The Effects of Green Tea (*Camellia Sinensis*) Probiotics on Broilers Exposed to Lead-Induced Oxidative Stress

[1]Yosef, T.A., [2]Al-Julaifi, M.Z. and [3]Kandeel, M.

[1]Department of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, Kafrelshiekh University
[2]Toxicology Laboratory, Management of Veterinary Laboratories, Ministry of Agriculture, Riyadh 11418, Saudi Arabia.
[3]Department of Pharmacology, Faculty of Veterinary Medicine, Kafrelshiekh University, Kafrelshiekh 33516, Egypt
Email: tarektoxicology@yahoo.com

Abstract: One-day-old broiler chicks were randomly divided into four groups (45 birds each) including three replicates for a 1-42 days as follows: (1) control: basal ration; (2): *Camellia sinensis* 1 g/kg basal ration; (3): lead acetate 200 mg/kg basal ration; (4): *Camellia sinensis* + lead acetate basal ration. Lead caused oxidative damage on blood and liver of the exposed birds as evidenced by a significant ($p<0.05$) increase in lead levels by 21.87 and 86.73 % and malondialdehyde (MDA) by 253.85 and 87.50 % respectively. Moreover decreased antioxidant enzymes activities. Co-supplementation of *Camellia Sinensis* to lead resulted in a significant ($p<0.05$) reduction in lead levels in the blood and liver by 42.85 and 58.82%; MDA by 45.45 and 50.00% respectively. SOD and CAT activities increased significantly ($p<0.05$), in addition to increasing in the GSH level. Results indicate that *Camellia Sinensis* may be beneficial in preventing lead-induced oxidative damage in poultry.

[Yosef, T.A., Al-Julaifi, M.Z. and Kandeel, M. **The Effects of Green Tea (*Camellia Sinensis*) Probiotics on Broilers Exposed to Lead-Induced Oxidative Stress.** *Journal of American Science*. 2012; 8(4): 499-506]. (ISSN: 1545-1003). http://www.americanscience.org. 66

Keywords: *Camellia Sinensis*; Lead; Oxidative Stress; MDA;GSH; SOD; CAT.

1. Introduction

Lead, one of the oldest known metals, is also one of the most widespread toxicants, and its poisoning remains a health threat. Lead toxicity occurs when an animal or a bird inhales or ingests a concentrated source of lead (Osweiler, 1996). The extent to which orally administered lead is absorbed is small. However, due to its slow rate of elimination, harmful levels of lead can accumulate in tissues after prolonged exposure to low quantities (Ercal et al., 2001). Lead produces acute and chronic poisoning and induces a broad range of physiological, biochemical and behavioral dysfunctions. The effect of lead on chicken, dove and wild animals is well-documented (Baykov et al., 1996 b). Lead is toxic to poultry at much lower levels than previously recognized, as little as 1.0 mg/kg lead in the diet can cause significant depression in the growth of broiler chickens (Bakalli et al., 1995). Studies have reported that lead has a potential for production of reactive oxygen species (ROS), disruption of tissue oxidant/antioxidant balance, and alteration of lipid metabolism therefore, the toxicities associated with this metal might be due to oxidative tissue damage and consequently a variety of pathological conditions, including lipid peroxidation, apoptosis, and tissue injuries (Ahamed and Siddiqui, 2007).

In order to protect against oxidative tissue damage, all living organisms have evolved an interdependent antioxidant system that includes enzymatic and non-enzymatic components in the liver (Ohtsuka et al., 1998) and erythrocytes (Orzechowski et al., 2000). Dietary supplementation with antioxidant improves the antioxidant status of poultry, lowering lipidperoxidation in the tissues and protecting cell from the harmful effects of ROS (Fellenberg and Speisky, 2006). Green tea (*Camellia sinensis*) are rich in flavanol monomer known as catechins consisting of various polyphenols which are 13.6 g/100 g in green tea and 4.2 g/100 g dry weight in black tea (Peterson et al., 2005). Tea catechins have a variety of pharmacologic effects including, antioxidant (Mohamadin et al., 2005), antimutagenic (Nagle et al., 2006), anticarcinogenic (Zaveri, 2006), and anti-inflammatory effects (Varilek et al., 2001).

Keeping the above facts in view, the present study was taken up to evaluate the effects of dietary lead exposure on lipid peroxidation; antioxidant status and lead concentrations in the blood and liver of broilers. We also aimed to determine whether the awful effects of lead, attributed to tissue peroxidation, could be reversed by adding *camellia sinensis* probiotics.

