

Research article

Impact of Water Supplementation With Turmeric and Tamarind Extracts on Broiler Liver Performance

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Abstract.

Turmeric and tamarind are medicinal plants that have a variety of health benefits, including laxative, antibacterial, anticancer, and hepatoprotective properties. The focus of this research was to look at the histology of the liver and the levels of SGPT-SGOT in broilers fed turmeric-tamarind extracts water at a rate of 2%. This study used a completely randomized design, with treatment options including a control, a mixture of turmeric rhizome and tamarind fruit (2%), and concentrations of 2% turmeric rhizome and 2% tamarind. The turmeric rhizome and tamarind extracts treatment was given in drinking water ad libitum. Quantitative data from the study were analyzed statistically, and if there was a significant effect, Duncan's test was used; qualitative data were presented with descriptions and images. The blood levels of SGPT in the control broilers were higher than in the broilers exposed to any of the other treatments. Also, in the control broiler livers, hydrofoc degeneration, nucleus pyknosis, and congestion were higher than in the treatment group broilers, but these were still within normal limits. Water supplementation with a mixture of turmeric rhizome and tamarind fruit extracts did not have a negative effect on the performance of the broiler livers.

Keywords: *Keyword: turmeric, tamarind, liver performance, broiler*

1. Introduction

The liver was an important part of the body's organs that function to secrete bile acids for fat metabolism, neutralize toxins, produce albumin protein, where cholesterol and triglycerides are produced and destroy old red blood cells. Disorders of the liver due to the consumption of drugs and antibiotics will affect the liver cells. The liver will respond to various diseases by becoming inflamed, which is called hepatitis. Liver damage was known to be damaged by performing some simple blood tests, such as checking the levels of the SGPT and SGOT enzymes. These enzymes were usually contained in liver cells. If the hepatocytes were damaged, these enzymes were released into the blood. Increased levels of SGPT-SGOT in the blood were indication of liver damage. [1].

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Turmeric (*Curcuma longa* L.) is a tuber containing phenolic essential oils, curcumin, flavonoids, tannin alkaloids, and terpenoids. Turmeric as a traditional medicine was often combined with other ingredients such as sembung leaves, cardamom and so on which have a gastroprotective effect [2]. Turmeric is anti-inflammatory [3] and antiproliferative [4]. Turmeric reduces liver cell damage in mice [5]. Turmeric rhizome could also be used as a medicine for wounds, rheumatism and acute wounds, by mixing turmeric with coconut oil. [6].

Tamarind whose scientific name is *Tamarindus indica* L. belongs to the type of tree and has a long life (annual). Parts of the *T. indica* L. plant such as stems, roots, and leaves could be used as medicine. [7] [8]. Tamarind fruit has potential as an anti-inflammatory drug and analgesic drug that has long been used in traditional medicine such as arthritis treatment, so it has great potential to be developed as a modern medicine [9]. Tamarind fruit is widely used in various therapies, including its potential as anticancer [10]. Tamarind mixed with other ingredients such as the camalia mulberry and ginger powder as a health drink can increase the immune response in white rats used as experimental animals [11]. Nanoparticles from tamarind peel have potential as therapeutic agents in the treatment of breast cancer in humans [12]. Tamarind juice at a dose of 40% is laxative but not as strong as Dulcolax [13]. Polysaccharides isolated from *Tamarindus indica* L. fruit had a hepatoprotective effect on rat liver, due to the antagonistic effect of thioacetamide which acts as a cell membrane stabilizer thereby preventing cell damage associated with thioacetamide saturation, or by preventing the interaction between thioacetamide in the cell transcriptional machinery [14]. Tamarind fruit has the potential as an antibacterial against Gram positive and Gram negative bacteria [15]. Antioxidant activity contained in tamarind fruit extract causes hypocholesterolemia in the in vivo test [16]. Tamarind fruit also significantly reduced body mass index (BMI), serum LDL levels in obese patients [17].

