Analysis of turmeric extract (*cucurmadomestica*) as natural preservative of tofu to SGOT levels and hepatic tissue structure of male white rats (*rattusnorvegicus*) wistar strain

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Abstract. The purposes of this research were: (1) to determine levels of SGOT (Serum Glutamate Oxaloacetate Transferase) of wistar rats after being given preserved tofu using water/aquadest, formalin, and turmeric extract with various concentrations, and (2) to identify histopathology images of rat liver after being given tofu which is preserved using formalin, turmeric extract, and soaked with water/aquadest. This true experimental research was designed with Randomized Post Test Only Control Group Design. The sample used was male white rat (*Rattusnorvegicus*) wistar strain of 40 individuals. SGOT levels were analysed using one way ANOVA statistic test and histopathology images of rat liver were analysed descriptively. The results of this research show that: (1) there has been different SGOT levels among male white rat (*Rattusnorvegicus*) wistar strain due to preserved tofu using turmeric extract with various concentrations of 20%, 40%, and 60%, (2) hepatic tissue structure of rats that is given tofu with turmeric extract 40% and 60% suffered from structural damage of its liver, while the rats given with tofu with turmeric extract 20% obtained a normal liver cell structure.

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 AFDS (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] that 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 (April, 3, 2017), from 27 samples that have been tested, have found 6 samples positive contains Rhodamin B dye namely “cake uli”, wet cake with pink dye, and shrimp paste. While 2 samples positive containing formalin are anchovy from Medan, whereas Medan is one of several provinces in Indonesia so that namely “anchovy Medan” and tofu. The cooperation that has been done between UniversitasPendidikanGanesha location di Singaraja Bali and the Sidemen village laboratory team, the Sidemen district of Karangasem regency, Bali province where the students of UniversitasPendidikanGanesha who has community advicor 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 using formalin to preserve of tofu against liver function disorder and free radical formation in mouse body by determining levels of SGOT, SGPT and MDA (malondialdehid) after administration using formalin to preserve of tofu for 25 days with 2 different groups mice are mice with administration using formalin to preserve of 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 tofu that preserve using formalin compared with the control group.

The results of routine sampling carried out by BPOM in recent years, there are types of hazardous materials that are often misused in food, namely formaldehyde, among other is tofu. Tofu preserved with formaldehyde has the characteristics not easily damaged for up to 3 days and can last up to 15 days at refrigerator temperatures. From physical appearance, tofu that is preserved using formalin seems too hard, chewy but not dense. At the end of 2005 and early 2006, publications on formalin abuse on food were very intense in the mass media and laboratory results in several major cities in Indonesia, it is known that 1.91% of tofu is preserved using formaldehyde with the largest percentage is 10.40% in the Kediri municipality of East Java province, Indonesia [2]. According to Minister of Health of Republic Indonesia, formaldehyde is chemical that is it prohibited used on food products. The use of formaldehyde is widely misused and is often used as a preservative for food and food items such as tofu which can cause liver damage. One the liver functions is to metabolize almost all substances that enter the body. Metabolism and detoxification of formaldehyde occure in the liver and produce toxic metabolites that can damage the liver.

Based on these problem, research was carried out on natural preservatives which are relatively safe for cells rather than synthetic preservatives, one of which is tumeric (Cucurma domestica).

Tumeric is a spice that contains typical antimicrobial substances that can inhibit bacterial growth, so it can be used to preserve food ingredients such as tofu. The results of research conducted by [11] stated that on the 3rd day, tofu which had been soaked with tumeric extract with concentration of 60% had inhibited the growth SPC (Standard Plate Count) of bacteria. The inhibition zone by the 60% concentration of tumeric extract has shown a significant difference compared to the inhibition zone by the three types of preservatives with concentrations of 20% and 40% to the growth of the test bacteria.
This study also showed that the active ingredients contained in turmeric were bacteriostatic to the test bacteria (*Staphylococcus aureus*) characterized by a decrease in inhibition zones from treatment on day 1 and day 3, each obstacle of 1 cm and 0.8 cm for turmeric extract with 40% concentration. Concentration of 60% turmeric extract in the 1<sup>st</sup> and 3<sup>rd</sup> day treatment inhibited bacterial growth by forming 1.5 cm and 1 cm barrier zones. And there has been a difference in the zone of inhibition of growth of test bacteria (*Staphylococcus aureus*) with polyculture bacteria isolated from tofu on exposure to turmeric extract. Greater inhibition zones have occurred in the growth of test bacteria compared to the growth of bacterial isolated from tofu. This is possible because the types of bacteria that have been isolated have not been identified and the possibility of polyculture working synergistically.

