Lipid Peroxidation in Broiler Chickens Under the Influence of Some Anticoccidial Agents

Maha M. El-Deib[1] and Ahlam E. Abd El-Lateif[2],

[1] Central Lab., Vet. Clinic, Faculty of Vet, Med., Zagazig Univ., [2]Institute of Animal Research, Zagazig,

ABSTRACT

Lipid peroxidation severely damage the cell membrane and compromises cell growth rate. The presence of malondialdehyde (MDA) in serum and tissues indicate a high level of lipid peroxidation with impaired antioxidant status. Determination of this marker, is of good aid in estimating the action of natural materials and xenobiotics potentially capable of exhibiting prooxidant or antioxidant properties on bio-membrane lipid system. We determined the level of MDA in serum, liver, kidney and brain tissues of broiler chickens to investigate and evaluate the antioxidant effect of *Artemisia cina* (A. cina) or its extracts as anticoccidial herbal treatments in comparison with other anticoccidials, drug (simiduramicin) and vaccine (Coccivac-B). In addition to estimation of serum albumin and globulin.

One hundred and eighty Hubbard chicks, were equally classified into six groups. The 1st group was kept as control. The groups from the 2nd till the 5th were medicated with A. cina powder (AP), watery extract (AW), alcoholic extract (AA) and or semiduramicin (Aviax) respectively from the 1st day till 42 days of age as food additive. The 6th group was vaccinated once with Cavvicac-B at 3rd day of age via eye drop. Coccidial infection was iniated at 21st day of age to half of these groups while the second half were kept as controls. Chicks were reared at high ambient temperature of summer season.

Among the used treatments, the antioxidant activity recorded only for AW and AA. Antioxidant effect was more potent for AW especially after 35 days. of AW supplementation. Where AW decreased MDA inspit of heat stress and coccidial infection in both serum and tissues by mean values of 3.3 - 26.6% in comparable to that of controls. Unfortunately, serum MDA was not indicator to MDA of other organs. Serum albumin significantly correlated only with MDA of brain tissue. Other treatments, AP, Avaix and Coccivac-B. were neither antioxidant nor prooxidant. On the light of the increasing need for natural antioxidants for human and animal health, the antioxidant activity of Artemisia cina watery extract was discussed.

INTRODUCTION

Lipid peroxidation is the process of oxidative degradation of polyunsaturated fatty acids. It was initiated when the generation of reactive oxygen species (ROS) such as H2O2, hydroxyl radical, superoxide, hydroperoxides or other free radicals exceeded the antioxidant capacities of cells or tissues *(1).* (ROS) are produced continuously in the body as a consequence of normal metabolic processes. Birds have a metabolic rate that is approximately 2-2.5 times higher than mammals of comparable body size potentially exposing the birds to more ROS *(2).* Benign functions of ROS have been reported including the body's responses to infection, the activation of nuclear transcription factors, gene expression and defense mechanism to target tumor cells *(3).* However, excessive ROS generation leads to cytotoxicity. Further more oxidative stress decreases protein synthesis, gen expression, interferes with the cell growth and induces apoptosis*(4-6)*.

Oxidation of lipids can have a marked negative effect on nutritional value, and may be responsible for the production of toxic compounds capable of triggering metabolic disorders such as mutagenesis, carcenogensis, circulatory disorders and ageing *(7).* The retardation of auto-oxidation is therefore a key

to high product quality. So the search for natural antioxidants, especially of plant origin, has greatly increased in recent years particularly since the use of synthetic antioxidants are suspected to be carcinogenic *(8)*.

Much renewed attention was paid to bioactive constituents of *Artemisia* genus. As reported, some substances of this large genus were shown to be antioxidant, antimalarial, antitumor, antipyretic, antiviral, antihepatitis and interferon-inducing. But the reported biological studies of *Artemisia* constituents and extracts were carried out *in vitro*. Meanwhile a lot of isolates from the genus have failed when tested biologically *(9)*. The preliminary success of *Artemisia cina* (A. cina) watery extract to be safe alternative to other anticoccidials either chemotherapeutical (Aviax) or immunological (Coccivac-B) *(10,11)*. Together with the shortage in studies dealing with the antioxidant effect of A. cina in animal tissues when added to animal diets particularly in broiler chickens, up till now, stimulate us to carry out this study aiming to:

1-Investigate and evaluate the antioxidant effect of A. cina and its extract *in vivo* in comparison with other different anticoccidials, either ionophorus antibiotic (Aviax) or vaccine (Coccivac-B). The antioxidant activity was evaluated by determination of lipid peroxidantion marker, malondialdehyde (MDA), in serum and tissues (liver, kidney and brain) of broiler chickens with or without one of the most important economic diseases in broiler industry, coccidiosis.

2-Whether serum MDA, as it available to measure in live bird, is an indicator to lipid peroxidation of other organs.

3-Whether serum albumin level correlated with MDA.

MATERIAL AND METHODS

Birds

One hundred and eighty Hubbard chicks were obtained as day-old from Ommat Company, Egypt. They were floor reared till 49 days of age and fed a balanced commercial ration. Routinely broiler vaccination program against Newcastle (Intervet), Infectious bronchitis (IZO S.P.A. Brescia, Italy) and Gumboro disease (Intervet) were applied according to instructions of manufactures.

Anticoccidial agents

Artemisia cina and it extract: A. cina plant was purchased and kindly identified and prepared by Prof. Dr Samih El-Dahmy, Pharmacognosy Dept., Faculty of Pharmacy, Zagazig University. The leaves of A. cina were air dried and pulverized to moderately coarse powder (Ap). For preparation of watery extract (AW) A. cina powder was soaked in a suitable amount of distilled water for 24 hours then filtered. The filtrate was freeze dried using a labconco, freeze dryer, Model 18. For preparation of alcoholic extract (AA), A. cina powder was extracted with ethanol (95%). The alcoholic extract was evaporated under reduced pressure to give gummy brownish residue.

