



Comparison between Ocimum Sanctum Hepatoprotector Extract and Curcuma Xanthorrhiza on the Histological Structure of Aspartame-Induced Wistar Rats

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Abstract: *The use of aspartame is still controversial, because there are studies stating that aspartame is safe to use, and there are studies suggesting aspartame has the potential to damage the liver, but aspartame has been approved by the FDA and BPOM in Indonesia with a daily dose of 50 mg/kg/day, the level of public knowledge we are still low to allow this dose to be overtaken, coupled with the presence of several food products that do not include the content of aspartame, basil leaves have been known to have hepatoprotector effect, but the dosage is still varied, and no researchers have compared curcuma Xanthoriza which is herbal medicine that has been quite well accepted in the community, Objective: to compare the hepatoprotector effect of basil leaf extract with xanthoriza curkuma, Method: Laboratory experimental study with posttest only with control group design. Wistar rats were divided into 5 groups and treated for 30 days. This study analyzed the histopathology of liver using paraffin histotechnics blocks, with HE staining and light microscopy. Analysis of degeneration degree using Kruskal-walis analysis post Hoc Mann-Whitney Results showed an increase in the degree of degeneration in the aspartame group at a dose of 100 mg/kgBB/day ($p < 0.05$) compared to the normal group and treatment. Use of aspartame past the ADI dose damaged the liver, kurkuma and basil leaf extract at a dose of 300 mg/kg has the same protective effect on aspartame-induced rat liver, conclusions: aspartame is toxic for the liver, the use of basil leaf extract at a dose of 300 mg/kg/day or kurkuma xhantoriza extract can reduce the toxicity of aspartame.*

Keywords: *xanthoriza; basil-curcuma; leaf; extracts*

I. Introduction

Aspartame is an artificial sweetener that has been consumed by hundreds of thousands of people in the world because it has been declared safe to consume at a dose of 50 mg/kgBB by the FDA, (Leon *et al.*, 1989) Likewise in Indonesia BPOM also states it is safe to consume if according to the Acceptable Daily Intake (ADI), (Menteri and Republik, 2009) dose, aspartame is also widely used as a type of sugar that is beneficial for patients Diabetes and weight loss in obese patients because they have no calories (Asif, 2013) After a long period of use of aspartame, it caused controversy among scientists, a meta-analysis study stated that aspartame did not have an effect on reducing blood sugar or body weight, but it could actually cause metabolic syndrome such as cardiovascular disease and hypertension, (Azad *et al.*, 2017) many recent studies in experimental animals have stated that this aspartame dose of ADI is very toxic, causing damage to kidney and liver function. (Marko D Prokić *et al.*, 2015) (Mourad, 2011), (Haliem and Mohamed, 2011), (Iyaswamy *et al.*, 2017)

Basil leaves have been tested with several studies to have a *hepatoprotector* effect because the content of *flavonoids* with a dose of 100-300 mg/kgBB/day, (Rahman, 2011), (Shah and Verma, 2012), from this study the dosage range is still too large, besides that no one has compared the effects of basil leaves with standard drugs which has been marketed like

turmeric which is used to protect the liver. Therefore researchers want to test the extent of the hepatoprotector effect of basil leaves compared with curcuma in maintaining liver cells from aspartame toxic substances

II. Materials and Methods

2.1 Research Design

This experimental study was carried out in the FKUMSU laboratory animal laboratory, using albino *wistar* rats with a double randomized design then divided into 5 groups, namely group 1: given standard food + *aguades*, group 2: given standard feed + aspartame 100 mg/kgBB), group 3: given standard feed + aspartame 100 mg/kgBB + basil extract 200 mg/kgBB, group 4: given standard feed + aspartame 100 mg/kgBB + basil ekstrak 300 mg/kgBB, group 5: given standard feed + aspartame 100 mg/kgBB + *curcuma xanthoriza* 200 mg/kgBB, all experimental animals were treated for 30 days by straining while water and food were given in an *adlibitum*.

2.2 Experimental Animals

Thirty-six male Wistar rats were divided into six groups of 6 animals each. Healthy albino *wistar* male rats weighing 100-200 g, came from UGM animal house. All the animals were kept under standard managemental conditions as per the norms of Committee for the purpose of control and supervision on experiments on animals. were maintained under a controlled environment with temperature at $23 \pm 2^{\circ}\text{C}$, relative humidity at $55 \pm 5\%$, and a 12-hours/12 hours light/dark cycle throughout the experiment. The animals were fed on a standard pellet diet. They were given ad-lib feed and wholesome drinking water throughout the experiment, before the treatment had received ethical approval from the FKUMSU ethical commission number 109/KEPK/FKUMSU/2018

