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Research Article – Biochemistry

Influence of hempedubumi (*Andrographis paniculata*) on serum biochemical parameters and liver morphology in broiler chickens

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Abstract

Serum biochemical parameters and liver morphology were evaluated in broilers fed diets supplemented with hempedubumi (*Andrographis paniculata,* AP) ground leaf. Birds (n = 160) were assigned in four treatment groups with five replications and each group contain eight birds. Broilers in the control group were fed the basal diet without additives, the group dietary treatments, Diet AP2 (Basal diet + 2g/kg *Andrographis paniculata*); Diet AP4 (Basal diet + 4g/kg *Andrographis paniculata*) and Diet AP8 (Basal diet + 8g/kg *Andrographis paniculata*). During day-21 and day-42, five birds from each treatment groups were randomly selected for serum biochemical analysis which involves pancreatic, renal, and hepatic functions (aspartate transaminase (AST), alkaline transaminase (ALT), alkaline phosphatase (ALP), glucose, cholesterol, triglycerides, total protein, albumin, urea, sodium, potassium, chlorine, and globulins). Liver morphology was determined after slaughtering two birds and collected the liver samples. Based on these results, AP at 8g/kg diet lowered serum cholesterol, glucose, and triglycerides. It also maintains the normal structure of liver indicating that no toxic effect from AP supplementation at a rate up to 8 g/kg, it also that AP lowered ALP and ALT in blood of chicken. In conclusion, supplementation of AP at 8g/kg in diet has the potential to contribute good health and safe for broiler chicken.

Keywords: *Andrographis paniculata*, broiler chicken, liver, serum

Introduction

In recent years, various spices and herbs have been extensively studied to search for natural sources which could be used as feed additives and growth promoters in animal nutrition (Abu-Dieyeh and Abu-Darwish, 2008; Najafi and Torki, 2010; Malahubban *et al*., 2013a). These phytogenic additives may have more than one mode of action, including improving flavour, stimulating the secretion of digestive enzymes, increasing gastric, antimicrobial, antihelminthic, immune stimulation, anti-inflammatory and anti-oxidative (Dhama*et al*., 2015). *Andrographis paniculata* Nees, (Acanthaceae) or local name known in Malaysia as '*HempeduBumi*'or King of bitter is common in Southeast Asia, India and China. *A. paniculata* has been traditionally used as an antioxidant, antiviral, anti-inflammatory, immune enhancing and hepatoprotective agents (Prajjal *et al*., 2003). Various reports stated the beneficial properties of *A. paniculata* such as on anti-cancer, anti-aging, and anti-dengue (Hsieh*et al*., 2015; Edwin*et al*., 2016; You *et al*., 2016).

However, limited number of publications reported the potential use of medicinal plants in broiler feeding. Mathivanan and Kalaiarasi (2007) reported that panchagavya, an ayurveda formulation supplemented with *A. paniculata* improved the immune status of broilers. Many studies have also been conducted on the effects of dietary herbs or spices on the growth performance of chicken, but very little studies are available on the effect of herbs on serum biochemical parameters and liver morphology in broiler chickens. While some reports (Mathivanan and Kalaiarasi, 2007; Kirkpinar *et al*., 2011; Malahubban and Zakry, 2016) demonstrated that adding herbs were not affecting serum biochemical status and liver morphology. However, according to Esonu *et al*. (2001), haemotological elements pointing to the physiological responsiveness of the animals and changes in diet will strongly affecting haematological traits (Church *et al*., 1984; Babatunde *et al*., 1987). Therefore, present study aimed to expand the information on the effect of ground *A. paniculata* leaf on serum biochemical properties and liver morphology of broiler chickens.

Materials and methods

Andrographis paniculata (AP) seedlings were grown in black polythene bags containing a mixture of soil, sand, and peat moss (2:1:1) under nursery conditions. At week-10 of planting, *A. paniculata* plants were harvested for leaves and were prepared as previously described (Malahubban *et al*., 2013a; Malahubban and Zakry, 2016).

One hundred and sixty (160) one-day old male broiler chickens (Cobb 500) obtained from a local hatchery were randomly assigned into 20 cages ((122 cm (length) x 91 cm (width) x 50 cm (height)) and warmed with 24 hours two-incandescent light bulb (60 watts) lighting for seven days located at each cage (approximate temperature at 32 °C with humidity recorded as 62 to 90%). From day-8, birds were maintained at ambient conditions (temperature at 28±2 °C, and humidity at 60 to 89%) with no lighting and *ad libitum* access to water and diet (commercial broiler starter (0–20 days) diets). At day-7 and day-21, the chicks were vaccinated to prevent Newcastle disease (Animal Health,

Table 1: Ingredients in the dietary treatments and nutritional analysis.

