

Dietary supplementation with artemisinin enhances growth performances but in high concentration has side effects on the hematologic system of broiler chickens

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Abstract

Artemisinin is the main biological compound from *Artemisia annua*, used with success in malaria treatment and with potential therapeutic effects in other parasitic diseases including coccidiosis in chickens. In the present study we aimed to assess the toxicity of artemisinin on the hematologic system and the effect on production performances of broiler chickens. For this purpose 18 days-old chickens were randomly divided into 4 groups of 30 chickens: one control group (C) and 3 experimental groups (Art5, Art50 and Art500). The control group received

standard growing feed while the diet of the experimental groups was supplemented with different concentrations of artemisinin (5 ppm, 50 ppm and 500 ppm) for 28 days. The chickens with 5 ppm of artemisinin in diet recorded a higher weight gain and feed intake and superior feed conversion ratio than the control group ($p \leq 0.01$). The performance characteristics of the chickens whose diet was supplemented with 50 ppm of artemisinin were similar with the control group, while 500 ppm of artemisinin negatively affected the chickens' production performances. The diet supplementation with 5 ppm of artemisinin didn't significantly affect the hematologic parameters of chickens, but in concentration of 50 and 500 ppm artemisinin induced a gradual decline of total leukocytes, lymphopenia, monocytosis, eosinopenia and caused anemia in case of the highest dosage. In conclusion, artemisinin in low concentrations could be used as a feed additive in poultry industry, by improving performances productions of broiler chickens and having no serious side effects.

Keywords: Broiler chickens, artemisinin, toxicity, hematology, production performances

1. Introduction

Artemisinin is a sesquiterpene lactone isolated from the herb *Artemisia annua*, which has been used for many centuries in traditional medicine to treat fever and jaundice, to heal wounds, eye infections and skin diseases. Artemisinin effects have been studied in many diseases like cancer, infectious or parasitic diseases and for many years it's being used with success as a combination therapy against drug-resistant malaria (Sadiq et al., 2014, Naeem et al, 2014). Different researchers studied artemisinin and *A. annua* effects in another apicomplexan infection namely the one responsible for coccidiosis in chickens (Allen et al, 1997;1998; Youn, 2001; Arab et al., 2006; del Cacho, 2010; de Almeida, 2012; Drăgan et al., 2014). Coccidiosis is an economically devastating disease for poultry industry. The development of drug resistance to all known anticoccidial drugs and the consumers concerns regarding meat residues have increased the interest of the scientific community for searching alternative means of control especially botanicals and natural feedstuffs, and artemisinin is one of the intensively studied compounds (Chapman, 1997; Allen and Fetterer, 2002, Abbas, 2012a; Abbas, 2012b).

Despite the extensive researches in using artemisinin in coccidiosis control, its effect on chickens' health has not been sufficiently investigated. Arab et al. (2009) shown that chickens

treated with a single oral dose of artemisinin exhibited neurological signs, liver, kidney and brain degenerations especially in very high dosages and presented a reduced feed intake dose dependently. Shahbazfar et al. (2011) showed that after chronic intake of artemisinin the chickens exhibited anemia, dose-dependent decreases in hematocrit and red blood cells, mild lesions in liver, kidney and brain, concluding that in therapeutic dosages artemisinin causes no serious side effects.

Although artemisinin has been proven to be safe in chickens in therapeutic dosages, the negative effect on hematologic system and the effect on chicken's production performances need to be further investigated. Thus, in the present study, we assessed the effect of 10-fold dosages of artemisinin (included for 28 days in chickens' diet) on hematological parameters, hematopoiesis and the influence on chicken's performance parameters.

2. Materials and methods

2.1 Animals

Broiler chickens ROSS 308 hybrids were purchased at one day old and kept in metal cages in dedicated facilities at the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Feed and water was provided *ad libitum* and the light program was continuous. From one-day-old until 18-day-old, they received standard feed without addition of anticoccidials.

Veterinary conditions regarding protection of animals used in this research are compiled according to national standards and legislation. All experiments were approved by the Research bioethics Commission of UASMV Cluj-Napoca (Registration no. of approval application: 4/19.09.2013)

2.2 Artemisinin

Artemisinin, powder (min. 98% purity) was purchased from Intatrade Chemicals GmbH, Germany, and was introduced in feed in concentrations of 5, 50 and 500 ppm (5, 50 and 500 mg artemisinin/kg feed).

