Study the Impact of EDTA and Vitamin E Supplementation in Diet on Physiological, Biochemical and Histopathological Pictures of Broiler Chicks

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Abstract: A total number of 540 broiler chicks one week old were used in five weeks study to detect the effects of gradual levels of dietary EDTA disodium and vitamin E alone or in combination on performance, physiological, biochemical, histopatholgical, heavy metals and trace elements changes of broiler chicks. The basal broiler diet was supplemented with EDTA and vitamin E to compose 12 experimental diets, namely as follows: T1 (control), T2 (0.5g EDTA/kg feed), T3 (1.0g EDTA/kg feed), T4 (2.0g EDTA/kg feed), T5 (56mg Vit. E/kg feed), T6 (112mg Vit. E /kg feed), T7 (0.5g EDTA+ 56mg Vit. E/kg feed), T8 (0.5g EDTA+ 112mg Vit. E/kg feed), T9 (1g EDTA+ 56mg Vit. E /kg feed), T10 (1g EDTA+ 112mg Vit. E /kg feed), T11 (2g EDTA+ 56mg Vit. E /kg feed), T12 (2g EDTA+ 112mg Vit. E /kg feed). The obtained results indicated that addition of EDTA and Vit. E to broiler diets had no significantly effect on body weight and carcass characteristics. Results showed that groups (T9), (T2) and (T4) were significantly ($P \leq 0.05$) improved feed conversion ratio compared with the control group. There are no clear effects of EDTA and Vit. E on thermoregulation parameters. Muscle crude protein % was significantly ($P \leq 0.05$) increased while, either extract % was significantly ($P \leq 0.05$) decreased by increasing the levels of EDTA, Vit. E and their combination in broiler diets compared with the control group. Serum ALT and AST activities increased significantly ($P \leq 0.05$) as EDTA levels increased alone or by combined high level of EDTA (T11 and T12) with Vit. E in the diets. While, ALT and AST activities decreased significantly ($P \leq 0.05$) as Vit. E individual increased in the diet compared to the control group. AP activity showed significant ($P \leq 0.05$) a higher variation with the use of high level of EDTA alone (T3 and T4) or with high level of EDTA combined with Vit. E (T11 and T12) compared to the control group. Increase Vit. E level in broiler diet was significantly ($P \leq 0.05$) decreased the AP. Serum Chol and TG concentrations were significantly ($P \leq 0.05$) decreased with increasing EDTA and Vit. E levels either individual or in a combination in the broiler diets and both have synergistic effects to reduce the serum Chol and TG levels. Both of TP and Glob increased significantly as EDTA and Vit. E levels or their combinations increased in the broiler diet. Ca and P levels in breast muscles and serum were significantly ($P \leq 0.05$) decreased as EDTA levels increased in broiler diet or by integrated high level of EDTA with Vit. E. Na concentration in breast muscles was significantly ($P \leq 0.05$) increased as EDTA levels increased in broiler diet or by combination of EDTA with Vit. E compared to the control group. However, K was significantly ($P \leq 0.05$) decreased in breast muscles as EDTA level increased in the diets. This study showed that addition of EDTA and Vit. E individual or in a combination to diets of broiler chicks, reduced significantly ($P \leq 0.05$) the Pb and Cd levels in both of breast muscles and serum, helped to eliminate heavy metals from the bird bodies as compared to non treated birds. But Cu concentrations in breast muscles was significantly ($P \leq 0.05$) increased as EDTA, Vit. E and their combination levels increased in the broiler diet. Results of histopathological examination indicated that the examined organs appear normal in treated chicks with 1g EDTA in addition to 0.5 g EDTA either alone or with 56 mg and 112 mgVit. E. Whereas, the treated chicks with high level of EDTA (2g) either alone or combined with 56 or 112 mg Vit. showed variable degree of damage in examined tissues including hemorrhage, vacuolization, congestion, focal necrosis, cell infiltration, edema, atrophy of lymphoid follicles and depletion as shown in Figures 1, 2, 3, 4, 5 and 6 respectively.

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1. Introduction

Antioxidants are the vitamins, minerals, enzymes, or other chemical compounds such as ethylene diamine tetra-acetic acid (EDTA) that give up an electron to stop free radicals from causing oxidation. Antioxidants are able to destroy free radicals in cells before they can attack DNA or cause lipids to oxidize. EDTA is a synthetic amino acid

used orally and intravenously to cleanse, detoxify, and remove heavy metals from the body. EDTA chelating therapy was approved for use by the Food and Drug Administration (FDA) in 1950 and since that date EDTA used to treat cardiovascular disease to improve circulation, removes plaque and improves oxygen flow to the brain since it was approved by the FDA in 1950. **(World Resources Institute, 2002; Vega-Lopez *et al.,* 2004, Pennathur and Heinecke, 2004; Pouls, *et al.,* 2005; Krishnaiah *et al.,* 2007; Flora *et al.,* 2008; Pham-Huy *et al.,* 2008; Flora, 2009; Roussel *et al.,* 2009).**

Nezhad *et al.*, (2010) stated that, EDTA is an organic acid which has similar potential with citric acid and it increases availability of some minerals. EDTA is a strong chelated and it improves the absorption rate of minerals in diets of poultry.

The main function of vitamin E (Vit. E) is to work as a biological antioxidant, but it may also function in membrane structure, prevention of heavy metal toxicity, blood clotting, and biological oxidation-reduction reactions **(McDowell, 2000).** Predominantly excess Vit. E is provided in feeds to prevent oxidation and rancidity of added fat **(Lauzon *et al.,* 2008).** So it acts as a chain-breaking lipid antioxidant and free radical scavenger in the membranes of cells and subcellular organs **(Niu *et al.,* 2009).** Vit. E is a biological antioxidant, soluble in fat which inhibits the oxidation of long chained unsaturated fatty acids of the cell membrane **(Gutteridge and Halliwell , 2000; McDowell, 2000).** Unsaturated fatty acids react with oxygen, and form superoxide, peroxide and hydroperoxides. These free radicals cause cell damage by disturbing the metabolism and structure of the biological membranes of those organs that contain excessive amount of unsaturated fatty acids **(Bast *et al.,* 1991).** Vit. E inhibits the effects of hydrogen protons and free radicals by saturating them, and so inhibits autooxidation **(McDowell, 2000).** It has been reported that lipid peroxidation is stopped in chicks fed with Vit. E supplemented diet **(Arslan *et al.,* 2001).** The nutritional requirements for Vit. E in many animal species and the risks related to its deficiency are well established **(Xiao *et al.,* 2011).** Diet supplemented with Vit. E could increase the content of α-tocopherol and reduce the rancidity levels in chicken meat **(Gao *et al.,* 2010).** Vit. E plays important role in enzyme system in the animal body. Vit. E added to levels beyond those needed to support optimal growth is beneficial in improving the immunocompetence of growing broilers **(Erf *et al.,* 1998)** also supplementation of Vit. E in diet at level of 100 and 200mg/kg improved the immune response of broiler under heat stress conditions **(Niu *et al.,* 2009). Bobade *et al.,* (2009)** reported that the inclusion of Vit. E and C together at 150 mg/kg and 200 mg/kg diet respectively helped in improving both the growth of chicken and their immune response to vaccination. **Ciftci *et al.,* (2005)** showed that these two anti-oxidant compounds, Vit. E and Vit. C, have some protective actions against heat stress-induced-deleterious effects. Therefore, dietary supplementation by 125 mg Vit. E plus 200 mg Vit. C/kg diet may increase egg production and improve egg quality in laying hens during heat stress.

The obtained results by **Ipek *et al.,* (2007)** showed that Vit. E and C can be used to attenuate the negative effects of heat stress of laying Japanese quail. Stress increases Vit. E and C requirements of poultry. A combination of 240 mg of Vit. E and 240 mg of Vit. C supplementation may also be useful for live weight, weight gain, feed conversion ratio and can offer a potential protective management practice in preventing heat stress related losses in performance of Japanese quails. Supplementation of Vit. E at 100mg/ day for 30 days in New Zealand White Rabbit reduced cadmium in blood serum, liver, kidney and reduces the oxidative stress and reduced lipid peroxidation generated by cadmium and protective against many of the adverse effects of cadmium (**Beytut *et al.,* 2003).**

The present study was carried out for a period of five weeks to investigate the effect of EDTA disodium and Vit. E supplementation levels alone and their combinations in the diets on performance, physiological, biochemical profile, eliminate of some heavy metals and histopathological changes in broiler chicks.

2. Materials and Methods

Location, Experimental Birds and Management of the Flock:

This study was conducted at Poultry Experimental Station belonging to Animal Production Department, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt. Most of experimental analyzes of this study were done in the Laboratories of Animal Health Research Institute, Agriculture Research Center, Ministry of Agriculture, El-Dokki, Giza, Egypt. A total number of 540 broiler Ross 308 chicks one week old were used in the present study. The experiment was lasted for 5 weeks during summer and birds were housed on floor and had free access to food and water *(ad libitum*). All experimental diets were isocaloric, isonitrogenous and were formulated to meet the requirements of the strain. Diet specifications and composition analysis are given in Table (1). All birds were reared under similar managerial and hygienic conditions. Temperature degree and humidity percentage were recorded daily through the experiment and were

ranged between 33.6°C to 37 °C and 75 to 80 % respectively as average.

Experimental Design and Procedures:

At the beginning of the experiment, the birds were randomly distributed into 12 experimental groups and each group was divided into three replicates 15 birds for each. Averages of body weight for the 12 experimental groups were apparently uniform. The basal broiler diet was supplemented with EDTA disodium and Vit. E (α-tocopherol) to compose 12 experimental diet groups, namely as follows:

T1 (control).
T2 (0.5g EDTA/kg feed).
T3 (1.0g EDTA/kg feed).
T4 (2.0g EDTA/kg feed).
T5 (56mgVit. E/kg feed).
T6 (112 mg Vit. E/kg feed).
T7 (0.5g EDTA+ 56mg Vit. E/kg feed).
T8 (0.5g EDTA+ 112mg Vit. E/kg feed).
T9 (1g EDTA+ 56mg Vit. E/kg feed).
T10 (1g EDTA+ 112mg Vit. E/kg feed).
T11 (2g EDTA+ 56mg Vit. E/kg feed).
T12 (2g EDTA+ 112mg Vit. E/kg feed).

Vit. E and EDTA were mixed with feed every other day to avoid decomposition. Weekly body weights of birds were recorded to the nearest (0.1g).

Carcass and immune organs test:

At the end of experiment, 72 birds were slaughtered 6 from each group for carcass and immune organs tests.

***Blood Sampling*:**

At the end of the experiment, 6 samples from each group were taken randomly to blood sample. Birds were fasted overnight before bleeding via jugular vein in unheparinzed tubes to determine the blood profiles and serum was separated and stored frozen at –20^{O} C until analyzed.

Main Data Collection

1- Productive data

a- Body weight.
b- **b-** Body weight gain.
c- Feed intake.
d- **d-** Feed conversion ratio.
e- Carcass yield data.

Body weight, body weight gain, feed consumption, feed conversion ratio were calculated weekly during the experimental time.

