The roles of vitamin E against free radicals during albendazole treatment in broiler chicken.

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Abstract

The present study was conducted to determine the effect of albendazole alone or in combination with vitamin E on antioxidant activity and histopathology, changes in the liver and kidney. Following oral administration of albendazole, 0.25 mg/kg body weight. Vitamin E 0.01 mg/kg body weight was used for 21 successive days on broiler chicken. The experiment was done on fifteen broiler chickens divided into three groups: one non-treated, two treated with albendazole 0.25 mg/kg body weight, and three treated with albendazole in combination with vitamin E 0.01 mg/kg body weight. The blood sample and tissue were taken at the end of the experiment, 12hrs after the last dose. The experimental result revealed that the significant decrease of liver enzymes caused by albendazole like serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ATP), when compared with control group. The experimental revealed the decrease in kidney parameters like urea creatinine level caused by albendazole and finally there was a significant increase in antioxidant enzymes activity like CAT, SOD, GPX and a significant decrease in MDA. Histopathology results in liver-treated animals with albendazole in combination with vitamin E showed dilated, congested portal blood vessels (PBV, arrow), mild to moderate biliary proliferation (BP, arrow), portal round cell aggregation (RCA, arrow), and focal hepatocellular degeneration (HCD, arrow). Histopathology result of kidney-treated animal with albendazole in combination with vitamin E showing a mild peritubular edema (PTE arrow), focal tubular degeneration (TD arrow), tubular regeneration (TR arrow) glomerular lobulation and atrophy (GL arrow), beside interstitial cell's aggregation (RCA arrow). H&E X200, 400. Therefore, vitamin E should be taken with albendazole to decrease its effect.

Keywords: Albendazole, vitamin E, and Antioxidant enzymes.

1 - INTRODUCTION

Oxygen is an indispensable element for the sustenance of living beings and many biological systems. Cells reduce oxygen and generate adenosine triphosphate (ALP) in the mitochondria. By-products known as free radicals are created during this process. These free radicals are beneficial at moderate levels but at higher concentrations can damage tissue by oxidative stress [1]. Antioxidants have been defined as substances that prevent the genesis of reactive oxygen species (ROS) or other oxidants, and repair the damage they cause [2]. Antioxidant defense systems act as a stable and symmetrical framework, and each depends on the activity of the other in health; the stability lies somewhat in support of the reactive species so that they can accomplish their biological roles. Repair systems protect against damage which happens at a low level in healing individuals[3]. Antioxidants are molecules that prohibit the oxidation of other molecules. Oxidation reaction means that chemical reaction transports electrons or hydrogen from a substance to an oxidizing agent. Oxidation reactions can output free radicals in turn these radicals can begin a chain reaction by removing free radical intermediates and prohibit other oxidation reactions. Antioxidants are often reducing agents such as anthills, ascorbic acid or polyphenols, tocopherols and toils [4]. The antioxidant defenses consist of a low molecular mass antioxidant such as vitamin. E and enzymes e.g. SOD, CAT, GPX the mission of antioxidant enzymes is to protect tissues and body fluids from damage by ROS, RNS whether produced physiologically or as a response to inflammation, infection or disease.[5] Vitamin E (vit.) is an important antioxidant in biological systems that decrease the peroxidation of un-structural lipids by a chain breaking free radical (FR),so it shares in the stability of cellular membranes ,Vitamin E α-tocopherol) is the most important lipid phase antioxidant .[6] Albendazole is one of the most widely used as anthelmintic in poultry. It is too active against most gastrointestinal and respiratory nematodes of cestodes and trematodes in the metabolism of AB gene generates several metabolites including two major ones. Albendazole Sulfoxide, which is active, and Albendazole sulfone which is inactive. Albendazole Mechanism of action. Albendazole Sulfoxide, the active metabolite, causes selective degeneration of cytoplasmic microtubules in intestinal and tegmental cells of intestinal helminths and larva. The metabolites bind to the B-tubulin subunit of helminthes microtubules polymerization. Albendazole Also causes impaired glucose utilization and causes a decrease in parasite glycogen stores. At high concentration albendazole inhibits parasite metabolic pathways such as Krebs cycle by inhibiting key enzymes such as malate dehydrogenase Subsequent decrease in Alp production occurs which cause energy depletion which leads to immobilization of parasite and subsequent death [8] this review covers key studies of effect of albendazole in combination with vitamin E on antioxidant than tissue and blood samples were collected and the protective effect of vitamin E on liver and kidney appreciated through measuring biochemical constituents is sera such as serum creatinine level, liver biomarkers as serum alanine aminotransferase (ALT),
phosphatase (ALP), total proteins, albumin and oxidative stress biomarkers such as catalase (CAT), superoxide dismutase, SOD and malondialdehyde (MDA) the obtained results vitamin. E. has protection through prohibiting the rise in liver and kidney injury biomarkers, and also the current pathological result enhances this effect.

