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Kidney histology and broiler serum creatinine levels supplemented with a mixture of water extract of turmeric and tamarind fruit

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Abstract

Turmeric (*Curcuma domestica* Val.) and tamarind are herbal ingredients that are widely used in traditional medicine. The study was conducted to determine the effect of continuous supplementation of turmeric extract and tamarind fruit on kidney performance. The experimental design used was a completely randomized design (CRD), with four treatments and five replications. The treatments consisted of: control, 2% water extract concentration of turmeric rhizome, 2% tamarind fruit water extract, 2% water extract mixture of turmeric rhizome and tamarind. The experimental units were 20 with 10 experimental animals in each experimental unit. There were 200 experimental animals were used. Renal histology was prepared using the paraffin method, and serum creatinine levels were tested by the spectrophotometric method. Qualitative data was presented in images, and quantitative data is analyzed using the SPSS for window program. The results showed that broiler serum creatinine levels were not significantly different between control and treatment. Kidney histology data showed no significant difference between the histology of the control kidney and the broiler kidney supplemented by water extract of turmeric rhizome and tamarind fruit. Water extract supplementation of turmeric and tamarind fruit 2% did not have a negative effect on kidney performance and serum creatinine level.

Keywords: Kidney histology, turmeric, tamarind fruit, creatinine

Introduction

The use of antibiotics in medicine for a long period could have negative effects on humans and animals. Likewise, contamination of toxins in feed or drinking water can also affect the performance of livestock organs such as the kidneys. (Tahirul *et al.* 2020) [20]. Reported that arsenic contamination in broiler drinking water caused an increase in serum creatinine levels, and fat degeneration and vacuolation in the glomerulus on histological features. The use of allopurinol 0.250mg / kg bw in broilers to treat gout was reported to cause mild disturbances in broiler kidneys (Sen, Gautam, and Yadav 2019) [16]. Contamination of micotoxin ochratoxin A and citrinin in feed causes degeneration and necrotic changes in tubular epithelial cells in broiler kidneys (Jayaramu *et al.* 2011) [6]. The use of herbal ingredients was believed by some people more safety and did not cause negative effects (Alok *et al.* 2013) [1]. Turmeric (*Curcuma domestica* Val.) and tamarind (*Tamarindus indica* L.) were herbal ingredients that were widely used in traditional medicine. A mixture of turmeric and tamarind fruit or combined with other ingredients was used as *jamu* (Indonesian traditional medicine) to cure various types of diseases.

Turmeric was thought to have originated from India, and had spread throughout Asia. In Indonesia, turmeric could grow from tropical coastal areas to mountains, with an altitude range of 0-1700 meters above sea level. Turmeric was easy to grow in various types of soil as long as it gets enough direct sunlight and enough nutrients and water. Turmeric rhizome mixed with coconut oil could be used as a medicine for ulcers, acute wounds, and rheumatism (Vimala and Gricilda Shoba 2014) [22]. Pulungan (2017) [15] stated that turmeric was a type of rhizome that contains active substances such as curcumin, phenol essential oils, flavonoids, alkaloids, terpenoids and tannins. These secondary metabolites were thought to inhibit the growth of fungi, especially *Candida albicans*. Curcumin contained in turmeric could cure osteoarthritis in patients by its ability to inhibit the NOS (nitric oxide synthase) enzyme from macrophages (Kertia *et al.* 2012) [7].

In addition, the methoxymethoxy in curcumin could also function as an antitumor (Kohyama *et al.* 2015) [8]. Turmeric could also reduce serum Low Density Lipoprotein (LDL) levels (Jantan *et al.* 2012) [5], lower cholesterol levels, reduce serum triglyceride and phospholipid levels (Jamaludin *et al.*, 2001). Turmeric function as a gastroprotector which could be seen from the decrease in the number of mast cells and eosinophils in the gastric histology of rats given turmeric extract (Mutmaimah *et al.* 2014) [13], as well as being estrogenic or could increase female fertility.