2. Materials and methods

Animals and experimental design

One hundred eighty "Ross" broiler chicks of one-day old were used for this study. The chicks were divided into four groups comprising one control and three experimental in 3 replicates of 15 birds each, following completely randomized design (CRD). The feed and drinking water were provided *ad libitum*. A lighting schedule of 23 L: 1 D was imposed throughout the experimental period. Ambient temperature was gradually decreased from 32°C on day 1 to 22°C at the end of the experiment. Newcastle disease vaccination was performed on day 10, whereas the Gumboro

vaccination took place on day 18. The basal ration (Table 1) was formulated according to NRC (1994) and analyzed by the AOAC (1995). Two phases were applied during the experiment: a starter (1-21 d) and finisher (22-42 d).The experimental groups were designed as: (1) control: basal ration; (2): *Camellia sinensis* 1 g/kg basal ration; (Sazedul et al., 2010); (3): lead (as lead acetate) 200 mg/kg basal ration (Seven et al., 2010); (4): *Camellia sinensis* 1 g/kg plus lead 200 mg/kg basal ration.

Production of Camellia sinensis probiotics

The chemical composition of fermented green tea probiotics are given in Table (2). Green tea probiotics were produced through 2 steps; the first step was producing solid culture with 5 h of static and 3 h of shaking fermentation process at 40°C by mixing 30% of green tea powder, 20% of wheat bran, and 50% of defatted rice bran inoculated by selected 2 strains Lactic acid bacteria (*Lactobacillus acidophilus* KCTC 3111 and *Lactobacillus plantarum* KCTC 3104). The second step was inoculating the selected strains (*Bacillus subtilis* KCTC 3239 and *Saccharomyces cerevisiae* KCTC 7915) with the solid culture and drying it (Ko and Yang, 2008). The chemical analysis of green tea probiotics showed crude protein, crude fat and crude fiber with the proportion of 19.20%, 2.92% and 11.08% respectively, and the concentrations of microbes were 4.1×10^7 cfu/g of *Lactobacillus acidophilus*, 5.6×10^6 cfu/g of *Lactobacillus plantarum*, 2.5×10^7 cfu/g of *Bacillus subtilis* and 6.3×10^9 cfu/g of *Saccharomyces cerevisiae*.

Sample preparation

On day 42 birds in the four groups were starved overnight for 12 h. Total 48 birds (four birds from each subgroup) were subjected to blood collection. Exactly 10 ml of blood was drawn from the wing vein of each bird into heparinized tubes and 2 ml aliquot of blood sample was transferred into another set of tubes for lead content determination. The remaining blood was centrifuged at 3000 rpm for 5 min; supernatants were discarded and the erythrocytes were washed thrice using 0.9% NaCl solution. The birds were sacrificed and liver was excised, and washed with ice-cold 0.9% NaCl solution to remove residual blood. Half of the liver was homogenized in ice-cold 50 mM sodium phosphate buffer and 0.1 mM Na2EDTA (pH 7.8). The soluble fraction was prepared by centrifugation at 4000 rpm for 10 min. The remaining half was stored frozen for lead content determination.

Lead content determination

Exactly 1 ml of blood was digested with 10 ml concentrated nitric acid and digests were brought to a constant volume of 25 ml with deionised water (Ademuyiwa, 1995). For liver, 1 g of sample was dried to a constant weight at 85°C. Dried samples were cold digested in 2 ml of nitric acid overnight. They were then hot digested on a block digester at 120°C until all the organic matter was dissolved. 2 ml of 30% (w/v) hydrogen peroxide were added during digestion to enhance oxidization. The digest was allowed to cool, and then diluted to 25 ml with deionised water (Alonso et al., 2000). Lead concentrations in the digests were determined by atomic absorption spectrometry (Perkin–Elmer Model 400, Shelton, CT, USA) at 217nm wavelength and 6 ma current. Values were expressed as µg/ml of blood or µg/g of tissue.

Measurement of indices of oxidative stress

The extent of lipid peroxidation was estimated in terms of thiobarbituric acid reactive substances (TBARS), using MDA as standard by monitoring the change of absorbance at 532 nm with the spectrophotometer (Placer et al., 1966). GSH levels were measured employing 0.04%-5,5% dithiobes-(2-nitrobenzoic acid) in 10% sodium citrate and recording at 410 nm as described by Dutta et al. (1995). SOD was assayed according to the procedure of Das et al., (2000).The activity of CAT was determined by measuring the breakdown of H_2O_2 at 240 nm according to the method of Aebi (1984). All chemicals used in the enzymatic activity were of analytical purity and were obtained from Sigma Chemical, Germany.

Statistical analysis

The data obtained from this study were analyzed by general linear models (GLM) of SAS Package Program (1990) to estimate variance components for a completely randomized design. Duncan's multiple comparison tests (1955) were used to examine significant differences between treatment means. Differences were statistically assessed at $P<0.05$.