2.3 Experimental design

At 18-days-old, broilers were divided into 4 experimental groups of 30 chickens each, with 3 replicates of 10 chickens, each individually marked. In the same day (day 0 of the experiment), the experimental feed was introduced in their diet during 28 days (until the age of 46 days). The experimental groups were: a control group (C) that received standard growing feed (without addition of anticoccidials) and 3 groups that received artemisinin in their diet (Art5, Art50 and Art500) in concentrations of 5 ppm, 50 ppm and 500 ppm. On day 0 and after 14 and 28 days of continuous administration of experimental feed, blood samples were aseptically collected from ulnar vein of each chicken from the first replicate, in heparin (50 UI/ml) containing tubes, using a 2.0 ml syringe and 23G needle.

2.4 Hematological measurements

Blood samples were tested for red blood cells (RBC) count, hemoglobin concentration, packed cell volume (PCV), the values of derived erythrocyte constants: mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and the mean corpuscular hemoglobin concentration (MCHC), total and differential white blood cells (WBC) counts.

The count of total number of RBC and WBC was carried out manually by hemocytometer method using Natt-Herrick modified by Prochaska dilution liquid (Ognean et al., 2011).

The concentration of hemoglobin was determined by spectrophotometer method (ELISA Bio-Rad 1100 microplate reader) measuring the absorbance at 540 nm, adding first 4% of ammonia solution to blood samples.

The PCV was established by using the microhematocrit method. Basically, capillary tubes were filled 2/3 with anticoagulated blood, the unfilled ends were sealed with clay and were centrifuged at 12000 rpm for 5 min. The PCV values were determined with the aid of a microhematocrit reader.

The MCV, MCH and MCHC were calculated using the following formulas:

$$\text{MCV } (\mu\text{m}^3) = [\text{PCV} \times 10] / \text{RBC};$$

$$\text{MCH (pg)} = [\text{Hb} \times 10] / \text{RBC};$$

$$\text{MCHC (g/dl)} = [\text{Hb} \times 100] / \text{PCV};$$

The differential WBC counts were performed by microscopic examination of blood smears, counting and identifying 100 successive WBC/sample, the results being reported in percentages of different WBC (Samour, 2006).

2.5 Performance characteristics

The consumed feed amount was monitored daily/cage and the chickens were weighted individually on days 0, 14 and 28 of the experiment for establishing the weight gain, feed intake and feed conversion ratio.

2.6 Histopathological and cytological evaluation of bone marrow

At the end of the experiment the chickens were euthanatized by cervical dislocation and chicken necropsy was performed.

Bone marrow samples were collected from the chickens in the first replicate for both histological and cytological evaluation (Elmore, 2006). For the histopathological examination the proximal tibiotarsus (Campbell, 1994) was bilaterally harvested, cleaned of connective tissue and skin and trimmed longitudinally into small pieces (about 1/1 cm with 0.2 cm thickness). After 72h of fixation in 10% neutral-buffered formalin the samples were decalcified for 3 weeks in 1/1 mixture of 8% formic acid and 8% hydrochloric acid (Prophet, 1992). When decalcification was completed, the tissues were dehydrated through successive baths of isopropyl alcohol (70%, 90%, 95%, and 100%), clarified in xylene and embedded in high-melting temperature paraffin wax following the routine processing protocol (Prophet, 1992). After solidification, the paraffin blocks were trimmed and kept at room temperature until used. Tissue sections were cut from each paraffin block at 2-3 μ m thickness with a rotary microtome (Leica RM 2125) and stored overnight at 37°C on a thermostatically controlled slide warmer. Tissue sections were routinely stained with hematoxylin and eosin (H&E) and examined under an Olympus BX41 microscope. During histopathological examination the proportions of granulocytic to erythroid cells (or Myeloid to Erythroid ratio) were assessed (Reagan, 2011; Wakenell, 2010).

The bone marrow smears were produced by touch imprints and push slide technique from sectioned proximal tibiotarsus before their trimming for histopathology. After fixation, the smears were stained by Wright's-Giemsa technique.

The bright field microscopy was acquired using a 3.2 megapixel resolution Olympus UC30 Digital Camera and processed by using the Olympus Stream Basic image analysis software (Olympus Stream Basic Package).

2.7 Statistical analysis

Statistical analysis was performed by Medcalc software version 15.8. Normal distribution of data was verified with Shapiro-Wilk test and in case of normally distributed data, Independent samples T-test was performed, which consisted in an F test for equal variances and depending on the variance the T-test (equal variances) or Welch-test (unequal variances) were used. In case of not normally distributed data Mann-Whitney test was applied. The statistical interpretation of histopathological and cytological data was made in Origin 8.5 software, ANOVA test. Differences were considered significant at a p value equal or lower than 0.05.