Tamarind was a type of plants that produce fruit that lives in tropical and subtropical areas. This plant was classified in the monotypic genus, subfamily Caesalpinioideae, family Leguminosae (Fabaceae). The names Tamarindus and tamarind were derived from Arabic which means Indian dates because of the fruit flesh that resembles dates. In India, this plant was a productive and beneficial plant (Bhadoriya *et al.* 2011) [3]. Every part of tamarind such as roots, stems, fruit and leaves had important benefits in traditional medicine (Kuru 2014) [9]. The sap from the seeds of tamarind also has the potential to be used as a medicinal coating/capsule (Huanbutta and Sittikijyothin 2018) [4]. The extract of tamarind/ tamarind seed husk was also very good for use as a natural dye for cotton, wool and silk which produces a very sharp color (Prabhu and Teli 2014) [14]. Water extract of tamarind seeds significantly reduced blood glucose levels of mice supplemented with streptomycin-induced diabetic rats (Maiti *et al.* 2004) [11]. Gums from tamarind seeds were also very well developed as an ingredient biogradebel capsule coating (Huanbutta and Sittikijyothin, 2017) [4].

The kidneys are excretory organs that function to filter blood, remove metabolic waste such as urea from the blood and excrete it together with water in the form of urine. The kidneys are susceptible to damage due to exposure to toxins or excess substances in the long term. If kidney function is impaired due to inflammation or kidney stone disease, the body becomes poisoned due to metabolic waste that cannot be removed. Indications of damage or decreased kidney function can be seen from the increased levels of Blood Urea Nitrogen (BUN) and plasma creatinine levels. This was due to the inability of the kidneys to excrete urea and creatinine into the urine. Large amounts of creatinine flow back into the blood so that the creatinine level in the plasma increases above normal limits (Thomas *et al.* 2017) [21]. This research was conducted to determine the effect of the combination of water extract of turmeric and tamarind fruit that was given continuously on kidney performance by observing the histology of the kidneys and serum creatinine levels.

Materials and Methods

Research material

The main ingredients of this research are turmeric and tamarind fruit obtained from traditional markets. The experimental animal used was Day Old Chick (DOC) broiler.

Research procedure

The experimental design used was a completely randomized design (CRD), using a concentration of 2% water extract of turmeric, 2% water extract of tamarind fruit, a mixture of water extract of turmeric and 2% tamarind fruit and control. Each treatment was repeated 5 times so that there were: 4 x 5 = 20 experimental units, and in each experimental unit there were 10 experimental animals. A total of 200 DOC boilers

were used and divided randomly into 4 groups, namely: A = control group; B = the water extract treatment group of turmeric 2%, C = the water extract treatment group of tamarind fruit 2%, D = the water extract treatment group of mixture of turmeric and tamarind fruit 2%. The experimental animals were initially measured for body weight (BW), given a standard formula diet which was weighed first and given drinking water ad libitum and placed in a battery cage.

2% water extract of turmeric rhizome was made by weighing 20 grams of fresh turmeric, adding 1000 ml of water then blending and filtering. Likewise, the procedure for making 2% tamarind fruit water extract and a mixture of turmeric and 2% tamarind. The water extract of turmeric and tamarind fruit was treated by giving it as drinking water ad libitum. The treatment was started at the age of 3 days until the age of 5 weeks.