Semiduramicin sodium (Aviax)®: (Pfizer U.S.A). It was used as a feed additive for controlling coccidiosis in broiler chickens at a level of 25 ppm per ton of ration.

Coccidial vaccine (Coccivac-B): It is a live oocysts vaccine for broiler vaccination and comprised four species of the wild type of Eimeria (E. acervulina, E. mivata, E. maxima, E. tenella) (Schering Plough Animal Health Corp. Millsboro, Delaware, USA). It was administered to chickens at 3rd day of age once through eye drops.

Eimeria species

Sporulated oocysts of mixed intestinal and cecal field Eimeria spp. isolates were obtained from the Dept. of Parasitology, Faculty of Vet. Med. Zag. Univ. Infection was made at 21 days of age with 7500 sporulated oocysts/bird using crop tube.

Experimental design

One hundred and eighty Hubbard day-old chicks were divided into 6 equal groups. The 1st group was kept as control. 2nd group

received AP in a dose of 10g/kg of commercial ration (1%) ***(10)***, 3rd and 4th groups received AW and AA extracts respectively in a dose equivalent to 1% of A. cina powder on ration, 5th group was medicated with Aviax at dose level 25 ppm in ration. Each of these treatments were added from the first day till 42 days of age (stopped one week before slaughtering). The 6th group was vaccinated with Coccivac –B at 3rd day of age once via eye drop.

On day 21 of age, all groups were sub-divided into two equal sub- groups, one of them was infected with suspension of mixed field 7500 sporulated oocysts/bird using crop tube, while the other one was kept as non infected control for each treatment. Our experiment was carried out during summer season at high ambient temperature (temperature reached to 36-42°C inside rearing house). Birds were supplied with ice in drinking water during higher day temperatures.

Sampling

Blood samples

The blood samples (n= 5 per treatment) were collected at days 21, 28, 35 and 49 after slaughtering and allowed to clot in a centrifuge tubes at room temperature. The tubes were centrifuged at 1500 rpm for 10 min. to obtain serum. The serum samples were subsequently stored at -20°C until analysis. The concentrations of serum MDA ***(12)***, total protein ***(13)***, abumin ***(14)*** and globulin were determined. Globulin concentration was calculated from the arithmatic difference between the total protein and albumin concentration.

Tissue samples

After slaughtering, postmortum examination was carried out, then chicken liver, kidney and brain were promptly excised and perfused with normal saline to reduce red blood cell contamination. Then kept in deep freezer at -20°C until assay. Liver, kidney and brain homogenates as described by ***Stroev (15)*** were used for determination of tissue MDA concentration using ascorbate-dependent nonezymic system for peroxide oxidation.

Statistical analysis

Analysis of variance (ANOVA) and correlation coefficient were carried out following the method described for one way classification ***(16)***.

RESULTS AND DISCUSSION

Free – radical oxidation reactions in lipid are facilitated by prooxidants and inhibited by antioxidant. Methods for determination of lipid peroxidation are of good aid in estimating the action of natural materials and xenobiotics, potentially capable of exhibiting prooxidant or antioxidant properties on biomembrane lipid system. These methods enable revealing peroxide oxidative properties in a variety of diverse materials, included drugs ***(15)***.

Markers of ROS – mediated damage in serum and tissues have been observed (in broiler) in the form of MDA, an end product of lipid peroxdation. The presence of MDA in the cells indicates a high levels of lipid peroxidation with impaired antioxidants status. Accumulation of these markers in cell membrane can react with DNA and are mutagenic, or with protein to cause structural and / or functional damage ***(17-19)***.

In this study, the MDA concentrations under the effect of *Astemesia cina* and its extracts, semiduramicin (Aviax), and Coccivace-B were determined. in serum, liver, kidney and brain of broiler chickens. Till the age of 35 days, the obtained results (Tables 1-3) revealed that MDA concentrations of AP treated groups were mostly equal to that of control groups in both coccidia infected or non infected cases for serum and all tested organs (liver, kidney and brain). This manners reflected no antioxidant properties for AP which may explain the lower renal and hepatic protective effect of AP in broilers ***(10)***. On other hand, supplementation of dried powder of *Artemisia inculcata* caused mild pathological lesions of different organs of broilers ***(20)***.

Table 1. MDA concentration in serum (µmol/L) and tissues (liver, kidney & brain, nmol/g tissue) of broiler chickens, controls and treated with anticoccidial agents Data are mean ± SE (n = 5)