Cucurmin contains antibacterial, antioxidant and essential oils which function to regulate gastric acid release so as not to overdo it so that it helps the intestine work [12]. Tumeric contains 5.8% essential oil composed of 6% sabien, 1% cineol, 0.5% borneol, 25% zingeberen and 53% sesquiterpenic. The yellow pigment of tumeric comes from the color of substance called cucurmin (3-4%). Curcumin derivates include demethoxy and cucurmin biodemetoxy. The content of cucurmin compounds varies depending on the tumeric variety. Curcumin shows anticoagulant activity by inhibiting which is by inhibiting collagen and adrenalin which induce platelet aggregation. The types of sesqiterpenic ketons found in tumeric rhizomes includes tumeron and antumeron, while types of curcumin are curcumin (diferuloilmetana), dimetoxycurcumin (hidroxyxinosamiloferuloilmetan), and bisdemetoxy-curcumin(hidroxysinamoiolmetana) [13].

Research conducted by [14] showed that administration of tumeric rhizome extract at all dosage levels was able to give a hepatoprotector effect on liver damage on the test animal due to administration acetaminophen which was measured by increasing SGOT levels. [15] stated that administration of various concentrations of tumeric traditional extract with varying concentrations have shown significant differences between each group and can prevent increasing SGOT levels.

2. Methods

This study uses a true experimental design with The Randomized Post Test OnlyControl Group Design. The population in this research were male white rats (*Rattusnorvegicus*) wistar strain with a body weight is 150-200 grams, the aged 2-3 months which is as a characteristic inclusion of this research. The sample used in this research was 40 male white rats. Treatment was divided into 3 groups and 2 control groups (positive and negative controls) were selected by randomization and have been done 8 times replications based on Federer formula [16]. Characteristics of exclusion of the research were: the body weight less than 150 and more than 200 grams, did not suffer from disability, received steroids. The independent variable of this research was the administration of tofu pellets which had been soaked using formalin and water/aquadest (control groups) and the administration of tofu pellets which had been soaked using tumeric with various concentrations (20%, 40% and 60%). The dependent variable in this study was the measurement of SGOT levels from blood serum of test animals and features of histopathological level of test animals.

2.1. Procedure

There are 8 stages in this procedure are:
1. Making turmeric extract based on [17]
2. Making tofu
3. Making tofu pellets based on [18]
4. Administration of tofu pellets in test animals
5. Collecting blood serum
6. Measuring SGOT serum
7. Making liver of preparations test animals according by [19].
8. Observing features liver histopathological
2.2. Data analysis
Data were analyzed using one-way ANOVA to analyze differences in serum levels in the treatment groups. If there is a difference from the analysis then continued by Least Significance Different test [20]. Histopathological analysis of liver descriptively.

3. Results and Discussion

3.1. Results

3.1.1. SGOT levels. The treatment in test animals by administering 120 grams of tofu pellets for 30 days was followed by collecting blood serum to measure SGOT levels as in table 1.

Table 1. Average results of SGOT levels

| Variable           | Mean  (U/L) | SD   |
|--------------------|------------|------|
| SGOT levels (PC +) (U/L) | 103.34.00 | 2.637|
| SGOT levels (NC -) (U/L) | 69.62    | 2.458|
| SGOT levels (T1) (U/L)    | 83.56.00 | 1.789|
| SGOT levels (T2) (U/L)    | 92.76    | 2.629|
| SGOT levels (T3) (U/L)    | 96.03.00 | 2.381|

Explanation:
PC +: Positive control: tofu which is soaked using formalin
NC -: Negative control: tofu which is soaked using aquadest/water
T1: treatment 1: tofu which is soaked using 20% turmeric extract
T2: treatment 2: tofu which is soaked using 40% turmeric extract
T3: treatment 3: tofu which is soaked using 60% turmeric extract

Based on treatment groups, where the highest levels of blood serum SGOT in positive control (PC +) with a mean 103.34 U/L. Average SGOT levels blood serum in test animals in negative control (NC -) amounted to 69.62 U/L. Average SGOT levels blood serum in treatments were 83.56 U/L in treatment 1 (T1), at 92.76 U/L in treatment 2 (T2) and at 96.03 U/L in treatment 3 (T3).

Several stages of data analysis have been carried out to test hypotheses including normality tests using Kolmogorov Smirnov (KS), homogeneity test using Levene test and hypothesis testing using one way ANOVA [20]. If there are differences in each treatment then the analysis is continued using least significance different level of 5% using the post hoc test. The results of normality test have been done and show normal data distribution shown as table 2.