2-MATERIAL AND METHODS
2.1: Drugs and chemicals.
A- Vitamin E (vitamin E. Capsule) was supplied by PHARCO pharmaceutical CO., Alex., Egypt, and vitamin E is dissolved in corn oil.
B- Albendazole 2-5 %pharm sweet Egypt.

2.2: Animals
Fifteen Broiler chicken. Twenty-one days old. Weighting about (400gm) used in this study. All chickens were maintained under similar conditions- the chickens were housed in batteries in a post- graduate research laboratory in the faculty of veterinary medicine. Zagazig University and a balanced ration with free access to water chicken were kept for one week for accommodation. Condition be for beginning of Experimental.

2.3: Experimental design
2.3.1: Chicken were classified to 3 group each one group 5 chicken
- The first group served as control non- tread.
- The second group received the therapeutic dose of albendazole 2- 5 mg/ kg orally for 21 days following the suction of the manufacturing company.
- The third group received albendazole in combination with vitamin E in therapeutic dose orally for 21 days.

2.3.2: Preparation of serum sample and tissue sampling
At the end of the experiment (12 hrs). After the last dose chicken was sacrificed and the following samples were collected. Blood collected from all chicken blood was taken on EDTA Coated tubes for hematological study serum separation to other samples for biochemical determination of liver function and kidney function test following necropsy tissue S specimens from liver and kidney for histopathological examination.
Biochemical markers of liver and kidney injury
Determination of serum alanine aminotransferase (ALT) aspartate aminotransferases (AST) activities was established according to the principles described previously (8,9), also evaluation of activity of serum alkaline phosphatase (ALP) was determined according to the principles mentioned before (LO) Evaluation of creatinine, urea and uric acid has been done according to the method previously (11). Determination of these parameters was carried out through commercial kits from spectrum diagnostics.

2.3.3: Hepatic and nephron his to pathological evaluation
Liver and kidney tissues were fixed in 10% neutral buffered for the main solution for 24 hrs. Then, the tissue processing and paraffin blocks preparation were done. Masson’s trichrome and hematoxylin eosin stains were used to evaluate circulatory disturbances, inflammation, degeneration, apoptosis, necrosis and any other pathological changes in the examined tissues according to method of (Suvarna et al., 2013).

2.3.4: Biochemical markers of antioxidant activity
Determination of catalase activity (CAT), superoxide dismutase activity (SOD), glutathione peroxidase activity (GPX) and malondialdehyde activity (MDA) by method according to previous principles (B), (14) and (15).

2.4: Statistical analysis
The data were analyzed using prism version 6, statistical evaluation of the result except as one analysis of variance (ANOVA).

3- RESULTS AND DISCUSSION
3.1: Effect of oral administration of albendazole and its combination with vitamin E
Table (1) below shows the effect of oral administration of albendazole and its combination with vitamin E once daily for 21 successive days on erythrocytes count, hemoglobin concentration, PCV and leukocyte count in broiler chickens post treatment (mean ± SE) n = 5

<table>
<thead>
<tr>
<th>Groups</th>
<th>RBCs (10⁶/µl)</th>
<th>Hb (g/dl)</th>
<th>PCV (%)</th>
<th>WBC (10³/µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.29 ± 0.24c</td>
<td>8.13 ± 0.22c</td>
<td>47.0 ± 0.31c</td>
<td>152.0 ± 4.2c</td>
</tr>
<tr>
<td>Albendazole</td>
<td>4.00 ± 0.36 b</td>
<td>7.00 ± 0.18b</td>
<td>42.00 ± 0.35b</td>
<td>149.00 ± 7.9b</td>
</tr>
<tr>
<td>Albendazole + vit. E</td>
<td>5.49 ± 0.24a</td>
<td>10.73 ± 0.17a</td>
<td>50.00 ± 0.32a</td>
<td>167.00 ± 7.8a</td>
</tr>
</tbody>
</table>

* Means with the same column carrying different superscripts are significantly different at P < 0.05.
3.1.1: Effect on RBcs, Hb, PCV, WBcs
Shown clearly from table (1) above that the oral administration of albendazole alone or in combination with vit. E in therapeutic dose for three week to broiler chicken induced significant increase in RBcs, Hb, PCV, WBcs level when compared with albendazole group.