Age / group	21 days				28 days				35 days				49 days			
	Serum	Liver	Kidney	Brain	Serum	Liver	Kidney	Brain	Serum	Liver	Kidney	Brain	Serum	Liver	Kidney	Brain
Control	b 8.02 ± 0.05	b 11.90 ± 0.13	a 7.57 ± 1.03	bc 18.78 ± 0.15	a 7.36 ± 0.02	bc 12.96 ± 1.86	a 8.92 ± 0.31	cd 16.24 ± 0.34	ab 7.62 ± 0.14	a 17.55 ± 1.95	a 9.46 ± 0.18	a 16.56 ± 0.16	ab 8.16 ± 0.05	a 14.36 ± 0.35	b 13.58 ± 0.19	c 19.37 ± 0.16
AP	b 7.69 ± 0.03	b 11.71 ± 0.08	b 5.51 ± 0.21	cd 18.15 ± 0.28	a 7.33 ± 0.02	bc 13.26 ± 1.03	a 8.78 ± 0.29	a 18.56 ± 0.44	a 7.65 ± 0.07	a 17.88 ± 0.46	b 8.56 ± 0.35	ab 15.47 ± 0.16	b 8.01 ± 0.3	ab 14.18 ± 0.19	b 13.29 ± 0.18	b 20.25 ± 0.17
AW	b 7.92 ± 0.04	b 12.00 ± 0.12	a 7.87 ± 0.18	d 17.7 ± 0.38	a 7.27 ± 0.01	d 8.46 ± 0.62	a 7.74 ± 0.21	d 15.57 ± 0.37	c 7.36 ± 0.03	b 12.97 ± 0.44	c 7.74 ± 0.09	ab 15.89 ± 0.60	c 7.52 ± 0.05	c 11.99 ± 0.17	b 14.52 0.40	ab 20.77 ± 0.40
AA	b 7.86 ± 0.17	b 11.93 ± 0.15	a 8.04 ± 0.55	a 20.37 ± 0.01	a 7.34 ± 0. 04	cd 10.16 ± 1.47	a 8.74 ± 0.39	abc 17.38 ± 0.24	bc 7.39 ± 0.03	B 14.67 ± 0.32	bc 8.16 ± 0.05	a 16.34 ± 0.53	b 8.00 ± 0.10	ab 14.19 ± 0.34	b 14.35 ± 0.71	ab 20.19 ± 0.19
Aviax	a 8.38 ± 0.02	a 13.67 ±0.18	a 9.04 ± 0.36	d 17.67 ± 0.25	a 7.39 ± 0.03	a 16.67 ± 0.59	a 8.85 ± 0.23	bc 16.95 ± 0.41	a 7.77 ± 0.04	ab 15.15 ± 0.65	a 9.34 ± 0.10	a 16.55 ± 0.26	ab 8.15 ± 0.10	b 13.43 0.22	a 16.82 ± 1.45	c 18.80 ± 0.19
Coccivac-B	b 7.81 ± 0.15	b 12.12 ± 0.07	ab 7.19 ± 0.88	b 19.32 ± 0.22	a 7.39 ± 0.03	ab 14.91 ± 1.1	a 8.46 ± 0.47	ab 18.04 ± 0.58	a 7.84 ± 0.08	a 17.64 ± 0.44	a 9.28 ± 0.22	a 15.71 ± 0.34	a 8.32 ± 0.05	a 14.9 ± 0.27	a 18.76 ± 0.20	a 21.26 ± 0.43

- Means within the same column carrying different letters are significant at 0.05.

AP = A. cina powder

AW = A. cina watery extract

AA = A. cina alcoholic extract

Table 2. MDA concentrations in serum (µmol/L) and tissues (liver, kidney & brain nmol/ g tissue) of Eimeria infected broiler chickens, controls and treated with anticoccidial agents. Data are mean ± SE (n = 5)

Age / group	28 days (1st week PI)				35 days (2nd week PI)			
	Serum	Liver	Kidney	Brain	Serum	Liver	Kidney	Brain
Control	a 7.60 ± 0.03	a 21.12 ± 0.98	ab 8.25 ± 1.02	a 18.41 ± 0.54	ab 7.97 ± 0.09	b 19.90 ± 0.65	c 9.43 ± 0.56	b 16.52 ± 0.28
AP	a 7.60 ± 0.03	a 23.45 ± 0.50	bc 6.36 ± 0.42	ab 17.51 ± 0.23	ab 7.96 0.03	a 23.88 ± 0.92	abc 12.39 ± 1.42	b 16.34 ± 0.08
AW	a 7.54 ± 0.05	b 16.09 ± 1.48	bc 6.75 ± 0.68	c 15.45 ± 0.18	c 7.71 ± 0.06	c 14.60 ± 0.48	bc 11.02 ± 0.37	c 15.60 ± 0.11
AA	a 7.44 ± 0.06	a 21.78 ± 1.57	c 5.44 ± 0.51	b 17.10 ± 0.22	bc 7.76 ± 0.05	c 14.63 ± 0.91	bc 10.41 ± 0.39	b 16.55 ± 0.11
Aviax	a 7.53 ± 0.04	a 20.38 ± 1.38	a 8.78 ± 0.55	b 16.90 ± 0.20	a 8.14 ± 0.05	c 16.65 ± 0.47	a 15.06 ± 1.05	b 16.47 ± 0.29
Coccivac-B	a 7.98 ± 0.01	a 20.13 ± 0.87	c 5.41 ± 0.59	a 18.13 ± 43	a 8.04 ± 0.09	b 20.80 ± 0.84	ab 13.16 ± 1.30	a 17.16 ± 0.15

- Means within the same column carrying different letters are significant at 0.05.

AP = A. cina powder
AW = A. cina watery extract
AA = A. cina alcoholic extract
PI = Post infection

Table 3. Significant difference of MDA content in all studied groups in comparable to that of controls with or without infection.

