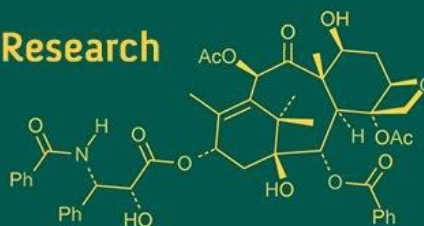


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Evaluation of antioxidant effect of *Zingiber officinale* on lead intoxicated broiler chicken

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Abstract

The present research investigation was carried out to study effect of 50% ethanolic rhizome extract of *Z. officinale* on lipid peroxidation & oxidative stress on Lead induced toxicity in broiler birds. 140 broiler chicks were divided into 7 groups each comprising of 20 birds. The toxicity was induced by administration of lead acetate @ 200 mg/kg feed from T₂ to T₇ and T₁ as normal control. T₃ & T₄ was given AA @ 200 mg/kg feed & Ext. @ 200 mg/kg b.w, respectively. T₅ was given AA @ 200 mg/kg feed & Ext. @ 200 mg/kg b.w. T₆ was given Ext. @ 300 mg/kg b.w. b.w, respectively. At the end of experiment, it was observed that, the extract of *Z. Officinale* @ 200 & 300 mg/kg alone and with L-ascorbic acid significantly reduced TBARS level; similarly the reduced levels of Glutathione were significantly increased towards the normal. The activities of antioxidant enzymes like glutathione peroxidase, superoxide dismutase and catalase were altered due to induced Pb toxicity which was improved in all treated groups. There was significant protective effect shown by *Z. officinale* and L-ascorbic acid by radical scavenging capacity with reduction in oxidative damage which illustrated the antioxidant effect of *Z. officinale* against lead toxicity in broiler chickens.

Keywords: Medicinal plants, *Zingiber officinale*, antioxidant, lead toxicity, broiler birds

1. Introduction

Heavy metals from manmade pollution sources are constantly released into aquatic and terrestrial ecosystems and therefore, the concern about the effect of anthropogenic pollution on the ecosystem is growing. Contamination with heavy metals is a serious threat because of their toxicity, bioaccumulation and biomagnifications in the food chain (Demirezen & Uruc, 2006) [11]. These pollutants often have direct physiological toxic effects because they are stored or incorporated in tissues, sometimes permanently (Bokori *et al.*, 1996) [6]

This heavy metal has been found to induce a wide range of behavioral, biochemical and physiological effects (Jackie *et al.*, 2011) [26]. The liver, kidneys, and brain are considered to be the target organs for the toxic effects of lead (Jackie *et al.*, 2011) [26]. Toxicity of lead is mainly attributed to the induction of oxidative stress by elevation of reactive oxygen species (ROS) such as superoxide radicals, hydrogen peroxide and hydroxyl radicals and lipid peroxides. Mechanism of heavy metal toxicity may be multi factorial. One of the primary mechanisms behind heavy metal toxicity has been attributed to heavy metal induced generation of the reactive oxygen species (ROS) leading to oxidative stress induced organ damage. Lead poisoning is one of the oldest and widely studied occupational and environmental hazards and because of its dense, ductile, malleable and corrosion resistant nature has found its pronounced use in human civilization. Exposure of livestock to lead and its derivatives in day-to-day life is unavoidable because of its extensive use and application. Lead, although one of the most useful metals is also one of the most toxic environmental pollutants which are detected in almost all phases of biological systems.

Ginger, the rhizomes of the plant *Zingiber officinale* (Family Zingiberaceae), is arguably one of the most widely used culinary agent and spice in the world (Baliga *et al.*, 2012) [4]. Phytochemical studies have shown that the unique culinary and medicinal properties of ginger are due to the presence of phytochemicals like zingerone, shogaols, gingerols, pardols, β -phellandrene, curcumen, cineole, geranyl acetate, terpheneol, terpenes, borneol, geraniol, limonene, β -elemene, zingiberol, linalool, α -zingiberene, β -sesquiphellandrene, β -bisabolene,

zingiberenol and α -farnesene (Palatty *et al.*, 2013) [40]. Therefore, the present research work was planned to investigate the antioxidant activity of *Zingiber officinale* along with L-ascorbic acid against lead acetate induced toxicity in broiler birds by analysing lipid peroxidation & oxidative stress parameters.