Table 2. The results of the test for normality of SGOT levels

| Variable            | Mean (U/L) | Standard Deviation | p Value | Explanation |
|---------------------|------------|--------------------|---------|-------------|
| SGOT levels (PC +)  | 103.34.00  | 2.637              | 0.200   | Normal      |
| SGOT levels (NC -)  | 69.62      | 2.458              | 0.201   | Normal      |
| SGOT levels (T1)    | 83.56.00   | 1.789              | 0.202   | Normal      |
| SGOT levels (T2)    | 92.76      | 2.629              | 0.203   | Normal      |
| SGOT levels (T3)    | 96.03.00   | 2.381              | 0.204   | Normal      |

The homogeneity test shows the data variant is homogenous shown in table 3.

Table 3. The results of the test for homogeneity of SGOT levels

| Variable | Levene test value | p value | Explanation |
|----------|-------------------|---------|-------------|
| SGOT levels | 0.461             | 0.764   | Homogen     |
Based on normality and homogeneity tests, continued hypothesis test as table 4 below. The one way anova test has shown that each treatment is different.

**Table 4.** The results of one way anova test

| Variable            | F value | p value | Explanation     |
|---------------------|---------|---------|-----------------|
| SGOT levels         | 234.251 | 0.0001  | Significance difference |

From the table mean : H1 is accepted and H0 is rejected, then continued Least significance difference (LSD) test using Post Hoc Test as table 5 below.

**Table 5.** The results of LSD test using posthoc test

| Variable              | Mean   | p Value | Explanation     |
|-----------------------|--------|---------|-----------------|
| SGOT levels (PC +) : NC (-) | 33.719 | 0.0001  | Significance difference |
| SGOT levels (PC +) : T1    | 19.781 | 0.0001  | Significance difference |
| SGOT levels (PC +) : T2    | 10.585 | 0.0001  | Significance difference |
| SGOT levels (PC +) : T3    | 7.312  | 0.0001  | Significance difference |
| SGOT levels (PC -) : T1    | 13.938 | 0.0001  | Significance difference |
| SGOT levels (PC -) : T2    | 23.135 | 0.0001  | Significance difference |
| SGOT levels (PC -) : T3    | 26.407 | 0.0001  | Significance difference |
| SGOT levels T1 : T2       | 9.196  | 0.0001  | Significance difference |
| SGOT levels T1 : T3       | 12.469 | 0.0001  | Significance difference |
| SGOT levels T2 : T3       | 3.273  | 0.01    | Significance difference |

Data interpretation is shown in figure 1.

![Diagram average SGOT levels](image-url)
3.1.2. Histopathological features of the liver in male white rats. The histopathological features of the negative controls indicate that normal liver cells with homogenous red cytoplasmic characteristics and polygonal cell forms. Histopathological picture of rat liver negative control group can be seen in figures 2 and 3.

In the liver lobule, hepatocytes spread from the central vein to the periphery radially and regularly. The form of hepatocytes is polygonal. The size varies; the cell nucleus is clear and round. Hepatocytes are the largest part of the liver and are responsible for their central role in metabolism.

In the positive control group showed that there was obvious damage that occurred in the liver cells in the form of hydropic degeneration and generation of fat. Histopathological features of rat liver that suffered liver structure damage due to the administration of formaldehyde containing tofu can be seen in figures 4 and 5.

In the treatment group 1 showed that the T1 group obtained results that had normal liver cell structure with visible appearance of rat liver hepatocytes with homogenous red cytoplasm, polygonal shaped cells, varying size, cell nucleus visible and round. The normal figure of normal rats in treatment group 1 can be seen in figure 6.
Histopathological figure of rat liver in group T2, which is rats given tofu pellets soaked with 40% tumeric extract obtained results contained hydropic degeneration and a mild generation of fat. This shows that there was mild damage the liver structure of T2 group rats can be seen in figures 7 and 8. Fatty tissue seen in some hepatocytes. The occurrence of degeneration which is a sign of hepatocyte damage. Cell degeneration phase which is a sign of hepatocyte damage include: cloudy swollen degeneration, hydropic degeneration, fatty degeneration and necrosis [21].

Fat degeneration occurs due to changes in fat triglyceride metabolism resulting in an increase in synthesis or decrease in fat secretion from cells. Degeneration of fat is reversible. Normal liver cells form exocytosis without vacuolization, normo chromatic cell nucleus and no bleeding. Cells are called degeneration if there is vacuolization in the cytoplasm but the cell nucleus is still normo chromatic [22].