	Dietary treatments[1]			
Ingredients	**C**	**AP2**	**AP4**	**AP8**
Corn	61.00	60.80	60.60	60.20
Soy Bean Meal (SBM) (44%)	25.00	25.00	25.00	25.00
Fish Meal	6.41	6.41	6.41	6.41
Palm Oil	5.00	5.00	5.00	5.00
Limestone	1.26	1.26	1.26	1.26
Salt	0.28	0.28	0.28	0.28
Dicalcium Phosphate (DCP)	0.10	0.10	0.10	0.10
Mineral Mix[a]	0.25	0.25	0.25	0.25
Vitamin Mix[b]	0.25	0.25	0.25	0.25
L-Lysine	0.20	0.20	0.20	0.20
DL-Methionine	0.15	0.15	0.15	0.15
Choline cloride	0.10	0.10	0.10	0.10
Andrographis paniculata (g/kg)	-	2.00	4.00	8.00
Calculated analysis (%)				
Metabolize Energy (ME) Kcal/kg	3201	3201	3201	3201
Crude Protein, %	20.00	20.00	20.00	20.10
Crude Fibre, %	4.35	4.36	4.36	4.38
Crude Fat, %	3.21	3.21	3.21	3.21
Calcium, %	0.99	0.99	0.99	0.99
Available P, %	0.33	0.33	0.33	0.33

[a]Premix provided per kg of diet: Mg = 56 mg; Fe = 20 mg; Cu = 10 mg; Zn = 50 mg; Co = 125 mg; I = 0.8 mg.
[b]Premix provided the following per kg of diet: Vitamin A = 50 MIU; Vitamin D3 = 10 MIU; Vitamin E = 75 MIU; Vitamin K = 20 g; Vitamin B1 = 10 g; Vitamin B2 = 30 g; Vitamin B6 = 20 g; Vitamin B12 = 0.10 g; Calcium D-Panthothenate = 60 g; Nicotinic acid = 200g; Folic acid = 5 g; Biotin = 235 mg.
[1]Diet C = Control (0 g/kg medicinal herb; A. paniculata); DietAP2 = 2g/kg *A. Paniculata* (146.8mg Andrographolide/kg); DietAP4 = 4g/kg *A. paniculata* (293.6mg Andrographolide /kg); Diet AP8 = 8g/kg *A. paniculata* (587.2 mg Andrographolide /kg).

Fort Dodge, Iowa, USA) at interocular and intranasal, respectively. At 21 days old, the chicks weight was averaged and assigned to four different dietary treatments where each treatment contained five replicates of eight broilers in each replicate. The feeding trial was started from day-21 and ended day-42 where in between imposed with the following dietary treatments: 1) Diet C (Control, basal diet); 2) Diet AP2 (Basal diet + 2g/kg *A. paniculata*); 3) Diet AP4 (Basal diet + 4g/kg *A. paniculata*) and 4) Diet AP8 (Basal diet + 8g/kg *A. paniculata*). Dietary treatments were in accordance with National Research Council (NRC, 1996) and level of inclusion was in quadratic increments (Table 1).

The daily dietary schedule and procedures during slaughtering at day-42 were described as in Malahubban *et al*. (2013a). Two birds (at day-42) per replicate were randomly selected, weighed to the nearest gram and slaughtered by severing the carotid artery and jugular veins (Halal method). Birds processing as described in the previous study (Malahubban *et al*., 2013a). After the process, ten birds in each treatment were selected for liver morphological examination.

Five birds from each treatments group were randomly selected and blood samples (4.0 mL) were collected from the wing vein using a 23-ga needle at day-21 and day-42. Immediately after collection, serum was separated by centrifugation at 3000 g for 10 min. The serum samples were stored at minus 20 °C for the analyses of serum glucose, cholesterol, triglycerides, albumin, total protein, sodium (Na), potassium (K), chlorine (Cl), urea, aspartate transaminase (AST), alkaline transaminase (ALT), and alkaline phosphatase (ALP) and were measured by specific commercial kits (Roche Diagnostica, Basal, Switzerland) using an auto-analyser (HITACHI 902, Automatic Auto-analyser). The serum globulin was calculated by subtracting serum albumin from serum total protein levels.