3. Results

3.1 RBC counts

At the first blood sample collection (day 0), the number of erythrocytes was higher in the groups treated with 5 and 500 ppm of artemisinin compared with C group ($p \leq 0.01$). At the second collection, the total number of erythrocytes increased significantly in all experimental groups, but there were no significant differences between groups. At the third blood sample collection, the RBC count had similar values in C and Art 5 groups, the group Art 50 had fewer erythrocytes compared with C group ($p=0.03$), whilst the chickens from group Art500 showed a very low number of erythrocytes ($2.23 \times 10^{12}/L$) (Table 1).

3.2 PCV

Before the administration of artemisinin, the group ART5 showed a higher value of PCV compared with C group ($p=0.01$). In day 14 of the experiment, all the groups showed similar percentages of PCV. In correlation with the values recorded for the RBC counts, at 28 days after administration of experimental feed, all the groups exhibited lower PCV values, the chickens of the group ART500 had the lowest value compared with the C group ($p=0.1$) (Table 1).

3.3 Hemoglobin concentration

In the first blood collection, the concentration of hemoglobin had similar values in all groups except group Art500 which recorded higher hemoglobin concentration ($p=0.0005$). This event persisted in the second blood sampling also ($p=0.02$), but in the third collection Art500 group showed a lower value for hemoglobin concentration compared with C group ($p=0.09$) and also compared with the other 2 samplings ($p\leq 0.02$) (Table 1).

3.4 Derived erythrocyte constants values

The value of MCV decreased significantly at the second sampling and slightly increased at the third blood collection in all experimental groups. In the first sampling, the chickens from all artemisinin treated groups showed a lower value of MCV compared with C group, and it was more significant in Art5 and Art500 groups ($p\leq 0.05$). At the second sampling the values recorded didn't differ significantly between groups. At the final recording, the highest value was registered in Art500 group ($106.49 \mu\text{m}^3$; $p=0.0008$). The same aspect was recorded for MCH. The MCHC values were similar in each group, except Art500 group with a higher MCHC value in first and second blood sampling ($p\leq 0.02$) (Table 1).

3.5 Total WBC count

In the first two blood sampling the total number of WBC was higher in experimental groups than in the C group, the WBC values that differ significantly only in Art 5 group in the first collection ($p=0.006$). After 28 days of administration of artemisinin in chickens' feed, the WBC values recorded for all experimental groups were lower than for C group ($p\leq 0.4$). In dynamics, the C group had similar values in all 3 blood samplings, while in the groups treated with artemisinin the total number of WBC showed a significant decrease from the first sampling until the last ($p\leq 0.05$). The Art5 group had the most dramatic decrease of WBC ($p<0.001$), although the lowest value recorded was in the group Art500 after 28 days of administration of experimental feed (Table 2).

3.6 Differential WBC count

Regarding the different types of leukocytes, the groups treated with 5 and 50 ppm of artemisinin showed a progressive decrease of heterophils percentage from first to last blood

sampling, with the lowest value recorded for the group Art50 (17.4%) in the third blood collection ($p=0.02$). However, the heterophils percentage of the chickens that received 5 ppm of artemisinin in their feed was similar with the C group in the second and third sampling. The Art500 group showed similar values of heterophils percentages in all three collections, but this values were significantly lower than those recorded for the control group only in the third blood sampling ($p<0.05$) (Table 2).

The percentage of lymphocytes showed a significant decrease from the first blood sampling until the last one in all groups treated with artemisinin ($p\leq0.05$). Although the control group recorded a lower percentage in the second collection, the drawback was recovered in the third blood sampling. The highest reduction of lymphocytes percentage was recorded for the group Art500, but this group showed a higher percentage of lymphocytes in the first blood sampling compared with the control group ($p=0.05$). After 28 days of administration of artemisinin the percentage of lymphocytes in the chickens from all experimental groups was significantly lower than the control group ($p\leq0.005$) (Table 2).

The percentage of monocytes in the control group recorded an increase in the second blood sampling and a dramatic decrease in the third sampling. This aspect was recorded also in the chickens treated with 5 ppm artemisinin but in the third blood sampling the percentage of monocytes of this group was significantly higher than the C group ($p=0.0004$). In the two other artemisinin treated groups the percentage of monocytes increased gradually from first to third blood sampling and had similar values, but much higher than the control group in the third collection ($p\leq0.0005$) (Table 2).