2- Physiological and biochemical data

***a- Thermoregulation measurements*:**

Respiration rate, skin, cloaca and feather temperatures were measured 2 times weekly till the end of experiment.

e- Chemical examinations of breast muscles:

Samples of breast muscles at the end of the experiment were analyzed for moisture, protein, fat and ash contents. Moisture, Protein and fat content samples were measured according to **AOAC (1995).** Ash was determined according to the person's chemical analysis of food ***(*Quasem *et al.*, 2009*).***

f- ***Serum biochemical parameters:***

Alanine and aspartate amino transaminase (ALT and AST) activities were determined colorimeterically according to the method of **Retiman and Francle, (1957).** Serum alkaline phosphatase was determined according to the modified methods of **Kind and King, (1954).** Serum creatinine was estimated according to **Husdan and Rapoport, (1968).** Serum uric acid was measured according to **Arliss and Entvistle, (1981).** Serum total protein was determined according to **Weichselbaum, (1946).** Albumin was measured according to **Doumas, (1971).** Serum globulin and albumin to globulin ratio were calculated mathematically. Serum cholesterol and triglycerides were measured using commercially available kits from Sigma Diagnostics Company, (Taufkirchen, Germany) on an auto analyzer apparatus.

Serum calcium was measured according to **Gindler and King, (1972)** and serum phosphorus was measured according to **Goldenberg, (1966).** Serum potassium and sodium was measured using flame photometer according to **Oser, (1979).** The concentration of lead, cadmium, zinc and copper in the serum samples were taken directly from digital scale reading of atomic absorption spectrophotometer according to method of **Amodio-Cocchieri and Fiore, (1987).**

g- ***Estimation of heavy metals in breast muscle*:**

At the end of the experimental period broiler chicks breast muscle of 6 birds from each group were prepared and digested according to the technique recommended by **Khan *et al.*, (1995).** The filtrated extract was collected in tubes and kept at room temperature until analyzed by using atomic absorption spectrophotometer. Calcium, phosphorus, sodium, potassium, lead, cadmium, zinc and copper were measured by using Air/acetylene flame atomic absorption spectrophotometer (UNICAM 969 AA spectrometer).

3- Histopathological examination:

At the end of experiment target organs were collected from 6 birds of each group for histopathological examination. Specimens were collected from the liver, kidney, spleen, brain, heart and thymus gland of the sacrificed birds and directly fixed in 10% neutral buffered formalin. Five nicron paraffin sections were prepared, stained with hematoxylin and eosin according to **Bancroft and Gamble, (2008)** and examined microscopically.

Statistical analysis:

Data were subjected to analysis of variance using the General Linear Models procedure of SPSS software program package **(SPSS, 2001, version 11.0).** All percentages were first transformed to arcsine being analyzed to approximate normal distribution before ANOVA. Also, significant differences among means were determined by Duncan's multiple range test **(Duncan, 1955)** at 5% level of significant. Data were analyzed by one way method using the following model. $Y_{ij} = u + N_i + e_{ij}$ Where Y_{ij} = the observed value, u = population means, N_i = the effect of treatment, e_{ij} = the standard error.

3. Results and Discussion

Productive results:

Results of body weight, body weight gain, feed intake and feed conversion ratio of broiler chicks fed diets supplemented with different levels of dietary EDTA and Vit. E are presented in Table (2). Results showed that the final body weight and daily gain were insignificant between treated groups, which mean that the dietary treatments had no effect on the body weight or daily gain. There was no clear trend for the EDTA and Vit. E effect on body weight and daily gain. However, treatment fed 1g EDTA plus 56mg Vit. E (T9) was recorded a higher numeric value of body weight and daily gain followed by group fed 2g EDTA plus 56mg Vit. E (T11) and group fed 0.5g EDTA plus 112mg Vit. E /kg feed (T8). These results may attribute to that EDTA is (an organic acid) a strong chelator which appeared to improve the absorption rate and availability of minerals in poultry diets **(Vohra and Kratzer, 1965; Liem *et al.*, 2008; Nezhad *et al.*, 2010).** The affinity to chelate with metal is quantitatively described as stability coefficient. EDTA has higher stability coefficient with all of the minerals and this is the reason for metal sweeper of EDTA and when EDTA is available in system it could bind all cations **(Kratzer *et al.*, 1959 and Nezhad *et al.*, 2010** Furthermore, the reactive oxygen generated from metabolism of extraneous chemicals in the body can be removed by the antioxidant defense system which the EDTA and Vit. E part of it **(Droge, 2002 and Ciftci *et al.*, 2005).** Also supplementation of Vit. E in diet at level of 100 and 200mg/kg improved the immune response of broiler under heat stress conditions **(Niu *et al.*, 2009). Bobade *et al.*, (2009)** reported that the inclusion of Vit. E and C together at 150 mg/kg and 200 mg/kg diet respectively helped in improving both the growth of chicken and their immune response to vaccination.

Total feed intake and daily feed intake were significantly ($P \leq 0.05$) lower for group fed 56mg Vit. E (T5) compared to control and other groups. At the same time, total feed intake and daily feed intake were significantly ($P \leq 0.05$) higher for group fed 0.5g EDTA and 112mg Vit. E (T8). The obtained results revealed that dietary treatments of group fed 1g EDTA and 56mg Vit. E (T9), group fed 0.5g EDTA (T2) and group fed 2g EDTA (T4) were significantly ($P \leq 0.05$) improved in feed conversion ratio compared with the control group. While, group fed 2g EDTA and 112mg Vit. E (T12), group fed 1g EDTA combined with 112mg Vit. E (T10) and group fed 56mg Vit. E (T5) recorded worst feed conversion ration compared with the control and other groups. **Ipek *et al.*, 2007)** reported that a combination of 240 mg of Vit. E and 240 mg of Vit. C in diet may be useful for live weight, weight gain, feed conversion ratio and can offer a potential protective management practice in preventing heat stress related losses in performance of Japanese quails. These results suggested that EDTA and Vit. E may tend to encourage effective conversion of feeds to weight. **Niu *et al.*, (2009)** found that there were no significant improvement in body weight and feed intake of broiler chicken fed diets contained 100 and 200mg Vit. E when birds were exposed to 32°C, and suggested that the reduced efficiency could be caused by changes in metabolic utilization of nutrients.

The effect of experimental treatments on the composition of the bird carcasses (in gram) and relative to live body weight (%) are given in Table (3). The results of some carcass variables such live body weight, dressing weight, dressing % heart % and abdominal fat pad% showing no significant effect of dietary EDTA and Vit. E treatments or their combination on carcass yield characteristics among all groups. Although, the live body weight and most of dressing weight improved for all treated groups compared to the control group but not significantly. While group fed 56mg Vit. E/kg diet (T5) were significantly higher in giblets and liver % compared with the control group. These results are in general agreements with those obtained by **Bobade *et al.*, (2009); Adebiyi *et al.*, (2011); El-Shafei *et al.*, (2013).**

Physiological results:

The obtained results of immunocompetent organ weights indicate that there were no significant differences in the weights of lymphoid organs between all experimental groups Table (4). There was no structural changes were observed in shape of bursa, thymus or the spleen of any group. However, group fed 112mg Vit. E (T6) recorded a higher value but not significant in spleen weight while group fed 2g EDTA plus 56mg Vit. E (T11) rerecorded a significantly ($P \leq 0.05$) higher value in bursa weight compared to the control group. At the same time, group fed 2g EDTA (T4) scored higher value in

thymus gland weight but not significant compared with the control group. In general, there was no clear trend of dietary EDTA and Vit. E supplementation in broiler diet on immunocompetent organs at 6 weeks of age. These results are agreement with results of **(Niu *et al.*, (2009)** found that supplementation of Vit. E with level of 100 and 200mg/Kg diet had no significant effects on ratio of weight for bursa, thymus and spleen to body weight. But these organs were decreased during heat stress and suggest that the decrease in lymphoid organ weights could have been a result of the reduction in feed intake, thereby providing less nutrients for proper development of these organs under heat stress conditions.

Effect of EDTA and Vit. E supplementation on (thermoregulation measurements) cloacal temperature (Tc), skin temperature (Ts), feather temperature (Tf) and respiration rate (RR) of broiler chicks are shown in Table (5). The addition of Vit. E to broiler diets of group fed 56mg and 112mg Vit. E /kg feed (T5 and T6) registered a higher value in Tc. While group fed 2g EDTA and 112mg Vit. E/kg feed (T12) was record a lower value in Tc. On the other hand, group fed 0.5g EDTA plus 56mg Vit. E (T7) was recorded a higher value in Ts. Group fed 2g EDTA plus 112mg Vit. E (T12) was higher in Tf. There was no significant effect of dietary EDTA and Vit. E on RR among all experimental groups. The obtained results of above traits were recorded no significant effect of dietary EDTA and Vit. E on those traits among all groups of experiment. These results are partially agree with the findings of **El-Shafei *et al.*, (2013)** found that no clear trend for the influence of EDTA and vitamin C or their integration on the above parameters.

Biochemical analysis of breast muscles:

Table (6) shows the results of biochemical analysis in breast muscle of broiler chicks fed different levels of EDTA and Vit. E. Moisture content was recorded from 71.25±0.66 to 72.90±1.04 % and ash content from 1.50±0.37 to 1.35±0.04 % without any significant difference among all treatments except group T11 was significantly ($P \leq 0.05$) lower in ash% compared to the control group. Crude protein % was significantly ($P \leq 0.05$) increased by adding different levels of EDTA alone (T2, T3 and T4) or by combination high levels of EADT with Vit. E (T10, T11 and T12) in broiler diets compared with the control group. While ether extract was significantly ($P \leq 0.05$) decreased by increasing the levels of EDTA, Vit. E and their combination in broiler diets compared with the control group. Group fed 2g EDTA (T4), group fed 56mg Vit. E (T5) and group fed 2g EDTA plus 112mg Vit. E (T12) had significantly ($P \leq 0.05$) lowest value in ether extract% compared to all other groups. These results suggest that the increase of crude protein in breast muscles of broiler fed diets contained levels of EDTA or Vit. E alone or combined together may be due to the increase N retention and decreased N excretion and respect to the effect of Vit. E on nutrient digestibility. This observation may suggest that Vit. E enhances nutrient retention. **Gopal *et al.*, (2009)** showed that the reduction in the protein content after exposure to nickel chloride may be due to its effect on protein synthesis, which considered as the primary biochemical indicator for stress. This synthesizing is influenced by a large number of exogenous substances that may be due to proteolysis, lack of protein biosynthesis or inhibition of translation. Also, Vit. E functions as an antioxidant, protecting the integrity of unsaturated bonds of cellular membrane phospholipids against free radical attack **(Boa-Amponsem *et al.*, 2000).** Heat stress stimulates the release of corticosterone from the adrenal gland, initiates lipid peroxidation in the cell membrane and leads to the generation of free radicals **(Etches *et al.*, 1995).** These free radicals can damage cell membranes by inducing lipid peroxidation of polyunsaturated fatty acids in the cell membrane **(Altan *et al.*, 2000; 2003; Bayraktar *et al.*, 2011).** However, the peroxidation induced by heat stress might disappear upon the stimulation of the antioxidation ability **(Lin *et al.*, 2000; Bayraktar *et al.*, 2011)** and may be this is the reason for decrease ether extract % in treated groups than control.