AP

MDA \ Age		21days	28days	35days	49days
Serum	-	=	=	=	=
	+		=	=	
Liver	-	=	=	=	=
	+		=	↑20%	
Kidney	-	↓	=	↓9.5%	=
	+		=	=	
Brain	-	=	↑	=	↑
	+		=	=	

AVIAX

MDA \ Age		21days	28days	35days	49days
Serum	-	↑	=	=	=
	+		=	=	
Liver	-	↑	↑	=	↓
	+		=	↓16.3%	
Kidney	-	=	=	=	↑
	+		=	↑59.7%	
Brain	-	↓	=	=	=
	+		↓	=	

AW

MDA \ Age		21days	28days	35days	49days
Serum	-	=	=	↓3.4%	↓
	+		=	↓3.3%	
Liver	-	=	↓	↓26.1%	↓
	+		↓	↓26.6%	
Kidney	-	=	=	↓18.2%	=
	+		=	=	
Brain	-	↓	=	=	↑
	+		↓	↓14.1%	

AA

MDA \ Age		21days	28days	35days	49days
Serum	-	=	=	=	=
	+		=	=	
Liver	-	=	=	↓16.4%	=
	+		=	↓26.5%	
Kidney	-	=	=	↓13.7%	
	+		↓	=	
Brain	-	↑	=	=	↑
	+		↓	=	

VACCINE

MDA \ Age		21days	28days	35days	49days
Serum	-	=	=	=	=
	+		=	=	
Liver	-	=	=	=	=
	+		=	=	
Kidney	-	=	=	=	↑
	+		↓	↑39.5%	
Brain	-	=	↑	=	↑
	+		=	↑9.8%	

AP A. cina powder
AW A. cina watery extract
AA A. cina alcoholic extract

\- without infection
\+ with infection

= Non significant with control
↑ Significantly higher than control
↓ Significantly lower than control

Contradictory, treating with AW extract leading to significant lowering effect on lipid peroxidation marker, MDA, in all tested organs and serum either with or without infection in comparison with control groups. The antioxidant effect of AW was more pronounced and constant at 35 days of age especially for liver tissue and serum while other determinations were similar or lower than that of the control, recording no elevation of MDA for anytime (Tables 1-3).

This effect revealing a powerful antioxidant action of AW that to our knowledge, has not been previously examined. Hence the mechanisms by which the AW inhibit lipid peroxdiation has not been previously elucidated. In our opinion the antioxidant power of AW may attributed to the bioactive fraction, flavonoids, which derived from A. cina in a *vitro* study ***(21)***. Flavonoids inhibit the activity of various enzyme systems; including cycloxygenase and lipoxygenase which are potentially pro-antioxidant and can generate radicals. By the previous inhibition flavonoids act as antioxidants, free radical scavengers and chelators of actions***(22-25).***

Antioxidant effects of flavonoids are even more potent than vitamin C and E. One study tested the effect of supplements of vegetables and fruits extracts, including sources of flavonoids, on lipid peroxidation. Plasma lipid peroxide concentration in the 15 subjects decreased from 16.85 to 3.13umo/l within 1-week and remained in this range through the additional 3-weeks of treatment ***(26).***

Flavonoids as subclass of polyphenols which are effective antioxidants in a wide range of chemical oxidation systems, being capable, for example of scavenging peroxyl radical, alkyl radicals, superoxide, hydroxyl radicals, nitric oxide and peroxynitrite in aqueous and organic environments. In similar manner to vitamin E, this activity is essentially due to the ease with which an hydrogen atom from an aromatic hydroxyl (OH) group can be donated to free radical and the ability of aromatic compound to support an unpaired electron due to delocalization around the n-electron system ***(8,27)***.

Treatment with A. cina in the form of alcoholic extract (AA) for broiler chicken characterized by lower antioxidant power than AW (Tables 1-3) without an effect on serum. In the light of our previous results it was viewed that efficacy of A. cina was optimally in watery extract and the antioxidant constituents of A. cina dissolve in water more than alcohol. In support of this view most flavonoids are present in plants bound to sugar as β-glycosides. Glycosides were considered too hydrophilic by passive diffusion in the small intestine ***(28,29).***

The beneficial effects of AW and AA were more obviously after 35days of age so we selected this age to study the correlation in non infected groups of AW and AA. The analysis of our data illustrated in Table 4 revealed no significant correlation between serum MDA and MDA of all testes organs (liver, kidney & brain). On contrary, MDA concentration in these organs significantly correlated with each other. Where a significant negative correlation between kidney tissue lipid peroxidantion and that of liver and brain tissues were obtained in Aw treated groups. And a highly significant positive correlation between MDA of liver tissue and brain tissues of AA treated groups were also obtained.

These results showed that serum MDA in this study was not indicator to lipid peroxidation of individual organs. Serum MDA reflected only the powerful antioxidant properties as noticed in 35days AW treated groups.

The levels of MDA in serum and other tested organs under the effect of Aviax (Tables 1-3) showed a high variation thus being hard to evaluate. Unlike antibiotics that depend on prooxidant activity of their therapeutic effects ***(30)***, the mode of action of Aviax as inophore antibiotic was upset the osmotic balance of the protozoan cell by altering the permeability of cell membranes for alkaline metal cations causing severe osmotic damage ***(31).***

Many authors ***(32-35)*** reported that ionphores (simidurmicin, monensin and maduramicin) and vaccine when they were given to the chickens as anticoccidials induced

Table 4. Correlations coefficient between MDA concentration in serum and tissues (liver, kidney & brain) and serum albumin levels and globulin in non infected AW & AA groups at 35 days of age in broiler chickens.

	Serum MDA		Liver MDA		Kidney MDA		Brain MDA	
	AW	AA	AW	AA	AW	AA	AW	AA
Liver	0.889	-0.585	-	-	-	-	-	-
Kidney	-0.620	-0.911	0.924*	0.831	-	-	-	-
Brain	0.294	-0.672	0.740	0.993**	-0.941*	0.864	-	-
Serum albumin	-0.276	0.000	0.645	0.792	-0.866	0.438	0.949*	0.721
Serum globulin	0.992	0.961	0.645	-0.332	-0.866	-0.431	0.949*	-0.427

* Correlation is significant at the 0.5 level.
** Correlation is highly significant at 0.01 level.
AW= A. cina watery extract
AA = A. cina alcoholic extract

many pathological changes in liver, kidney, intestine and spleen. These degenerative changes may explain the elevated level of MDA in serum and tissues (liver, kidney & brain) that observed sometimes in Aviax and vaccine groups and illustrated in Tables 1,2. The concentration of MDA in the liver and heart of diseased broiler was found to be twise the MDA in healthy birds ***(16)***. Although Aviax and vaccine appeared sometimes as pro-oxidant in this work we can not considered them as prooxidant since they behaved in most times as controls.

