Analysis of galangal (*Alpinia galanga*) extract as natural preservative tofu against serum glutamate pyruvate transaminase (SGPT) levels and hepatic tissue structure of white rat (*Rattus norvegicus*) wistar strain

N L P M Widiyanti*, S Mulyadiharja, I M P A Santiasa and K R I Anjasmara

Biology Department, Mathematics and Science, Universitas Pendidikan Ganesha, Bali-Indonesia, 81116

*E-mail: manikwidiyanti@gmail.com

Abstract. Tofu is traditional food that contains lots of protein and water that are demand by the people of Indonesia. Tofu quickly damaged by microorganisms and to preserve tofu using natural preservatives include soaking tofu in galangal (*Alpinia galanga*) extract. Galangal rhizoma contain essential oil and methanol fraction that inhibition activity growth of microbial. The purpose of the research that has been done is: to analyze the differences of SGPT levels and to identify the hepatic structure of *Rattus norvegicus* with male sex after administration of tofu which is soaked using aquadest, formalin, and galangal extract with various concentrations. This research was a true experimental research which designed with the randomized posttest only control group design. SGPT levels were analyzed using one way Anova and the histopathologic of white rat liver were analyzed descriptively. Research results have shown: there has been a difference in SGPT levels in white rats (*Rattus norvegicus*) with male sex after being given tofu soaked using aquadest, formalin, and galangal extract with various concentrations and tofu which is preserved using formalin and galangal extract 20%, 40% and 60% concentrations cause damage to the structure of hepatic tissues in the form of hydropic and fatty degeneration.

1. Introduction

Tofu is traditional Indonesian food that much in demand by the people of Indonesia. The composition of tofu which is consist lots of protein and water causes tofu is a medium suitable for the growth of microbes so that tofu becomes quickly damaged [1]. Many tofu entrepreneurs use chemicals substances that are harmful to health to prevent tofu damage. Discovery has been done by BPOM/AFDS (Badan Pengawas Obat dan Makanan/ Agency for Food and Drugs Supervisor) on food indicated using formalin and borax among others tofu. Preserved tofu using formalin usually has a characteristic not only easily damaged up to 3 days and can be store up to 15 days at refrigerator temperature. In terms of physical, preserved tofu using formalin appear too hard, chewy but not solid.

Sampling and laboratory tests several large cities in Indonesia have found that 1.91% tofu...
contains formalin with the largest percentage in municipality of Kediri, East Java province of Indonesia that is 10.42% [2]. Research that has been done by [3] have found one of the tofu samples originating from the traditional market in Bandung containing dye synthetic metallic yellow.

According to regulation of minister of health number 1168/Menkes/Per/X/1999, formalin is chemical whose use is prohibited for food [4]. Formalin is chemical that is carcinogenic and mutagenic. Statement by [5] formalin is known to be strong disinfectant against bacteria and fungi. Formalin can also harden the tissue so it is used as a corpse preservative and used in the process of examination of biological materials and pathology. According to [6] has reported that from the inspection that has been done by BPOM Bali province of Indonesia, in the district market Kediri regency Tabanan Bali province of Indonesia, from 5 samples tested formalin, 2 samples has been detected positively containing formalin that is anchovy and tofu. According to [7] writes has been inspected by BPOM Bali province, in the market regency Badung in street Cokroaminoto the city of Denpasar, Bali Province of Indonesia, on Wednesday (August, 31, 2016) have found that from about 50 samples has been tested by the team BPOM laboratory, 7 samples positives has been containing Rhodamin B namely 1 sample “cake abug”, 1 sample “cake bendu” 1 sample “cake red gipang” 2 samples “cake uli”, and 2 samples “cake begina” respectively. The official website [8] that has been published April, 7, 2017, has stated by the operational team labs of BPOM province of Bali, in Kereneng market, city Denpasar province of Bali, on Friday (3/4/2017). From 27 samples that have been tested, have found 6 samples positive contains Rhodamin B dye namely “cake uli”, wet cake with pink dry, and shrimp paste. While 2 samples positive containing formalin are “anchovy Medan”, whereas Medan is one of several provinces in Indonesia so that namely “anchovy Medan” and tofu. The cooperation that has been done between Ganesha University of Education and the Sidemen village laboratory team, the Sidemen district of Karangasem regency, Bali province where the students of Ganesha University of Education who has community service with advicer is [9] stated that from some snack food samples of children trafficked around elementary school number 1 Sidemen, only 1 sample is ice sugar containing Rhodamin B dye (unpublished).