The eosinophils percentage was significantly lower than the control group in the third blood sampling in the chickens treated with 50 and 500 ppm of artemisinin ($p\leq0.002$) (Table 2).

Regarding basophils percentage, there were significant differences between control group and experimental groups only in the third blood sampling ($p\leq0.01$), in this case there were no basophils detected in artemisinin treated groups (Table 2).

3.7 Performance characteristics

For all the periods recorded the weight gain of the chickens treated with 5 ppm of artemisinin was higher than of the chickens from the control group, but it was significant only in the first and third periods ($p=0.01$). The group treated with 50 ppm artemisinin recorded similar

values with the control group whilst the chickens treated with 500 ppm of artemisinin recorded significantly reduced weight gains compared with the control group ($p \leq 0.05$) (Table 3).

During the experiment the chickens treated with 5 ppm of artemisinin consumed a higher amount of feed than the C group, but it was significant only in the second and third periods of recording ($p \leq 0.001$). The group Art50 recorded values similar with the control group during the experiment. The chickens treated with 500 ppm of artemisinin had a lower feed intake compared with the control group in all the three periods ($p \leq 0.03$) (Table 3).

In the first period recorded the feed conversion ratio was superior for the group treated with 5 ppm artemisinin ($p = 0.0005$) whilst the group treated with 500 ppm of artemisinin had an inefficient use of feed compared with the control group ($p = 0.02$). In the period 14-28 days all the groups showed similar values of the FCR. For the total period of recording, the groups treated with 500 ppm of artemisinin had the worst FCR ($p = 0.2$) whilst the chickens that received 5 ppm of artemisinin in feed had a better FCR compared with the control group ($p = 0.1$) (Table 3).

3.8 Histopathological and cytological evaluation of bone marrow

Concerning the M:E ratio, this was higher for the group ART5 respectively for ART50 (2:1 and 2.1:1 compared with 1.6:1 for control group and 1.7:1 for ART500 group), but without statistical significance ($p = 0.4$). Except for the moderately increased number of myeloid precursors no other changes were noticed (fig. 1, fig. 2).

4. Discussions

The poultry industry has benefited of a rapid development mainly due to advances in genetics, nutrition and health management. The diet formulations are especially important because they can increase broiler performances or if incorrectly formulated they can cause great economic losses for the meat industry (Alvarenga et al., 2015).

Despite of advances in drug development broilers are still exposed to many infectious or parasitic diseases which produce also losses with morbidity or even mortality. The searching of alternative substances for disease control, particularly natural products, is very encouraged not only for solving the problem of meat drug residues but also for their beneficial effect on the gut microbiota or their immunomodulatory properties, having as result the improvement of production performances (Sugiharto, 2014).

Artemisinin, the main bioactive compound of the herb *A. annua*, and a powerful antimalarial drug, has been extensively studied for its effects against coccidiosis in broiler chickens. Although, it has been proven that can be used as an alternative in coccidiosis control, its effects on chickens health are not well known.

In the present study we supplemented the diet of broiler chickens with 5 ppm, 50 ppm and 500 ppm of artemisinin for 28 days in order to evaluate the possible toxicity of this compound on the hematologic system of chickens or its potential to enhance the production performances.

In the lowest concentration, artemisinin improved weight gain and feed conversion ratio, the chickens having a higher intake of feed. The chickens which received in the diet 50 ppm of artemisinin recorded similar weight gains and feed conversion compared with the chickens from the control group fed with standard feed. However, artemisinin in 500 ppm concentration, caused reduced weight gain, inefficient feed conversion and a lower intake of feed. This negative effect is probably due to the higher quantity of the compound that may give a bitter taste of the feed and reduce the palatability just as is happening in children treated for malaria with artemisinin based combination therapies (Yeka and Harris, 2010). Arab et al. (2009) in a single oral dose study, administrating 10, 50, 250, 1250 and 2500 mg of artemisinin/kg feed to 30 day old chickens, noticed the same reduction in food consumption which was dose dependent. However, Shahbazfar et al. (2011) didn't notice any negative effect on the weight gain or food consumption, but they used lower dosages: 17, 34, 68, and 136 ppm for 36 days. In this study, the decreasing erythrocytes number tendency, the hemoglobin concentration and PCV in the chickens supplemented with 500 ppm of artemisinin suggest the evolution of anemia. Based on the increased values of MCV we can conclude that it is a macrocytic anemia (Aslinia et al., 2006). Shahbazfar et al. (2011) noticed the clinical signs of anemia in chickens fed with 68 and 136 ppm artemisinin, the significant reduction of hematocrit in blood tests, and of RBC count especially in higher concentrations of artemisinin.