MDA at 49days of age was determined to study the effect of drug withdrawal (one week before marketing) on serum and other organs. A significant lower levels of MDA in comparison to control and other treated groups was only for AW treated groups (Tables 1-3) in serum and liver. The continuous antioxidant effect of AW post drug withdrawal is of major clinical importance not only for bird health but also for the consumer health through the main aim of broiler industry, meat production. Lipid peroxidation adversely affects the overall quality of foods including flavor, taste, and nutritional value. Moreover lipid peroxidation products such as peroxyl and hydroxyl radical are related to the development of cardiovascular diseases and other metabolic disorders such as mutagensis, carcinogenesis and aging ***(36,37)***.

Nutritive antioxidants (such as ∝ tochopherol, β carotene and vitamin C), spice extract and muscle dipeptides has been investigated as potential antioxidant in meat production ***(38)***. On the light of this concept and our results we suggest to use AW as food additives till the age of slaughtering aiming to add AW to the list of nutritive antioxidants that used for meat production but more studies are required.

It is worthy to mention that birds in this experiment has been exposed to heat stress as they reared at high ambient temperature of summer season temperature reached to 36-42°C. MDA in both serum and tissues (liver, brain and kidney) of rabbit was found to be increased under heat stress exposure ***(39)***. Heat stress increased MDA by increasing free radicals (which initiate lipid peroxidation) and decreaing cellular antioxidant defences either enzymatic as catalase and superoxide dismutase or nonenzymatic as glutathione ***(40,41)***.

The powerful antioxidant lowering effect of AW on MDA in the serum and tissues of broiler chickens inspit of heat stress as reported herein may characterized AW as anti heat stress agent. Moreover ***Tan (9)*** reviewed that genus *Artemisia* have antipyretic activity and some members of this genus contain ∝ and β santonin and asurbin caused a decrease in rectal temperature of rats in a way similar to dopamine. Supporting our results, diet supplementation with antioxidants as vitamin E and/or chromium reduced the elevated MDA in serum and tissues of heat stressed rabbit ***(39)***. Also such supplementation in broiler prevented the negative effects of heat stress ***(42)***..

Temperature stress considers one of physiologic factors that may change the protein concentration of birds ***(43)***. The mean values of total protein and their fractions were found to be decreased in serum of heat stressed rabbit ***(39)***. In comparable to reference ranges ***(44)***, our results recorded lower mean values in serum protein fractions, albumin and globulin, in all heat stressed broiler chickens herein, controls and treated (Tables 5,6).

This reduction of protein functions with raising temperature my be due to dilution of plasma proteins caused by increase in water consumed and decrease of protein synthesis as a result of the depression of anabolic hormonal secretion ***(45)***. Another explanation is the decrease in feed nitrogen intake which occurs under heat stress condition ***(46)***. Moreover, the increase in serum cortisol during heat stress inhibits protein synthesis in tissues and increase protein and lipid catabolism ***(40,41)***.

Blood plasma albumin particibate in the protection of erythrocyte membrane against lipid peroxidation by binding lipid peroxides ***(15)***. Table 4 showed significant positive correlation only between brain tissue MDA

Table 5. Serum albumin and globulin concentrations (mg/dl) in six studied non infected groups. Data are mean ± SE (n = 5)

Age / group	21 days			28 days			35 days			49 days		
	Albumin	Globulin	A/G ratio	Albumin	Globulin	A/G ratio	Albumin	Globulin	A/G ratio	Albumin	Globulin	A/G ratio
Control	a 1.46 ± 0.13	a 1.42 ± 0.15	bc 1.08 ± 0.16	a 1.33 ± 0.13	ab 1.46 ± 0.20	b 0.95 ± 0.09	a 1.52 ± 0.14	ab 1.56 ± 0.015	bc 0.99 ± 0.08	a 1.62 ± 0.03	a 2.44 ± 0.22	c 0.68 ± 0.05
AP	a 1.56 ± 0.19	a 1.40 ± 0.07	bc 1.11 ± 0.11	a 1.28 ± 0.08	ab 1.40 ± 0.19	b 0.97 ± 0.11	a 1.40 ± 0.13	a 1.96 ± 0.06	c 0.72 ± 0.08	a 1.70 ± 0.08	a 2.70 ± 0.23	c 0.65 ± 0.08
AW	a 1.52 ± 0.17	a 1.62 ± 0.15	c 0.93 ± 0.03	a 1.52 ± 0.08	a 1.70 ± 0.11	b 0.90 ± 0.04	a 1.90 ± 0.26	ab 1.80 ± 0.10	bc 1.06 ± 0.12	a 1.82 ± 0.03	c 1.42 ± 0.12	a 1.31 ± 0.09
AA	a 1.94 ± 0.24	a 1.24 ± 0.09	a 1.61 ± 0.25	a 1.28 ± 0.09	c 0.90 ± 0.04	a 1.43 ± 0.11	a 1.56 ± 0.06	c 1.12 ± 0.08	a 1.43 ± 0.14	a 1.78 ± 0.08	bc 1.88 ± 0.15	b 0.97 ± 0.09
Aviax	a 1.62 ± 0.13	a 1.36 ± 0.21	abc 1.24 ± 0.08	a 1.66 ± 0.14	bc 1.06 ± .07	a 1.58 ± 0.14	a 1.62 ± 0.16	bc 1.38 ± 0.12	ab 1.19 ± 0.11	a 1.68 ± 0.09	ab 0.26 ± 0.07	bc 0.75 ± 0.06
Coccivac-B	a 1.38 ± 0.11	a 0.96 ± 0.16	ab 1.53 ± 0.17	a 1.30 ± 0.08	c 0.88 ± 0.06	a 1.48 ± 0.03	a 1.48 ± 0.12	ab 1.80 ± 0.24	bc 0.88 ± 0.14	a 1.52 ± 0.08	c 1.54 ± 0.02	b 0.99 ± 0.06