***Serum biochemical analysis*:**

Results of ALT, AST, alkaline phosphatase, creatinine and uric acid are given in Table (7). Serum ALT and AST activities increased significantly ($P \leq 0.05$) as EDTA levels increased alone or by combined high level of EDTA (T11 and T12) with Vit. E in the diets. While, ALT and AST activities decreased significantly ($P \leq 0.05$) as Vit. E individual increased in the diet compared to the control group. The significant changes in activities of these enzymes in blood serum indicate that tissue impairment caused by stress **(James *et al.*, 1991).** Also**,** alteration in serum enzymes activity under stress conditions occur due to malfunctioning of liver, as degenerating and necrotic cells leak enzymes from cytoplasm **(Khan and Sardar, 2005).** The increase of serum ALT and AST may be attributed to hepatocellular damage or cellular degradation (hemorrhage, fibroplasia and infiltration of portal triad, congestion and cytoplasmic vacuolization of hepatocytes and edema) were noticed in T4, T11 and T12 (which have 2.0 g EDTA), Figure (1) in liver and perhaps in heart and muscle as also shown by (**Yamawaki *et al.*, 1986; El-Shafei *et al.*, 2013**). High level of EDTA in broiler diet increased the ALT and AST enzymes activity which mean that this level is not optimal for

broiler chicks that may be led to adverse effect by increasing lipid peroxidation caused by oxygen free radicals produce damage to liver tissue which is revealed by increase ALT and AST concentration in serum, which indicate inability of liver to metabolize the ALT and AST and may also, attribute to the outflow of these enzymes from the liver cytosol to the blood stream **(Bharavi *et al.*, 2010; Cinar *et al.*, 2011; El-Shafei *et al.*, 2013).** In addition, (**Bhatti *et al.*, 2003: Bhatti and Dil, 2005)** believed that alteration in serum enzymes activity under stress conditions occur due to malfunctioning of liver, as degenerating and necrotic cells leak enzymes from cytoplasm. These findings are supported by the obtained results of liver histopathological examination presented in Fig. 1 to Fig. 6. The reduction occurred in serum ALT and AST of broiler groups fed levels of Vit. E may be due to the anti-oxidants activities of Vit. E as reported by (**McDowell, 2000; Xiao *et al.*, 2011).**

The alkaline phosphatase (AP) activity showed significant ($P \leq 0.05$) a higher variation with the use of high level of EDTA alone (T3 and T4) or with high level of EDTA combined with Vit. E (T11 and T12) compared to the control and other groups. While, increase Vit. E level in broiler diet was significantly ($P \leq 0.05$) decreased the AP. Alkaline phosphatase is one of the most frequently used biochemical markers of osteoblast activity and differentiation **(Magnusson *et al.*, 1999).** The higher activity of AP may attribute to that AP is a hydrolase enzyme for removing many types of molecules, including nucleotides, proteins and alkaloids, and therefore its activity is high in tissue involved in high level of metabolism. Increased AP activity is not limited to liver damage only but it is also associated with pathological changes in the bone, kidneys and biles. Therefore, the high level of ALT activity may be due to damage or disturbance in any of these organs. Increased AP activity has been associated with lead and other heavy metal poisonings **(Gursu *et al.*, 2004; Ambali *et al.*, 2011).** On the other hand, **Arslan *et al.*, (2001)** found that the increase in serum AP levels of broilers fed with excess dietary Vit. E may be related to osteoblastic activity.

Creatinine level was significantly ($P \leq 0.05$) decreased for group fed 112mg Vit. E/kg diet (T8) compared with the control group (T1). However, group fed 2g EDTA/kg diet (T4) recorded a significantly ($P \leq 0.05$) higher value in creatinine concentration compared to the control group, which means that this level of EDTA (2g/kg diet) had deleterious effect on kidney function causing kidney congestion of renal BVs, focal necrosis and necrosis of renal tubules as shown in (Fig. 2). Creatinine is a chemical waste molecule that is generated from muscle metabolism. The kidneys maintain the blood creatinine in a normal range. The lower values derived that no muscular wastage which might have been possibly cause by inadequacy of protein in animals. Vit. E at level of 200 mg/kg diet may cause amendatory effect for renal and hepatic functions **(Polat *et al.*, 2011).** Results of uric acid (UA) show no significant changes in serum UA for all groups, whilst the control group was numerical higher in UA compared to treated group. Both of creatinine and UA indicate better condition for kidney functions especially with Vit. E alone or with low level of EDTA combined with Vit. E supplementation. **Lin *et al.*, (2004)** found that treated broiler chicken with corticosterone at level of 30mg/kg diet induced muscle proteolysis resulting in high UA level in blood. These results indicated that the supplementation of Vit. E may improve the function of kidney to the normal case. **Sahin *et al.*, (2001[a])** reported that supplementation of Vit. E and vitamin A in broiler diet lead to decreased UA while protein and albumin concentrations increased. This results were probably due to the higher catabolic effect (or concentration) of ACTH, yielding more glucose, uric acid, and triglycerides in the serum with supplemental dietary Vit. E and vitamin A **(Sahin *et al.*, 2001[a]).** These results are agreed with the results obtained by above authors.

Serum cholesterol (Chol) and triglycerides (TG) concentration were significantly ($P \leq 0.05$) decreased with increasing EDTA and Vit. E levels either individual or in a combination in the broiler diets Table (8). Also, combination of EDTA with Vit. E in the broiler chick diets has synergistic effects to reduce the serum Chol and TG levels significantly ($P \leq 0.05$) at 6 weeks of age compared to the control group. The reduction in cholesterol concentrations has been explained by elevated faecal excretion of cholesterol, impaired liver cholesterol uptake **(Hocgraf *et al.*, 2000)** and increased plasma thyroxin levels **(Bayraktar *et al.*, 2011).** However, the oxidised oil and Vit.E-induced decline in triglyceride concentrations might be due to a reduced synthesis of fatty acids **(Bayraktar *et al.*, 2011).** On the other hand, **Ajakaiye *et al.*, (2010)** reported that the serum uric acid, creatine, cholesterol, triglycerides, free fatty acids, AST and ALT increased significantly ($P<0.05$) from respective control mean values in heat-stressed chicken. It is well documented that heat stress overtaxes the thermoregulatory mechanism of birds, which leads to alteration of biological function and a shift in biological resources as response solution. The mechanism of this alteration has been reported to be through increased generation of free radicals at the cell level. Several authors have documented **(Altan *et al.*, 2003, Imik *et al.*, 2009)** that free radical generation affects blood serum

metabolites of AST, AP, TP, cholesterol and glucose which is manifested in bird's adaptation response through decreased production performance. Decreased Chol and TG concentrations found in the present study were in agreement with previous reports of **(Altan *et al*., 2003; Sahin *et al*., 2003[a]; Imik *et al*., 2009; Ajakaiye *et al.,* 2010; Bayraktar *et al.,* 2011).** This experiment was performed in summer and temperature degree was ranged between 33.6-37^{O}C (Mean= 35.3^{O}C).

There was significant ($P \leq 0.05$) increase in serum total protein (TP) and globulin (Glob) for groups treated with EDTA and Vit. E alone or in a combination between them as presented in Table (8). Both of TP and Glob increased significantly ($P \leq 0.05$) as EDTA and Vit. E levels or their combinations increased in the broiler diets. Elevate the value of Glob in serum of treated groups than control may reflect the enhanced effect of EDTA and Vit. E on the immunity status of these groups especially group T12 (2g EDTA +112mg Vit. E), T10 (1g EDTA + 1112mg Vit. E) and T8 (0.5g EDTA + 112mg Vit. E) which recorded a significantly ($P \leq 0.05$) increased in Glob value than the control and other groups. The results of serum protein concentration as compared to control group may be attributed to the hormonal regulation of protein metabolism, for example growth hormone increased the synthesis of cellular protein, glucocosteroids increased break down of most tissue proteins. The increasing of corticosterone hormone and glucocorticiods which are secreted by the adrenal cortex increased the quantity of protein in most tissues while decreased the amino acids concentration in the plasma, as well as decreased both liver protein and plasma proteins, or may be due to the decrease of thyroxin secretion, which thyroxin increases the rate of metabolism of all cells and, as a result indirectly affects protein metabolism **(Guyton and Hall, 2006; Al-Daraji and Amen, 2011).** These results are similar to the results of **Sahin *et al*., (2001[a] and 2003[C])** found that Vit. E supplementation increased plasma protein concentration while it markedly decreased glucose and cholesterol concentrations in broiler chicken under heat stress. The increases of serum Alb and Glob concentrations observed in experimental groups could be partially explained, by the reduction of synthesis and secretion of corticoids in birds received Vit. E and EDTA. Regarding the A/G ratio, the obtained results indicated that there was significant ($P \leq 0.05$) decrease in A/G ratio with groups combined EDTA with Vit. E in the diets compared to the control.

Trace elements in blood and breast muscles:

Table (9) shows the trace elements in breast muscles and serum of broiler chicks fed diets supplemented with different levels of EDTA and Vit. E. Calcium (Ca) and phosphorus (P) levels in breast muscles and serum were significantly ($P \leq 0.05$) decreased as EDTA levels increased in broiler diet or by integrated high level of EDTA with Vit. E compared to the control and other groups. Meanwhile, broiler groups fed Vit. E alone appeared slightly increase in Ca and P concentrations but not significant in both of muscles and serum. These results may be attributed to that Vit. E enhance the digestibility of Ca and P. Similar findings were obtained by **Sifri *et al.,* (1978)** recorded that, plasma Ca level was significantly decreased by added Na_2EDTA in adult quail. The reduction in Ca concentration in breast muscles and serum may refer to that additional dietary EDTA in rat at level of 300 and 800mg/kg diet had no influence on Ca absorption and retention but increased urinary Ca excretion **(Hurrell *et al.,* 1994).** These results are in agreement with those of **Sahin *et al*., (2001[b])** found that plasma Ca and P concentrations increased in heat-stressed Japanese quails fed a diet supplemented with Vit. E. This finding may be referring to the properties of EDTA as a chelating agent to minerals. The results of Ca and P either in breast muscles or serum are in harmony with those established by **Kabuage *et al.,* (2002)** which recorded that there were significantly decreased in these parameters when broiler fed diets contained 2% EDTA. Furthermore, **Liem *et al.,* (2008)** demonstrated that adding EDTA up to 3.65% in broiler diets decreased Ca and increased P concentrations significantly in plasma of broiler chicks.

Sodium (Na) concentration levels in breast muscles was significantly ($P \leq 0.05$) increased as EDTA levels increased in broiler diet or by combination of EDTA with Vit. E compared to the control group. In contrast, Na concentration in serum was significantly ($P \leq 0.05$) decreased as EDTA levels increased in broiler diet or by combination of EDTA with Vit. E compared to the control group. This finding may be refer to muscular edema and degeneration as shown in histopathological pictures of heart and liver muscles in group T4, T11 and T12 (Fig. 1 and 3). The reduction in serum level of Na may due to heat stress (33.6-37 ^{O}C) occurred during the experimental time and may also probably as a result of hemodilution following increased water consumption, also high levels of Na may reduce the negative effect of heat stress **(Ribeiro *et al.,* 2008).** Furthermore, the variation levels of serum Na and other minerals could refer to the disturbances in acid-base balance of birds due to serve heat stress during experimental period **(Khattak *et al.,* 2012).** These results are similar with the results obtained by **(Khattak, *et al.,* 2012; El-Shafei *et al.,* 2013).**

Likewise, **Khattak *et al.*, (2012)** reported that birds under continuous panting result in respiratory alkalosis. Respiratory alkalosis is characterized by excessive removal of blood carbon dioxide. Carbone dioxide in the blood is a source of H_2CO_3. This is a source H ion in the blood a lack of H ion can lead to poor Na and K reassertion, causing more Na and K in the urine and leaving less Na and K in the blood.