3. Results

The mean lead level in the blood of lead-exposed birds was 1.22 times higher than that of control birds. Similarly, in the liver, there was a 1.87 fold increase in lead level of exposed birds compared with control group (Tables 3 and 4). *Camellia Sinensis* supplementation brought about a significant reduction ($p<0.05$) in the lead level, both in the blood and liver of exposed birds. A 42.85% reduction in the blood and a 58.82% reduction in the liver were observed (Tables 5 and 6).

Lipid peroxidation as determined by MDA level, was found to be markedly higher in the blood and liver of test birds when compared with the control ($p<0.05$), with values being 3.54 and 1.88 times higher than control, respectively (Tables 3 and 4). *Camellia Sinensis* supplementation resulted in a significant reduction ($p<0.05$) in lipid peroxidation in both tissues,

which amounted to 45.45% in the blood and 50% in the liver (Tables 4 and 5). Exposure to lead in the diet for six weeks decreased GSH level in the blood (58.04%) and liver (47.52%). The *Camellia Sinensis* supplementation led to a significant increase ($p<0.05$) in the GSH of exposed birds. The increase was more remarkable in the liver (67.27%), while in the blood it was 47.69%. Tables 3 and 4 also show the activities of SOD and CAT in the blood and liver of birds for all groups. Lead exposure decreased SOD and CAT activities significantly ($p<0.05$) when compared with the control. The decrease in the blood was 48.15 and 52.26%, while it was 35.71 and 36.31% in the liver for SOD and CAT respectively. *Camellia Sinensis* attenuated the effects of lead on SOD and CAT in the blood and liver. The increase in the activity of SOD and CAT in the blood was 59.62 and 61.51% however, it was 43.30 and 44.98 % in the liver respectively.

Table 1. Composition of basal diets and nutrient levels

Feed components	Starter phase (d 1 to 21)	Finisher phase (d 22 to 42)
Ingredient, % as-fed		
Corn	55.59	60.91
Soybean meal (44%CP)	32.21	26.60
Fish meal (64%CP)	4.00	4.10
Soybean oil	3.87	4.40
Limestone	1.28	1.30
Dicalcium phosphate	1.30	1.10
Salt	0.36	0.35
Vitamin-mineral premix*	1.00	1.00
L-Lysine_HCl, 78%	0.12	0.09
DL-Methionine, 98%	0.27	0.15
Nutrient content (calculated)		
ME, Mcal/kg	3.00	3.10
CP	21.49	19.52
Calcium	1.00	0.88
Available phosphorus	0.43	0.41
Lysine	1.31	1.14
Methionine	0.67	0.50

* Vitamin and mineral premix provided per kilogram of diet: Vitamin A, 12,000 IU; cholecalciferol 1,500 IU; vitamin E, 30 mg; vitamin K3, 5 mg; vitamin B1, 3 mg; vitamin B2, 6 mg; vitamin B6, 5 mg; vitamin B12, 30 µg; Ca-D-pantothenate, 10 mg; Folic acid, 0.75 mg; D-biotin, 0.08 mg; Mn, 80 mg; Zn, 60 mg; Fe, 40 mg; Cu, 5 mg; Se, 0.15 mg; Co, 0.1 mg; I, 0.4 mg.

Table 2. Composition of *Camellia sinensis* probiotics supplement

Component	**Value**
crude protein	19.20%
crude fat	2.92%
crude fiber	11.08%
L. acidophilus	4.1 × 107 cfu/g
L. plantarum	5.6 × 106 cfu/g
B. subtilis	2.5 × 107 cfu/g
S. cerevisiae	6.3 × 109 cfu/g

Table 3. Effects of *camellia sinensis* (1 g/kg diet) on blood lead levels, MDA, SOD, CAT and GSH concentrations of broilers exposed to lead (200 mg/kg diet) for 6 weeks (n=45).

	Treatment groups			
	Control	*Camellia sinensis*	Lead	Lead + *Camellia sinensis*
Lead burden (mg/l)	0.032 ± 0.00^{c}	0.032 ± 0.00^{c}	0.039 ± 0.00^{a}	0.035 ± 0.00^{b}
MDA, (µmol/g Hb)	0.39 ± 0.08^{c}	0.37 ± 0.05^{c}	1.38 ± 0.17^{a}	0.84 ± 0.06^{b}
GSH, (mmol/mg protein)	1.12 ± 0.12^{a}	1.15 ± 0.15^{a}	0.47 ± 0.03^{c}	0.81 ± 0.33^{b}
SOD,(Unit/g Hb)	2.16 ± 0.07^{a}	2.35 ± 0.06^{a}	1.12 ± 0.12^{c}	1.54 ± 0.07^{b}
CAT,(Unit/mg Hb)	5.32 ± 0.47^{a}	5.86 ± 0.14^{a}	2.54 ± 0.31^{c}	3.61 ± 0.23^{b}

Results are expressed as mean ± standard deviation.