2. Methodology

2.1. Research procedure

This study used a completely randomized design (CRD), with 4 treatments, namely control, a mixture of 2% turmeric - tamarind, 2% turmeric, and 2% fruit tamarind. With 5 replications, so $4 \times 5 = 20$ experimental units, 1 unit = 10 experimental animals, so that $20 \times 10 = 200$ DOC broilers were used. The DOC used were weighed (BB), fed standard formula and given drinking water ad libitum and kept in a battery cage. 2% turmeric

rhizome water extract was made using 20 grams of turmeric rhizome added 1 liter of water, blended and filtered. The same method was used to make 2% tamarind fruit water extract and a mixture of 2% turmeric-tamarind water extract. Supplementation is given ad libitum as drinking water. The treatment was given until the broiler was 5 weeks old.

After treatment until the broilers were 35 days old, blood was taken with a 3 ml syringe from the wing brachial vein, the blood taken was separated between blood cells and serum. Then the broilers were slaughtered, the liver was cleaned and washed with 0.9% NaCl solution, then macroscopically observed liver texture, color and other appearances, then photographed with a camera. Next The liver was put into a glass bottle containing 10% NBF, then histology preparations were made. Histological preparations were carried out as carried out by Sudatri et al., 2016 using the paraffin method with Hematoxylin-Eosin staining[18].

2.2. The process of making liver histology preparations

Liver organs that have been soaked in 10% NBF fixative solution, then put in a tissue cassette, followed by a dehydration process with ethanol solution with graded levels of concentrations of 70%, 80%, 95%, and absolute alcohol. Furthermore, for 60 minutes the clearing process was carried out with xylol solution at room temperature. After that, the paraffin infiltration process was carried out by placing the liver pieces into liquid paraffin (temperature 60°C) for 45 minutes. After that, the paraffin infiltration process was carried out by inserting the liver pieces for 45 minutes into liquid paraffin at 60°C. Then the preparation was cooled so that it became a paraffin block.

Pieces of liver in paraffin blocks were sliced using a rotary microtome with a thickness of 5µm, then the incision was attached to a glass object that had been coated with gelatin, placed on the surface of warm water with a temperature of 45°C. The preparations are placed vertically, so that they dry quickly and the slides stick to the object glass.

2.3. Hematoxylin Eosin (HE) staining

The next process was staining with hematoxylin-eosin for 45 minutes and incubated at 60°C. Then the deparaffinization process was carried out to dissolve the paraffin using xylol 3 times, then the rehydration process was carried out by adding the preparations

in 100%, 95%, 80%, and 70% alcohol for 5 minutes, then put into distilled water. for 10 minutes, until all the alcohol was dissolved.

The process of staining hematoxylin by immersing the slide in hematoxylin solution for 5 minutes then washed with running water until clean for 5 minutes, and continued with staining using eosin for 3 minutes, washed with running water, after which the preparations were covered with a glass cover with Canadian balsam media. And liver histology preparations are ready to be observed under a microscope.

The liver histology preparations were observed under a microscope connected to an optical camera and a laptop with 100x and 400x magnification. The number of normal and abnormal cells per visual field was counted, and observations were repeated 5 times per preparation with different visual fields. The results of the observations are then averaged and the percentage is made.

2.4. Determination of Plasma SGOT-SGPT Levels

Determination of serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) concentration was carried out at the Bali Denpasar Regional Health Lab in accordance with standard procedures using a standard kit [19].

Quantitative data obtained from the study will be analyzed statistically using SPSS software and if there is a real or very significant effect, it will be continued with the Duncan test level 5%. And if the data is not normally distributed, the data will be tested with the Kruskal Wallis Test and the Mann Whitney follow-up test. Qualitative data is presented in the form of pictures and descriptions.

