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Serum glutamate pyruvate transaminase (SGPT) levels and histopathological of KUB chicken liver given water extract of neem leaves (*Azadirachta indica*) in drinking water

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Abstract
Azadirachta indica A. Juss or neem is herbal that contains antioxidants and bioactive compounds of alkaloids, steroids, flavonoids, saponins and tannins that can play a role in various biological activities. The purpose of this study was to determine the effect of water extract of neem leaves mixed in drinking water on the liver health performance of KUB chickens. The observed variables were serum glutamate pyruvate transaminase (SGPT) levels and liver histopathology. The experimental animals used were KUB chickens (Kampung Unggul Balitbangtan), using a Completely Randomized Design (CRD) with four treatments and five replications. The treatment group consisted of P0 (control or 0%), neem leaf water extract 1% (P1), 3% (P2) and 5% (P3) administered during the starter phase (42 days). The experimental animals used were 200 tails. Preparation of liver histology preparations by paraffin method. SGPT levels were tested by the IFCC2 Enzymatic Kinetic method (International Federation of Clinical Chemistry and Laboratory Medicine). The results showed that KUB chicken SGPT levels were not significantly different between control and treatment. Liver histology data showed inflammatory cell infiltration damage, degeneration, necrosis, and vessel congestion but there was no significant difference between control and treatment chicken livers. Water extract supplementation of neem leaf has no negative effect on liver performance and SGPT levels

Keywords: Neem, *Azadirachta indica*, liver histology, SGPT, KUB chicken

Introduction
Health is one of the determining factors for the success of livestock. The use of antibiotics to control disease can stimulate livestock growth, but the use of antibiotics can be dangerous from antibiotic residues that will enter products including chicken meat which has an impact on the emergence of resistant microbes. To avoid this, natural antibiotics are safer but more effective. Neem (*Azadirachta indica*) is a plant that is often used as an herbal plant for both medicine and additional feed. The crude protein content of neem leaves is 15.8%, crude fiber is 14.6% and BETN 56.6% [1]. Neem leaves contain about 20.69% crude protein and 4.1% fat after being processed into neem flour through drying and milling processes [2]. This percentage varies depending on the nutrient composition in the soil where the neem plant grows. Neem contains phytochemicals such as Azadirachtin, Nimbidin, Nimbin, Nimbinin, Nimbidinin, Nimbolide, Nimbidic acid, Nimbidin and Sodium Nimbidate which have various pharmacological effects such as antipyretic, antiviral, analgesic, antibacterial, contraceptive and hepatoprotective effects and many more [3]. Some of these ingredients are toxic, such as nimbin, nimbidin, salannin and azadirachtin [4]. Neem leaves contain bioactive compounds of alkaloids, steroids, flavonoids, saponins and tannin [5, 6], Research by adding neem leaf powder to broiler feed at a rate of 2.5%; 5%; 7.5% and 10%, showing the results that there are significant differences in the concentrations of cholesterol, glucose, SGPT (Serum Glutamic Pyruvate Transaminase) and SGOT (Serum Glutamic Oxaloacetic Transaminase) but there is no significant difference between total protein, calcium, and sodium [7]. Another study on the addition of neem leaf powder to laying birds concluded that the addition of neem leaf meal up to 15% could increase egg production but did not affect physiological parameters,

hematological structures. Relatively small body weight can be caused by nutritional imbalances and nutrient utilization [8]. Research conducted on 7-day-old broiler chickens given 10% aqueous extract of neem leaves after being infected with *Escherichia coli* O78 showed that neem leaves act as an antibiotic by suppressing the growth of *E. coli* and affect the value of Serum Glutamic Pyruvic Transaminase (SGPT) [9]. Serum Glutamic Pyruvic Transaminase (SGPT) is an aminotransferase enzyme that is more sensitive to acute liver damage. This enzyme will be released into the blood if the liver cells are disturbed, irritated or damaged in the liver cells. One of the roles of SGPT compounds is that they are needed in the process of gluconeogenesis. This gluconeogenesis process will convert energy sources such as fatty acids and amino acids into glucose. Glucose is a source of energy needed for cell activity, development and growth [10].