Values in the same row with different superscript are significantly different at $p < 0.05$.

Table 4. Effects of *camellia sinensis* (1 g/kg diet) on liver lead levels, MDA, SOD, CAT and GSH concentrations of broilers exposed to lead (200 mg/kg diet) for 6 weeks (n=45).

	Treatment groups			
	Control	*Camellia sinensis*	Lead	Lead + *Camellia sinensis*
Lead burden (mg/kg tissue)	0.98 ± 0.23c	0.92 ± 0.24c	1.83± 0.14a	1.48 ± 0.19b
MDA, (µmol/g tissue)	0.32 ± 0.03c	0.33 ± 0.11c	0.60 ± 0.08a	0.46 ± 0.06b
GSH, (mmol/mg protein)	4.63 ± 0.55a	4.97 ± 0.25a	2.43 ± 0.36c	3.15 ± 0.22b
SOD,(Units/mg protein)	8.15 ± 1.13a	8.89 ± 1.12a	5.24 ± 0.96c	6.89 ± 0.45b
CAT,(Units/mg protein)	17.02 ± 1.50a	17.79± 0.69a	10.84 ± 1.78c	14.24 ± 0.81b

Results are expressed as mean ± standard deviation.

Values in the same row with different superscript are significantly different at $p < 0.05$.

Table 5. Effects of Lead exposure (200 mg/kg diet) for 6 weeks, compared to control, on blood and liver lead levels, lipid peroxidation, SOD, CAT and GSH concentrations of broilers (n=45).

Tissue / Parameter	Lead	
	Blood %	Liver %
Lead burden (mg/kg tissue)	↑ 21.87	↑ 86.73
MDA, (µmol/g tissue)	↑ 253.85	↑ 87.50
GSH, (mmol/mg protein)	↓ 58.04	↓ 47.52
SOD,(Units/mg protein)	↓48.15	↓ 35.71
CAT,(Units/mg protein)	↓ 52.26	↓ 36.31

Table 6. Ameliorating effects of *camellia sinensis* probiotics (1 g/kg diet) ,compared to lead-exposed group, on blood and liver lead levels, MDA, SOD, CAT and GSH concentrations in broilers exposed to lead (200 mg/kg diet) for 6 weeks (n=45).

Tissue / Parameter	Lead + *Camellia sinensis*	
	Blood %	Liver %
Lead burden (mg/kg tissue)	42.85	58.82
MDA, (µmol/g tissue)	45.45	50.00
GSH, (mmol/mg protein)	47.69	67.27
SOD,(Units/mg protein)	59.62	43.30
CAT,(Units/mg protein)	61.51	44.98

4. Discussion

This study was conducted in order to investigate the role of *camellia sinensis* probiotics in alleviating the oxidative stress status produced after lead-intoxication in broilers. Researchers have demonstrated the toxic effects of lead in poultry (Shafiqur and Joshi, 2009; Ratan et al., 2010). Oxidative damage associated with the presence of lead has been proposed to indicate a possible role of free radicals in the pathogenesis of lead toxicity (Adonaylo and Oteiza, 1999). The cytotoxic effects of lead have been interconnected to the ability of lead ions to trigger formation of free radicals which in turn cause oxidative stress, reducing cellular antioxidant defense system and/or increasing susceptibility of cells to oxidative attack by altering the membrane integrity and fatty acid composition (Igor et al., 2011). Studies have shown that superoxide, hydrogen peroxide and hydroxyl radicals are produced after exposure to lead in various cellular systems (Gurer et al., 1999).

The present study showed that dietary exposure to 200 mg/kg diet of lead acetate for six weeks induced oxidative injury monitoring by increase levels of lead and MDA, as well as decreased levels of GSH and activities of SOD and CAT in both blood and liver. Blood lead concentration was slightly changed, however, lead was significantly accumulated in the liver of lead-treated broilers compared with the control and *camellia sinensis* plus lead groups. This is in agreement with previous reports which declared that lead concentrations in the liver may be high in animals that have no toxicity symptoms and a nearly normal blood lead level (Humphreys, 1991). Further support for our findings comes from Khan et al.(1993), which reported that toxic doses of lead administrated orally accumulated in the liver. The observed elevation in MDA level of birds exposed to lead in this study may be due to either overproduction or accumulation of ROS resulting from lead exposure. Lead altered lipid metabolism and enhanced lipid peroxidation directly through increasing fluxes of superoxide, hydrogen peroxide and hydroxyl radicals (Gurer et al., 1999) and indirectly through damage the protective antioxidant barrier (Patra et al., 2001). An inhibition of δ-ALAD activity leads to accumulation of δ-ALA, which undergoes auto-oxidation inducing free radicals and so lipid peroxidation (Flora et al., 2008). Previous studies had reported that levels of lead and MDA were increased as a result of lead supplementation (Hamed et al., 2010).