- Means within the same column carrying different letters are significant at 0.05.
AP = A. cina powder
AW = A. cina watery extract
AA = A. cina alcoholic extract

Table 6. Serum albumin and globulin concentrations (mg/dl) in six studied coccidia infected groups. Data are mean ± SE (n = 5)

Age / group	28 days (1st week PI)			351 days (2nd week PI)		
	Albumin	Globulin	A/G ratio	Albumin	Globulin	A/G ratio
Control	a 1.56 ± 0.09	d 1.64 ± 0.15	b 0.99 ± 0.11	a 1.50 ± 0.11	c 1.60 ± 0.14	a 0.96 ± 0.09
AP	a 1.38 ± 0.05	bc 1.16 ± 0.13	ab 1.27 ± 0.19	a 1.50 ± 0.15	bc 1.82 ± 0.12	a 0.83 ± 0.08
AW	a 1.58 ± 0.07	d 1.82 ± 0.15	b 0.90 ± 0.11	a 1.70 ± 0.17	a 2.20 ± 0.13	a 0.079 ± 0.05
AA	a 1.36 ± 0.10	ab 1.48 ± 0.21	b 0.96 ± 0.09	a 1.64 ± 0.05	ab 2.00 ± 0.04	a 0.82 ± 0.03
Aviax	a 1.64 ± 0.07	bc 1.10 ± 0.04	a 1.51 ± 0.12	a 1.40 ± 0.07	c 1.56 ± 0.09	a 0.90 ± 0.04
Coccivac-B	a 1.40 ± 0.07	c 1.02 ± 0.06	a 1.39 ± 0.07	a 1.50 ± 0.10	abc 1.92 ± 0.13	a 0.79 ± 0.04

- Means within the same column carrying different letters are significant at 0.05.

AP = A. cina powder
AW = A. cina watery extract
AA = A. cina alcoholic extract
PI = Post infection

and serum albumin and globulin after 35days of AW supplementation. Such correlation may be through cerebrospinal fluid as it approximates an ultrafilterate of plasma.

It could be concluded that:

1- Antioxidant activity achieved only for watery and alcoholic extract of A. cina.

2- Antioxdiant power was more potent in watery extract.

3- Other treatments AP, Aviax and vaccine were neither antioxidant nor prooxidant.

4- Unfortunately, serum MDA was not indicator to MDA of other individual organs.

5- Serum albumin significantly correlated only with MDA of brain tissue.

Based on the beneficial effect of AW as natural antioxidant we suggest to use AW as food additive till the age of marketing to protect the broiler chickens against bad effects of lipid peroxidation that may be helpful to decrease the load of climatic stress and produce meat of high quality.

REFERENCES

1. ***Halliwell B and Gutteridge J (1993):*** Free Radicals in medicine and Biology, 2nd ed. Clarendon Press, Oxford.

2. ***Iqbal M, Probert, L, Alhumadi N and Klandorf H (1999):*** Protein glycosylation and advanced glycosylated endproducts (AGEs) accumulation: an avian solution? Journal of Gerontology, 54A (4): 171-176.

3. ***Simon, H H, Haj-Yehia A and Lives-Schaffer F (2000):*** Role of reactive oxygen species (ROS) in the apoptosis induction", apopotosis 5:415-18.

4. ***Donati Y R A, Slosman D O and Polla B S (1990):*** Oxidative injury and the heat shock response. Biochem. Pharmacol. 40: 2571-2577.

5. ***Lin Y F, Chang S J and Hsu A L (2004):*** Effects of supplemental vitamin E during the laying period on the reproduction performance of Taiwan native chickens. Br. Poult. Sci. 45: 807-814.

6. ***Mujahid A, Yoshiki Y, Akiba, Y and Toyomizu M (2005):*** Superoxide radical production in chicken skeletal muscle induced by acute heat stress. Poultry Science October 5, 84: 307-314.

7. ***Ruiz J A, Perez-Vendrell A M and Esteve-Garacia E (1999):*** Effect of β-carotene and vitamin E on oxidative stability in leg meat of broilers fed different supplemental fats. J. Agric. Food Chem., 47 (2): 448-454.

8. ***Sandak R N and El-Hadidy E M (2004):*** Utilization of natural antioxidants and their effects on human health. Advances in Agricultural Research in Egypt, Special Issue. Egypt J. Agric. Res. 5(1) 1-97.

9. ***Tan R X, Zheng W F and Tang H Q (1998):*** Biologically active substances from the genus artemisia (Review). Planta Med. 64: 295-302.

10. ***Eid A M, Abd-El-Lateif A E, El-Dieb M M and El-Said M A (2004):*** Anticoccidial efficacy of *Artemisia cina* and its extracts in broiler chickens. Zag. Vet. J, 32 (2): 163-176.