In the present study, the white blood cells followed a decreased trend in all chickens that were fed with the artemisinin diets which corresponded with lymphopenia, monocytosis and eosinopenia in the chickens supplemented with 50 ppm and 500 ppm of artemisinin. This leukogram pattern is consistent with a stress leukogram due to endogenous steroid release in

stressful conditions (Maxwell, 1993, Schmidt, 2015). This may imply that the dietary supplementation with higher concentration of artemisinin may represent a stress to the chickens.

In animal experiments, artemisinin and its derivatives produced frequently inhibition of erythropoiesis, this seems to represent a sensitive target for artemisinins (Efferth and Kaina, 2010). In the present study there were sufficient numbers of erythroid precursors at the bone marrow evaluation for all experimental groups. Contradictory studies show that artemisinins can enhance or inhibit leukocyte functions in human or animal trials (Efferth and Kaina, 2010).

5. Conclusions

Artemisinin improved performances characteristics of broiler chickens and had no serious side effects, thus in low concentrations could be used as feed additive in poultry industry.

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400

Table 1

The effect of different concentrations of artemisinin on erythrogram parameters of broiler chickens compared with control group

Blood sampling	Group	RBC ($\times 10^{12}/L$)	PCV (%)	Hb (g/dl)	MCV (μm^3)	MCH (pg)	MCHC (g/dl)
I	C	1.88 $\pm$ 0.70 ^a	27.3 $\pm$ 0.67 ^b	5.12 $\pm$ 0.15 ^a	155.99 $\pm$ 12.96 ^a	28.86 $\pm$ 2.28 ^a	18.71 $\pm$ ^a
	Art5	3.37 $\pm$ 0.36 ^b	29.7 $\pm$ 0.58 ^a	5.18 $\pm$ 0.15 ^a	91.54 $\pm$ 9.14 ^b	17.03 $\pm$ 2.00 ^b	18.67 $\pm$ ^a
	Art50	2.06 $\pm$ 0.36 ^{ac}	27.5 $\pm$ 0.70 ^b	5.37 $\pm$ 0.33 ^a	102.32 $\pm$ 25.87 ^{ab}	20.32 $\pm$ 5.65 ^b	19.71 $\pm$ ^{ab}
	Art500	3.04 $\pm$ 0.44 ^{bc}	27.9 $\pm$ 0.64 ^{ab}	6.28 $\pm$ 0.23 ^b	114.51 $\pm$ 15.22 ^b	24.23 $\pm$ 3.49 ^{ab}	21.08 $\pm$ ^b
II	C	5.94 $\pm$ 1.11 ^a	28.6 $\pm$ 0.55 ^{ab}	5.14 $\pm$ 0.26 ^a	62.08 $\pm$ 9.75 ^a	11.40 $\pm$ 2.02 ^a	17.94 $\pm$ ^a
	Art5	4.94 $\pm$ 0.77 ^a	29.0 $\pm$ 0.50 ^a	6.51 $\pm$ 0.50 ^b	72.43 $\pm$ 9.91 ^a	16.03 $\pm$ 2.47 ^a	22.49 $\pm$ ^{ab}
	Art50	4.55 $\pm$ 0.99 ^a	26.9 $\pm$ 0.85 ^b	5.85 $\pm$ 0.57 ^{ab}	96.01 $\pm$ 22.15 ^a	19.80 $\pm$ 4.16 ^a	21.66 $\pm$ ^{ab}
	Art500	5.04 $\pm$ 1.05 ^a	28.7 $\pm$ 0.68 ^{ab}	6.63 $\pm$ 0.54 ^b	81.58 $\pm$ 14.24 ^a	18.78 $\pm$ 3.83 ^a	23.20 $\pm$ ^b
III	C	3.76 $\pm$ 0.25 ^a	26.0 $\pm$ 1.12 ^a	5.46 $\pm$ 0.36 ^a	72.48 $\pm$ 5.11 ^a	15.26 $\pm$ 1.63 ^a	20.87 $\pm$ ^a
	Art5	3.35 $\pm$ 0.32 ^{ab}	25.8 $\pm$ 0.70 ^a	4.68 $\pm$ 0.26 ^a	84.13 $\pm$ 9.11 ^{ab}	15.86 $\pm$ 2.53 ^{ab}	18.22 $\pm$ ^a
	Art50	3.13 $\pm$ 0.23 ^b	25.2 $\pm$ 0.80 ^a	4.51 $\pm$ 0.39 ^a	83.90 $\pm$ 5.63 ^a	14.91 $\pm$ 1.55 ^{ab}	18.03 $\pm$ ^a
	Art500	2.23 $\pm$ 0.14 ^c	23.3 $\pm$ 1.12 ^a	4.28 $\pm$ 0.71 ^a	106.49 $\pm$ 6.20 ^b	20.61 $\pm$ 3.49 ^b	18.67 $\pm$ ^a