Potassium concentration (K) was significantly ($P \leq 0.05$) decreased in breast muscles as EDTA level increased in the diets as shown in T2, T3 and T4. Also, the K concentration increased gradually with increasing Vit. E level in the diet, whereas combined EDTA with Vit. E was significantly ($P \leq 0.05$) decreased K concentration in breast muscle. Serum K was decreased as EDTA level increased in diets or EDTA combined with Vit. E. Addition of Vit. E with EDTA improves the EDTA application by decreasing depletion of trace elements from breast muscles and serum. Researchers have reported a reduction in plasma concentration of K and Na due to heat stress and probably as a result of hemodilution following increased water consumption of birds (**Belay and Teeter, 1993; Ribeiro, *et al.*, 2008).** The reason for the variation in serum minerals may be referred to the disturbances in acid-base balance of birds due to serve heat stress during experimental period **Khattak *et al.*, (2012)**. The lower serum K concentration in treated groups compared to the control group could be due to the fact that K ions shift between muscle and extra cellular fluid during heat stress to maintain cellular atmospheric pressure **(Keskin and Durgun, 1997)** or it may be due to reason that K excretion from kidney was increased and also the uptake was increased by erythrocyte and skin **(Simth and Teeter, 1987).**

Heavy metals in blood and breast muscles:

Heavy metals in breast muscles and serum of the broiler fed diets supplemented with different levels of EDTA and Vit. E are specified in Table (10). Lead *(*Pb) and cadmium (Cd) in breast muscles and serum were significantly ($P \leq 0.05$) decreased as increasing the EDTA and Vit. E percent in the diet compared with the control group. But the decreasing of Pb and Cd in breast muscle and serum was significantly ($P \leq 0.05$) lower and was more effective when high levels of EDTA was combined with any level of Vit. E compared with those of control group. Addition of EDTA and Vit. E in this study may prevent the accumulation of these heavy metals in the analyzed tissue and serum. Furthermore, Vit. E and EDTA may decrease of the toxic effect of Pb and Cd by producing complexes with heavy metal cations which facilitates their elimination from the organism. Also, Vit. E and EDTA probably reduce the absorption of Pb or Cd from the gastrointestinal tract **(Cinar *et al.*, 2011; El-Shafei *et al.*, 2013).** This reduction in Pb and Cd in serum under influence of Vit. E might be due to the excess excretion of Pb and Cd through urine and feces **(Cinar *et al.*, 2011).** Also, the reduction in Pb and Cd concentration in breast muscles and serum of treated groups may be due that Vit. E and EDTA act as antioxidants that give up an electron to inhibit production of free radicals by blocking lipid peroxidation. Antioxidants play a very important biological role in the body by protecting against oxidative damage particularly oxidative damage to DNA **(Sahin *et al.*, 2002; Erdogan *et al.*, 2005; Ribeiro *et al.*, 2008; Seven *et al.*, 2009; Khattak *et al.*, 2012).**

On the other hand, zinc (Zn) in breast muscles was not affected throughout the experimental period by the supplementation of EDTA, Vit. E and their combination compared to the control group. The results of Zn in this study show that slightly increment but not significant in muscle concentration of Zn for all treated groups compared to the control. While, serum Zn was significantly ($P \leq 0.05$) decreased as EDTA levels increased in broiler diets compared to the control group. But serum Zn did not changes than control group by supplementation of either Vit. E alone or by combined with EDTA. **Hurrell *et al.*, (1994)** found that addition $NaFe^3$+EDTA at level of 50 and 100mg/kg in rat diet might improve Zn and Cu absorption from low- Zn-bioavailability diet. However, **Hill *et al.*, (1987 [a]) and Hill *et al.*, (1987 [b])** established that EDTA had no effect on zinc absorption in rats, swine or chickens whether in breast or serum. EDTA is a strong chelator which appeared to improve some of the minerals absorption in poultry diet. Previous investigations showed that addition of EDTA to broiler (**Kabuage *et al.*, 2002 and Nezhad *et al.*, 2010)** and layer chickens (**Nezhad *et al.*, (2008**) that contains plant proteins improved zinc absorption. The affinity to chelate with metal is quantitatively described as stability coefficient. EDTA has higher stability coefficient with all of the minerals and this is the reason for metal sweeper of EDTA and when EDTA is available in system it could bind all cations (**Nezhad *et al.*, 2010).** It is known that Vit. E has similar effects to those of ascorbic acid **(Linder, 1991).** It has been reported that zinc stabilizes the red cell membrane against cellular changes caused by peroxidations and that zinc plays a role similar to that of Vit. E in reducing peroxidative damage on cellular membrane **(Pond *et al.*, 1995).** Vit. E protects the cell from the detrimental effects of peroxidation. Vit. E is present in the membrane components of the cell and prevents peroxide formation **(Mc Dowell, 2000).** The absorption of Vit. E is facilitated by the formation of micelles which are then solubilised by the action of

bile salts and pancreatic juices in the intestine **(Putnam and Comben, 1987; Sahin *et al.*, 2001[b]).** These results are compatible with findings of **Hill *et al.*, 1987[a,b]; Hurrell *et al.*, 1994; Kabuage *et al.*, 2002; Nezhad *et al.*, 2008; Nezhad *et al.*, 2010; El-Shafei *et al.*, 2013).**

Copper (Cu) concentrations in breast muscles was significantly ($P \leq 0.05$) increased as EDTA, Vit. E and their combination levels increased in the broiler diet of all tested groups compared with the control group. In contrast, Cu concentration in serum was significantly ($P \leq 0.05$) decreased as EDTA levels increased in broiler diet compared with the control group. There was no significant effect of Vit. E and combination of EDTA with Vit. E on serum Cu compared to control group. Similar finding was reported by **Nezhad *et al.*, (2010)** when studied the interaction effect of EDTA+MP (microbial phytase) in broiler chick diets on concentration of Cu which was significantly ($P \leq 0.05$) increased. Also, found that addition of different levels of EDTA to diets not supplemented with MP and contained low level of available phosphorus increased concentration of serum Cu of broiler chicks. **Nezhad *et al.*, (2008)** reported that EDTA is strong chelatore and improved some minerals absorption such as Cu and Zn in poultry. Also, **Nezhad *et al.*, (2010)** documented that addition of different levels of EDTA to diets which contained low level of available phosphorus decreased concentration of Cu in serum. But, when Vit. C added to EDTA, the Cu depletion prevented and sometimes Cu content increased. **Waters *et al.*, (2001)** expressed that there was significantly increased in urinary losses of lead, cadmium, zinc and calcium following EDTA chelating therapy. Vit. E may be play a beneficial role in preventing copper-induced oxidative damage in poultry and shows potential for veterinary use. These results are in agreement with those discovery by **El-Shafei *et al.*, (2013)** found that Cu concentration increased in breast muscle as EDTA, Vit. C and their combination levels increased in broiler diets.

Histopathological examination:

Macroscopically the examined organs appear normal in treated broiler chicks with 1g EDTA in addition to 0.5g EDTA either alone or with 56 mg and 112mg Vit. E. But the treated chicks with 2g EDTA either alone or with 56 mg or 112mg Vit. E showed variable degree of damage in examined tissues. Liver showed hepatic hemorrhage, vacuolization of hepatocytes, portal infiltration congestion and edema (**Figs. 1: b, c, d and f**). The hepatic damage may be attributed to increase of serum ALT and AST in T4, T11 and T12 (which have 2.0g EDTA/kg diet), (**Figs. 1: b, c, d and f**) or may be attributed to the outflow of these enzymes from the liver cytosol to the blood (**Yamawaki *et al.*, 1986: Cinar *et al.*, 2011).** The histopathological changes of chicks liver treated with high level of EDTA might be due to the formation of highly reactive radicals and subsequent lipid peroxidation. The accumulated hydroperoxidase can cause cytotoxicity, which is associated with the peroxidation of membrane phospholipids by lipid hydroperoxidase, the basis of hepatocellular damage **(Renugadevi and Prabu, 2010).** Meanwhile, these changes are in nearly agreement with those recorded by **Reuber, (1969)** found that these changes could be due to accumulation of metabolites and led to increase the cytoplasmic osmotic pressure and inhibition of water into the cell.

The examined kidney of control chicks appears normal (**Fig.2a**). Meanwhile in T4 and T12 there were congestion of renal blood vessels, focal necrosis of renal tubules associated with inflammatory cells infiltration and leukocytic cell infiltration were noticed (**Figs. 2: b, c, d, e** and **f**). These results are in moderate agreement with that recorded with **Wynn, *et al.*, (1970)** that failed to show any renal damage, and found that EDTA was not well tolerated at dietary levels above 5% and in fairly agreement with that reported by **(Ahrens and Aronson, (1971).** The examined heart of treated groups with high levels of EDTA showed perivascular and intermuscular edema, congestion of myocardial blood vessels, myolysis of focal myocytes and intermuscular heterophiles infiltration (**Figs. 3: b, c, d, e and f**). These changes may be due to high level of EDTA in diet of group T4 and T12. However, the examined control brain appears normal (**Fig.4a**), but in T4 and T12 showed necrosis and pyknosis of neurons were detected (**Figs. 4: b, c and d**). The examined control spleen appears normal (**Fig.5a**). While in T4, T11 and T12 hyperplasia of reticular cells, atrophy of lymphoid follicle and lymphocytic depletion were observed (**Figs. 5: b, c and d**). Also, the examined thymus gland of control group, chicks appear normal (**Fig. 6a**). However, T4, T11 and T12 showed lymphocytic necrosis, congestion of BVs and hemorrhage (**Figs. 6: b, c, and d**). These changes may be due to high level of EDTA in the chick diets. The same results were found with those obtained by **El-Shafei *et al.*, (2013)** when fed broiler chicks diet contained high level of EDTA either alone or mixed with vitamin C. Unfortunately, there were no enough literature are available in this point to explain clearly the effect of EDTA and Vit. E either alone or in their combination on the previous examined organs (Liver, kidney, heart, spleen and thymus gland).

Conclusion:

According to the obtained results, it is concluded that the growth performance and feed conversion were improved but not significantly due to EDTA and Vit. E application and they were more pronounced with supplementation of low level of EDTA with Vit. E, that considered as an optimum levels which could improve the health status and growth parameters of broiler chicks in this experiment. Also, addition of EDTA and Vit. E to broiler chick diets, reduced significantly the Pb and Cd levels in both of breast muscles and serum helped to eliminate heavy metals from the bird body and in turn improved the biochemical parameters ALT, AST, AP, Chol, TG, Creatinine, TP, globulin and UA as compared to non treated birds, but in certain levels to keep the body chemical parameters in balance without any adverse effects. Histopathological examination indicated that the examined organs appear normal in treated chicks with 1g EDTA in addition to 0.5 g EDTA either alone or with 56 mg and 112 mg of Vit. E. But treated chicks with high level of EDTA (2g) either alone or combined with 56 or 112 mg Vit. E showed variable degree of damage in examined tissues including hemorrhage, vacuolization, congestion, focal necrosis, cell infiltration, edema, atrophy of lymphoid follicles and depletion.

Table (1): Compositions of experimental diets of broiler Ross 308 chicks.