2.3 Test Drug

Alcohol extract of basil leaves (*Ocimum sanctum*) is obtained in the following ways: One kilogram of fresh basil leaves was identified at USU's herbarium laboratory, then washed, after which it was dried at room temperature, so that a dry weight of 150 gr was obtained, then extraction was carried out in the biochemical laboratory FKUMSU with maceration method obtained 38 grams of thick green extract, suspensions used in doses of 200 mg/kg and 300 mg/kg in the group induced by liver damage with aspartame

Standard hepatoprotective

Curcuma extract in capsule form, purchased from Distributors: Sejahtera Mandiri, POM TR. 133 373 471. Made into suspension at a dose of 200 mg/kgBB according to the previous research dose

Hepatotoxin

Asian Aspartame is purchased from www.jualbahanfarmasi.blogspot.com. Used in the form of a suspension at a dose of 100 mg/kg, for the induction group.

Macroscopic changes

Macroscopic: differences in body weight between groups will be compared and weight of organs also compared between groups, and observations also see if there are changes in color in the organs observed.

Histopathology

On the 30th day, rats were euthanized and their liver organs washed and washed with 0.9% NaCl to then be fixed in 10% formalin solution. After processing, washing, dehydration with alcohol concentration is increased, clearing, infiltration, *embedding*, cutting, attaching, *deparafinization*, *de-alcoholization* and staining with *hematoxylin* eosin, then the preparation is observed under a light microscope. The assessed is the change in *hepatocyte* cell structure that is assessed by enlargement of 40 xs in the *periportal* zone and the middle zone that is between the portal area and central vein, then counted the number of degenerated cells in 100 cells in 1 field of view, the average of degenerated cells is calculated in 5 fields of view. The degree of damage was optimized by scoring namely:

- 0: no *hepatocyte* cell damage
- 1: liver cell damage reaches 0.1-5%
- 2: liver cell damage reaches 6-25%.
- 3: liver cell damage reaches 26-50
- 4: liver cell damage more than 50%

Statistical analysis:

Comparison of body weight every week during treatment, liver weight, analyzed by ANOVA test if it does not meet the requirements of an alternative *kruskal walli* test, the significance value $p < 0.05$, while the degree of degeneration between groups will be analyzed by the *Kruskal Walis* test but it will also be assessed descriptively.

III. Result

3.1 Weight and liverweight of rat

It appears that turmericand basil can improve weight loss caused by aspartame, and can affect the weight of the rat's liver to normal, although it is not statistically significant (Table 1)

Table 1. Comparison of body weight and liver weight between treatment groups

Group	N	Week 1 (g)	Week 2 (g)	Week 3 (g)	Week 4 (g)	Difference in body weight	unwilling(g)
1	6	177.71±10.4	223.91±18.8	223.91±18.8	222.46±22.3	56.37±14.8	7.64±1.0
2	5	168.26±17.1	210.85±20.7	210.85±20.7	217.40±22.8	49.14±30.1	8.06±1.3
3	5	166.88±18.7	211.79±25.4	211.79±25.4	214.64±23.4	59.09±27.5	7.94±1.1
4	6	157.20±22.8	205.70±19.1	205.70±19.1	204.34±19.2	47.14±20.2	6.55±0.9
5	6	167.43±11.2	216.27±16.5	205.70±16.5	222.01±14.1	54.57±23.1	6.98±1.0

3.2 Comparison liver degeneration between group

Table 2. Comparison of degrees of liver degeneration between groups

Group	N	Maximum	Minimum
1	6	0	0
2	5	2	3
3	5	2	3
4	6	0	2
5	6	0	1

The group's analysis of Mann Whitney showed significant differences between the negative control groups with positive control ($p = 0.002$), between negative controls (group 1) and group 3 ($p = 0.002$), between negative controls (group 1 and group 4 ($p = 0.02$), positive control group 2 with group 5 (0.04), positive controls group 2 with group 4 ($p = 0.01$), positive control group 2 with group 4 ($p = 0.01$), group 2 with group 4 ($p = 0.01$), between treatment groups 3 and group 4 ($p = 0.02$), and no Significant difference between group 1 and group 5 ($p = 0.13$), the positive control group 2 with group 3 ($p = 0.5$), between group 4 and group 5 (0.11).