The liver of 42-day-old birds was removed, weighed, and preserved. The liver was preserved in 10% buffered formaldehyde for at least 48 hours and then undergone a series of dehydration and clearing process using a histokinette (Leica® ASP 300). As small fraction of the liver was transferred into tissue cassette, the sample was rinsed in graded series of alcohol baths beginning with 70% alcohol and increasing the concentration up to 100% alcohol. The whole procedures of tissue processing in the histokinette took 16 hours (pre-set time) to complete. To stabilize tissue structure, the liver was transferred into warmed wax (pre-set temperature at 58 °C) and immediately frozen. Subsequently, processed liver was embedded in paraffin (wax) to form paraffin blocks and serially sectioned using a microtome (Leica® Model RM2155) at five µm thickness. Paraffin was first removed from the tissue sections using xylene and then soaked in a descending series of 100%, 95% and 70% alcohol baths forxylene and formalin fixative removal. These sections were stained with differential double stained haematoxylin and eosin and then visually examined using light microscope (Leica®) at 10X, 20X and 40X magnifications and observed and recorded for any histological changes and/or abnormalities.

Statistical data analysis was carried out using the SPSS software (IBM SPSS version 21). Differences between means for serum biochemical properties among treatments were analyzed for ANOVA followed by Duncan's test at the 0.01 level of significance.

Result

At day-21, data were recorded as a baseline as no dietary treatments inclusion except commercial feed, therefore, no significant differences of serum samples were detected among broiler chickens (Table 2). At day-42, birds fed on Diet AP2 (2.96 mmol/L) and Diet AP8 (2.85 mmol/L) showed significantly lowered serum cholesterol

Table 2: Serum biochemical parameters of broilers at initial (day-21) and at the end (day-42) of dietary treatments (Mean±SE).

Parameters	Day	Diet[1]			
		C	AP2	AP4	AP8
Cholesterol (mmol/L)	D_{21}	2.69±0.27	2.63±0.31	2.74±0.16	2.68±0.24
	D_{42}	3.78±0.20^{a}	2.96±0.18^{b}	3.58±0.18^{a}	2.85±0.01^{b}
Glucose (mmol/L)	D_{21}	5.40±0.40	5.10±0.32	5.31±0.34	5.60±0.39
	D_{42}	5.52±0.46^{a}	1.94±0.22^{b}	2.91±0.32^{b}	2.30±0.05^{b}
Triglycerides (mmol/L)	D_{21}	0.96±0.11	0.90±0.09	1.10±0.15	13.70±1.32
	D_{42}	0.99±0.12^{a}	0.63±0.06^{ab}	0.64±0.07^{b}	0.49±0.22^{c}
Albumin(g/L)	D_{21}	11.50±0.87	10.97±0.30	10.64±1.00	14.42±0.88
	D_{42}	16.27±1.17	13.23±1.24	12.85±1.31	19.19±2.36
Total protein (g/L)	D_{21}	24.30±1.83	22.18±1.10	19.14±1.08	0.94±0.13
	D_{42}	26.63±2.62	26.84±2.14	22.53±1.37	23.48±1.96
Globulin (g/L)	D_{21}	12.90±5.15	11.21±1.64	8.50±1.41	5.49±2.53
	D_{42}	10.37±2.20	13.61±2.52	9.68±1.53	9.05±2.10

Different letters within the same column differ significantly (P<0.01).
[1]Diet C = Control (0 g/kg medicinal herb; *A. paniculata*); DietAP2 = 2 g/kg *A. paniculata* (146.8mg Andrographolide/kg)*;* DietAP4 = 4 g/kg *A. paniculata* (293.6mg Andrographolide/kg)*;* Diet AP8 = 8 g/kg *A. paniculata* (587.2 mg Andrographolide/kg).

Table 3: Sodium, potassium, chlorine, and urea levels in serum of broiler fed on diets supplemented with different rates of *A. paniculata* ground leaf (Mean ± SE).