Ingredients	Diets of broiler chicks		
	Starter (1-10 day)	Grower (11-24 day)	Finisher (25-42 day)
Ground yellow corn 7.7%.	58.00	59.02	61.50
Corn glutten meal 61%.	9.14	9.00	8.00
Fish meal herring 72%.	3.50	3.20	3.00
Soybean meal 42%.	23.65	22.69	21.00
Sunflower oil 8500.	1.00	2.67	3.30
Dicalcium phosphate.	2.00	1.50	1.40
Limestone .	1.59	1.11	1.10
Premix[1].	0.30	0.30	0.30
Sodium Chloride.	0.30	0.30	0.30
Lysine.	0.39	0.21	0.10
Methionine.	0.13	-	-
Total (Kg).	100	100	100
Calculated			
Crude protein%.	22.99	22.14	20.72
ME.Kcal/Kg.	3029.77	3157.60	3206.95
C/P ratio.	131.79	142.62	154.78
Calcium%.	1.20	0.90	0.87
Available Ph. %.	0.55	0.45	0.43
Lysine%.	1.46	1.24	1.06
Methionine %.	0.63	0.46	0.42
Methionine + Cystine %.	0.98	0.82	0.79

[1]Composition of vitamins and minerals premix. Each 3Kg of vitamin and minerals mixture contain: Vit. A 10.000.000 IU, Vit. D_3 2.000.000 IU, Vit. E 10.000 mg, Vit. K_3 1.000 mg Vit. B_1 1.000 mg, Vit. B_2 5.000 mg, Vit. B_6 1.500 mg, Vit B_{12} 10 mg, Niacin 20.000 mg, Pantothenic acid 10.000 mg, Folic acid 1.000 mg, Biotin 50 mg, Choline chloride 500.000 mg, Copper 4.000 mg, Iodine 300 mg, Iron 30.000 mg, Manganese 60.000 mg, Zinc 50.000 mg, Cobalt 100 mg and Selenium 100 mg.

Table (2): Effect of different dietary levels of EDTA and vitamin E on final body weight (g) at 6th weeks of age, daily gain during the total period (2-6 weeks of age), total feed intake, daily feed intake and feed conversion of broiler chicks.

Treatment	Final body weight (g/bird)	Daily gain (g/bird. day)	Total feed intake[2]	Daily feed intake[3]	Feed conversion[4]
T1	1962.86^{abcde1}±44.03	47.13^{abcd}±1.11	4026.33^{c4}±1.00	115.04^{c}±0.03	2.051^{d}±0.0006
T2	1996.07^{abcd}±61.16	47.97^{abcd}±1.68	3842.83^{k}±0.83	109.80^{k}±0.02	1.925^{l}±0.0008
T3	1912.31^{bcde}±44.51	45.81^{abcd}±1.30	3893.33^{j}±0.67	111.24^{j}±0.02	2.035^{e}±0.0009
T4	2024.17^{abc}±59.62	49.15^{abc}±1.83	3912.50^{h}±1.17	111.79^{h}±0.03	1.932^{h}±0.0006
T5	1829.00^{e}±44.29	44.47^{cd}±1.35	3839.63^{l}±0.70	109.70^{l}±0.02	2.098^{c}±0.0008
T6	1843.21^{de}±57.80	43.06^{d}±1.72	3977.87^{f}±0.93	113.65^{f}±0.03	2.157^{a}±0.0008
T7	2006.54^{abc}±53.38	49.31^{ab}±1.45	4014.46^{d}±1.61	114.70^{d}±0.05	1.999^{f}±0.0005
T8	2048.57^{ab}±58.12	49.08^{abc}±1.75	4204.17^{a}±0.83	120.12^{a}±0.03	2.052^{d}±0.0003
T9	2081.43^{a}±37.21	51.18^{a}±1.12	3904.50^{i}±0.83	111.56^{i}±0.03	1.875^{j}±0.0004
T10	1909.23^{bcde}±61.01	46.44^{abcd}±1.83	4008.50^{e}±0.83	114.53^{e}±0.03	2.098^{c}±0.0008
T11	2050.00^{ab}±32.05	50.40^{a}±1.21	4048.00^{b}±1.00	115.66^{b}±0.03	1.974^{g}±0.0005
T12	1873.33^{cde}±37.00	44.75^{bcd}±0.86	3943.17^{g}±0.83	112.66^{g}±0.02	2.104^{b}±0.0005

[1] Least squares means ± pooled standard error.

a,b,c,d,e,f,g,h,i,j,k,l, Means having different letter exponents among columns are significantly different (*P≤0.05*).

[2] (g/bird. 5 weeks) from 2-6 weeks of age. [3] (g/bird. day). [4] (g feed/1g weight gain. bird) from 2-6 weeks of age.

Table (3): Effect of different dietary levels of EDTA and vitamin E on carcass characteristics of broiler chicks at 6th weeks of age.

Treat.	Live body weight (g)	Dressing weight (g)	Dressing %	Giblets %	Gizzard %	Liver %	Heart %	Abdominal fat pad %
T1	2145.00[1]±172.90	1520.62±146.27	70.65±1.78	4.11^{b}±0.18	1.47^{ab}±0.21	2.24^{b}±0.18	0.39±0.01	1.86±0.16
T2	2332.50±174.23	1633.74±142.07	69.87±1.53	4.20^{b}±0.17	1.45^{ab}±1.12	2.32^{b}±0.13	0.43±0.04	1.58±0.27
T3	2315.00±150.40	1630.38±131.71	70.19±1.21	4.25^{b}±0.16	1.51^{ab}±0.03	2.29^{b}±0.16	0.44±0.05	1.40±0.22
T4	2161.25±163.41	1471.41±128.62	67.91±0.81	3.93^{b}±0.19	1.46^{ab}±0.23	2.04^{b}±0.19	0.43±0.03	1.16±0.15
T5	2170.00±79.61	1563.82±72.39	70.63±0.97	4.96^{a}±0.24	1.51^{ab}±0.10	2.98^{a}±0.29	0.47±0.02	1.52±0.22
T6	2306.25±194.43	1646.25±146.39	71.32±0.81	4.01^{b}±0.22	1.19^{b}±0.06	2.44^{ab}±0.22	0.38±0.03	1.29±0.04
T7	2318.75±140.41	1617.90±126.86	69.55±1.31	4.40^{ab}±0.14	1.65^{a}±0.13	2.28^{b}±0.21	0.46±0.05	1.44±0.10
T8	2301.25±142.88	1626.12±108.87	70.60±0.73	4.02^{b}±0.10	1.41^{ab}±0.04	2.15^{b}±0.13	0.45±0.05	1.30±0.25
T9	2300.00±181.56	1638.11±150.67	71.00±1.10	4.34^{ab}±0.19	1.46^{ab}±0.12	2.46^{ab}±0.16	0.41±0.03	1.32±0.16
T10	2350.00±149.72	1652.27±122.21	70.16±0.79	3.95^{b}±0.20	1.35^{ab}±0.02	2.20^{b}±0.15	0.40±0.04	1.16±0.19
T11	2357.50±206.18	1662.75±168.90	70.29±1.42	4.36^{ab}±0.14	1.43^{ab}±0.06	2.47^{ab}±0.13	0.46±0.03	1.45±0.24
T12	2256.25±151.42	1579.41±107.38	69.99±0.88	4.57^{ab}±0.39	1.57^{a}±0.06	2.53^{ab}±0.33	0.47±0.04	1.63±0.03

[1] Least squares means ± pooled standard error.

a,b, Means having different letter exponents among columns are significantly different (*P≤0.05*).

Table (4): Effect of different dietary levels of EDTA and vitamin E on absolute immune organ weights (g) of broiler chicks at 6th weeks of age.

Treatments	Spleen weight	Bursa weight	Thymus weight	Cecal tonsil weight
T1	2.11[1]±0.43	1.96^{bc}±0.30	7.98^{abc}±0.82	0.68^{ab}±0.08
T2	2.67±0.18	2.58^{abc}±0.87	10.99^{ab}±1.29	0.89^{a}±0.08
T3	2.09±0.16	2.14^{abc}±0.33	8.61^{abc}±1.18	0.60^{ab}±0.09
T4	2.46±0.27	3.09^{ab}±0.41	11.90^{a}±2.08	0.62^{ab}±0.11
T5	3.05±0.70	1.30^{c}±0.39	6.43^{c}±0.50	0.61^{ab}±0.10
T6	3.44±1.48	1.87^{bc}±0.50	7.33^{bc}±1.27	0.55^{b}±0.04
T7	2.63±0.61	2.37^{abc}±0.10	10.79^{abc}±2.00	0.70^{ab}±0.05
T8	2.40±0.34	2.14^{abc}±0.39	9.70^{abc}±1.22	0.78^{ab}±0.16
T9	1.72±0.17	2.97^{abc}±0.83	8.50^{abc}±1.55	0.76^{ab}±0.07
T10	3.28±0.63	2.91^{abc}±0.75	8.38^{abc}±0.62	0.69^{ab}±0.13
T11	2.87±0.14	3.75^{a}±0.39	9.58^{abc}±1.25	0.72^{ab}±0.09
T12	2.66±0.21	1.91^{bc}±0.29	7.05^{bc}±1.23	0.68^{ab}±0.03

[1]Least squares means ± pooled standard error.

a,b,c Means having different letter exponents among columns are significantly different (*P≤0.05*).

Table (5): Effect of different dietary levels of EDTA and vitamin E on cloacal temperature (Tc), skin temperature (Ts), feather temperature (Tf) and respiration rate (RR) of broiler chicks at different times during the experiment.

Treatments	Tc (°C)	Ts (°C)	Tf (°C)	RR (r.p.m)
T1	40.62^{abc}[1]±0.08	40.03^{ab}±0.23	34.45^{abc}±0.57	76.33±3.95
T2	40.57^{abc}±0.12	39.37^{b}±0.28	33.83^{bc}±0.91	74.00±5.73
T3	40.62^{abc}±0.07	39.98^{ab}±0.24	33.03^{c}±0.56	83.00±5.88
T4	40.40^{bc}±0.09	40.25^{a}±0.21	34.67^{abc}±0.74	77.00±4.75
T5	40.77^{a}±0.14	39.98^{ab}±0.34	34.68^{abc}±0.39	77.83±2.69
T6	40.75^{a}±0.12	39.96^{ab}±0.33	34.67^{abc}±0.36	77.72±2.61
T7	40.67^{abc}±0.06	40.50^{a}±0.09	35.72^{ab}±0.60	86.00±2.00
T8	40.70^{ab}±0.08	40.08^{ab}±0.22	35.75^{ab}±0.94	83.00±4.49
T9	40.42^{bc}±0.12	40.42^{a}±0.12	35.43^{ab}±0.49	85.00±2.41
T10	40.37^{bc}±0.10	39.95^{ab}±0.23	33.77^{bc}±0.47	78.00±2.68
T11	40.45^{abc}±0.14	40.07^{ab}±0.24	34.70^{abc}±0.41	76.33±2.89
T12	40.33^{bc}±0.08	40.15^{a}±0.15	35.92^{a}±0.68	80.00±3.69

[1]Least squares means ± pooled standard error.

a,b,c, Means having different letter exponents among columns are significantly different (*P≤0.05*).

Table (6): Effect of different dietary levels of EDTA and vitamin E on chemical analysis of breast muscles of broiler chicks at 6th weeks of age.

Treatments	Moisture%	Crud protein %	Ether extract %	Ash%
T1	72.00±0.55	20.00^{de}±0.55	2.15^{a}±0.08	1.50^{a}±0.37
T2	72.80±0.77	23.00^{a}±0.40	1.78^{c}±0.07	1.45^{ab}±0.05
T3	71.25±0.66	22.20^{abc}±0.37	1.44^{d}±0.05	1.44^{ab}±0.05
T4	71.36±0.62	22.20^{abc}±0.33	0.37^{h}±0.01	1.40^{ab}±0.04
T5	72.08±1.33	21.00^{bcd}±0.66	0.39^{h}±0.01	1.44^{ab}±0.05
T6	72.45±0.29	18.90^{e}±0.62	0.57^{g}±0.02	1.42^{ab}±0.04
T7	72.22±1.15	21.20^{bcd}±0.33	0.85^{e}±0.03	1.47^{ab}±0.04
T8	71.63±110	20.70^{cd}±0.47	1.43^{d}±0.04	1.45^{ab}±0.04
T9	72.90±1.04	19.80^{de}±0.40	1.98^{b}±0.06	1.43^{ab}±0.05
T10	71.85±1.04	22.00^{abc}±0.47	0.62^{fg}±0.02	1.39^{ab}±0.04
T11	71.49±0.65	22.50^{ab}±0.44	0.72^{f}±0.01	1.35^{b}±0.04
T12	71.78±0.98	22.00^{abc}±0.55	0.41^{h}±0.10	1.38^{ab}±0.04

[1] Least squares means ± pooled standard error.

a,b,c,d,e,f,g,h, Means having different letter exponents among columns are significantly different (*P≤0.05*).