3.1.2: Effect on serum AST, ALT, ATP
It was clearly evident from table below (2) that the oral administration of albendazole alone or in combination with vitamin E in therapeutic dose for three week to broiler chicken induced significant decrease in AST, ATP, ALT level when compared with albendazole group.

Table 2: Effect of oral administration of albendazole and its combination with vitamin E once daily for 21 successive days on serum AST, ALT and ALP in broiler chickens post treatment (mean ± SE) n = 5

<table>
<thead>
<tr>
<th>Groups</th>
<th>AST (U/L)</th>
<th>ALT (U/L)</th>
<th>ALP (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>33.35 ± 1.58c</td>
<td>37.60 ± 2.70c</td>
<td>33.00 ± 2.20b</td>
</tr>
<tr>
<td>Albendazole</td>
<td>75.33 ± 11.40a</td>
<td>62.36 ± 6.47a</td>
<td>44.70 ± 2.51a</td>
</tr>
<tr>
<td>Albendazol + vit. E</td>
<td>68.35 ± 3.08b</td>
<td>42.55 ± 5.12b</td>
<td>30.6 ± 2.70</td>
</tr>
</tbody>
</table>

* Means with the same column carrying different superscripts are significantly different at P < 0.05.

3.1.3: Effect on creatinine urea
It was clearly evident from table (3) below that the oral administration of albendazole alone or in combination with vitamin E in therapeutic dose for three week to broiler chicken induces significant decrease in creatinine urea level when compared with albendazole group.

Table 3: Effect of oral administration of albendazole and its combination with vitamin E once daily for 21 successive days on serum creatinine and uric acid in broiler chickens post treatment (mean ± SE) n = 5

<table>
<thead>
<tr>
<th>Groups</th>
<th>Creatinine (mg/dl)</th>
<th>Uric acid (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.79 ± 0.07b</td>
<td>3.900 ± 0.32b</td>
</tr>
<tr>
<td>Albendazol</td>
<td>1.52 ± 0.07a</td>
<td>4.87 ± 0.63a</td>
</tr>
<tr>
<td>Albendazol + vit. E</td>
<td>0.89 ± 0.01c</td>
<td>3.00 ± 0.33c</td>
</tr>
</tbody>
</table>

* Means with the same column carrying different superscripts are significantly different at P < 0.05.

3.1.4: Effect on serum protein, albumin and globulin
It was clearly evident from table (4) bellow that the oral administration of albendazole alone or in combination with vitamin E in therapeutic dose for three week to broiler chicken induces significant decrease in serum protein, albumin, globulin, level when compared with albendazole group.

Table 4: Effect of oral administration of albendazole and its combination with vit. E once daily \21 successive days on serum total proteins, albumin and globulins in broiler post treatment (mean ± SE) n = 5

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total proteins (g/dl)</th>
<th>Albumin (g/ dl)</th>
<th>Globulins (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.73 ± 0.39c</td>
<td>4.10 ± 0.35c</td>
<td>3.21 ± 0.22c</td>
</tr>
<tr>
<td>Albendazol</td>
<td>8.72 ± 0.63a</td>
<td>5.30 ± 0.51a</td>
<td>4.10 ± 0.21a</td>
</tr>
<tr>
<td>Albendazol + vit. E</td>
<td>8.11 ± 0.55b</td>
<td>4.90 ± 0.51b</td>
<td>3.75 ± 0.22b</td>
</tr>
</tbody>
</table>

* Means with the same column carrying different superscripts are significantly different at P < 0.05.