Values with no common superscript in a column and periods of blood sampling were significantly different ($p < 0.05$); results are expressed as means $\pm$ SEM;

Table 2

The effect of different concentrations of artemisinin on leukogram parameters of broiler chickens compared with control group

Blood sampling	Group	WBC (x10 ⁹ /L)	Differential WBC (%)				
			Heterophils	Lymphocytes	Monocytes	Eosinophils	Basophils
I	C	13.9±1.03 ^a	24.6±4.20 ^a	40.5±4.81 ^a	20.8±3.93 ^a	13.5±1.86 ^a	0.6±0.40 ^a
	Art5	22.3±2.32 ^b	28±3.56 ^a	36.6±3.66 ^a	23.3±4.53 ^a	11.3±1.85 ^a	0.8±0.61 ^a
	Art50	15.7±1.43 ^{ab}	25.3±3.20 ^a	45.7±2.64 ^{ab}	14.7±1.77 ^a	13±1.51 ^a	1.3±0.65 ^a
	Art500	17.1±1.06 ^b	19.9±3.34 ^a	52.3±2.89 ^b	16.3±2.32 ^a	10.4±1.54 ^a	1.1±0.57 ^a
II	C	13.8±2.39 ^a	23.2±2.98 ^a	27.8±4.77 ^a	31.5±4.17 ^a	17.5±4.37 ^a	0 ^a
	Art5	16.7±2.20 ^a	22.6±3.49 ^a	22.3±3.96 ^a	33.4±3.96 ^a	21.7±2.70 ^a	0 ^a
	Art50	18.3±2.78 ^a	20.6±3.62 ^a	31.3±4.44 ^a	23.9±2.54 ^a	24.1±4.27 ^a	0.1±0.10 ^a
	Art500	15.5±1.74 ^a	21.5±3.06 ^a	25.7±4.67 ^a	30.1±2.76 ^a	22.7±2.86 ^a	0 ^a
III	C	13.0±1.45 ^a	22.0±1.18 ^a	38.38±1.95 ^a	12.5±2.07 ^a	26±2.23 ^a	1.13±0.40 ^a
	Art5	11.2±0.39 ^a	23.0±1.06 ^a	29.8±1.00 ^b	23.9±1.00 ^b	23.3±2.54 ^{ab}	0 ^b
	Art50	11.3±0.56 ^a	17.4±0.60 ^b	30.8±1.44 ^b	38.2±1.32 ^c	13.6±1.35 ^c	0 ^b
	Art500	10.2±0.99 ^a	19.11±0.70 ^b	25.33±1.69 ^c	38.89±1.26 ^c	16.67±1.41 ^{bc}	0 ^b

Values with no common superscript in a column and periods of blood sampling were significantly different ($p < 0.05$); results are expressed as means $\pm$ SEM;

Table 3

The effect of different concentrations of artemisinin on production performances of broiler chickens compared with control group

Group	Weight gain			Feed intake			Feed conversion ratio		
	0-14 d	14-28 d	0-28 d	0-14 d	14-28 d	0-28 d	0-14 d	14-28 d	0-28 d
C	43.44±1.30 ^a	42.41±1.60 ^{ab}	42.92±1.26 ^a	114.52±0.04 ^a	124.41±0.12 ^a	119.47±0.07 ^{ab}	2.71±3.10 ^a	3.07±1.43 ^a	2.89±1.85 ^a
Art5	48.40±1.42 ^b	46.71±1.59 ^a	47.56±1.28 ^a	117.35±0.03 ^b	143.30±0.12 ^a	130.33±0.06 ^a	2.43±1.40 ^a	3.09±2.85 ^b	2.75±1.47 ^b
Art50	42.76±1.70 ^a	41.49±2.11 ^{ab}	42.33±1.64 ^a	113.34±0.05 ^a	126.40±0.11 ^a	119.87±0.06 ^{ab}	2.66±1.88 ^a	3.12±4.02 ^a	2.88±2.59 ^a
Art500	33.71±1.21 ^c	38.07±1.47 ^b	35.89±1.16 ^a	98.13±0.11 ^c	111.48±0.12 ^a	104.81±0.11 ^b	3.02±5.16 ^b	3.07±4.52 ^c	3.04±4.38 ^c