The decreased GSH levels after exposure to lead observed in our results may comes from high affinity of this metal to SH groups (Gurer et al., 1998). In this respect, Saxena and Flora (2004) had reported a significant decrease in GSH content of erythrocytes as a result of lead exposure. It has been revealed that lead may affect antioxidant barrier via inhibiting activities of enzymes involved in GSH metabolism, such as GST and SOD by blocking their SH groups (Gurer et al., 1998). The lower activities of SOD founded in this study in lead exposed birds may be partly explained by interaction between lead and essential metals such as copper and zinc that are essential cofactors for SOD (Patil et al., 2006). Another possible explanation is the massive production of superoxide anions, which overrides enzymatic activity and so leads to fall in SOD concentration. Various reports regarding influence of lead on SOD activities have given similar results (Mohammad et al., 2008; Yin et al., 2008). The observation of low activity of CAT in the blood and liver of lead-exposed birds in this study could be due to the down regulated synthesis or over-utilization of the antioxidant enzymes due to persistent toxicant misuse (Irshad and Chaudhuri, 2002). Our results are in agreement with other studies in literature (Liao et al., 2004; Lamsal et al., 2007).

The potential role of oxidative stress injury, which is associated with lead, suggests that antioxidants may enhance the efficacy of treatment designed to lessen lead-induced toxicity. Antioxidants and vitamins are very important in the treatment of heavy metal poisoning. Vitamin C (El-Tohamy and El-Nattat, 2010); vitamin E (Abdalla, 2009). vitamin B6, β-carotene, zinc, and selenium, (Ping and Yueliang, 2002) have been shown to protect against lead-induced oxidative stress. Our results indicate that

supplementation of *camellia sinensis* probiotics (1 g /kg diet) resulted in a significant decrease in blood and liver lead levels. In addition, alteration induced in the level of MDA, GSH, SOD and CAT by lead were upturned.

The benefits mechanisms of *camellia sinensis* effects are mainly attributed to its antioxidant properties and the ability of its polyphenolic catechins to scavenge reactive oxygen species (Yang, 1999). The *camellia sinensis* catechins have been shown to be more effective antioxidants than Vitamins C and E (Rice-Evans et al., 1995). The metal-chelating properties of *camellia sinensis* catechins are also important contributors to their antioxidant activity (Kumamoto et al., 2001). The reduction in lead levels of blood and liver as a result of *camellia sinensis* supplementation could be due primarily to the chelating property of its catechins which can decrease lead lipophilicity and so its absorption from gastrointestinal tract and consequently reducing the tissue lead burden (Mehana et al., 2010). These findings are in agreement with other investigators who have reported protective effect of *camellia sinensis* in different metal-induced toxicity as arsenic (Chandronitha et al., 2010) chrome, mercury, cadmium (Everygreenherb.com/poisons. 2010). *Camellia sinensis* supplementation resulted in a significant reduction in the elevated level of MDA in the lead-exposed birds. *Camellia sinensis* is effective in scavenging free radicals, including superoxide, hydrogen peroxide and hydroxyl radicals by acting as a two electron reducing agent and confers protection by contributing an electron to reduce free radicals, thus neutralizing these compounds in the extracellular aqueous environment prior to their reaction with biological molecules initiating lipid peroxidation (Halliwell and Gutteridge, 1999). *Camellia sinensis* flavanol, in the cell membrane, protect the unsaturated fatty acids against oxidants and decreased MDA levels by blocking ROS production (Hosnuter et al., 2004).

The ability of *camellia sinensis* to reverse the GSH depletion observed in the lead-exposed birds might be due to its ability to quench ROS. *Camellia sinensis* acts as an alternative sulphydryl nucleophile to GSH, thereby preventing its oxidation to GSSG in detoxification reaction against free radicals (Oda et al., 2010). in addition to its ability to regenerate other small molecule antioxidants such as glutathione, α-tocopherol and β-carotene (Halliwell and Gutteridge, 1999). The increase in SOD and CAT activities after *Camellia sinensis* supplementation to lead-exposed birds, was attributed to induction or mutually protective interactions especially superoxide dismutase, which present at low level only but highly inducible under oxidative stress (Gonzalez et al., 2000). *Camellia sinensis* increased total antioxidant activity while peroxides and oxidative stress-induced damage were decreased (Erba et al., 2005). Also, Yin et al., (2008) found that *Camellia sinensis* could reverse oxidative stress which had been impaired in birds receiving lead. These results put forward that *Camellia sinensis*, an antioxidant and chelator, may be beneficial in preventing lead-induced oxidative stress. This study is a contribution to the potential for veterinary use of *Camellia sinensis* in poultry.

