon leaf extract is toxic with an LC_{50} value of 200.2 ppm. (Mangirang F, 2019). The results of research conducted by Temarwut FF, in 2022, on shrimp larvae showed that the aqueous extract of bitter gourd has high toxicity with an LC_{50} value of 0.82 ppm. Test results on seeds of similar plants show that dumbaya (*Momordica cochinchinensis*) seed extract in *Mus musculus* causes liver cell damage and is unsafe when used in the long term or for 14 days (Mampa AS. 2017). The doseof bitter melon extract (EBP) 250 mg/kg BW, EBP given to experimental rats is still considered safe for liver cells and testicular organs. As for the kidneys up to a dose ofEBP 1000mg/kg BW, EBP is still relatively safe. EBP doses of 500, 750, and 1000 mg/kg BW were considered unsafe for liver cells and testicular organs. As for the kidneys up to a dose of EBP 1000mg/kg BW, EBP is still relatively safe. The doses of EBP 500, 750, and 1000 mg/kg BW, which were given were considered unsafe for liver cells and testicular organs. The EBP given is still considered safe for liver cells and testicular organs. As for the kidneys up to a dose of EBP 1000mg/kg BW, EBP is still relatively safe. EBP doses of 500, 750, and 1000 mg/kg BW, are considered unsafe for liver cells and testicular organs (Adimunca, Cornelis, 2000)

3. Research methodology

Young bitter melon fruit and seeds obtained from the Traditional Market / Pasar Rawu Serang City. It is dried and blended to get powder/flour.

Extract Making

The extraction method used is the maceration method in which 400 grams of simplicia seeds and bitter melon powder are put into an inert or glass topless container and then 1000 mL of ethanol solvent is added. Stir using a stirrer and occasionally shaken. Let stand for 1-2 days after that, separated residue and filtrate using filter paper. The obtained filtrate was collected and concentrated. Furthermore,evaporation is carried out by means of evaporation to separate the required substance from the solvent.

Test Animal Setup

Experimental animals (Mus musculus mice) with a weight range of 23-28 g were acclimated for 10 days in order to adapt to the environment and during the adaptation process the mice were fed corn, carrots and water from cucumbers. Micewere also fasted to eat for 8 hours but were still given water before treatment.

Dosing

The dose used was based on research by Dwi Ari Nugrahani and Vivi Sofia (2011) on the ethanol extract of bitter melon fruit and seeds given orally to experimental animals, namely the highest dose: 8 g/kg BW, it was found that it had no significant effect on the SGPT-SGOT value. The doses used in this study were as follows:

Extract dilution

Dilution	***Dose 1 : 100 mg***	***Dose 2 : 50 mg***	***Dose 3 : 25 mg***	***Dose 4 : 12,5 mg***
Extract	*4000 mg*	*2 ml (Dose 1)*	*1 ml (Dose 1)*	*1 ml (Dose 1)*
DMSO (solvent)	*1 ml*	-	-	-
Aquabidest steril	*9 ml*	*2 ml*	*3 ml*	*7 ml*
Final volume	*10 ml*	*4*	*4*	*8*

Test Animal Grouping

As many as 23 mice were randomly divided into five treatment groups, namely one control group that was given aquadest and four treatment groups that were given a dose of extract so that each group of test animals consisted of 5 male mice.

Acute Toxicity Test (LD_{50})

In the LD_{50} acute toxicity test, each treatment group was given extracts of bitter melon fruit and seeds which had been dissolved into distilled water orally using a probe with different dose levels, namely 4 dose level groups and 1 control group.

4. Result and discussions

The extraction process does not use hot methods such as reflux or cold heat such as soxhlet because it is feared that there are groups of compounds that are not resistant to heating such as flavonoids which are easily oxidized at high temperatures (Koirewoa, 2012). According to Sundari (2010) the possibility of damage to chemical compounds contained in a natural material can be avoided because it is not accompanied by heat.