Table (7): Effect of different dietary levels of EDTA and vitamin E on serum constituents (ALT, AST, AP., Ceat., and UA) of broiler chicks at 6th weeks of age.

Treatment	ALT (GPT) Iu/ml	AST (GOT) Iu/ml	AP. u/ml	Creat. g/dl	Uric a.mg/dl
T1	39.00^{bcd}±0.68	11.00^{de}±0.54	10.00^{cd}±0.15	1.25^{bcd}±0.08	5.50±0.42
T2	39.00^{bcd}±0.89	11.00^{de}±0.59	10.50^{c}±0.24	1.30^{abc}±0.06	5.00±0.48
T3	41.00^{ab}±0.78	13.50^{bc}±1.16	12.50^{b}±0.24	1.38^{ab}±0.06	4.85±0.43
T4	43.50^{a}±0.93	19.00^{a}±1.37	17.03^{a}±0.88	1.40^{a}±0.05	4.50±0.45
T5	35.00^{ef}±0.44	9.50^{de}±0.40	10.00^{cd}±0.12	1.20^{cde}±0.02	4.85±0.07
T6	32.00^{f}±0.84	9.00^{e}±0.52	8.00^{e}±0.18	1.15^{de}±0.03	4.70±0.10
T7	37.50^{bcde}±1.51	11.00^{de}±0.55	10.00^{cd}±0.16	1.15^{de}±0.02	4.95±0.40
T8	36.00^{de}±0.83	9.50^{de}±0.75	9.00^{de}±0.52	1.10^{e}±0.03	4.90±0.38
T9	36.50^{cde}±1.68	11.50^{cd}±0.73	10.50^{c}±0.32	1.20^{cde}±0.01	4.85±0.39
T10	35.00^{ef}±2.30	10.00^{de}±0.92	10.50^{c}±0.25	1.15^{de}±0.04	4.75±0.42
T11	40.00^{bc}±1.20	14.00^{b}±0.20	13.00^{b}±0.45	1.35^{ab}±0.03	4.55±0.37
T12	40.00^{bc}±1.06	14.00^{b}±0.07	12.52^{b}±0.25	1.30^{abc}±0.01	4.35±0.39

[1] Least squares means ± pooled standard error.

a,b,c,d,e,f, Means having different letter exponents among columns are significantly different (*P≤0.05*).

Table (8): Effect of different dietary levels of EDTA and vitamin E on serum constituents (Chol., Trig., TP, Alb, Glob, and A/G ratio) of broiler chicks at 6th weeks of age.

Treatment	Chol. mg/dl	Trig. mg/dl	Tp. g/dl	Alb. g/dl	Glob. g/dl	A/G ratio
T1	106.00^{a}±2.05	85.00^{a}±2.41	5.43^{d}±0.20	3.25^{cd}±0.06	2.18^{d}±0.17	1.49^{a}±0.17
T2	102.50^{ab}±1.47	82.50^{ab}±1.29	5.70^{c}±0.11	3.25^{cd}±0.07	2.45^{c}±0.04	1.33^{bc}±0.02
T3	99.17^{bc}±2.32	72.50^{c}±1.41	5.90^{bc}±0.09	3.25^{cd}±0.11	2.65^{bc}±0.03	1.23^{cde}±0.05
T4	88.50^{e}±2.08	63.50^{de}±1.08	6.05^{bc}±0.16	3.55^{abc}±0.18	2.50^{c}±0.06	1.42^{a}±0.44
T5	102.50^{ab}±1.47	85.00^{a}±2.29	6.30^{ab}±0.14	3.60^{ab}±0.10	2.70^{b}±0.05	1.33^{bc}±0.03
T6	99.00^{bc}±2.12	82.50^{ab}±1.26	6.50^{a}±0.15	3.70^{a}±0.08	2.80^{ab}±0.07	1.32^{bc}±0.01
T7	102.50^{ab}±1.54	80.00^{ab}±2.36	6.05^{bc}±0.12	3.30^{cd}±0.11	2.75^{ab}±0.08	1.21^{def}±0.06
T8	101.50^{ab}±2.24	78.50^{b}±1.85	6.13^{ab}±0.14	3.20^{d}±0.10	2.93^{a}±0.06	1.09^{f}±0.03
T9	97.50^{bcd}±1.59	68.00^{cd}±1.43	6.05^{bc}±0.09	3.26^{cd}±0.09	2.79^{ab}±0.04	1.17^{def}±0.04
T10	94.00^{cde}±2.29	67.50^{d}±1.49	6.20^{ab}±0.14	3.25^{cd}±0.08	2.95^{a}±0.08	1.10^{f}±0.05
T11	92.50^{de}±1.44	65.00^{d}±0.78	6.00^{bc}±0.10	3.35^{bc}±0.08	2.65^{bc}±0.05	1.27^{cd}±0.03
T12	89.00^{e}±1.39	60.00^{e}±0.60	6.20^{ab}±0.21	3.25^{cd}±0.08	2.95^{a}±0.14	1.11^{ef}±0.04

[1] Least squares means ± pooled standard error.

a,b,c,d,e,f, Means having different letter exponents among columns are significantly different (*P≤0.05*).

Table (9): Effect of different dietary levels of EDTA and vitamin E on trace elements in breast muscles and serum of broiler chicks at 6th weeks of age.

Treat.	Ca		P		Na		K	
	Breast M. ppm	Serum mg/dl	Breast M. ppm	Serum mg/dl	Breast M. ppm	Serum mEq/L	Breast M. ppm	Serum mEq/L
T1	12.60^{a}±0.66	11.19^{ab}±0.03	4.30^{ab}±0.16	4.80^{a}±0.08	119.50^{f}±1.10	119.50^{a}±0.71	5.40^{a}±0.18	5.25^{ab}±0.25
T2	10.30^{b}±0.14	10.35^{bc}±0.47	4.20^{abc}±0.12	4.80^{a}±0.08	122.00^{ef}±0.73	117.50^{ab}±0.81	4.20^{cd}±0.14	5.10^{abc}±0.20
T3	9.80^{b}±0.26	9.05^{d}±0.27	4.00^{abcd}±0.11	4.40^{ab}±0.18	125.00^{e}±1.10	112.50^{cd}±1.74	4.00^{d}±0.11	4.90^{abc}±0.26
T4	7.60^{c}±0.10	8.10^{e}±0.37	3.70^{d}±0.25	4.12^{b}±0.21	133.00^{a}±0.73	109.00^{d}±1.07	4.00^{d}±0.10	4.60^{c}±0.22
T5	12.70^{a}±0.34	11.65^{a}±0.32	4.30^{ab}±0.08	4.90^{a}±0.08	122.00^{ef}±0.34	120.00^{a}±0.88	5.50^{a}±0.08	5.35^{a}±0.11
T6	12.70^{a}±0.27	11.70^{a}±0.38	4.40^{a}±0.05	5.05^{a}±0.13	124.00^{cd}±0.44	119.50^{a}±0.95	5.60^{a}±0.07	5.40^{a}±0.16
T7	12.50^{a}±0.30	11.27^{ab}±0.08	4.30^{ab}±0.20	4.80^{a}±0.19	122.00^{ef}±0.58	119.67^{a}±0.91	5.30^{a}±0.10	5.20^{ab}±0.16
T8	12.60^{a}±0.09	11.45^{a}±0.29	4.20^{abc}±0.09	4.85^{a}±0.10	123.00^{de}±0.77	119.00^{ab}±0.77	5.50^{a}±0.15	5.23^{ab}±0.06
T9	11.90^{a}±0.44	10.90^{ab}±0.29	4.10^{abcd}±0.14	4.79^{a}±0.25	124.00^{cd}±0.69	116.33^{ab}±2.19	4.80^{b}±0.12	5.00^{abc}±0.13
T10	11.80^{a}±0.33	10.95^{ab}±0.33	4.10^{abcd}±0.22	4.90^{a}±0.11	124.00^{cd}±0.66	115.50^{bc}±1.64	4.80^{b}±0.15	5.05^{abc}±0.14
T11	10.30^{b}±0.27	9.85^{cd}±0.42	3.90^{bcd}±0.05	4.60^{a}±0.22	132.00^{a}±0.47	112.00^{cd}±0.88	4.40^{c}±0.11	4.75^{bc}±0.18
T12	10.20^{b}±0.20	9.88^{cd}±0.32	3.80^{cd}±0.08	4.65^{a}±0.21	130.00^{b}±0.47	112.50^{cd}±0.73	4.50^{bc}±0.07	4.85^{abc}±0.13

[1] Least squares means ± pooled standard error.

a,b,c,d,e,f, Means having different letter exponents among columns are significantly different (*P≤0.05*).

Table (10): Effect of different dietary levels of EDTA and vitamin E on heavy metals in breast muscles and serum of broiler chicks at 6th weeks of age.

Treat.	Pb (ppm)		Cd (ppm)		Zn (ppm)		Cu (ppm)	
	Breast muscles	Serum	Breast muscles	Serum	Breast muscles	Serum	Breast muscles	Serum
T1	2.60^{a}±0.04	3.50^{a}±0.06	1.40^{a}±0.02	1.25^{a}±0.08	4.33±0.05	6.65^{a}±0.18	0.95^{d}±0.04	1.15^{bc}±0.04
T2	1.40^{e}±0.10	3.15^{ab}±0.09	1.00^{d}±0.03	0.78^{bc}±0.07	4.40±0.12	5.90^{bc}±0.33	0.98^{d}±0.07	0.90^{e}±0.06
T3	1.00^{e}±0.07	2.60^{cd}±0.18	0.80^{e}±0.03	0.40^{ef}±0.09	4.40±0.20	5.25^{cd}±0.57	1.20^{c}±0.04	0.75^{f}±0.07
T4	0.62^{f}±0.03	1.70^{ef}±0.11	0.77^{e}±0.09	0.16^{g}±0.03	4.50±0.09	5.00^{d}±0.46	1.30^{c}±0.07	0.55^{g}±0.07
T5	2.40^{b}±0.07	3.05^{bc}±0.10	1.30^{b}±0.08	0.90^{b}±0.06	4.50±0.11	6.85^{a}±0.18	1.20^{c}±0.04	1.25^{ab}±0.04
T6	2.30^{b}±0.12	2.55^{d}±0.21	1.20^{c}±0.05	0.75^{bc}±0.07	4.70±0.20	6.95^{a}±0.23	1.30^{c}±0.06	1.25^{ab}±0.03
T7	1.20^{d}±0.07	2.70^{bcd}±0.23	1.00^{d}±0.04	0.75^{bc}±0.04	4.40±0.09	6.45^{ab}±0.14	1.90^{a}±0.04	1.15^{bc}±0.03
T8	1.00^{e}±0.05	2.80^{bcd}±0.24	0.80^{e}±0.01	0.70^{bcd}±0.05	4.50±0.04	6.50^{ab}±0.14	1.70^{b}±0.03	1.20^{abc}±0.03
T9	0.90^{e}±0.03	2.05^{e}±0.07	0.77^{e}±0.03	0.45^{e}±0.07	4.50±0.27	6.60^{ab}±0.12	1.70^{b}±0.05	1.10^{bcd}±0.05
T10	0.90^{e}±0.03	2.10^{e}±0.07	0.62^{f}±0.01	0.52^{cde}±0.08	4.60±0.19	6.60^{ab}±0.13	1.80^{ab}±0.03	1.20^{abc}±0.02
T11	0.45^{f}±0.03	1.20^{g}±0.04	0.60^{f}±0.01	0.17^{fg}±0.04	4.70±0.06	6.15^{ab}±0.19	1.70^{b}±0.05	0.95^{de}±0.03
T12	0.47^{f}±0.02	1.30^{fg}±0.10	0.55^{f}±0.01	0.18^{fg}±0.03	4.81±0.06	6.20^{ab}±0.22	1.19^{c}±0.05	1.08^{cd}±0.06

[1] Least squares means ± pooled standard error.
a,b,c,d,e,f,g, Means having different letter exponents among columns are significantly different (*P≤0.05*).