Corresponding author
Tarek A. A. A. Yosef
Department of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, Kafrelshiekh University, Kafrelshiekh 33516, Egypt. Management of Veterinary laboratories, Toxicology Laboratory, Ministry of Agriculture, Riyadh, Saudi Arabia
tarektoxicology@yahoo.com

References

Abdalla, A.E. (2009). The role of antioxidant (vitamin E) in the control of lead (pb) pollution and enhancement of growth within Nile Tilapia (*Oreochromis Niloticus*). Intern J Appl. Res, Vet. Med., 7:3-12.

Ademuyiwa, O. (1995). Zur Toxikologie der Arsen-Kupfer Wechsekwirlung im Saugetierorganimimus. Untersuchunge an Ratten. Ph.D. Thesis, University of Munich, Munich. Federal Republic of Germany.

Adonaylo, V.N., & Oteiza, P.I. (1999). Lead intoxication: antioxidant defenses and oxidative damage in rat brain. Toxicology, 135:77-85.

Aebi, H. (1984). Catalase in vitro methods in enzymology (Ed. B. J. Willam), Academic Press, New York, ABD, 105,121-126.

Ahamed, M., & Siddiqui, M.K.J. (2007). Environmental lead toxicity and nutritional factors. Clin. Nutr., 26:400-408.

Alonso, M.L., Benedito, J.L., Miranda, M., Castillo, C., Hernandez, J., & Shore, R.F. (2000). Arsenic, Cadmium, Lead, Copper and Zinc in cattle from Galicia, NW Spain. Sci. Total Environ, 246:237-248.

AOAC (1995). Official methods of analysis, 16th ed. Association of Official Analytical Chemists, Arlington, VA. 4.1-4.17.

Bakalli, R.I., Pesti, G.M., & Ragland, W.L. (1995). The magnitude of lead toxicity in broiler chickens. Vet Hum Toxicol., 37:15-19.

Baykov, B., Stayanov, M., & Gugova, M. (1996 b). Cadmium and Lead bioaccumulation in male chickens for high food concentrations. Toxicoligicl and Environmental Chemistry. 54:155-159.

Chandronitha, C., Ananthi, S., Ramakrishnan, G., Lakshmisundaram, R., Gayathri, V., & Hannah, R. (2010). Protective role of tannin-rich fraction of *Camellia sinensis* in tissue arsenic burden in

Sprague Dawley rats. [Online] Available: http://greentealovers.com/greenteahealthcatechin.htm.

Das, K., Samanta, L., & Chainy, G.B.N. (2000). A modified spectrophotometric assay of superoxide dismutase using nitrite formation by superoxide radicals. Ind. J. Biochem. Biophys, 37:201-204.

Duncan, D.B. (1955). Multiple range and multiple F test. Biometrics. 11: 1 Fuller R (1989). Probiotics in man and animals A Review. J. Appl. Bacterol., 66:365-378.

Dutta, P., Seirafi, J., Halpin, D., Pinto, J. & Rivlin, R. (1995). Acute ethanol exposure alters hepatic glutathione metabolism in riboflavin deficiency. Alcohol, 12:43–47.

El-Tohamy, M.M., & El-Nattat, W.S. (2010). Effect of Antioxidant on Lead-Induced Oxidative Damage and Reproductive Dysfunction in Male Rabbits. Journal of American Science, 6(11):264-272.

Erba, D., Riso, P., Bordoni, A., Foti, P. Biagi, P.L., & Testolin, G. (2005). Effectiveness of moderate green tea consumption on antioxidative status and plasma lipid profile in humans. J Nutr Biochem., 16:144-149.

Ercal, N., Gurer-Orhan, H., & Aykin-Burns, N. (2001). Toxic metals and oxidative stress. Part I: Mechanisms involved in metal-induced oxidative damage. Curr. Top. Med. Chem., 1:529-539.

Everygreenherb (2010). Using herbs to neutralize poisons. [Online] Available: http://everygreenherb.com/poisons.html.

Fellenberg, M.A., & Speisky, H. (2006). Antioxidants: their effects on broiler oxidative stress and its meat oxidative stability. Worlds Poultry Science Journal, 62:53-70.