[10] has tested administration of formalin tofu against liver function disorder and free radical formation in mouse body by determining SGOT, SGPT and MDA (malondialdehid) levels after administration of formalin tofu for 25 days to 2 different groups mice with administration of formalin tofu in different levels are 0.25% and 0.50%. The results of his research have shown that there is a tendency of increased levels of SGOT, SGPT and MDA in blood of rats given formalin tofu compared with the control group.

Because of the danger of using formalin, it is necessary to find natural preservatives. According to [11] spices Indonesia contains many active substances that are antimicrobial and has great potential to be used as a natural preservative, one of them is galangal (Alpinia galangal) plant. In addition to having many content of antimicrobial active substances, the existence of this galangal is also easy to obtain in the community and has affordable economic value so that its potential as a natural preservative can be maximized.

Galangal rhizoma (Alpinia galanga) contain flavonoid, several identified are kaempferide, galangin, alpinin and 3-dioxy-4-methoxy flavone [12]. The presence of microbial growth inhibition activity by essential oil and methanol fraction of galangal rhizomes in some species of bacteria and fungi [13]. Infusion of galangal ethanol extract contained in essential oil can inhibit some pathogenic fungi [14]. The main chemical compound of galangal is essential oil which is composed eugenol, seskuerpen, pinen, metil-sinamat, kaemferida (yellow crystal), galangan, and galangol (resin) [15]. [16] stated the galangal rhizome isolated chemicals such as 1-S-1-acetoxychavicol acetate, 1-S-1-acetoxyeugenol acetate, 1-S-1-hydroxychavicol acetate, trans-p-hydroxycinnamaldehyde, trans-p-coumaryl alcohol, trans-p-hydroxycinnamyl acetate and trans-p coumaryldiacetate.

Role of galangal as antimicrobial has evidence in several research, one of them is research
have been done by [17], that the results tofu that soaked with extract galangal natural preservative for 3 days forming inhibition bacterial zone with range 0.5-1 cm. Based on the research, the researchers want to analyze galangal extract as natural preservative tofu against SGPT and hepatic tissue structure in white rat (Rattus norvegicus) wistar strain with male sex.

2. Methods

Sample in this research was using 40 white rat (Rattus norvegicus) wistar strain with male sex, age was 8-12 weeks and body weight was 150-200 grams. Number of sample followed formulation \( t(r-1) \geq 20 \) [18], \( t \) is treatment, \( r \) is replication. Sampling design of this research were using true experimental design with The Randomized Posttest Only Control Group Design and Completely Randomized Design (CRD) used simple random sampling. Research subject was administration of tofu which is soaked used aquadest, formalin and various concentrations galangal extract towards 40 of male white rat (Rattus norvegicus) wistar strain until 1 month.

2.1 Procedure

Several procedures in this research are :
1. The making the galangal (Alpinia galanga) extract as a natural preservative (Alpinia galanga) has been done according to [19]
2. The making of the tofu has been done according to [17].
3. The making of tofu pellets has been done according to [20].
4. Provision of tofu pellets to mice up to 1 month
5. Have taken the blood serum through the heart and continued to take the liver organ
6. Testing SGPT levels that have been done according to [21].
7. The manufacture of liver tissue preparations has been performed on the basis from [22] and location have done made preparation in Balai Besar Veteriner Wilayah VIII Denpasar Bali Indonesia

2.2 Data analysis

1. Data analysis has been done using Anova one way [23] with significance level 0.05. If there were significant differences in each treatment, will be analyzed using with Least Significance Different (LSD) test.
2. Identification of cells in liver tissue preparation has been compared with [24], [25] and [26]