The liver has three basic functions, namely forming and secreting bile into the intestinal tract; plays a role in various metabolisms related to carbohydrates, lipids and proteins; filter the blood and get rid of bacteria or foreign objects that enter the blood. The liver synthesizes heparin which is an anticoagulant substance and has an important detoxification function [11]. In carrying out the detoxification function, compounds that have toxic properties to body cells are converted by hepatocyte enzymes through oxidation, hydrolysis, or conjugation [12] into compounds that are no longer toxic, and are then carried by the blood to the kidneys for excretion [13]. Liver cell damage starts from the degeneration process, namely cell swelling with reversible changes [14]. The next stage of liver cell damage is necrosis which is characterized by irreversible changes or cannot return to normal. Cell necrosis is characterized by swelling and cell leakage, infiltration of inflammatory cells and changes in the nucleus due to specific DNA breakdown in the form of karyolysis, pyknosis and karyorrhexis [15]. The liver is the main organ of metabolism that is often damaged but has a high regeneration capacity [13]. One of the herbal medicines that work as a hepatoprotector is neem leaves. This has been investigated by [16] who examined the effect of giving neem leaf extract on SGPT activity in male wistar rats induced by high doses of paracetamol. Neem is able to function as a hepatoprotector.

Materials and Methods

The research design used was a Completely Randomized Design (CRD) with treatment with neem leaf extract (*Azadirachya indica*) mixed into drinking water. The neem leaves are taken from the neem plant that grows around the Udayana University Bukit Jimbaran campus in Bali. The experimental animal used was Day Old Chick (DOC) KUB (Kampung Unggul Balitbangtan).

Research procedure

The leaves of *A. indica* used were mature leaves, namely leaf number 3 from top to bottom [17]. The leaves are removed from the petiole then washed and blended with water in a ratio of 1:1. Filtered to obtain 100% aqueous extract. Then diluted to a concentration of 1%, 3% and 5%. This water extract is made every time it is going to give water to the treated KUB chickens. The study used 4 treatment groups, namely P0 (Control or without the addition of neem leaf water extract), P1 (given 1% neem leaf water extract in drinking water), P2 (given 3% neem leaf extract in drinking water) and P3 (Given extract Neem leaf water 5% in drinking water). Each group consisted of 5 replications and each replication consisted of 10 chickens. So the total number of chickens used was 200. DOCs were first kept for 1 week for adaptation and selection, then their initial body weight was weighed to be selected randomly and grouped. KUB chicken weight will be weighed every week to see the weight gain. The reared chickens were placed in a plotted litter system cage and each plot was separated by a wire screen. The chicken feed given was feed for the starter phase chicken, namely CP511B Charoen Pokphand ad libitum. KUB chickens are kept until the age of 6 weeks (42 days) by always paying attention to health, cage sanitation and environmental conditions.

After the KUB chickens are 42 days old, it will be continued with blood sampling. Blood was taken from the wing vein using a 3ml syringe and the blood was collected in the Vaculab tube without EDTA. Then it is left until the blood cells settle and a clear part is obtained which is called serum. To obtain the liver, the chicken is slaughtered first, then dissected and the liver is separated and washed with 0.9% NaCl solution, macroscopically observed for color, texture or abnormalities in the liver and documented in the form of photos. Then the organs were put into a collection bottle containing 10% NBF fixative solution for further histological preparations. Preparation of liver histology preparations using paraffin method and hematoxylin-Eosin staining [18]. Histological observations of liver incision preparations were observed under a microscope with a magnification of 10 x 40. The number of all liver cells per field of view was counted, both normal and abnormal. To calculate the percentage of abnormal cells, the number of abnormal cells was calculated divided by the number of all cells per field of view, then multiplied by 100%. Observations were repeated 5 times the field of view for each preparation. The results obtained were then averaged. For qualitative data, images of liver histology preparations were photographed with an optilab camera connected to a computer [19]. The quantitative data obtained were analyzed statistically using SPSS Software and if there was a significant effect, it would be continued with Duncan's test at levels α=0.05 and α=0.01 and qualitative data is presented in the form of images. Liver histology variables observed were abnormalities in liver cells such as degeneration, pyknotic cell (necrosis), inflammatory cell infiltration and vessel congestion. SGPT examination is carried out at UPTD. Bali Provincial Health Laboratory Center using the IFCC2 Enzymatic Kinetic method (International Federation of Clinical Chemistry and Laboratory Medicine). This research has obtained approval from the Animal Ethics Commission, Faculty of Veterinary Medicine, Udayana University with Certificate of Approval of Animal Ethics No: B/209/UN14.2.9/PT.01.04/2021

Results

Based on the results of laboratory tests, the results of serum glutamate pyruvate transaminase (SGPT) values are as follows (Table 1)

Table 1: Average SGPT levels of KUB chickens treated with extract neem leaf water followed by Duncan's test.

Treatment of water extract of neem leaves (*A. indica*)	SGPT level (µ/L)
P0 (control)	11,20±1,48a
P1 (1%)	11,60±3,65a
P2 (3%)	12,60±3,13a
P3 (5%)	13.60±8,02a
Normal value	23,8-52,8

Description: Different letters behind the average value in the same column showed a significantly different value (p<0.05) between treatments.