Flora, S.J.S., Mittal, M., & Mehta, A. (2008). Heavy metal induced oxidative stress and its possible reversal by chelation therapy. Indian J Med Res, 128:501–523.

Gonzalez, E., Rosello-Catafau, J., Jawerbaum, A., Sinner, D., Pustovrh, C., & Vela, J. (2000). Pancreatic nitric oxide and oxygen free radicals in the early stages of streptozotocininduced diabetes mellitus in the rat. Braz. Med. Biol. Res., 33: 1335-1342.

Gurer, H., Neal, R., Yang, P., Oztezcan, S., & Erçal, N. (1999). Captopril as an antioxidant in lead-exposed Fischer 344 rats. Hum Exp Toxicol., 18:27–32.

Gurer, H., Ozgunes, H., Neal, R., Spitzand, D.R., & Ercal, N. (1998). Antioxidant effects of N-acetyl cystein and succimer in red blood cells from lead exposed rats. Toxicology,128:181–189.

Hamed, E.A,. Abdel-Raheim, M.A., Meki, A.A., & Abd El-Mottaleb, A.A. (2010). Protective effect of green tea on lead-induced oxidative damage in rat's blood and brain tissue homogenates. J Physiol Biochem., 66:143–151.

Halliwell, B., & Gutteridge, J.M.C. (1999). Free Radicals in Biology and Medicine.3rd ed, Oxford University press.1-543.

Hosnuter, M., Gurel, A., Babuccu, O., Armutcu, F., Kargi, E., & Isikdemir, A. (2004). The effect of CAPE on lipid peroxidation and nitric oxide levels in the plasma of rats following thermal injury. Burs, 30:121-125.

Humphreys, D.J. (1991). Effects of exposure to excessive quantities of lead on animals. Br. Vet. J, 147: 18-30.

Igor, P., Isabelle, P., Brigitte, B., Mona, T., Etienne, D., Céline, O., & Béatrice, A. (2011). Cytotoxicity and oxidative stress induced by different metallic nanoparticles on human kidney cells. Particle and Fibre Toxicology, 8:10.

Irshad, M., & Chaudhuri, P.S. (2002). Oxidant-antioxidant system: role and significance in human body. Indian J. Exp. Biol., 40:1233-1239.

Khan, M.Z., Szarek, J., & Markiewicz, K. (1993). Effects of oral administration of toxic levels of lead and selenium upon concentration of different elements in the liver of broiler chicks. Zentralbl. Veterinarmed. A, 40: 652-664.

Ko, S.Y., & Yang, C.J. (2008). Effect of green tea probiotics on the growth performance, meat quality and immune response in finishing pigs. Asian-Australasian Journal of Animal Sciences, 2:352-359.

Kumamoto, M., Sonda, T., Nagayama, K., & Tabata, M. (2001). Effects of pH and metal ions on antioxidative activities of catechins. Biosciences Biotechnology Biochemistry, 65 (1),126-132.

Lamsal, M., Gautam, N., Bhatta, N., Toora, B.D., Bhattacharya, S.K., & Baral, N. (2007). Evaluation of lipid peroxidation product, nitrate and antioxidant levels in newly diagnosed and two months follow-up patients with pulmonary tuberculosis. Southeast Asian. J. Trop. Med. Pub. Health, 38 (4):695-703.

Liao, M.F., Chen, C.C., & Hsu, M.H. (2004). Evaluation of the serum antioxidant status in asthmatic children. Acta. Paediatr. Taiwan, 4 (45):213-217.

Mehana, E. E., Abdel Raheim, M.A., & Meki, K.M. (2010). Ameliorated effects of green tea extract on lead induced liver toxicity in rats. Experimental and toxicologic pathology. 13,173–180.

Mohamadin, A., El-Beshbishy, H., & El-Mahdy, M. (2005). Green tea extract attenuates cyclosporine A-induced oxidative stress in rats. Pharm. Res., 51:51-57.

Mohammad, I.K., Mahdi, A.A., Raviraja, A., Najmul, I., Iqbal, A., & Thuppil, V. (2008). Oxidative stress in painters exposed to low lead levels. Arh Hig Rada Toksikol, 59(3):161-169.

Nagle, D.G., Ferreira, D., & Zhou, Y. (2006). Epigallocatechin-3-gallate (EGCG): Chemical and biomedical perspectives. Phytochemistry, 67:1849-1855.

NRC (1994). Nutrient requirements of poultry, National Research Council, National Academy Press, Washington, DC.

Oda, M., Al- Zamely-Amira, M., Jasim, A., & Mufeed, J.E. (2010). Effect of Desferal Drug and Green Tea on the Levels of Lipid Prophile in Sera of Rabbits Induced Diabetes Mellitus by Iron Over load. International Journal of Biotechnology and Biochemistry, 6(6):969-982.