3. Results and Discussion

3.1 Results

Serum Glutamate Piruvate Transaminase (SGPT) level of white rat (Rattus norvegicus) after the treatment has been done measurement as Table 1 below.
| Groups                      | SGPT (µ/L) levels | Average (µ/L) |
|-----------------------------|-------------------|---------------|
| Control (-)                 |                   |               |
|                             | 25.32             |               |
|                             | 21.83             |               |
|                             | 24.44             |               |
|                             | 27.00             |               |
|                             | 23.57             |               |
|                             | 22.70             |               |
|                             | 20.95             |               |
|                             | 29.68             |               |
| Control (+)                 |                   |               |
|                             | 38.41             |               |
|                             | 41.03             |               |
|                             | 34.05             |               |
|                             | 41.90             |               |
|                             | 47.14             |               |
|                             | 38.41             |               |
|                             | 40.16             |               |
|                             | 39.29             |               |
| Extract of galangal 20%    |                   | 36.12         |
|                             | 33.17             |               |
|                             | 39.29             |               |
|                             | 35.79             |               |
|                             | 38.41             |               |
|                             | 34.92             |               |
|                             | 32.30             |               |
|                             | 41.03             |               |
|                             | 34.05             |               |
| Extract of galangal 40%    |                   | 37.98         |
|                             | 37.54             |               |
|                             | 34.92             |               |
|                             | 36.67             |               |
|                             | 39.29             |               |
|                             | 38.41             |               |
|                             | 36.67             |               |
|                             | 40.16             |               |
|                             | 40.16             |               |
| Extract of galangal 60%    |                   | 38.41         |
|                             | 37.54             |               |
|                             | 40.16             |               |
|                             | 41.90             |               |
|                             | 32.30             |               |
|                             | 38.41             |               |
|                             | 41.03             |               |
|                             | 39.29             |               |
|                             | 36.37             |               |

Based on Table 1. The average SGPT(µ/l) level in each group can be described in the form of bar chart in Figure 1.
The average value of SGPT that have been shown by the treatment group and the control group have exceeded the normal limit of SGPT levels is 17.5 – 30.2 µ/L.

3.2 Hypothesis test

Result of normality test was showed normality of data. And test of homogeneity was showed data is homogen. Hypotessis test for H0 (Hypothesis nul) and H1 (Hypothesis alternative) accepted or rejected, determined by Anova. The result analysis of Anova is p<0.05. That means H0 is rejected and H1 is accepted. This means there has been a difference in SGPT levels in white rats (Rattus norvegicus) blood serum wistar strain with male sex due administration of tofu soaked with galangal extract with various concentrations. Test the hypothesis continued to test LSD using post hoc test to know significance difference each galangal concentrations. The results is control (-) that tofu have been soaked used aquadest toward control (+) and all of the treatments was significantly different. Control (+) that tofu have been soaked used formalin, the treatments used 20%, 40% and 60% of extract galangal against control (-) was significantly different. All of the treatments toward control (+) and toward each the treatment was not significantly different.

3.3 Histopathology of hepatic tissue of white rats (Rattus norvegicus) wistar strain

The results of examination of liver tissue of white rats both in the control group and the treatment shown by Figure 2, 3, 4, 5, 6.

![Liver tissue structure of white rats (control (-))](image)

**Figure 2** Liver tissue structure of white rats (control (-)) (enlargement 1000x). a) central vein, b) sinusoid congestion, c) normal hepatocytes, d) normal sinusoid
**Figure 3** Liver Histopathology Structure of white rats (control +) (enlargement 1000x). a) central vein, b) sinusoid congestion, c) Hydropic degeneration, d) degeneration of fat.

**Figure 4** Liver Histopathology Structure of white rats (treatment of galangal extract 20%) (enlargement 1000x). a) central vein, b) sinusoid congestion, c) Hydropic degeneration, d) degeneration of fat.

**Figure 5** Liver Histopathology Structure of white rats (treatment of galangal extract 40%) (enlargement 1000x). a) central vein, b) sinusoid congestion, c) Hydropic degeneration, d) degeneration of fat.
Figure 6 Liver histopathology Structure of white rats (treatment of galangal extract 20%) (enlargement 1000x). a) central vein, b) sinusoid congestion, c) Hydropic degeneration, d) degeneration of fat.

Liver tissue structure of white rat in the negative control group has shown normal tissue such as central vein, sinusoid congestion, hepatocyte cells and sinusoid. The control positive group’s tissue structure and treatment has shown the presence of defects such as fatty tissue and hydropic degeneration in hepatocyte cells. Sinusoid congestion has been present in all liver tissues in both control and treatment groups.