In the experimental group 3 (T3) is the administration to male white rats, tofu pellets which tofu soaked using 60% tumeric extract showed a clear result of liver cell damage, namely in the form of hydropic degeneration and a low generation of fatty fats, can observe as figures 9 and 10.
3.2. Discussion

3.2.1. White rat sgot levels after being given tofu which has been soaked with water/aquadest, formalin and turmeric extract with various concentrations.

This study used male rats as test animals because they had a more stable biological condition compared to female rats. Male rats at 2-3 months of age were young adult rats that had optimum physiological conditions. The mouse was first adapted for 14 days in order to adjusting from the environment during the study. Based on the results of the study and the average results of the levels of SGOT of rats given tofu pellets which is tofu soaked using formaldehyde as much as 103.014 U/L, where the level exceeds the normal level of SGOT when associated with normal levels of SGOT according to [23], the normal SGOTin rats is 45.7-80.8U/L.

In this study, increased levels of SGOT in male white rats were caused by exposure to formalin-containing tofu given for 30 days at a dose of 0.25%. This form because formalin is an irritating substances, where formaldehyde enters the body to be detoxified and metabolized in the liver and produce xenobiotic substances then can damage liver function. Formalin which enters the body will be metabolized into formic acid compounds and circulated through the portal of vein so that it enters the liver tissue. There is a Kuffer in the liver tissue that will immediately activate Reactive Oxygen Species (ROS) which is a toxic compound. Removal of ROS results damage to fat tissue which can cause damage to the cell structure that is on the mitochondrial membrane. Formic acid is produces in the metabolic process of formaldehyde in the body which will inhibit the metabolic activity of mitochondrial oxidase activity in P450cytochrome resulting tissue hypoxia in the liver cells. Prolonged hypoxia will cause damage to the tissue of the liver cells. Damage can result liver injury resulting increased levels of SGOT then released into serum [24][25].

The results of the measurement of SGOT levels in the treatment group showed lower SGOT levels compared to positive controls and higher SGOT levels compared to negative controls, with an average of 83.56U/L (tofu soaked using 20% turmeric extract) 92.76 U/L (tofu soaked using 40% turmeric extract), 96.03U/L (tofu soaked using 60% turmeric extract). Tofu soaked using 20% turmeric extract showed SGOT levels still in the normal range that has shown that concentration of 20% is the optimum concentration that can be exposed to the body of mice every day.

Tumeric (Curcumadomestica) is a type of plant that contains curcuminoinds consisting of cucurmin compounds and their derivatives include demethoxycurcumin and biodesmethoxycurcumin. Besides tumeric contains essential oils which consist of chetonesesquiterpen, turmeron, tumeon, zingiberen, felandren, sabinen, borneol andsineil. Curcumin is a compound that is used as an antioxidant. The body needs antioxidants that can help protect the body from free radical attacks, including protecting liver cells from toxic substances.
The hepatoprotective mechanism occurs because curcumin is an antioxidant that is able to capture superoxide ions, thus preventing liver cell damage due to lipid peroxidation in a way mediated by the antioxidant enzyme is superoxide dismutase (SOD), which is converts $O_2^-$ into a less toxic product [26].

According to [27] hepatoprotective activity due to antioxidant activity. Cucurmin is a potential protection against various reactive oxygen including superoxide anion radicals, hydroxy radicals, nitrogen dioxide radicals, singlet oxygen, and the other. Cucurmin shows potential inhibitory activity against P450 in rat liver. One of the cucurmin metabolites is tetrahidrocucurmin has a better protective effect compared to silymarin, because it contains hydroxyl and methoxyl groups from phenil rings and 1,3-dicheto. Antioxidant activity increases when there is phenolic hydroxy in ortho the methoxyl group.

Tumeric also contains cucurminoide and flavonoid compounds. Both compounds are known to be antioxidants. Various antioxidant activity compounds contained in tumeric rhizome which causes at a higher concentration level or certain conditions its activity actually turns into prooxidant, namely a condition where an antioxidant will change its activity into an oxidant or oxidators. This causes the emergence of cytotoxic activity from tumeric, especially derivatives ofgernacrena,curcumanoida, and andflavonoids [28].

[14] stated that the white rats given tumeric rhizomes before the administration of acetaminophen, the average level of SGOT was much lower than the group of rats without the administration of tumeric rhizome. [15] reported that giving different concentrations of tumeric juice showed significant differences between each group.