3. Result and Discussion

3.1. Liver histology

The ANOVA assay results showed that there was a very significant difference between control group and treatment group in the hydrophic degeneration cell abnormality variable ($P = 0.003$) and there was no significant difference in the inflammatory cell infiltration variable ($P = 1.36$), (Table 1). Hepatosis cells that undergo hydrophic degeneration were characterized by the presence of many vacuoles in the cytoplasm so that the liver cells experience swelling and the cells look pale in color. Meanwhile, inflammatory cell filtration is the presence of inflammatory cells such as leukocytes, in the tissue due to inflammation in the tissue (Figure 1).

The Kruskal Wallis assay on several measured liver histological variables was presented in Table 1. The abnormalities of fat cell degeneration and cell necrosis did not show a significant difference ($P = 0.227$). Fat degeneration was a continuation of hydrophic degeneration which is characterized by the accumulation of fat in the cytoplasm. Under the cell microscope, there was a clear colored spotting. If the cells are continuously exposed to toxic substances, the cells will experience necrosis (cell death).

TABLE 1: Mean hydrophic degeneration and inflammatory cell infiltration of broiler livers that were supplemented by turmeric-tamarind water extract.

Variable	Treatment			
	A	B	C	D
Hydrophic degeneration (%)	3,08± 1,02 a	1,29 ±0,45 b	1,62 ±0,82 b	1,34± 0,36 b
Inflammatory cell infiltration (%)	0,36 ± 0,33 a	0,86 ± 0,64 a	0,91 ± 0,20 a	0,50 ± 0,29 a

Note: different letters behind the mean value and on a column indicate significant differences
 A: control B: broilers fed with 2% turmeric C: broilers fed with 2% ac tamarind D: broilers fed a mixture of turmeric and tamarind

Pycnotic abnormalities and congestion showed significant differences with P values of 0.012 and 0.003, respectively. The pycnotic showed dark due to the accumulated chromatin. This occurs when cells become necrotic. Where as congestion was the rupture of capillaries so that blood enters the sinusoids. To find out the differences between treatments, the pycnotic and congestion variables were followed by the Mann Whitney assay. The percentage of cells that experienced picnotics between control and 2% turmeric treatment did not show a significant difference, while the congestion abnormalities between control and turmeric treatment showed very significant differences ($P = 0.005$). Meanwhile, pycnotic core abnormalities and congestion between control and 2% acid treatment and 2% acid turmeric treatment showed significant differences. Meanwhile, the percentage of cells became picnotic nuclei and congestion between 2% turmeric treatment and 2% tamarind turmeric treatment did not show a significant difference. Meanwhile, the percentage of cells experiencing pycnotic core abnormalities between 2% acid treatment and 2% tamarind turmeric showed a significant difference and congestion abnormalities between 2% acid treatment and 2% sour turmeric showed no significant difference.

The histological abnormalities of the liver found in this study included: hydrophic degeneration, cell necrosis, picnotic nuclei, inflammatory cell infiltration, fatty degeneration, congestion (Figure 1).

TABLE 2: Broiler livers histology that were supplemented with turmeric water- tamarind and were tested by Kruskal Wallis test.

Treatment	Fat degeneration (%)	Necrosis (%)	Picnotic (%)	Congesti (%)
A	7,93 a	10,74 a	13,3 a 8,07 a 7,13 a 4,13 a	2,13 a 1,00 b 1,00 b 1,27 a b
B	1,13 a	8,00 b		
C	5,40 a	9,67 b		
D	7,60 a	8,80 c		

Note: different letters behind the mean value and on a colom indicate significant differences
 A: control B: broilers fed with 2% turmeric C: broilers fed with 2% ac tamarind D: broilers fed a mixture of turmeric and tamarind