After the broilers were 35 days old, blood was collected from the wing veins, separated from the blood cells to obtain serum. Then the broilers were dissected, the kidneys were separated, washed with 0.9% NaCl solution, macroscopically observed the color of the kidneys, the texture or the presence of abnormalities in the kidney organs, documented with a camera, then put in a collection bottle filled with 10% NBF fixative, then the kidneys were made histological preparations. Histological preparations were made by the paraffin method with Hematoxylin-Eosin staining as was done by (Sudatri *et al.* 2016). Pieces of the kidney that have been fixed with 10% NBF solution, put into a tissue cassette, then do the dehydration process with ethanol solution with a grade of 70%, 80%, 95%, and absolute alcohol with two repetitions, followed by the clearing process with xylol solution for 60 minutes at room temperature. The next process is paraffin infiltration by putting the kidney pieces in liquid paraffin (temperature 60 °C) three times for 45 minutes. Furthermore, the preparation was put in a mold containing liquid paraffin, cooled at room temperature so that it becomes a paraffin block. The next process is paraffin infiltration by inserting the tissue into liquid paraffin (temperature 60°C) three times for 45 minutes each. Paraffin blocks were cut 5µm thick using a rotary microtome, then the incisions were placed on the surface of warm water with a temperature of 45 °C and attached to a object glass that had been coated with gelatin. The preparation is dried by placing it vertically, then placing it on the warmer slide until it sticks to the object glass. Slice of kidney tissue in paraffin stained with Hematoxylin-Eosin were arranged in a rack for staining, then incubated at 60 °C for 45 minutes, then placed at room temperature until cold. Subsequently, deparaffinization was carried out with the stages of dissolving paraffin in xylol 3 times, continued with the rehydration process in alcohol with 100%, 95%, and 80% levels, 70% each stage lasted for 5 minutes, then put in distilled water for 10 dip or until the alcohol dissolves. The next process was staining in hematoxylin by immersing the slides with a solution of hematoxylin for 5 minutes, then washing it in running water for 5 minutes, and then staining it with eosin for 3 minutes. After staining with eosin, the slides were put in an alcohol solution with a grade of 70%, 80%, 90%, to 100% each for 10 dips, then continued with the clearing process using xylol twice for 2 minutes, after which the preparations were covered with a cover glass with a Canadian balsam medium. And the preparations were ready to be observed under a microscope that has been linked to an opticlub camera and a

laptop. The renal histology variables observed were abnormalities in kidney cells, such as tubular degeneration, tubular protein deposits, picnotic cell nuclei, inflammatory cell infiltration, and tubular congestion. Observations were made in 5 visual fields and repeated 3 times. The results were presented as a percentage.

Results

Blood serum creatinine

The mean of broiler blood serum creatinine level test results is presented in Table 1. Homogeneity test of broiler blood serum creatinine level showed that the data distribution was not homogeneous, so the data was tested by using the Kruskal-Wallis test. There was no significant difference in serum creatinine levels in the control and treatment groups.

Table 1: Results of broiler blood serum creatinine analysis using the Kruskal-Wallis test

Variable	Group	Mean Rank	Mean (U/L)	Chi- Square	P
Creatinine	A	13,10	0,76	2,933	0,420
	B	9,90	0,70		
	C	7,30	0,66		
	D	11,70	0,74		

Note: P value <0.05 indicates a significant difference.

The same letter behind the mean value and standard deviation on the same row indicates that the values are not significantly different ($P > 0.05$) between treatments

A: broilers were given drinking water without extract (control)

B: broilers who are given 2% turmeric rhizome water extract

C: broilers who were given drinking 2% tamarind fruit water extract

D: broilers who are given a 2% concentration of water extract mixture of turmeric and tamarind fruit

Kidney histology

The ANOVA test results showed that there was no significant difference in tubular degeneration variables between kidney histology of the control broiler group and histology of the kidneys of the broiler group that were supplemented with water extract of turmeric rhizome and water extract of

tamarind fruit ($P = 0.775$). Likewise, the variable protein deposits in the tubules, picnotic nuclei, inflammatory cell filtration and tubular congestion were tested with the Kruskal Wallis test because the data distribution was not homogeneous, showing no significant differences ($P > 0.05$) between the control group and treatment group.