Blood profile examination was carried out at the Bali Provincial Health Laboratory (2021).

Histological description of KUB chicken liver to see any abnormalities or damage that occurs due to the administration of neem leaf water extract in drinking water shows the picture as shown in Figure 1. Histological preparations were made by paraffin method and Hematoxylin-Eosin staining, then observed with a microscope connected to an optical lab camera. And a laptop at 100x and 400x magnification. Histopathological evaluation to assess abnormalities using a grading system or a score based on percentage of cells damaged.

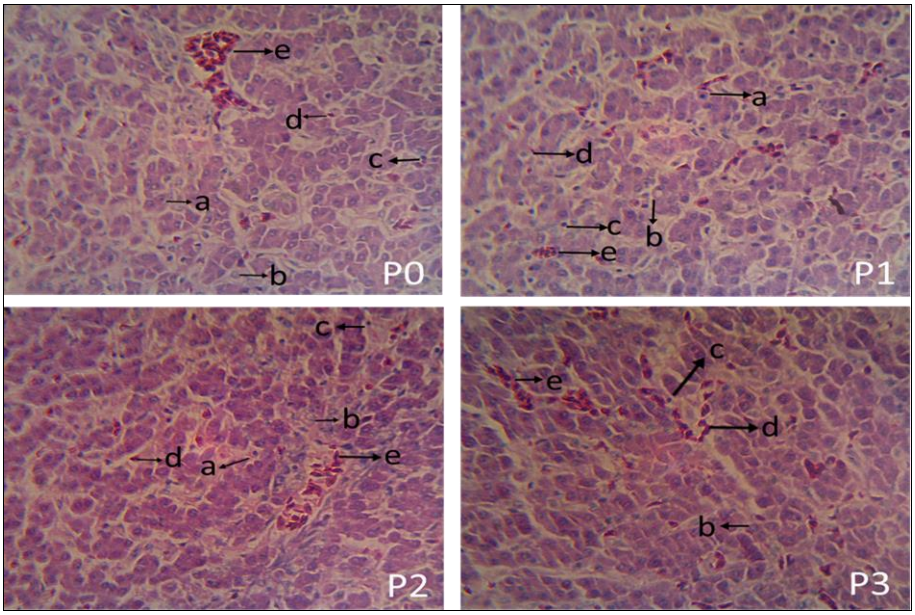

Fig 1: Histology of KUB chicken liver stained with Hematoxylin-Eosin stain, observed with a microscope connected to an opticallab camera and laptop with 400x magnification. P0 = control (0%), P1 (water extract of neem leaves 1%), P2 (water extract of neem leaves 3%), P3 (water extract of neem leaves 5%). a. Normal cells, b. degeneration c. necrotic/pyknotic cells, d. inflammatory cell infiltration, e. vessel congestion

Based on histological observations on KUB chicken liver preparations treated with neem leaf extract (*A.indica*) it was seen that there was some damage, namely cell degeneration, cell necrosis or pyknotic, inflammatory cell infiltration and vessel congestion. Observations of damage in each group were observed and counted in four fields of view of the preparations and five replications. The results of the analysis of KUB chicken liver histology observations are shown in Table 2.

Table 2: Histological analysis results of KUB chicken liver treated with leaf water extract Neem (*A. indica*)

Variable	Treatment			
	P0 (control)	P1 (1%)	P2 (3%)	P3 (5%)
Inflammatory cell infiltration (%)	8, 20±2, 95b	4, 60±1, 14a	5, 80±1, 10ab	7, 60±1, 14b
Degeneration (%)	9, 00±1, 00a	8, 80±0, 84a	13, 60±3, 36b	14, 00±3, 81b
Necrosis/pyknotic cell (%)	19, 60±5, 03a	22, 40±4, 34a	31, 00±6, 52b	29, 80±2, 59b
Vessel congestion (%)	4, 20±1, 30b	1, 80±0,45a	2, 40±0, 55a	4, 20±1, 64b

Description: Different letters behind the average value in the same column showed a significantly different value (p<0.05) between treatments.

Histological observations of inflammatory cell infiltration, degeneration and vessel congestion were found to be the least in the treatment with 1% neem leaf extract (P1), while the least necrosis was found in the control (P0). The degree of damage was classified into 4 groups based on the nonalcoholic steatohepatitis (NASH) scoring system, namely normal when the damage occurred was in the range of 0-5% of all hepatocytes; mild (5-33%), moderate (33-66%) and massive (p>66%) [20]. Based on this scale, all liver abnormalities that occurred in this study were still in a condition of mild damage (5-33%).