Ohtsuka, A., Kojima, H., Ohtani, T., & Hayashi, K. (1998). Vitamin E reduces glucocorticoid-induced oxidative stress in rat skeletal muscle. J. Nutr. Sci. Vitam., 44:779-786.

Orzechowski, O., Ostaszewski, P., Brodnicka, A., Wilczak, J., Jank, M., Balasinska, B., Grzelkowska, K., Ploszaj, T., Olczak, J., & Mrowczynska, A. (2000). Excess of glucocorticoids impairs whole-body antioxidant status in young rats. Relation to the effect of dexamethasone in soleus muscle and spleen. Horm. Metab. Res, 32:174-180.

Osweiler, G.D. (1996). Lead toxicosis. In: Toxicology, ed., 191–197. Williams and Wilkins, Philadelphia,PA.

Patil, A.J., Bhagwat, V.R., Patil, J.A., Dongre, N.N., Ambekar, J.G., Jailkhani, R., & Das, K.K. (2006). Effect of lead (Pb) exposure on the activity of superoxide dismutase and catalase in battery manufacturing workers (BMW) of western Maharashtra (India) with reference to heme biosynthesis. Int J Environ Res Public Health, 3:329–337.

Patra, R.C., Swarup, D., & Dwidedi, S.K. (2001). Antioxidant Effects of α-tocopherol, ascorbic acid and l-methionine on lead-induced oxidative stress of the liver, kidney and brain in rats. Toxicology, 162:81-88.

Peterson, S., Dwyer, J., Bahgwat, S., Haytowitz, D., Holden, J., Eldridge, A., Beecher, G., & Aladesanmi, J. (2005). Major flavonoids in dry tea. J. Food Composition and Analysis, 18: 487-501.

Ping, C.H., & Yueliang, L. (2002). Antioxidant nutrients and lead toxicity. Toxicology, 180(1) 33-44.

Placer, Z.A., Cushman, L.L., & Johnson, B.C. (1966). Estimation of product of lipid peroxidation (Malonyl Dialdehyde) in biochemical systems. Anal. Biochem., 16:359-364.

Ratan, M., Kumar, A., Gopala, R., Anjaneyulu, Y., & Dilip, G. (2010). Oxidative Stress Induced by Lead and Antioxidant Potential of Certain Adaptogens in Poultry. Toxicol Int., 17(2):45–48.

Rice-Evans, C.A., Miller, N.J., Bolwell, P.G., Bramley, P.M., & Pridham, J.B. (1995). The relative antioxidant activities of plant-derived polyphenolic flavonoids. Free Radical Research, 22 (4): 375–383.

SAS (1990). SAS® User's guide: statistics. Version 6, Fourth edition. SAS Institute Inc., Cary, NC.

Saxena, G., & Flora, S.J.S. (2004). Lead induced oxidative stress and hematological alterations and their response to combined administration of calcium disodium EDTA with a thiol chelator in rats. J Biochem & Molecular Toxicology, 18:21–233.

Sazedul, K.S., Yong, K.G., & Chul, J.Y. (2010). Effects of *Camellia sinensis* and mixed probiotics on the growth performance and body composition in broiler. Journal of Medicinal Plants Research., 4(7):546-550.

Shafiqur, R., & joshi1, M.V. (2009). Effect of lead toxicity on growth and performance of broilers. Tamilnadu J. Veterinary & Animal Sciences, 5(2):59-62.

Seven, I., Aksu1, T., & Tatli, P. (2010). The Effects of Propolis on Biochemical Parameters and Activity of Antioxidant Enzymes in Broilers Exposed to Lead-Induced Oxidative Stress. Asian-Aust. J. Anim. Sci., 23(11):1482-1489.

Varilek, G.W., Yang, F., Lee, E.Y., de Villiers, W.J.S., Zhong, J., Oz, H.S., Westberry, K.F., & McClain, C.J. (2001). Green tea polyphenol extract attenuates inflammation in interleukin-2-difeicient mice, a model of autoimmunity. J. Nutr., 131:2034-2039.

Yang, C.S. (1999). Tea and health. Nutrition, 15 (11–12): 946–949.

Yin, S.T., Tang. M.L., Su, L., Chen, L., Hu, P., Wang, H.L., Wang, M., & Ruan, D.Y. (2008). Effects of epigallocatechin-3-gallate on lead-induced oxidative damage. Toxicology, 249(1)45–54.

Zaveri, N.T. (2006). Green tea and its polyphenolic catechins: medicinal uses in cancer and noncancer applications. Life Sci., 78:2073–2080.