3.4 Discussion
3.4.1. Influence soaking of tofu with galangal preservative toward SGPT value

The SGPT enzyme is known to exit in the liver cells and in small amounts present in kidney, heart and skeletal muscle cells. The presence of the most SGPT enzyme in the liver makes this enzyme as a specific indicator of liver cell damage. The liver has one of the functions of detoxification that plays an important role in secreting and inactivating foreign substances that come from outside the body (xenobiotic), one of which is formalin. Formalin that enters the body of mouse through the feed will be metabolized into a formic acid compound which may be passed by the portal vein to the liver tissue. Portal veins vary dirty blood and metabolic residues that are toxic to the central vein of the lobes of the liver through the liver sinusoid. In sinusoidal endothelium there is blood exchange into hepatocyte cells. The exchange will bring the formic acid to enter the hepatocyte cells. Formic acid entering through the liver sinusoid will be affect all liver cells such as liver cells, stellate cells, sinusoid (endothelial cells and kuffer cells).

The Kuffer cells will be trigger the release of Reactive Oxygen Species (ROS) that are free radicals and are toxic. This statement is in line with [10] that formalin entering the body will cause the formation of toxic free radicals. ROS triggers the opening of mitochondrial permeability transition pore or canal in mitochondrial membranes. This opening causes the release of proteins in the cells to the cytosol, one of them cytochrome. Cytochrome discharges activates cascade is a chemical reaction process in which one reaction triggers another series of activation reactions and reactivates another chemical reaction continuously. The activation has the function of regulating systematic cell death or apoptosis. In that process the cell becomes deficient in Adenosin Tri Phosphate (ATP) so that the cell will slowly cause hypoxia and end up with cell damage [27]. Damage of these cells structure will cause the SGPT enzyme will be released from the liver cells to the blood circulation intracellular and result in increased levels of SGPT in the blood.

Data analysis has shown that the feeding of tofu soaked with formalin with a concentration of 0.25% for 30 days increased the SGPT level in blood serum of white rats wistar strain significantly. This result is in line with [10] study who reported that there was an increase in SGPT levels in white rats treated with tofu contain formalin with various concentrations of 3 ml/200 grams of body weight. [28] studied
reported that there was an increase in SGPT levels in white rats of wistar strains on 0.1 ml formalin administration orally for 14 days. The increase of galangal extract concentrations as natural preservative of tofu is directly proportional with the average SGPT levels in mice given tofu soaked with galangal extract. Study by [13] has been shown the presence of microbial growth inhibition activity by essential oil and methanol fraction of galangal rhizomes in some species of bacteria and fungi. Studies by [14] has been shown infusion of galangal ethanol extract contained inessential oil can inhibit some pathogenic fungi among other: Tricophyton, Mycosporumgypseum, and Epidermofloccasum.

The main chemical compound of galangal is essential oil which is composed of eugenol, sesquiterpen, pinen, metil-sinamat, caemferida, galangan, and galangol [15]. According to [29] the application of rhizomes extract should be careful because it has the effect of cytotoxicity and capable of damaging DNA in six human cell line among other: normal cells, fibroblast, inactivated p53 cells, normal epithelium cells, breast tumor cells and lung adenocarcinoma. According to [16] the galangal rhizome isolated chemicals such as 1· S-1-acetoxychavicol acetate, 1· S-1-acetoxyeugenol acetate, 1· S-1-hydroxychavicol acetate, trans-p hydroxycinnamaldehyde, trans-p-coumaryl alcohol, trans-p hydroxycinnamyl acetate and trans-p coumaryldiacetate, and according [30] that chemicals inhibit xanthin oxidase enzyme. [31] investigated the effect of eugenol on the growth of Gram positive and Gram negative bacteria, at 1,000 ppm, eugenol inhibited the growth of the bacteria and at high concentration of 2,000 ppm was obtained against P.aeruginosa and comparison to amphicillin (1 mg/ml) used as a positive control.

Plant extract and essential oils may exhibit different modes of action against bacterial strains, such as interference with the phospholipids bilayer of the cell membrane which has as a consequence a permeability increase and loss of cellular constituents, damage of the enzymes, involved in the production of cellular energy and synthesis of structural components, and destruction or inactivation genetic material. In general, the mechanism of action is considered to be the disturbance of the cytoplasmic membrane, disrupting the protonotive force, electron flow, active transport and coagulation of cell content [32].

Eugenol (C10H12O2) or 2-metoxi-4-fenol (allibenzena) is includesfenol compound. Eugenol is aromatic compound, an allyl chain substituted guaiacol of the phenylpropanoids, found in essential oil. While phenol (phenyl alcohol) is organic compound a coustic, poisonous with formula C6H5OH derived from benzene and used in resins, plastics and pharmaceuticals and in dilute form as a disinfectant and antiseptic; once called carbolic acid.