2. Materials and Methods

2.1 Preparation of 50% Ethanol cold extract

The plant material i.e. roots of plant *Zingiber officinale* was collected from the local market of Nagpur and were dried at room temperature. 500 gram of powder of dried rhizome of *Zingiber officinale* was mixed with 2000 ml of 50% Ethanol in Stoppard flask and allowed to stand at room temperature for 72 hrs with frequent agitation until the soluble matter get dissolved. After 72 hrs, the mixture was filtered through muslin cloth, so as to remove the insoluble material. The filtrates were again filtered through filter paper and then poured in clean and already weighed petri plate and allowed for complete evaporation at room temperature and finally stored in desiccators in cool and dry place.

2.2 Experimental animals

The Institutional Animal Ethics Committee (IAEC) (CPCSEA Reg. No. 244/GO/ReBi/S/2000/CPCSEA Dated 01.08.2000) approved the experimental protocol. The experimental protocol met the national guidelines as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. The present research work was carried out 140 Broiler chicks were purchased from M/s. Atharvaraj Hatcheries, Nagpur. Broiler chicks were kept under standard managemental conditions as per the norms of BIS. All the birds were maintained on deep litter system at Poultry Farm, Department of Poultry Sciences, Nagpur Veterinary College, Nagpur and were fed on commercial diet and provided free access of drinking water during experimental period. On arrival, all chicks were weighed and randomly distributed in to seven treatment groups viz, T₁, T₂, T₃, T₄, T₅, T₆, and T₇ of 20 chicks in each group. Brooding with respective treatment group was carried out with electric hover brooders and continued until 14th day of age. All the Broiler chicks were fed on commercial feed purchased from M/s Supreme Agrovet Industries, Nagpur and provided free access of drinking water during experiment.

2.3 Experimental protocol

Broiler chicks (140) were divided into 7 groups T₁, T₂, T₃, T₄, T₅, T₆, and T₇ each group comprising of 20 birds. The lead toxicity was induced by administration of lead acetate @ 200 mg/kg (Ibitoye *et al.*, 2011) [25] of feed for 30 days. Group T₁ served as normal control, Group T₂ served as lead acetate control. Group T₃ and T₄ were received L-Ascorbic acid @ 200 mg/kg feed and plant extract @ 200 mg/kg b.w along with Lead acetate, respectively. Group T₅ was received L-Ascorbic acid @ 200 mg/kg feed and plant extract @ 200 mg/kg b.w along with Lead acetate. Group T₆ was received plant extract @ 300 mg/kg b.w along with Lead acetate. Group T₇ was received L-Ascorbic acid @ 200 mg/kg feed and plant extract @ 300 mg/kg b.w along with Lead acetate. The experiment was conducted for period of thirty days.

2.4 Estimation of the extent of lipid peroxidation

a. Estimation of Thiobarbituric Acid Reactive Substances (TBARS)

TBARS were estimated by thiobarbituric acid assay method of Niehaus and Samuelson (1968). 0.16ml of 3.0 M solution of standard tetramethoxy propane was made up to 100 ml with double distilled water. 1.0 ml of this was taken and made up to 100 ml with double distilled water, which served as working standard.

Procedure: The liver tissue homogenate was prepared in Tris Hydrochloric acid buffer (pH 7.5; 0.025 M). To 1.0 ml of the tissue homogenate or 0.5ml of plasma, 2.0 ml of Thiobarbituric acid-Trichloroacetic acid-Hydrochloric acid (TBA-TCA HCl) reagent was added and mixed thoroughly and kept in boiling water bath for 15 minutes. The flocculent precipitate was removed by centrifugation at 1000 rpm for 10 minutes. A series of standard solutions in the concentration of 2-10 mmoles were treated in a similar manner. The absorbance of the sample was read at 535nm against a reagent blank. TBARS were expressed as mmoles/100 g of tissue.

b. Estimation of glutathione

Reduced glutathione was determined by the method of Ellman (1959) [14]. 10 mg of reduced glutathione was dissolved in 100 ml of distilled water.