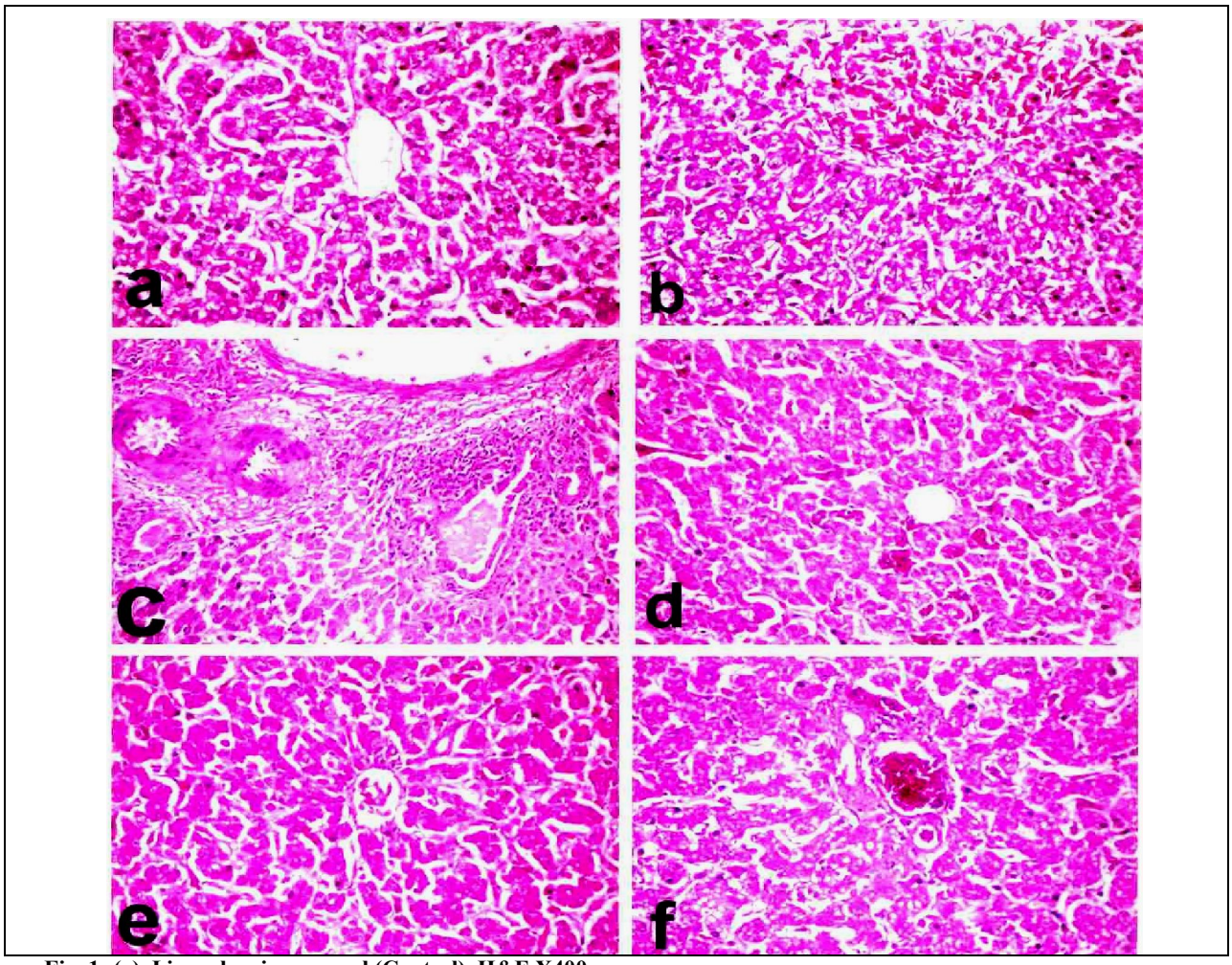

Fig. 1: (a), Liver showing normal (Control). H&E X400.
(b), Liver showing hepatic haemorrhage and vacuolization of hepatocytes (T4&12). H&E X400.
(c), Liver showing fibroplasia of portal triad and portal infiltration with leukocytes (T4&12). H&E X400.
(d), Liver showing slight congestion of hepatic sinuscides (T11). H&E X400.
(e), Liver showing no histopathological changes (T6). H&E X400.
(f), Liver showing cytoplasmic vacuolization of hepatocytes and edema (T4&T12). H&E X 400.

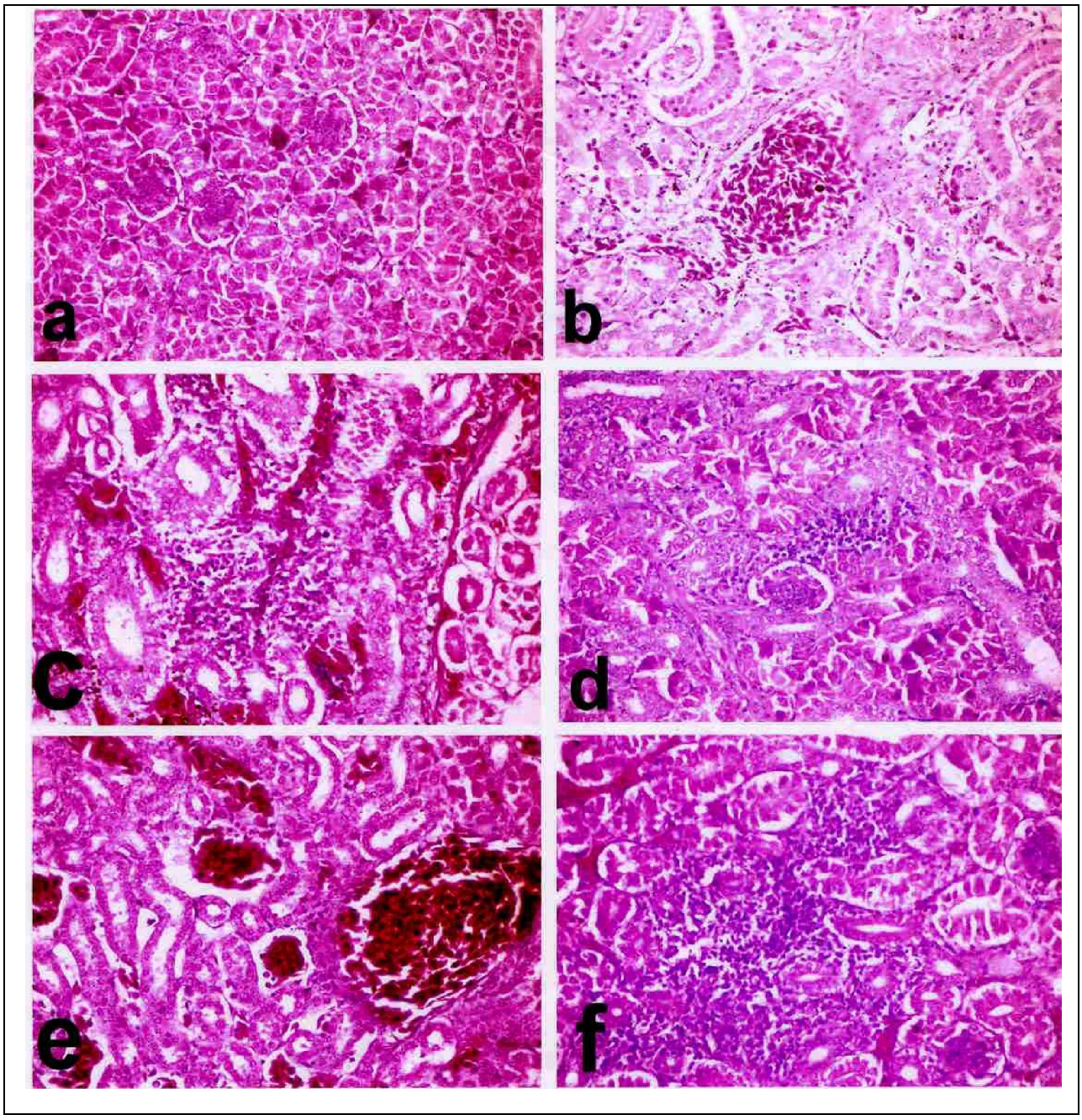

Fig. 2: (a), Kidney showing normal histological structure of renal parenchyma (control). H&E X400.
(b), Kidney showing congestion of renal BVs (T4). H&E X400.
(c), Kidney showing focal necrosis of renal tubules associated with leukocytic cells infiltration (T4). H&E X400.
(d), Kidney showing focal tubular necrosis associated with inflammatory cells infiltration (T4&12). H&E X400.
(e), Kidney showing congestion of renal BVS (T4&12). H&E X400.
(f), Kidney showing necrosis of renal tubules associated with leukocytic cells infiltration (T12). H&E X400.