Discussion

Glutamic Pyruvic Transaminase (GPT) is an enzyme that is

more sensitive to acute liver damage. This enzyme will be released into the blood if the liver cells are disturbed, irritated or damaged in the liver cells. Referring to the results in table 1, it can be seen that the serum SGPT levels of control KUB chickens were the lowest when compared to the group of KUB chickens treated with neem leaf extract. SGPT levels in the treatment group P1 11.60µ/L, P2 12.60µ/L and P3 13.60µ/L were all higher than P0 11.20 /L, but all SGPT levels were still below the normal range (23.8-52.8µ/L) and statistically not significantly different between control (P0) and treatment. Increased levels of SGPT in the treatment groups P1 (1%), P2 (3%) and P3 (5%) could be caused by stress factors, because animals were treated with drinking water mixed with neem leaf water extract every day. The P3 treatment which received an additional 5% of neem leaf water extract showed the highest levels of SGPT. This can be interpreted that the higher the concentration of water extract of neem leaves given will because the experimental animals to experience more stress. The phytochemical content of neem leaves such as flavonoids, phenols and tannins will cause drinking water to be more concentrated and the taste to be bitterer. This condition is thought to cause chickens to become stressed. In addition, it can also be caused by other factors that cannot be controlled by researchers such as room temperature and humidity of the cage [21]. Stress conditions can lead to an increase in ACTH (adrenocorticotropic hormone) secretion which will reach the adrenal glands through blood circulation and stimulate the formation of corticosteroids which play a role in stimulating the release of glucocorticoid hormones. Glucocorticoid hormones will affect the function of the liver by increasing metabolic processes in the liver, namely through the process of gluconeogenesis. This gluconeogenesis process will convert energy sources such as fatty acids and amino acids into glucose in an effort to produce more energy [10]. SGPT enzyme is one of the enzymes needed in the process of gluconeogenesis which will catalyze chemical reactions in liver cells. If the liver is overworked, it can cause liver cell damage.

Factors that affect the work of the liver include repeated intake such as substances contained in neem and dosage. Excessive doses and repeated intakes have the potential to cause damage to body organs, especially the liver which acts as a detoxification organ [22]. Based on the histopathological observations of KUB chicken liver, it can be seen that liver abnormalities were found in all treatment groups. In treatment P1 (1%) obtained the smallest percentage of inflammatory cell infiltration, degeneration and congestion of vessels. So it can be stated that the administration of 1% water extract of neem leaves is the best concentration because it is able to act as a hepatoprotector. Likewise with necrosis, administration of 1% water extract of neem leaves was not significantly different from the control and still has less value than the 3% and 5% ones. The range of values for all variables of liver damage was between 1.80±0.45% (vessel congestion P1) to 31.00±6.52% (necrosis P2). Based on the nonalcoholic steatohepatitis (NASH) scoring system, it is normal if the damage that occurs is in the range of 0-5% of all hepatocytes; mild (5-33%), moderate (33-66%) and massive (>66%) [20]. Based on this scale, all liver abnormalities that occurred in this study were still in a condition of mild damage (5-33%). This is also corroborated by the research of [21] which found that mice given ethanol extract of neem leaf 1.35 mg/grBB had more liver damage than mice given 0.7 mg of neem leaf ethanol extract. /grBW and 0.9 mg/gBW. It is suspected that excessive doses of neem leaf ethanol extract also have an effect on causing liver cell damage. The ethanol extract of neem leaves (*Azadirachta indica*) contains active compounds such as flavonoids (quercetin and rutin) [3]. Flavonoids are one of the antioxidant groups, which can inhibit the oxidation process due to free radicals. Hepatocytes may still detoxify toxic substances because changes in hepatocytes are relatively mild so they do not interfere with the detoxification process [23]. This mild damage can be returned to normal if the treatment is stopped, but if this situation continues it will result in cell death (necrosis). Giving an herbal ingredient containing flavonoid compounds, phenols and tannins in the right amount can suppress cell damage. *Azadirachta indica*, is also able to track levels of Glutamate Oxalolate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT), Alkaline Phosphatase (ALP), Serum Bilirubin and increase total protein. Thus, this plant clearly informs the functional improvement of liver cell status [24]. This damage occurs due to the presence of free radicals, disease or toxic agents that can cause bleeding in the blood capillaries or inflammation occurs because the blood vessels are dilated so that the vascularity at the injury site widens and contains blocked blood [25].

Conclusion

The addition of 1% neem leaf water extract (*A. indica*) into the drinking water of KUB chicken starter phase was able to act as a hepatoprotector by providing protection to the function and histological structure of the liver. The level of water extract of Neem leaves (*A. indica*) from a concentration of 1% to 5% had no negative effect on liver performance and SGPT levels of KUB chicken.

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