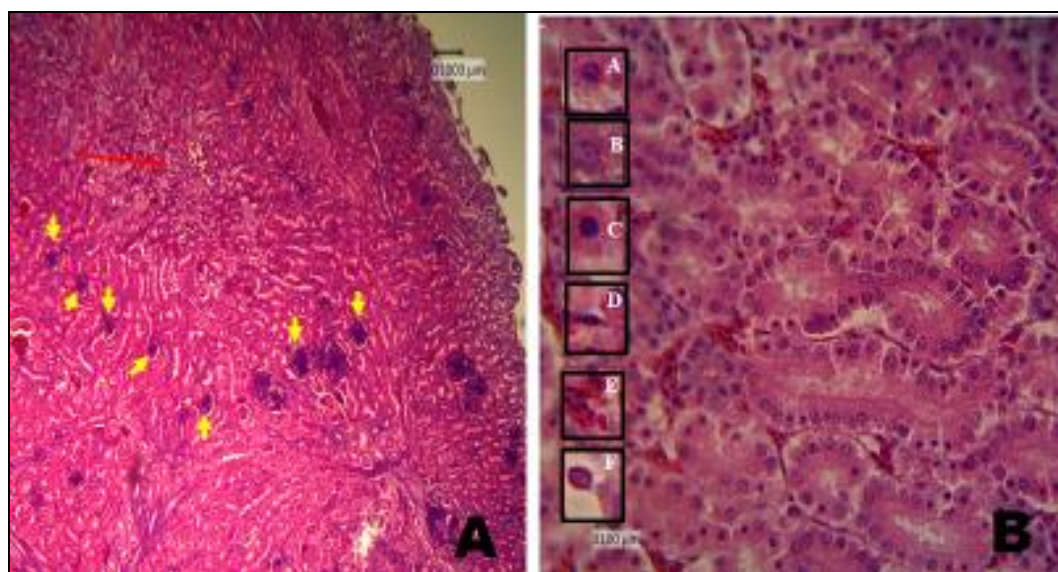
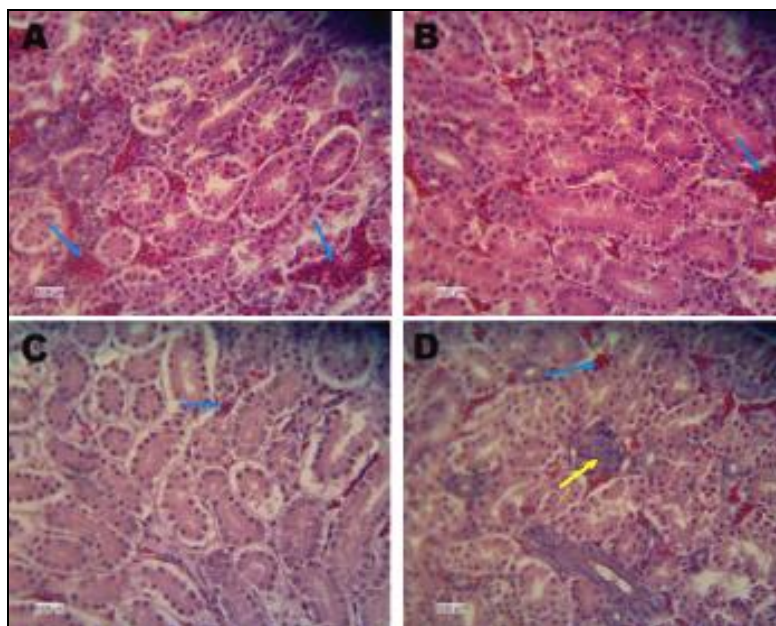


Fig 1: Histology of broiler kidney stained with Hematoxylin-Eosin dye, observed with a microscope connected to an opticlub camera, A=50x magnification, B=400x magnification thumbnail 250 (A=normal, B=hidrofoc degeneration, C=picnotic, D=inflammatory cell infiltration, E=congestion, F=protein deposits) yellow arrow = renal glomerulus, orange arrow = renal tubule



A: renal histologi of broilers supplemented drinking water without extract (control)
 B: renal histology of broilers supplemented drinking water with 2% turmeric water extract
 C: renal histology of broilers supplemented drinking water with 2% tamarind fruit water extract
 D: renal histology of broilers were supplemented with drinking water with a mixture of 2% water extract of turmeric and tamarind fruit