Parameters	Day	Diet[1]			
		C	AP2	AP4	AP8
Na (mmol/L)	D_{21}	114.2 ± 6.9	110.9 ± 6.6	126.0 ± 8.9	127.3 ± 8.4
	D_{42}	124.3 ± 9.6^{b}	135.9 ± 3.2^{ab}	140.4 ± 0.3^{ab}	150.0 ± 0.2^{a}
K (mmol/L)	D_{21}	15.2 ± 1.3	13.2 ± 1.4	16.9 ± 2.2	17.9 ± 2.0
	D_{42}	19.3 ± 0.4	19.2 ± 2.3	19.8 ± 0.4	19.1 ± 1.2
Cl (mmol/L)	D_{21}	79.8 ± 2.4	69.3 ± 3.0	74.6 ± 3.7	75.6 ± 4.4
	D_{42}	77.1 ± 6.8^{b}	86.6 ± 2.2^{ab}	97.6 ± 0.1^{a}	89.9 ± 0.3b^{a}
Urea (mmol/L)	D_{21}	0.33 ± 0.04	0.28 ± 0.04	0.24 ± 0.03	0.24 ± 0.02
	D_{42}	0.77 ± 0.04^{a}	0.39 ± 0.02^{b}	0.44 ± 0.03^{b}	0.37 ± 0.03^{b}

Different letters within the same column differ significantly (P<0.01).
[1]Diet C = Control (0 g/kg medicinal herb; *A. paniculata*); DietAP2 = 2 g/kg *A. paniculata* (146.8mg Andrographolide/kg)*;* DietAP4 = 4 g/kg *A. paniculata* (293.6mg Andrographolide/kg)*;* Diet AP8 = 8 g/kg *A. paniculata* (587.2 mg Andrographolide/kg).

Table 4: Serum enzymes in broiler fed different dietary treatments (Mean ± SE).

Parameters	Day	Diet[1]			
		C	AP2	AP4	AP8
AST (U/L)	D_{21}	199.4±10.6	179.4±20.7	195.0±20.7	178.7±17.3
	D_{42}	246.0±17.3	247.9±10.9	234.0±11.1	213.7±7.8
ALT (U/L)	D_{21}	4.72 ±0.71	4.00±0.34	4.20±0.65	5.09± 0.58
	D_{42}	5.07 ±0.51^{a}	3.67±0.52^{b}	2.95±0.50^{b}	2.94±0.27^{b}
ALP (U/L)	D_{21}	1876.0±209.6	1685.3±129.0	1892.3±184.2	1314.4±124.6
	D_{42}	1745.8±211.9^{a}	1438.2±413.6^{ab}	764.0±60.3^{b}	653.0±109.9^{b}

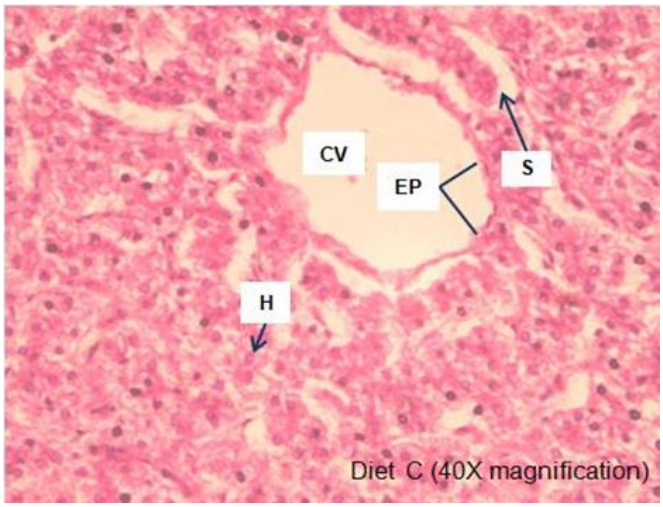
Different letters within the same column differ significantly (P<0.01).
[1]Diet C = Control (0 g/kg medicinal herb; *A. paniculata*); DietAP2 = 2 g/kg *A. paniculata* (146.8mg Andrographolide/kg)*;* DietAP4 = 4 g/kg *A. paniculata* (293.6mg Andrographolide/kg)*;* Diet AP8 = 8 g/kg *A. paniculata* (587.2 mg Andrographolide/kg).

level as compared to Diet C (3.78 mmol/L). In the present study, serum glucose of broilers fed Diet AP2 (1.94 mmol/L), Diet AP4 (2.91 mmol/L) and Diet AP8 (2.30 mmol/L) at day-42 were significantly lower as compared to Diet C (5.52 mmol/L). In general, serum glucose level of broilers fed on AP was decreased. Serum triglycerides level of broilers decreased significantly in broilers fed Diet AP4 (0.64 mmol/L) and Diet AP8 (0.49 mmol/L) as compared to those fed Diet C (0.99 mmol/L). The albumin, total protein, and globulin in serum of broilers fed on different dosages of AP leaves were unchanged (Table 2).