Values with no common superscript in a column were significantly different ($p < 0.05$); results are expressed as means $\pm$ SEM;

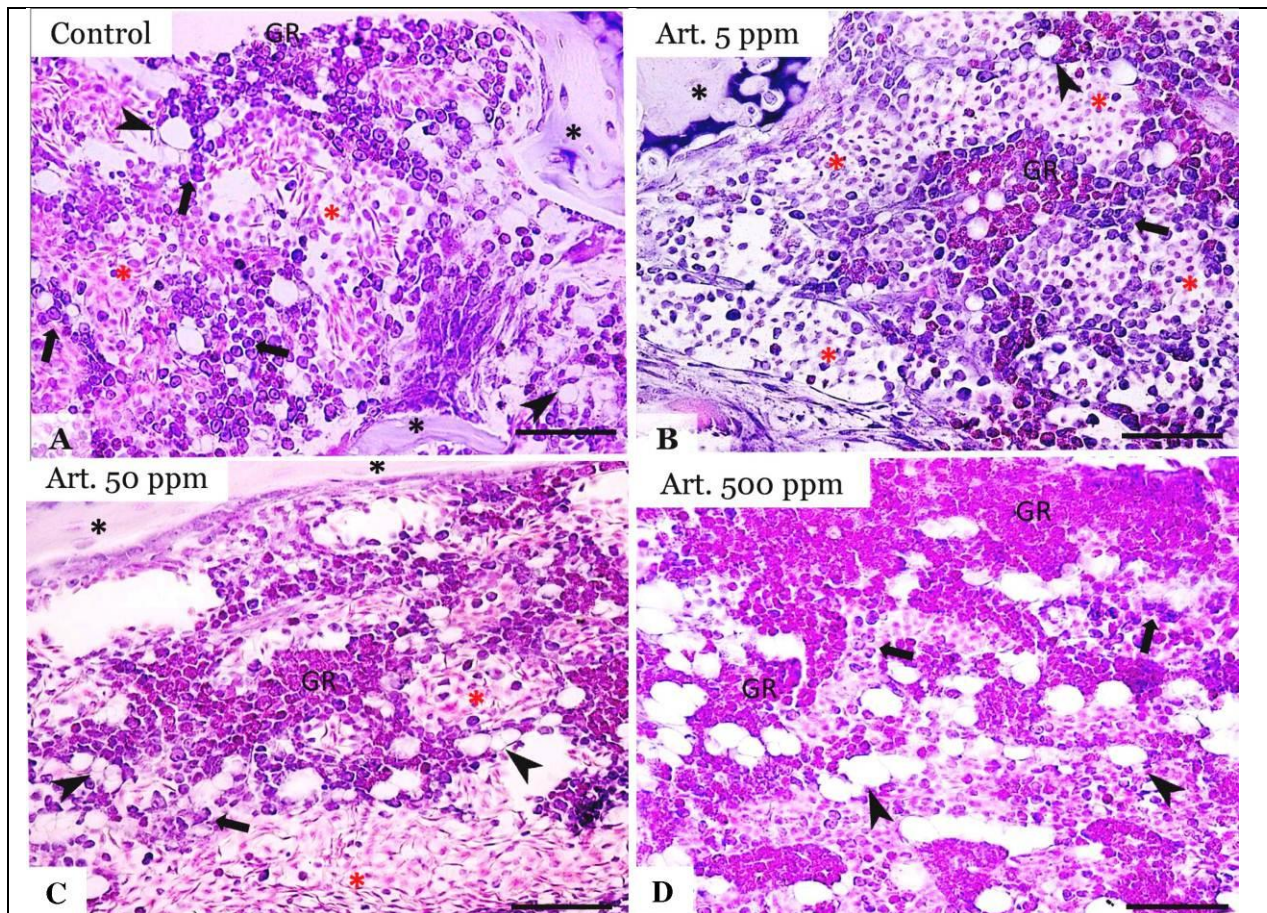


Fig.1. Representative histological images of bone marrow following chronically oral intake of 5, 50 and respectively 500 ppm of atemisin compared with the control group.

In all images abundant venous sinuses (indicated by red asterisks) delimitates islands and sheets of hematopoietic cells which are admixed in various proportions with mature adipocytes (indicated by arrow heads). Moderately increased number of myeloid precursors can be observed for the Art. 50 (image C) and Art. 500 (image D) groups compared with the control (image A).