3.1.5: Effect on antioxidant enzymes CAT, SOD, GPx, MDA.
It was clearly evident from table (5) bellow that the oral administration of albendazole alone or in combination with vitamin E in therapeutic dose for three weeks to broiler chicken induces significant increase in antioxidant enzymes like CAT, SOD, GPx, and significant decrease in MDA enzymes.
Table (5): Effect of oral administration of albendazole and its combination with vitamin E once daily for 21 successive days on serum SOD, CAT, GPX and MDA in broiler chickens post treatment (mean ± SE) n = 5

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD (U/ML)</th>
<th>CAD (U/ML)</th>
<th>GPX (U/ML)</th>
<th>MDA (U/ML)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>33.68 ± 0.069 a</td>
<td>256.21 ± 6.62 a</td>
<td>120.33 ± 1.50 a</td>
<td>7.45 ± 0.41 b</td>
</tr>
<tr>
<td>Albendazol</td>
<td>27.21 ± 0.021 b</td>
<td>200.00 ± 7.10 b</td>
<td>1110.21 ± 2.30 b</td>
<td>20.32 ± 0.11 b</td>
</tr>
<tr>
<td>Albendazol + vit. E</td>
<td>30.31 ± 0.21 c</td>
<td>240.11 ± 3.30 c</td>
<td>118.21 ± 2.10 c</td>
<td>9.11 ± 0.21 c</td>
</tr>
</tbody>
</table>

* Means with the same column carrying different superscripts are significantly different at P < 0.05

3.3: Histopathological result of liver
Liver of albendazole – treated animals 21-day sacrifice showing dilated portal blood vessels (RCA) and biliary proliferation proliferation hyperplasia (BP). Marked interstitial round cells aggregation. Hepatocellular degeneration (HCD) and individual cellular apoptosis (HCA). Hα E200.

![Figure 1. Liver of albendazole treated animals 21-days sacrifice](image1)

3.3.1: Liver of albendazole + vit. E) treated animals 21 day sacrifice showing Dilated, congested portal blood vessels (PBV) arrow), mild to moderate biliary proliferation hyperplasia (BP). Marked interstitial round cells aggregation, (RCA) arrow). And focal hepato cellular degeneration (HCD), arrow) Hα EX 200.

![Figure 2. Livers of albendazole+vitamin E treated animals 21days sacrifice](image2)

3.4: Histopathological result of kidney in 21 day
Fig.3 Photomicrograph of kidney (A, B).G2 Showing peritubular edema (PTE, arrow), marked glomerular lobulation and atrophy ( GA, arrow), interstitial round cell aggregation (RCA, arrow), and renal tubular degeneration( RTD, arrow) with focal early necrotic changes.

![Figure 3. kidney of albendazole treated animals 21days sacrificed.](image3)

Albendazole + vit E treated animal 21day sacrifice showing Amidper tubular edema (PTE, arrow), focal tubular degeneration (TL, arrow), tubular regeneration (TR, arrow), glomerular lobulation and atrophy (GL, arrow) beside interstitial round cells aggregations (RCA, arrow). HXE 200, 400.
3.5: Discussion
The present study conducted to investigate the effects of albendazole alone or in combination with vitamin E on broiler chicken and evaluate its impact on antioxidant enzymes and on some biochemical parameters as well as histopathological changes. The study result reported that the level of liver enzymes ALT, ATP, AST, were significant decrease when compared with albendazole group the study finding were in agreement with some previous study by (Nishikim, et al 1972) who reported that the antioxidant mainly using natural and synthetic antioxidant performs asensible therapeutic approach for prevention and treatment of liver disease due to role of oxidative stress in contributing to initiation and progression of hepatic damage. The result reported that the level of urea and creatinine were significant decrease when compared with albendazole group. The result reported that the significant increase in antioxidant enzymes like CAT, SOD, GPx activities with significant decrease in MDA when compared with albendazole group. These results are in agreement with (Outurk-urek et al 2001) who found that the antioxidant enzymes SOD, CAT, GPx activities in chicken were increased as lipid peroxidation levels were reduced upon supplementation with vitamin E.

4-CONCLUSION
It could be concluded that vit. E has a protective effect against hepatonephrotoxicity of albendazole which contributes to decreasing the harmful effect of albendazole by inhibiting free radical formation and by restoration of the antioxidant system in the combination of vit. E and albendazole showed a better result than albendazole alone;

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5-REFERENCES
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