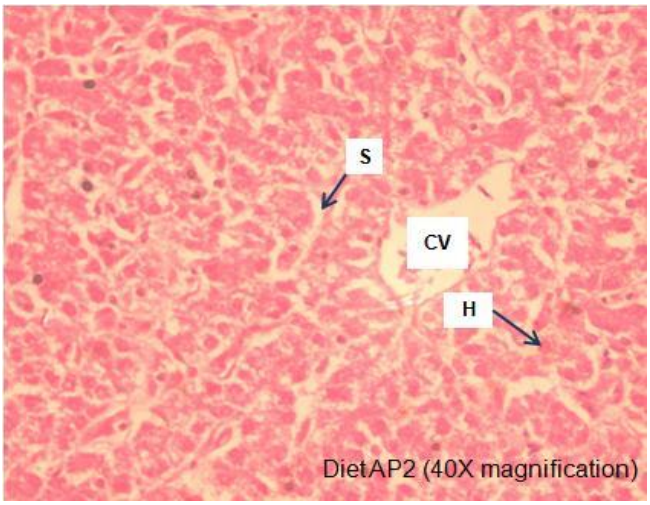
The serum sodium level in broilers was significantly higher in birds fed Diet AP8 (150 mmol/L) compared to Diet C at 124.3 mmol/L (Table 3). Chlorine serum was also significantly higher in AP4 and AP8 diets over control basal diet. Serum urea was significantly reduced (P<0.01) in herbal dietary treatments at varying dosages as compared to the control diet (0.77 mmol/L). In general, present study indicated that herbal diets could lowering serum enzymes (Table 4). Birds fed on Diet AP2, Diet AP4 and Diet AP8, showed significantly lowered alanine transaminase (ALT) level as compared to Diet C. The serum alkaline phosphatase (ALP) level in broilers was significantly lower in birds fed Diet AP4 and Diet AP8 compared to Diet C. Histological studyon the liver using light microscopic showed a normal structure and no alteration in the liver of the treated broiler chicken fed on diets treated with AP (Fig. 1).

Discussion

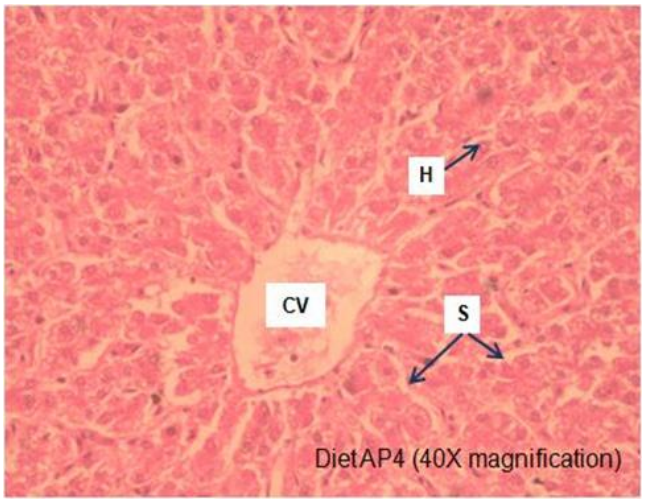
Diet supplemented with 2 g/kg AP and 8 g/kg AP in broiler chicken feeding lowered serum cholesterol level which recorded at normal range as 2.59 to 3.78 mmol/Lindicating that consuming *A. paniculata* ground leaf exhibited cholesterol lowering activity. The normal value of cholesterol in chicken varies from 2.59 - 3.88 mmol/Las suggested by Sturkie *et al.* (2000). Several factors could contribute to variable levels of cholesterol such as chicken breed, nutritional pattern, feed type, and environment.



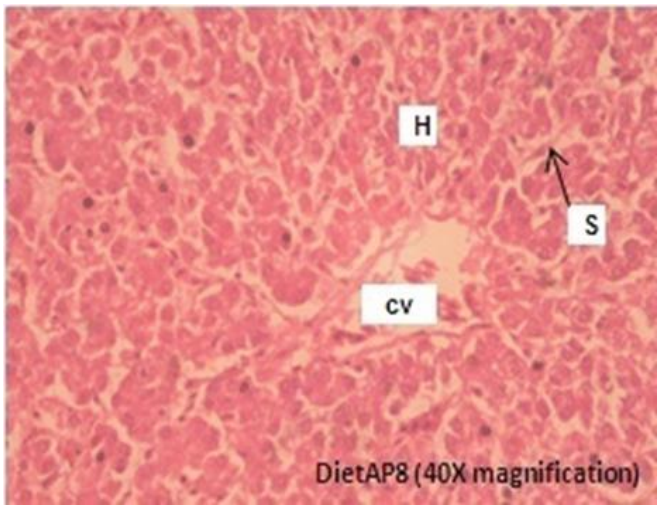
Micrograph of liver (Diet C) the lumen of central vein (CV) is lined by a simple squamous epithelium (EP), and observed also hepatocytes (H), and sinusoid (S) at normal architectures, (40X magnification).