GR-Myeloid cells (granulocytic/myeloid); Arrows indicates cells from the erythroid cell line.

Black asterisks indicates the trabecular bone lined by flattened or cuboidal osteoblasts and sparse osteoclasts. H&E stain, objective x40. Scale bar=75 μ m.

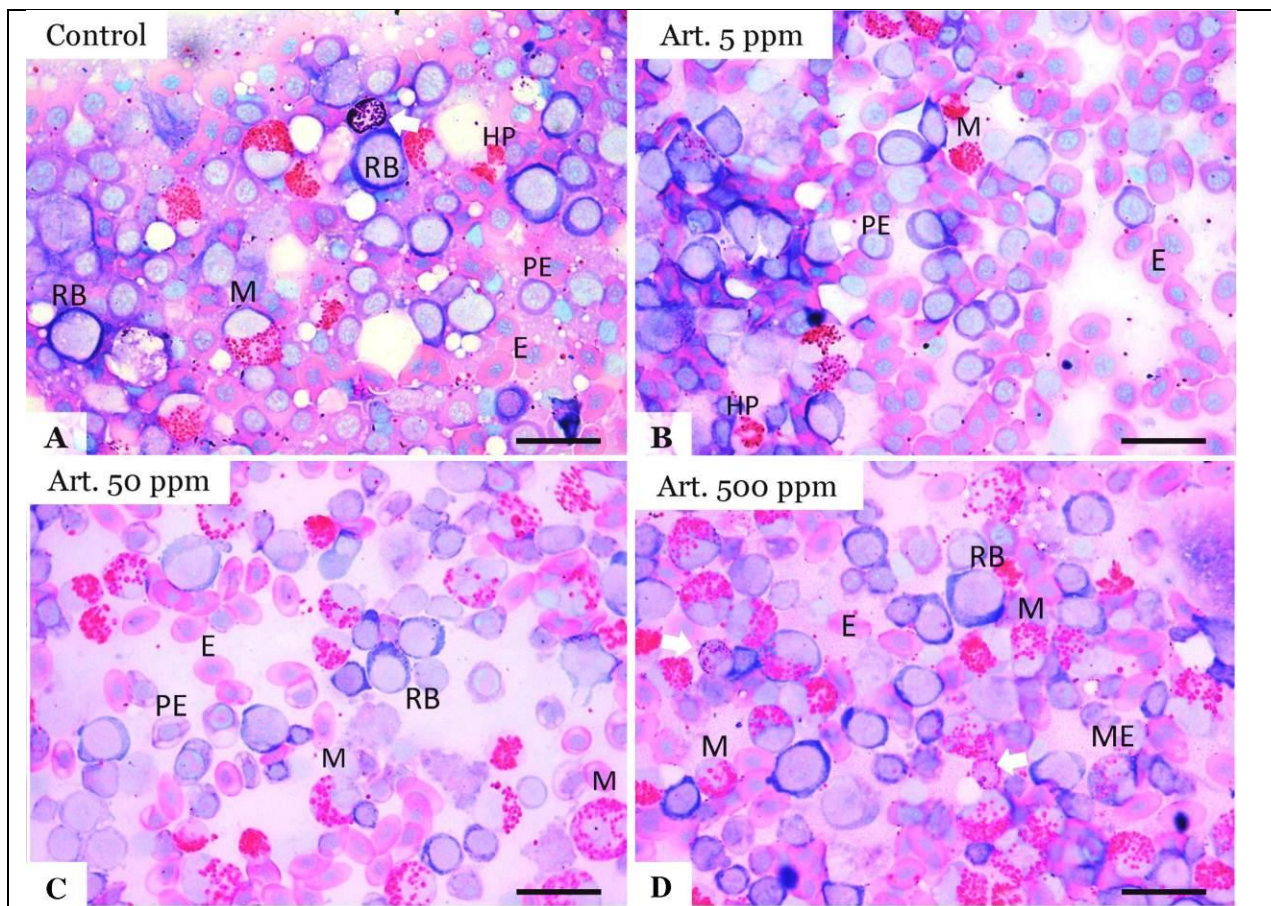


Fig.2 Cytological images of bone marrow following chronically oral intake of 5, 50 and respectively 500 ppm of atemisin compared with the control group; E- Mature erythrocyte, PE- polychromatic rubricytes and erythrocytes, M- myelocyte, ME- eosinophilic myelocytes, RB- rubriblast. White arrow- basophilic myelocytes; Wright's-Giemsa stain, objective x100. Scale bar=20 μ m.