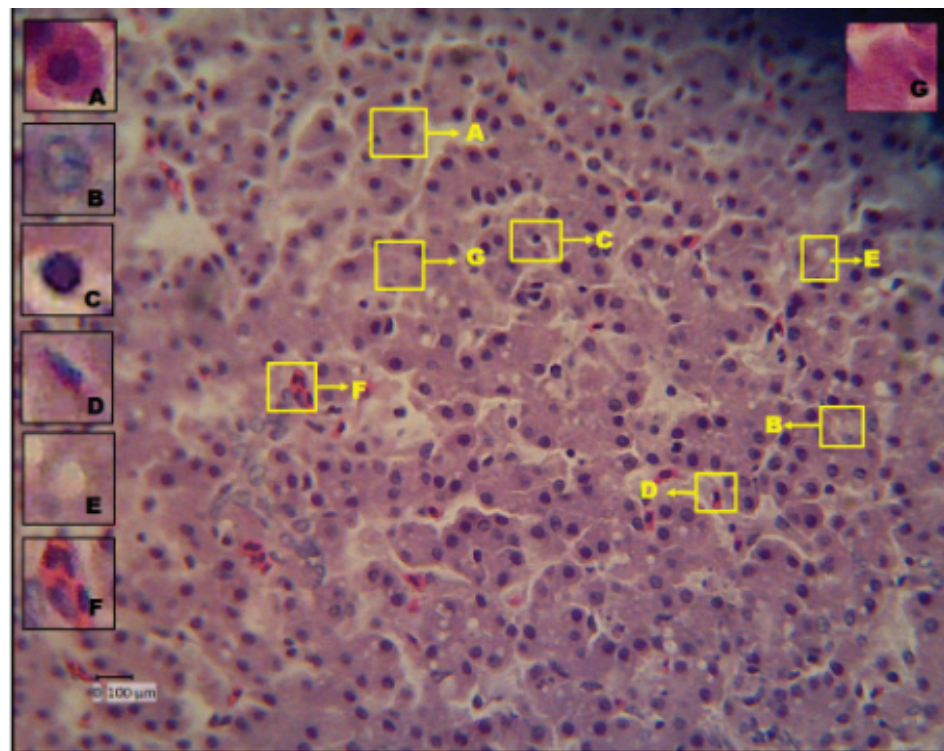


Figure 1: The histology of broiler liver stained with Hematoxylin-Eosin dye, was observed with a microscope connected to an opticlabb camera and a laptop with 400x magnification and the cells zoomed in with the Image Raster program with a thumbnail size of 250. A = normal cells, B = hydrophic degeneration, C = nucleus picnotic, D = inflammatory cell infiltration, E = fatty degeneration, F = congestion, G = necrotic.

3.2. SGPT and SGOT concentration

The histological abnormalities of the liver found in this study included: hydrophic degeneration, cell necrosis, picnotic nuclei, inflammatory cell infiltration, fatty degeneration, congestion (Figure 1).

The Kruskal-Wallis test showed that the concentration of SGOT broiler blood serum was not significantly different ($P = 0.339$) between treatment group and control group. Meanwhile, SGPT levels showed a significant difference ($P = 0.035$), so to determine the

TABLE 3: The results of the *Kruskal-Wallis* test and the *Mann Whitney* assay of SGPT and SGOT broiler blood serum levels were given water extract of turmeric and tamarind fruit.

Variable	Treatment	Mean Rank	Mean (U/L)	Normal
SGPT	A	16,40	88,25 b	23,8-52,8
	B	5,60	50,60 a	
	C	10,40	73,20 a	
	D	9,60	81,40 a	
SGOT	A	13,80	324,80 a	37,8-311,0
	B	9,60	330,29 a	
	C	7,20	258,80 a	
	D	11,40	325,40 a	

Note : different letters behind the mean value and on a column indicate significant differences between groups A: control B: broilers fed with 2% turmeric rhizome water extract C: broilers fed with 2% acidic fruit water extract D: broilers who were fed a mixture of 1% turmeric rhizome water extract and 1% tamarind fruit

differences between treatments continued with the Mann-Whitney assay. The Mann-Whitney assay showed that there was very significant difference between the SGPT levels of the control broilers and the broilers group that was given a mixture of water extract of turmeric and tamarind fruit ($P = 0.008$). The control broiler had SGPT levels of 88.25 / U / L, which was the highest compared to the treatment. And SGPT levels in all broiler groups were slightly higher than the normal range.