11. ***El-Said M A, Abd El-Lateif A E, Abdien, H M F and El-Deib M M (2008):*** Comparative study on the efficacy of watery extract of *Artemisia cina*; semiduramicin and Coccivac-B as anticoccidial agents in broiler chickens. Zag. Vet. J. 36 (5):182-196.

12. ***Yoshioko T N, Kawada T, Shimoda M and Mori (1979):*** Lipid peroxidation in maternal and cord blood and protective mechanisms against activated oxygen toxicity in the blood". Am. J. Obstet. Gynecol. 135: 372-6.

13. ***Henry R G (1976):*** Clinical Chemistry. Hoeber, N.Y. 413.

14. ***Gindler E M and Westegard, J O (1973):*** Clin. Chemi. 6,7.

15. ***Stroev E A and Macarova V G (1989):*** Laboratory Manual in Biochemistry: study

in peroxide oxidation of biological membrane lipids. 1st ed. English translation, Mir Publishers, Moscow, 244-255.

16.Snedecore, G W and Cochran, W G (1967): Statistical methods. The Iowa State Uni. Press. Ames, Iowa USA, P. 593.

17.Diaz-Cruz A C , Nava, R , Villanueva M , Serret, R, Guinzberg and Pina, E (1996): Heptaic and cardiac oxidative stress and other metabolic changes in broilers with the ascites syndrome. Poultry Science, 75: 900-904.

18.Trudy, M and James, R M (2003): "Oxidative stress" Biochemistry, the molecular baseis of live. 3rd Ed.: 319-320.

19.Ozturk O and Gumulsu S (2004): Age related changes of antioxidant enzyme activities, glutathione status and lipid peroxidation in rat erythrocytes after heat stress. Life Science, 75: 1551-1556.

20.Gehan N A E (2005): Pathological studies on some feed supplement in chickens infected with coccidiosis. A Thesis Ph.D., (Pathology), Faculty of Vet. Med., Zagazig University.

21. Ryahovskaya T V, Ushbaeva G G and Zhemaletdinov F G (1989): Rastit. Resur. 25, 249-253. Chem. Abst. 111, 70445h. Cited in Biologically active substances from the genus Artemisia (Review). Planta Med. 64: 295-302.

22.Chu Y H, Chang C L and Hsu H F (2000): Flavonoids content of several vegetables and their antioxidant activity. *J. Sci. Food Agric., 80: 561-6.*

23.Knekt P, Jarvinen R, Reunanen A and Maatela J (1996): Flavonoid intake and coronary mortality in Finland; a cohort study. Brit Med. J., 312 : 478-481.

24.Knekt P, Kumpulainen J, Jarvinen R, Rissanen H, Heliovaara M, Reunanen A, Hokulinen T and Aromaa A (2002): Flavonoid intake and risk of chronic diseases. *Am. J. Clin. Nutr., 76: 560-8.*

25.Parr AJ and Bolwell G P (2000): Review: Phenols in plant and in man. The potential for possible nutritional enhancement of the diet by modifying the phenols content or profile. 3. Sci. Food. Agric., 80 : 985-1012.

26.Lampe J W (1999): Health effects of vegetables and fruit: assessing mechanism of action in human experimental studies. *Am. J. Clin. Nutri., 70: 4755-4905.*

27.Duthie G G and Crozier A (2000): Pant-derived phenolic antioxidants. *Current Opinion Lipidol., 11: 43-47. (C.F. Duthie et al., 2000, Nutr. Res Reviews, 13: 79-106).*

28.Cook N C and Samman S (1996): Flavonoids-chemistry, metabolism, cardio-protective effects and dietary sources, *J. Nutr. Biochem., 7: 66-76.*

29.Plumb G W, Price K R, Garcia-Conesa M T and Williamson G (1998): Effects of glycosylation, esterification and polymerization of flavonoids and hydroxycinnates on antioxidant properties: A structure-function study, *Polyphenols Communications 98 (pp 35, 36). International Conference of Polyphenols, Lille, 1-4 Sept.*

30.Yoshie Y and Oshima H (1998): Synergistic induction of DNA strand breakage by catechol, estrogen and nitric oxide: implications for hormonal carcinogenesis. Free rad. Boil. Med; 24(2): 341-8.

31. Calnek B W, Barnes H J, Beard C W, McDougakd L R and Saif Y M (1997): Diseases of poultry. 10th Ed. Editorial Board for the American Association of Avian Pathologists. Mosby-Wolfe.

32.Saber H M (1995): Pharmacological studies on some anticoccidial drugs in chickens. Thesis for Ph.D., Fac. of Vet. Med. Zagazig University.

33.Halla M R (1995): Pharmacological studies on some anticoccidial drugs in chickens. A thesis for M.V.Sc. (Pharmacology) Fac. of Vet. Med., Zagazig University.

34.Mervat T (2000): Some pharmacological studies on some recent anticoccidial agents in poultry. A thesis for M.V. Sc. (Pharmacology) Fac. of Vet. Med. Zagazig Univ.

35.Sanaa M M S (2005): Pharmacological studies on the effect of some coccidial vaccines in replation with clostridia infection in chickens. A thesis, Ph.D. (Pharmaclogy), Fac. Vet. Med. Zagazig. University.

36.Ruiz J A, Perez-Vendrell A M and Esteve-Garacia E (1999): Effect of B-carotene and vitamin E on oxidative stability in leg meat of broilers fed different supplemental fats. *J. Agric. Food Chem., 47(2): 448-454.*

37.Galobart J, Barroeta A C, Baucells M D, Codony R and Ternes W (2001): Effect of dietary supplementation with Rosemary extract and α-Tocopheryl acetate on lipid oxidation in eggs enriched with ω3-fatty acids., Poultry Science 80:460-467.