3.3 Histopathological observations

Histopathology observation of the negative control group showed no abnormalities in the histological structure of the liver, both in the *periportal* region and around the central vein, (Figure 1), in the positive control group (aspartame) *hepatocyte* cells were seen swelling around the *perifortal* with normal nucleus, cytoplasm which vacuole, necrosis seen with a *cariolysis* nucleus in the portal area and a large number of buffer cells (figure 2) liver in the basil leaf extract group at a dose of 200 mg /kgBB, showing the presence of cytoplasm that has granules and the presence of *hepatocyte* cells undergoing *karyolysis*, the cupffer cells appear to be quite a lot (figure 3) The liver in the treatment group of basil leaf extract with a dose of 300 mg/kgBB/day shows a normal structure in the portal area and there are still a few granular cells in the cytoplasm in the mid portal area, while the area around the normal central vein, also appear quite a lot of cupffer cells (figure 4), liver in the extract group turmeric shows a normal picture of *hepatocytes* from the portal area to the central vein. And there are quite a lot of cupffer cells (figure 5)

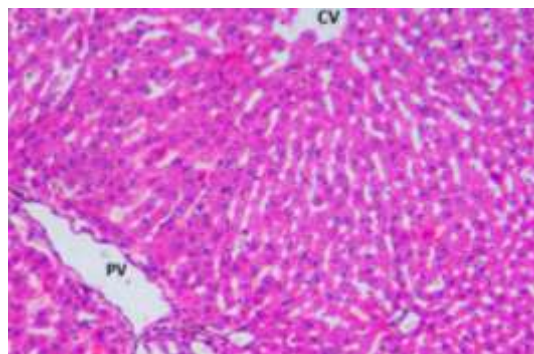


Figure 1. PV: portal vein, CV = central vein, normal hepatocyte cells appear

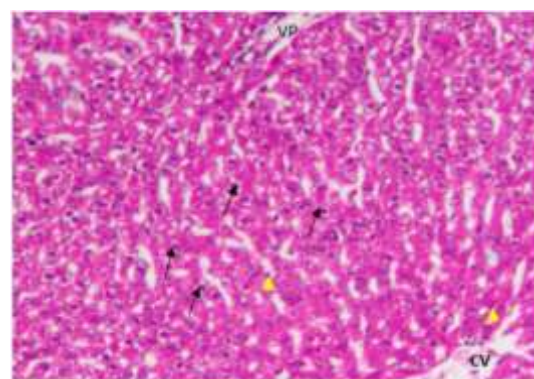


Figure 2. CV = central vein, VP = portal vein. The arrows indicate degenerated hepatocyte cells, = cupffer cells

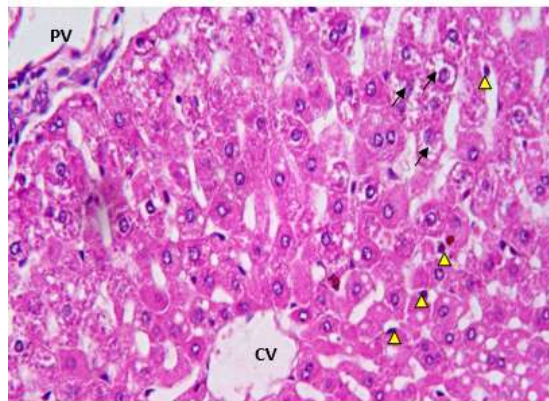


Figure 3. CV = central vein, PV = portal vein, arrow: degeneration of hepatocyte cells, = cell cupffer

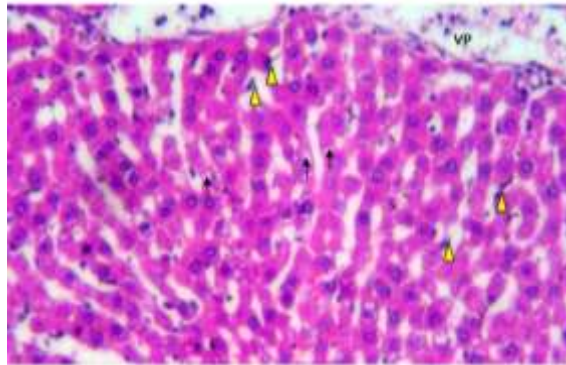


Figure 4. VP = portal vein, arrow = degenerated cell = cupffer cell

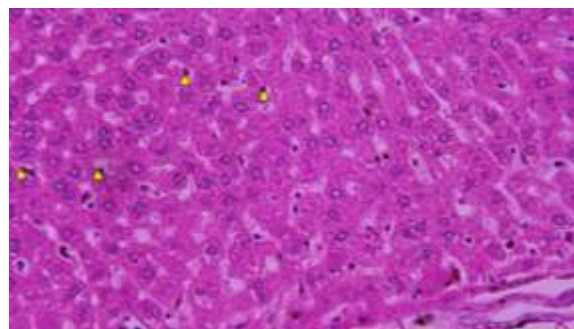


Figure 5. VP = portal vein, arrow = cupffer cell

IV. Discussion

In this study, the administration of basil extract 300 mg/kgBB/day of rats given aspartame dose 2x ADI dose was seen to have a protective effect against *hepatocytes*, as measured by body weight, liver organ weight and histology by light microscopy observation, Aspartame which has a *hepatotoxic* effect has been reported: after aspartame is eaten it will be metabolized in the digestive tract to *aspartadic* acid, phenylalanine and methanol and the end product is formal dehyde and aspartic acid. Methanol is oxidized more slowly and has many effects on *hepatocyte* cells (Marko D Prokić *et al.*, 2015) In this study *cytoplasmic vacuolation*