Fig 2: Comparison of the histological appearance of the broiler kidney between control and treatment stained with Hematoxylin-Eosin dye, observed with a microscope connected to an opticlub camera and a laptop with 400x magnification, yellow arrow = glomerulus, blue arrow = kidney tubule

Degeneration is the loss of normal cell structure before cell death which is a sign of the initiation of cell damage due to toxic substances. Cells appear swollen due to changes in cell permeability. Then, the cell nucleus becomes pyknotic, the cell nucleus condenses, looks smaller and darker. Infiltration of inflammatory cells was also found in the renal tubules by approximately 0.1 to 0.6% in both the control and treatment groups. Inflammatory cell infiltration is the presence of

leukocytes into the tissue due to inflammation. In this study also found the presence of protein deposits in the tubules and congestion, but the percentage was very low. This protein deposition was caused by an increase in osmotic pressure in the interstitial fluid so that filtration and reabsorption in the tubule is disturbed. Whereas congestion was an increase in blood cells in tissues undergoing a pathological process (Assiam *et al.* 2014) [2].

Table 2: Histological analysis results of broiler kidney treated with water extract of turmeric and tamarind fruit using the Kruskal-Wallis test

Variable	Group	Mean Rank	Mean (%)	Chi-Square	P
Protein deposits in the tubules	A	14,40	1,27	7,72	0,052
	B	13,30	1,07		
	C	4,40	0,13		
	D	8,90	0,22		
Inflammatory cell infiltration	A	9,50	0,22	5,10	0,164
	B	10,20	0,22		
	C	15,10	0,67		
	D	7,20	0,19		
Picnotic cell	A	9,30	1,08	1,76	0,624
	B	11,90	2,69		
	C	12,50	2,62		
	D	8,30	2,05		
Tubular congestion	A	11,30	0,33	0,75	0,861
	B	8,80	0,87		
	C	10,40	0,93		
	D	11,50	0,96		

Note: P value <0.05 indicates a significant difference. The same letter behind the mean value and standard deviation on the same row indicates that the values are not significantly different (P> 0.05) between treatments

A: broilers were given drinking water without extract (control)

B: broilers who are given 2% turmeric rhizome water extract

C: broilers who were given drinking 2% tamarind fruit water extract

D: broilers who are given a 2% concentration of water extract mixture of turmeric and tamarind fruit

Discussions

To analyze the effect of giving water extract of turmeric and tamarind fruit continuously on the performance of broiler kidneys, it was measured from data on serum creatinine levels and kidney histology. Creatinine is the result of muscle metabolism which is filtered by the kidneys and excreted in the urine. If the kidneys are damaged / damaged, creatinine cannot be filtered and excreted in the urine so that the levels in the blood increase (Thomas *et al.* 2017) [121]. Serum creatinine levels also correlate with glomerular filtration rate (Lane *et al.* 2010) [110]. In this study, creatinine levels and renal histological features between control broilers and treated broilers did not show any significant differences. Although there are some kidney cells becoming degeneration, pycnosis nuclei and inflammatory cell filtration, these were only mild symptoms. The same symptoms also occurred in the kidney histology of the control broiler group. This indicates that giving water extract of turmeric and tamarind fruit continuously until harvest did not interfere with the performance of the broiler kidneys. In fact, turmeric was actually renoprotective of the kidneys (Shalaby and Hammouda 2014) [117]. The same thing was also said by Kertia *et al.* (2012) [7] that therapy using turmeric extract could reduce levels of BUN and creatinine in osteoporosis sufferers. The content of flavonoids in tamarind fruit is antioxidant, able to protect cells from free radicals that cause damage to cells (Martinello *et al.* 2006) [112]. The continuous application of turmeric rhizome extract and tamarind water extract did not cause serious damage to the histopathology of the broiler kidneys. The use of natural ingredients for treatment for a long term was much safer without side effects (Alok *et al.* 2013) [1].

Conclusion

Supplementation of water extract of 2% turmeric rhizome and water extract of 2% tamarind fruit continuously on broilers until harvest, did not have a negative effect on kidney performance and serum creatinine levels.

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