4. Discussion

SGPT and SGOT are enzymes found in liver cells. If the liver cells were damaged, this enzyme would be released into the blood. In this study, the SGPT level of control broiler group serum was the highest compared with the treatment broiler group. The elevated ALT levels indicate a disturbance, irritation or injury to the liver cells, and the range of SGPT values in all broiler groups was higher than the normal range. This was probably because the assay animals are a little stressed because they receive treatment every day. Serum SGPT levels were less sensitive in detecting liver damage, because this enzyme was also released by other damaged organs such as the heart, kidneys and brain [20]. In this study, there was no significant difference ($P > 0.05$) between the SGOT levels of control group and treatment group.

Serum SGOT-SGPT level test is an indicator of liver function. SGPT and SGOT are enzymes found in liver cells. If the liver cells are damaged, this enzyme will be released into the blood. In this study, serum SGPT levels in control broilers were the highest compared to the treatment. Increased levels of SGPT indicate disturbance, irritation or

injury to liver cells, and the range of SGPT values in all broiler groups is higher than the normal range. This is probably because the test animals are a little stressed because they receive treatment every day. Serum SGOT levels are less sensitive in detecting liver damage, because this enzyme is also released by other damaged organs such as the heart, kidneys and brain [20]. In this study, there was no significant difference ($P>0.05$) between control and treatment SGOT levels.

In liver histology, the control variable hydrophyseal degeneration was the highest compared to the treatment. Hydropic degeneration is a temporary change, if microscopically observed, it can be seen that there are vacuoles in the cytoplasm of the cells, so that the liver cells look swollen, and the liver cells look paler in color. Hydropic degeneration includes minor damage because it can heal and liver cells become normal again (reversible). The low number of liver cells with hydrophilic degeneration in the treated broiler group was probably caused by the content of phenolic compounds in turmeric rhizome and tamarind fruit which act as antioxidants, which repair the damage experienced by cells. Fat degeneration is damage to hepatocytes characterized by morphological changes and decreased liver function due to the accumulation of fat in the cytoplasm of liver cells. Microscopically, the cells were seen as small, clear fat spots (Figure 1). This can occur due to conditions of ischemia, anemia, disorders of toxic substances, excess consumption of fat and protein. And if liver cells are continuously exposed to toxic substances, it will cause cell death or necrosis [21]. Cell necrosis begins with pyknotic changes in the cell nucleus. Cells whose core is very dark and compact and also covers all parts of the cell nucleus are pyknotic cells. Cells that will undergo pyknosis will see the presence of chromatin that collects as single globules and the nucleus looks dark. Pyknosis can occur due to damage in the cell, namely damage to the membrane which is then followed by the Golgi apparatus and mitochondria which later the cell can no longer eliminate water and triglycerides so that it accumulates in the cytoplasm of the cell. Congestion occurs due to rupture of capillary blood vessels which can cause blood to enter the sinusoids. Inflammatory cell infiltration is the entry of white blood cells into the tissue that occurs as a result of the cells undergoing [22]. In this study, fat degeneration, inflammatory cell infiltration, and cell necrosis showed no significant difference between the histology of treated and control broilers. This means that the continuous administration of turmeric rhizome water extract and tamarind juice extract until the end of harvest did not have a negative effect on broiler liver performance. This is confirmed by the histological picture of the broiler liver (Figure 1).

5. Conclusion

Based on the research results, it could be concluded that supplementation of turmeric rhizome and tamarind fruit water extracts and their combination until harvest on broiler has no significant effect on the livers performance.

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