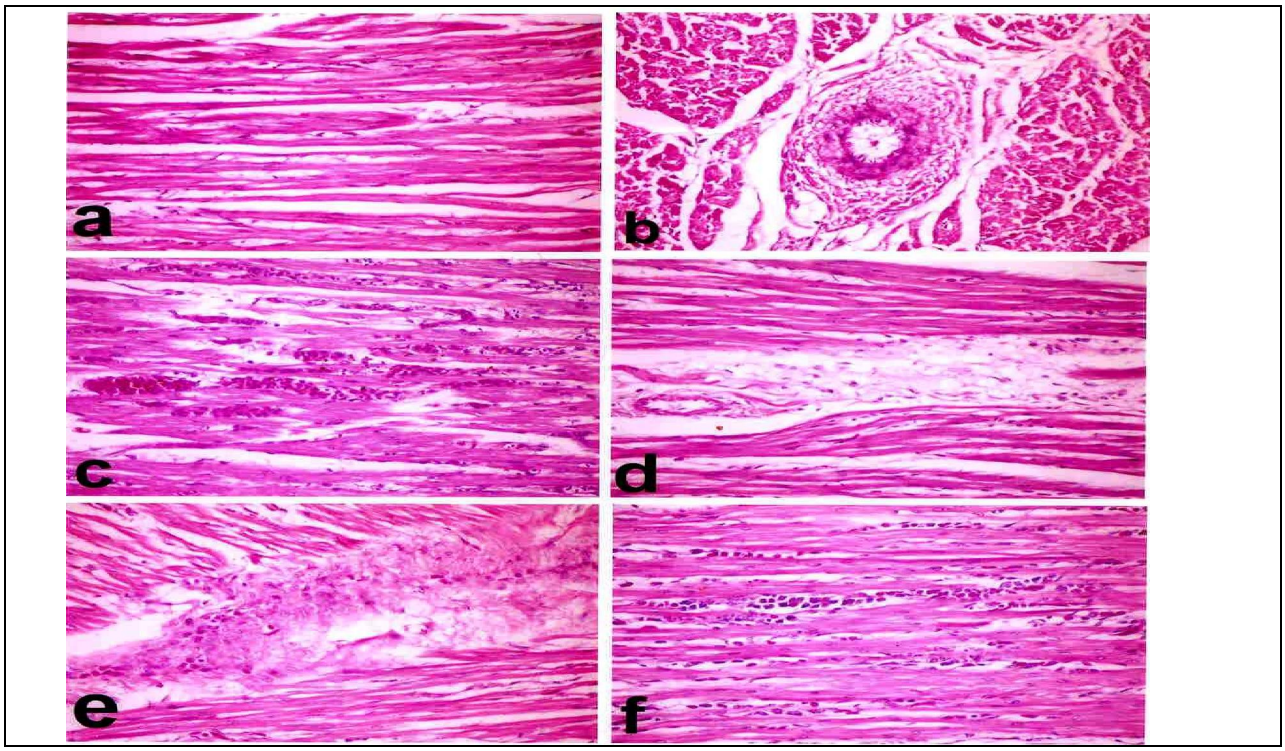

Fig. 3: (a), Heart showing normal (control). H&E X400.
(b), Heart showing perivascular edem (T4). H&E X400.
(c), Heart showing congestion of myocardial BVs (T4, 11&12) H&E X400.
(d), Heart showing intermuscular edema (T12). H&E X400.
(e), Heart showing myolysis of focal myocytes (T4). H&E X400.
(f), Heart showing intermuscular heterophiles infiltration (T4 &12). H&E X400.

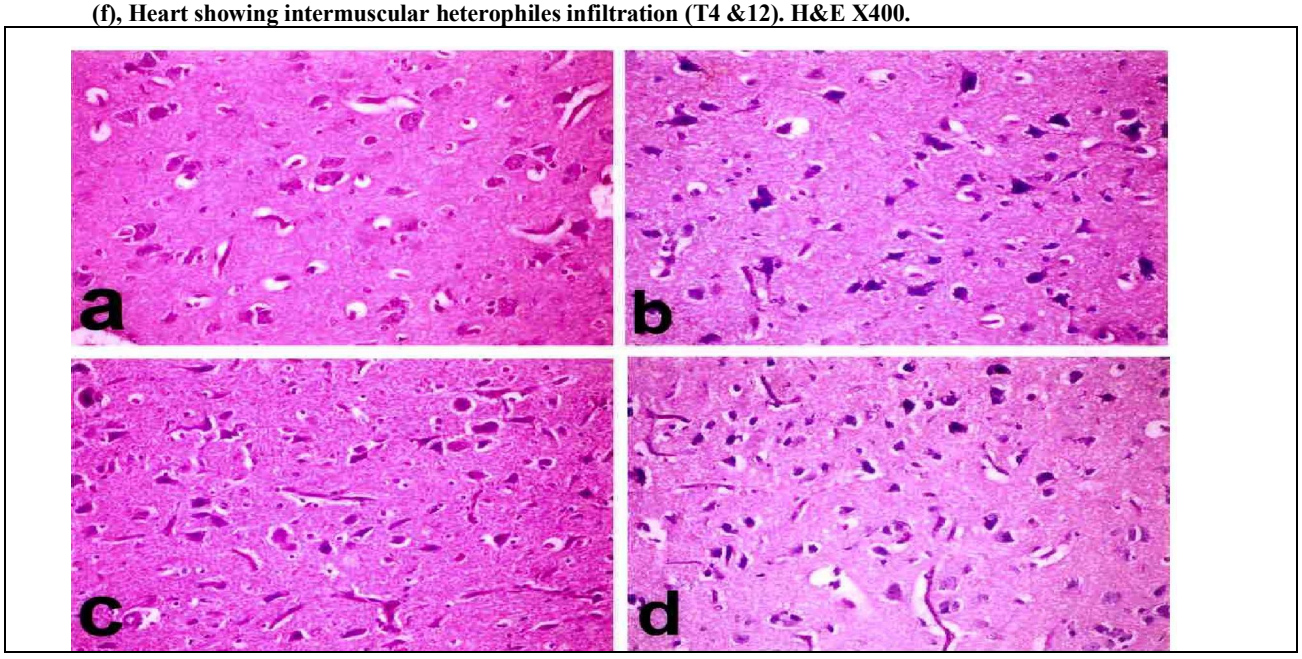

Fig. 4: (a), Brain showing normal (control). H&E X400.
(b), Brain showing necrosis and pyknosis of neurons (T4). H&E X400.
(c), Brain showing pyknosis of some neurons (T12). H&E X400.
(d), Brain showing necrosis and pyknosis of some neurons (T4 and 12). H&E X400.

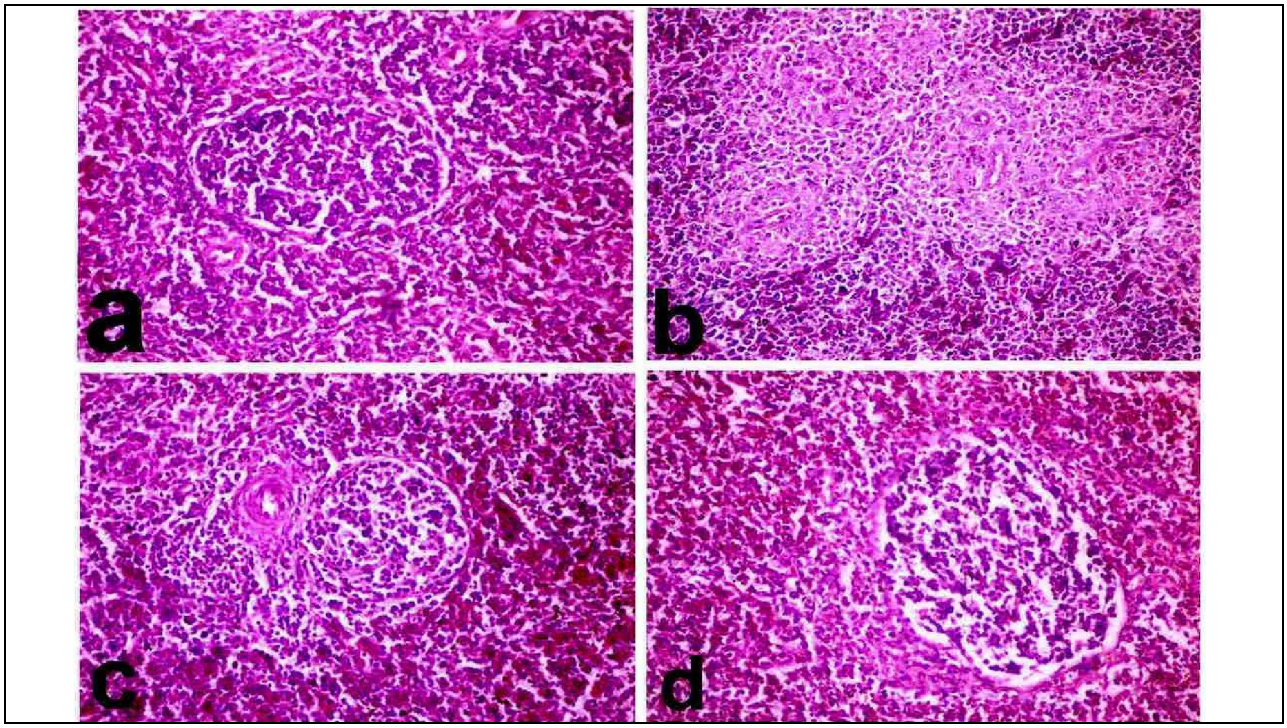

Fig. 5: (a), Spleen showing normal (control). H&E X400.
(b), Spleen showing hyperplasia of reticular cells (T4). H&E X400.
(c), Spleen showing atrophy of lymphoid follicle (T11&12). H&E X400.
(d), Spleen showing lymphocytic depletion (T4 and T12). H&E X400.

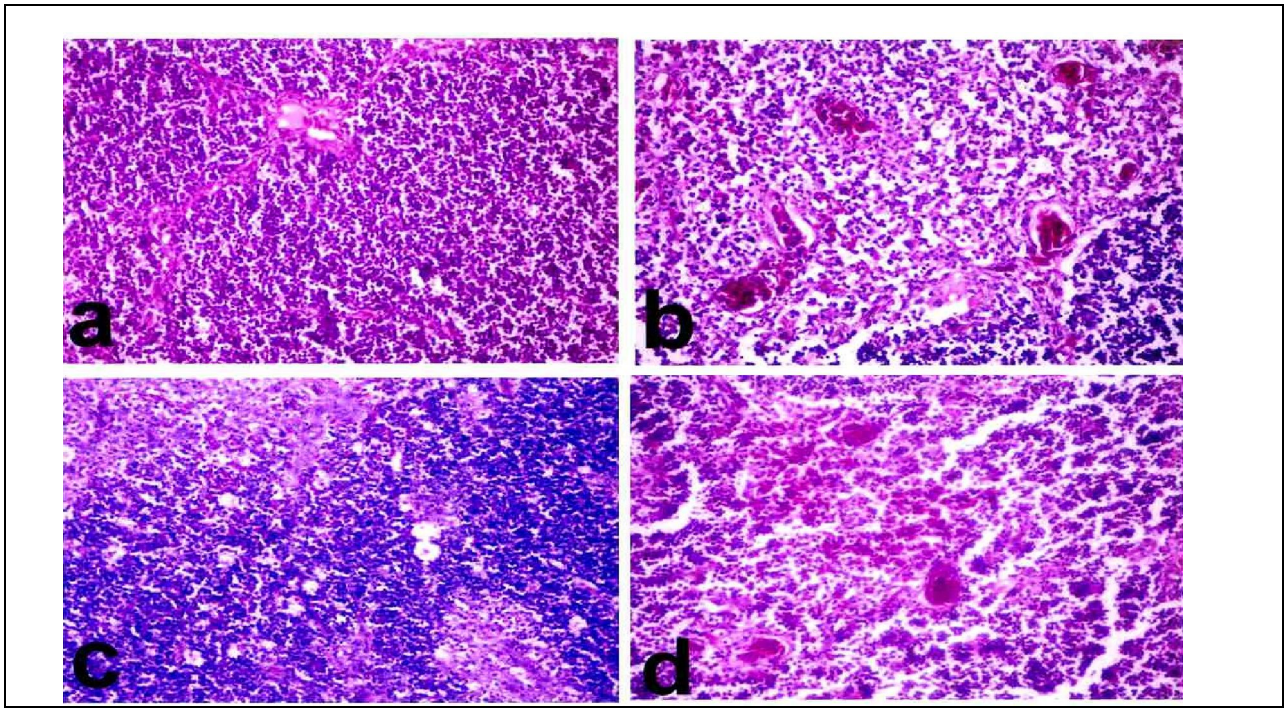

Fig. 6: (a), Thymus gland showing normal (control). H&E X400.
(b), Thymus gland showing lymphocytic necrosis and depletion in the thymic (T11&12). H&E X400.
(c), Thymus gland showing congestion of BVs (T4&12). H&E X400.
(d), Thymus gland showing thymic congestion and haemorrhage (T4 and T12). H&E X400.

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