is likely to occur due to a response to changes in the structure of the plasma membrane consisting of proteins and lipids that affect the function of the sodium and potassium pumps that cause sodium accumulation and the transfer of water into cells, this is likely due to the formation of secondary free radicals that occur because methanol and aspartic acid after consumption. (Kumar Choudhary, Selvaraj and Sheela Devi, 2015), which is in accordance with this study which suspects that methanol might increase lipid *peroxidation* production which affects cell membranes, resulting in vacuolization in the cytoplasm. Some researchers say that vacuolization of the cytoplasm is the initial defense mechanism of cells against toxins that enter the cell

The kupffer cells that appear to be more dominant in the aspartame treatment group, and the aspartame + basil leaf extract group and in the aspartame group with turmeric show that there is an accumulation of formaldehyde which damages protein molecules, this molecule will be detected by kupffer cells, according to the literature that macrophages) This can destroy protein. In this study hepatocyte cell damage may also be caused by the activity of these buffer cells, which results in tumor necrosis factor alpha, interleukins, reactive oxygen, nitrogen species, proteases, and prostaglandins. This mediator can affect hepatocytes which cause cell death. However, some researchers claim that the kupffer cell acts as a protective. (Haliem and Mohamed, 2011)

Based on its effect on body weight, it appears that there is no effect of giving aspartame with the addition of basil leaf extract ($p > 0.05$). This is the same as previous research where aspartame has no effect on body weight. (Azad *et al.*, 2017) which has been considered that aspartame can act as a therapeutic management in patients' obesity (Leon *et al.*, 1989), more surprisingly lately aspartame actually has a risk of liver and heart damage, namely increased lipid peroxide due to reduced glutathione levels from the liver, (Ashok *et al.*, 2014), (Marko D. Prokić *et al.*, 2015), (Azad *et al.*, 2017) basil leaf extract in this study does not seem to have an effect on the body weight of *aspartam*-induced rats. This happens because the basil leaves have an anti-oxidant effect, no effect on body weight, when viewed with a comparison with negative controls do not differ significantly ($p > 0.05$)

Basil extract also appears to have no effect on organ weight compared to negative control and positive control. In previous studies it was suggested that toxic substances influence organ weight loss such as the liver, (Jain, 2015) studies it was suggested that toxic substances influence organ weight loss such as the liver,¹⁶ this study differs from previous studies possibly due to the inhomogeneous sample weight between groups that were It might also affect the ratio of organ weights between groups, so this is a weakness in this study.

Histological observation of light microscopy analysis of degeneration of *hepatocyte* cells showed a protective effect of basil leaf extract at a dose of 300mg/kgBB and extract of turmeric in rat liver. That might be due to the antioxidant ability of basil leaf extract and curcuma extract, which binds to reactive oxygen species (ROS) Natural antioxidant compounds contained in basil leaf extracts are *phenolic* compounds (*tocopherols*, *flavonoids*, *phenolic acids*), nitrogen compounds (alkaloids, chlorophyll derivatives, amino acids, and amines) and beta carotene. Beta carotene contained in basil is an antioxidant compound that can prevent damage from cells by increasing levels of antioxidant molecules such as glutathione and increasing the activity of antioxidant enzymes such as superoxide dismutase and *catalase* to prevent organelles or cell membranes from binding to free radicals with toxic substances. (Rahman, 2011) while curcuma *xanthoriza* can protect by modulating various signaling molecules, including inflammatory molecules, transcription factors, protein *kinase* enzymes,

protein *reductase*, adhesion molecules, growth factors, receptors, cell cycle regulation proteins, *chemokines*, DNA, RNA and metal ions. (Palipoch *et al.*, 2014), (Masoud, 2017) in accordance with the allegation that aspartame can cause a decrease in *superoxid* dismutase and *catalase* in tissues.

The protective effect is seen with the improvement of cell structure in the area around the portal vein, as it is known that toxic substances will affect the area around the portal vein more heavily, as well as the protective agent that is given will also have the most powerful influence in the area around the portal vein, because circulation is bleeding to the liver lobules the first is from this portal vein.

V. Conclusion

From the research results it can be seen that aspartame causes changes in the histological structure of the liver, and basil leaf extract at a dose of 300 mg/kgBB/day has the same *hepatoprotective* effect as curcuma. Aspartame intake should be reduced and not consumed in the long term; basil leaf extract might be developed as a new *hepatoprotector*. Analysis of the active substance from basil leaf extracts which acts as a *hepatoprotector* needs to be carried out for its development.

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