Micrograph of liver (Diet AP2) central vein (CV) of the liver lobule, collects blood from the sinusoid (S), and the liver plate are composed of hepatocytes (H) appear to radiate as spokes of a wheel from the central vein, (40X magnification).



Micrograph of liver (Diet AP4), central vein (CV), which is continuous with the endothelial lining of the hepatic sinusoid (S), and the liver plate are composed of hepatocytes (H), (40X magnification).



Micrograph of liver (Diet AP8), central vein (CV), which is continuous with the endothelial lining of the hepatic sinusoid (S). Observed also that the liver plates are composed of hepatocytes (H), (40X magnification).

Fig. 1: Histological examination on liver of 42-day old broilers fed control diet, Diet AP2, Diet AP4, and Diet AP8. All figures show normal liver histology where hepatocytes (H), sinusoid (S), central vein (CV) and portal area conditions were at normal (40X magnification).

Moreover, this phenomenon possibly related to the hypolipiaemic effect which might be attributed to the presence of saponins and fibre that bind to serum lipids especially cholesterol, thereby easing their excretion from circulation as reported by Matawalli *et al*. (2004) on the methanolic leaf extract of *Adansonia digitata* in rat. In addition, the presence of diterpene lactones in *A. paniculata* which are also reported as the cholesterol-lowering components (Chen *et al.*, 2011; Choi *et al.*, 2012). In contrast, several reported that *A. paniculata* leaf ground was not significantly reduced the serum cholesterol in rabbits and rats (Dwivedi *et al.* 1987; Zhang and Tan, 2000; Mathivanan and Kalaiarasi, 2007). Feeding *A. paniculata* ground leaf on lowering serum cholesterol level in broiler chicken might be the first report.

In the present study, serum glucose of broilers fed on 2, 4, and 8 g/kg AP at day-42 was significantly lower as compared to control diet. In general, serum glucose level of broilers fed on *A. paniculata* was lowered. The *A. paniculata* effect in the present study was similar with Reyes *et al.* (2006) and Zhang and Tan (2000) who reported that *A. paniculata* led to a significant reduction of blood glucose levels indicating a hypoglycaemic effect. In contrast, Mathivanan and Kalaiarasi (2007) did not observe a significant difference in glucose level between *A. paniculata* and control groups.

Serum triglycerides level in broilers was lowered when fed diets supplemented with AP at 4 g/kg and 8 g/kg, indicating that the serum triglycerides level may be correlated with dosage of *A. paniculata*. A similar phenomenon as in serum cholesterol, triglyceride-lowering activity of broiler fed on *A. paniculata* ground leaf could be related to the presence of bioactive diterpene lactones (andrographolide) which was successfully extracted from *A. paniculata* in the previous study (Malahubban *et al*., 2013b). Thus, this herb might be useful in preventing cardiovascular

diseases, however, the role of triglycerides on heart diseases still unclear and questionable (Goldberg *et al.*, 2011). Albumin, total protein, and globulin in serum of broilers fed on different dosages of *A. paniculata* ground leaf do not altered. However, Elagib *et al.* (2012) reported that cinnamon (*Cinnamomum verum*), cumin (*Cuminum cyminum*), fenugreek (*Trigonella foenum-graecum*) and ginger (*Zingiber officinale*) inclusion in feed diets of broiler chicks exhibited some changes by increasing the serum total proteins and globulins, but albumin remains unchanged. Broilers fed the AP supplement showed lowered ALT and ALP rates, suggesting that a normal liver function. This was further evidenced by reduced lipid peroxidation in liver.

In the present study, histopathological study on the liver using light microscopy showed that a normal structure and no alteration in the livers of the treated broiler chickens with *A. paniculata* at varying dosages.

This study suggests that AP at 8g/kg diet lowered serum cholesterol, glucose, and triglycerides. It also maintains the integrity of liver indicating that no toxic effect from AP supplementation at a rate up to 8 g/kg, while AP lowered ALP and ALT in blood of chicken. Thus, the use of AP ground leaf at 8g/kg diet is recommended to improve health and safe for broiler chicken production.

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