In this study, the experimental animal used was male white mice (*Mus musculus*). Mice were chosen because of their small size, easy maintenance and care. Male mice are not affected by the estrus cycle which can cause unstable hormone activity which will later affect the observation process (Lu, 1995). In this test, the administration is done orally using a probe. This route is adapted to people's habits in consuming bitter melon.

After the intervention of bitter melon extract in experimental animals with a predetermined dose orally, the results obtained:

LD_{50} Test Results of Pare Fruit Extract(Dosage 0.5 g/Kg Bw)

No	*Dosage*	*M1*	*M2*	*M3*	*M4*	*M5*	*% Death*	*Description*
1	*100mg/25 g*	*-*	*-*	*-*	*-*	*-*	*0*	
2	*50mg/25 g*	*-*	+	*-*	*-*	*-*	*20*	
3	*25mg/25 g*	*-*	*-*	*-*	*-*	*-*	*0*	*Non-toksic*

4	*12,5mg/25 g*	*-*	*-*	*-*	*-*	*-*	*0*
5	*Control*	*-*	*-*	*-*	*-*	*-*	*0*

In the table above, it can be seen that at a dose of 50 mg/kg bw, one mice died 24 hours after treatment, although their physical activity seemed normal. This is due tointerference from the dominant mice in the group.

After treatment of bitter melon extract test material, all mice had normal activities and did not show any toxic symptoms. The results of the toxicity test were obtained,namely in group I after treatment of bitter melon extract there were no deaths and mice were active as usual. From these results it can be concluded that a dose of 100 mg/kgbw is not toxic or does not cause toxic symptoms.

The results of the intervention of bitter melon seed extract in experimental animalswith doses that have been determined orally, the results obtained are:

LD_{50} Test Results of Pare Seed Extract(Dosage 0.5 g/Kg Bw)

No	*Dosage*	*M1*	*M2*	*M3*	*M4*	*M5*	*% Death*	*Description*
1	*100mg/25 g*	*-*	*-*	*-*	*-*	*-*	*0*	
2	*50mg/25 g*	*-*	*-*	*-*	*-*	+	*20*	
3	*25mg/25 g*	*-*	*-*	*-*	+	+	*40*	*Non-toksic*
4	*12,5mg/25 g*	*-*	*-*	*-*	*-*	+	*20*	
5	*Control*	*-*	*-*	*-*	*-*	*-*	*0*	

According to Marlinda et al (2012), the active compounds contained in medicinal plants are almost always toxic when given in high doses. All poisonings occur due to reactions between toxic substances and receptors in the body (Katzung, 2002). Oral administration of ethanol extract of fruit and bitter melon seeds causes the active substance contained in the extract to be absorbed in the digestive tract and then undergo a process of distribution and metabolism.

Death of mice at a dose of 50 mg/kg bw after treatment of bitter melon seed extract test material within 24 hours. At a dose of 25 mg/kg bw there were mice that died after being given the extract. This occurs because the mice experience shortness of breath due to choking. At a dose of 12.5 mg/kg bw, there were mice that dead onday 6. In general, all mice were active as usual (normal) and no toxic

symptoms were seen.Factors that affect the LD_{50} value include species, strains, gender, age, weight, gender, nutritional health, and stomach contents of experimental animals. Administration technique (time of administration, ambient temperature, humidity and air circulation) and human error

5. Conclusion

The results of the research on bitter melon extract found that 1 mice died at a dose of 100 mg/kgbw (20%), while at other doses there were no mice that died. The results of the research on bitter melon seed extract found that 1 mice died at a dose of 100 mg/kg body weight (20%), at a dose of 50 mg/kg body weight 2 mice died (40%), and at a dose of 12.5 mg/kg body weight it was found 1 mice died (20%),while at other doses no mice died. This indicates that the extract of bitter melon fruitand seeds is included in the non-toxic category. The treatment of the extract test material did not cause toxic symptoms in which all mice had normal activities. So that the fruit and seeds of bitter gourd are safe for consumption.

Limitation and study forward

Further research is needed on the toxicity test of bitter melon fruit and seed extracton white rats of the Wistar strain so that the dose used can be higher.
It is necessary to perform SGOT-PT examination and histological examination of theliver tissue

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