38.Chan W K, Decker E A, Lee J B and Butterfiled D A (1994): EPR spin-trapping studies of the *hydroxyl* radical scavenging activity of camosine and related dipeptides, *J. Agric. Food Chem., 42: 1407-1410.*

39.El-Sayed G R, El-Sherbini S. and Gamil M (2009): Reduction of heat stress in rabbits by some antioxidatnts., 6th Int. Sci. Conf., Mansoura, 22-23 April 2009: 811-827.

40.Sahin K, Kucuk O and Sahin N (2001): Effect of dietary chromium picolinate supplementation on performance, insulin and corticosetrone in laying hens under low ambient temperature. Anim. Physiol. Anim. Nutr. 85, 142-147.

41.Mohamed T I and El-Sayed G R (2007): Performance, biochemical parameters and oxidation status of heat-stressed growing rabbits in response to dietary of chromium picolinate and vitamin E supplementation. Mansoura, Vet. Med. J. 9 (1), 109-128.

42.Sahin K, Sahin N and Kucuk O (2003): Effects of chromium, and ascorbic acid supplementation on growth, carcass traits, serum metabolites, and antioxidant status of broiler chickens reared at a high ambient temperature. Nutr. Res. 23, 225 - 238.

43.Hochleithner M (1994): Biochemistries in: Avian Medicine: Principles and Application. Ritchie B. R., Harrison, G.J. and Harrison, Linda Rds. Wingers Publishing Inc. Lake Woth, Florida, USA. PP. 223-245.

44.Farahat, A A (1975): Morphological and biochemical constituents of the blood in the farm and experimental animals. 1st ed. Al-Kilany Press, Cairo P. 7-8.

45.El-Masry K A and Habeeb A A (1989): Thyroid functions in lactating Frisian cows and water buffaloes under winter and summer Egyptians conditions. In: Proceedings of 3rd Egyptian-British Conference on Animal, Fish and Poultry Production, Alexandria, Egypt 2, 613-620.

46.Marai I F M, Habeeb A A M and Gad A E (2002): Rabbits productive, reproductive and physiological performance traits as affected by heat stress: a review Livestock Production Science. 78, 71-90.

الملخص العربي

دراسة التأكسد الفوقي للدهون الخلوية في بداري التسمين تحت تأثير بعض مضادات الكوكسيديا

[1]مها مصطفى علي الديب، [2]أحلام السيد عبد اللطيف

[1]مستشفى كلية الطب البيطري – جامعة الزقازيق – كيمياء حيوية

[2]معهد بحوث صحة الحيوان – فرع الزقازيق – علم الأدوية

نظراً لأهمية الأكسدة الفوقية للدهون الخلوية في تقييم استخدام أي مواد دخيلة على الجسم سواء من مصدر طبيعي أو كيميائي أجري هذا البحث بهدف دراسة وتقييم التأثير المضاد للأكسدة أو البادئ للأكسدة لكل من الشيح الخرساني ومستخلصيه المائي والكحولي كمضادات للكوكسيديا ومقارنتهم بمضادات الكوكسيديا الأخرى الشائعة الاستخدام مثل عقار السيميديورواميسين أو لقاح الكوكسيديا الحي (كوكسيفاك – ب) في بدارى التسمين المصابة أو الغير مصابة بالكوكسيديا.

أجري هذا البحث على ١٨٠ من بداري التسمين تم تقسيمهم إلى ستة مجموعات الأولى تركت كمجموعة ضابطة والمجموعات من الثانية حتى الخامسة تم إعطائهم مسحوق الشيح الخرساني أو مستخلصيه المائي أو الكحولي أو عقار السيميديورواميسين كإضافات أعلاف على التوالي منذ اليوم الأول وحتى عمر ٤٢ يوم، أما المجموعة السادسة فقد تم تحصينها بالكوسيفاك–ب مرة واحدة عند عمر ثلاثة أيام، وقد تم عدوى نصف عدد طيور هذه المجموعات بالكوكسيديا عند عمر ٢١ يوم.

تم قياس المالوندايالديهايد (MDA) في مصل الطيور وفي كل من أنسجة الكبد والكلى والمخ وكذلك تم قياس البروتين الكلي والألبيومين وتقدير الجلوبيولين في مصل الطيور.

أوضحت الدراسة التالي:

(١) انخفاض الأكسدة الفوقية للدهون الخلوية في المجموعات المعالجة بالمستخلص المائي أو الكحولي للشيح الخرساني فقط دون غيرها من العلاجات الأخرى المستخدمة في هذه الدراسة، والتى ظهرت بوضوح بعد ٣٥ يوم من إضافتها لعليقة الطيور سواء المصابة أو الغير مصابة بالكوكسيديا.

(٢) إن هذا الانخفاض في الأكسدة الفوقية للدهون الخلوية كان أقوى مع المستخلص المائي سواء في المصل أو في الأنسجة مما يشير إلى قوة المستخلص المائي (AW) كمضاد طبيعي للأكسدة.

(٣) أن باقي العلاجات المستخدمة (مسحوق الشيح الخرساني، الأفياكس، أو الكوكسيفاك ب) لم تكن مضادة للأكسدة ولا بادئة لها بل كانت تحاكي المجموعة الضابطة في أغلب الأحوال دون فروق معنوية.

وفي ظل الاحتياجات المتزايدة لمضادات الأكسدة المأخوذة من مصادر طبيعية تم مناقشة أهمية استخدام المستخلص المائي للشيح الخرساني كمضاد طبيعي للأكسدة سواء لصحة الطيور أو لصحة الإنسان المستهلك للحوم هذه الطيور.