SGOT is an indicator that is sensitive to liver cell damage. If there is damage to the hepatocyte cell membrane, if there is damage to the hepatocyte cell membrane, the permeability of the liver cell will increase then the sot enzyme will be released into the blood circulation. Antioxidants are compounds that can neutralize and fight toxic or free radicals and inhibit oxidation in body cells, thereby reducing damage. In certain damages there can be an imbalance between free radicals and antioxidants which can cause oxidative stress [29].

Oxidativestress is a condition of balance disturbance between the production of free radicals and antioxidants that have the potential to cause cell damage resulting in increased levels of SGOT [30]. The physical health condition of mice is also very influential on the value of SGOT activity; therefore increasing levels of SGOT can also be caused by rats experiencing stress during the research.

According to [31], the use of tumeric cannot be ascertained to be safe or toxic especially in the liver because there are as many as 64 compounds in the tumeric which are thought to be toxic, but also because the liver is main target of drugs and xenobiotics. The compounds contained in tumeric which are hepatotoxic if consumed continuously are sesquiterpen, difurocumeneandparvitlorene.Sesquiterpenis the main component which is contained in essential oil.

From the present study, the workability of curcumin 20% is able to inhibit the increase in SGOT levels. When liver cells are not damaged, the enzyme SGOT does not enter to blood stream in large amount. The concentration of 20% tumeric extract is able to inhibit SGOT increase and be able to safe damage of liver.

3.2.2. Histopathology of rat liver after being given tofu pellets which is tofu has been soaked with water/aquadest, formalin and tumeric extract.

From the observation results of the preparations showed that the liver cells of mice that had been given tofu pellets with tofu which had been soaked using formalin, 40% and 60% tumeric extract had shown liver cell damage in the form of hydropic degeneration and fat generation. Whereas tofu soaked with water and 20% tumeric extract showed normal liver cell structure.

Normal liver cell anatomical structure that is polygonal shaped cells and homogenous red cytoplasm. Liver cell damage characterized by hydropic degeneration and the generation of fat shows that hydropic degeneration is temporary liver damage caused by cell experiencing nutritional...
deficiencies, cell aging and as a way of adapting liver cells to increased metabolism in the liver. Microscopically the cells are seen by many vacuoles in the cytoplasm and the cell size looks larger than normal cells [32]. If the hydropic degeneration damage does not return to normal, the liver cells will experience fat degeneration before permanent damage occurs.

Fat degeneration occurred in mice given tofu pellets containing formaldehyde and treatment in mice given tofu pellets which had been soaked using 40% and 60% tumeric extract. This was due to the fact that formaldehyde and excessive antioxidant inhibited the action of intracellular lipase enzymes.

According to [33], the liver is said to be damage if the amount of sgot enzyme in the plasma is greater than its normal level. The mechanism is toxic substances or excess substances that enter the body will be metabolized by cytochrome P450 enzymes in the liver to become free radicals and the bind to hepatocyte cells in the liver so that the liver membrane changes its permeability or increases. Changes in the liver cell membrane can have consequences, namely substances from inside the cell come out freely so that the liver undergoes shrinkage and necrosis occurs. Otherwise substances that are outside the liver cell can enter and cause the liver to become large or hydropic degeneration and to develop apoptosis.

According to [34] fat degeneration occurs due to abnormal accumulation of fat in the cytoplasm of hepatocytes. In normal circumstances, fat is taken in the form of fatty acids through pinocytosis. Fatty acid are synthesized tryglyceride, and bound to phospholipids and proteins then transported by blood as lipoproteins. Fat needed for binding of substrate facilitates electron transfer and provides a place for molecular interaction of cytochromes P450 reductase. Administration of chemical compounds will cause the formation of free radicals in the liver, which then causes lipid prooxidation in the cell membrane and will disrupt the activity of cytochrome P450 which functions in the biotransformation process. This will result in inhibition of phase I reactions in the biotransformation process which will cause the failure of the liver detoxification mechanism.

4. Conclusion
Based on the results of research and discussion, it can be concluded that this research is as follows.

1. There is a difference in SGOT levels and description of the structure of the liver tissue of male white rats due to the administration of tofu pellets which have been soaked using tumeric extract with various concentrations of 20%, 40% and 60%.
2. The description of hepatic cell structure which has been administration of tofu pellets which have been soaked using 20% tumeric extract with the result observation of normal structure of liver. Whereas tofu which has been soaked using 40% and 60% tumeric extract and made in form of pellets is exposed the white rat experiencing liver cell damage in the form of hydropic degeneration and the generation of fat which is the same condition as tofu which has been soaked using formalin.

Recommendation
For people can use natural preservatives such as turmeric at a certain amount.

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