A study conducted by [33] reported that the essential oil of galangal rhizomes at concentrations of 100 ppm and 1000 ppm inhibited Escherichia coli growth with diameter zone 7 mm up to 9 mm. While the growth of Staphylococcus aureus bacteria inhibited at 1000 ppm with diameter zone is 7 mm. [17] reported that the galangal extract at 60% used to preserve the tofu for 3 days of immersion inhibited the growth of bacteria isolated from tofu by forming a 0.575 cm diameter zone, whereas against Staphylococcus aureus bacteria inhibited bacterial growth of 0.8cm. The natural preserved of tofu using the concentration variations (20%, 40% and 60%) of the galangal extract given to white rats wistar strain for 30 days resulted in an average increase of SGPT levels, whose values have exceeded the normal SGPT of white rats with range 17.5-30.2 µ/L [34]. All active chemicals contained in galangal can cause damage to liver cells such as methanol, essential oil, 1· S-1-acetoxychavicol acetate, 1· S-1-acetoxyeugenol acetate, 1· S-1-hydroxychavicol acetate, trans-p hydroxycinnamaldehyde, trans-p-coumaryl alcohol, trans-p hydroxycinnamyl acetate and trans-p coumaryldiacetate.
3.4.2 Structure of hepatic tissue of white rat wistar strain (Rattus norvegicus).

Based on data analysis of hepatic tissue structure of white rat wistar strains, in the negative control group had shown no damage to liver cells. This is characterized by absence of fatty tissue in the liver, a neatly arranged sinusoid empties into the central vein, the liver cell structure in normal circumstances with spherical nucleus and is in the central, no degeneration, necrosis, fibrosis and cirrhosis are found to be directly proportional to normal SGPT levels is 24.44 µ/L. In contrast to the rat group of negative control, the rat group of positive control and the treatment groups, there was found damage to the liver cells such as hydrophic degeneration and degeneration of fat. Hydroptic degeneration, has been shown by the formation of a clear fluid-filled vacuole around the nucleus cell that slowly shifts the nucleus cells to the edge of the liver cell. This statement, according to [35], that the degeneration of liver cells resulted from the accumulation of toxic substances and other metabolites in the liver cells.

Hydropic degenerations occurred shift to intracellular to form clear vacuoles containing substances that resemble liquids in cells. According to the [36], the vacuoles formed in hydropic degeneration can unite and form larger vacuoles that occupy the cytoplasm and replace of the nucleus cell. Hydropic degeneration is reversible lesion, which when toxic exposure is stopped, the tissue structure will be return to its original state. If toxic exposure continues, will be cause necrosis. Degeneration of fat has been demonstrated in the presence of clear vacuoles that fill the cytoplasm and cause the nucleus cell to squeeze into the edges of cell. Fatty degeneration occurs because of the accumulation of fat, which has been characterized by intracytoplasmic fat vacuoles that can enlarge and push the nucleus cell to the edge of cell. According to [37], the formation of intra cytoplasmic vacuoles is caused by presence of metabolic disorder and deficiencies of lipolytic factors.

Previous authors reported that an increase in SGPT levels by 1-3 times the normal SGPT is caused by conditions such as pancreatic degeneration, liver fatigue, Laennec cirrhosis and biliary cirrhosis [38]. In hepatic tissue structure of rat, in all mouse tissue both control and treatment groups, sinusoid congestion is present. This congestion is an excessive contained by blood in the veins of a tissue. This is characterized by the presence of blood found the hepatic sinusoids. In general congestion occurs in the central veins and sinusoids surrounding sinusoid. This causes the sinusoid to become dilated. Such changes are general response of blood vessels as a result of the use of chloroform or ether anesthetic agents prior to necropsy. This occurs because chloroform is powerful anesthetic that causes vasodilatation of blood vessels [39]. Because it is a general response, congestion is not categorized as liver damage.

4. Conclusion
1. There was a difference of SGPT level in white rats wistar strain that was given tofu with various concentrations (20%, 40% and 60%).
2. Hepatic tissue of white rats wistar strain has shown normal tissue in the negative control group, whereas in the positive control group and treatment there is found damage of liver cells such as hydroptic degeneration and fatty degeneration

5. Recommendation
For the community recommended to use a concentration lower than 20%, considering galangal (Alpinia galangal) extract is used as a traditional food ingredient of Indonesian and also as a natural preservative.
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