Procedure: A 1 gm of liver tissue was homogenized in phosphate buffer (pH 8.0; 0.2 M). From this 0.5ml was pipette out and precipitated with 2.0 ml of 5% Trichloro acetic acid (TCA) 1.0 ml of the supernatant was taken after centrifugation and added to it 0.5 ml of Ellman's reagent and 3.0 ml of phosphate buffer. The yellow colour developed was read at 412 nm. A series of standards were treated in a similar manner along with a blank containing 3.5 ml of buffer. The amount of glutathione was expressed as mmoles/100 g of tissue.

2.5 Determination of Level of Enzymatic antioxidant

a. Estimation of Glutathione Peroxides (GPx)

Glutathione peroxidase was assayed by the method of Rotruck *et al.* (1973).

Reagents: 0.4 M Tris buffer, pH 7.0, 10 mM Sodium azide, 2.5 mM Hydrogen peroxide, 4-mM Reduced glutathione, 10%TCA, 0.3 M Phosphate solution, 4m MEDTA . Ellman's reagent: 0.04% DTNB in 1% sodium citrate Reduced glutathione standard: 20 mg reduced glutathione/100 ml of distill water.

Procedure: To 0.2 ml of tris buffer, 0.2 ml of EDTA, 0.1 ml of sodium azide and 0.5 ml of plasma of different samples were added and labeled accordingly. To this mixture, 0.2 ml of glutathione and 0.1 ml of hydrogen peroxide were added. The contents were mixed well and incubated at 37 °C for 10 minutes along with a tube containing all the reagents. After 10 minutes the reactions were arrested with the addition of 0.5 ml of 10% TCA, centrifuged and the supernatant were assayed for glutathione by Ellman's method. To 2.0 ml of the supernatant, 3.0 ml disodium hydrogen phosphate solution and 1.0 ml of DTNB reagent were added. The colors developed were read at 412 nm. The activity was expressed in term of μ mol/L.

b. Estimation of Superoxide Dismutase (SOD)

Superoxide dismutase (SOD) activity was determined by the method of Kakkar *et al.* (1984) [28]

Reagents: 50 mM Phosphate buffer, pH 7.4, 20 mM L-Methionine, 1% (v/v) Triton X-100, 10 mM Hydroxylamine hydrochloride, 50 mM EDTA, 50 μM Riboflavin. Greiss reagent: 1% sulphanilamide, 5% phosphoric acid and 0.1% naphthylethylene diamine dihydrochloride.

Procedure: Reaction mixture was prepared by adding 50 mM Phosphate buffer, 20 mM L-Methionine, 1% (v/v) Triton X-100, 10 mM Hydroxylamine hydrochloride, 50 mM EDTA, among 1.4 ml aliquots were pipette out from the reaction mixture in a test tube. 100 μl of the plasma sample was added followed by pre incubation at 37 °C for 5 min. 80 μl of riboflavin was added and the tubes were exposed for 10 min to 200 W Philips fluorescent lamps. The control tube contained equal amount of buffer instead of sample. The sample and its respective control were run together. At the end of the exposure time, 1.0 ml of Greiss reagent was added to each tube and the absorbance of the color formed was measured at 543 nm. One unit of enzyme activity was defined as the amount of SOD capable of inhibiting 50% of nitrite formation under assay condition. The activity of SOD was expressed as U/ml

c. Estimation of catalase

The catalase estimation was assayed by the method of Sinha (1972) [46].

Reagents: 0.01M Phosphate buffer, pH 7.0, A: 0.1M Monobasic sodium phosphate, B: 0.1M Dibasic sodium phosphate, Mixed 39 ml of A and 61 ml of B that was diluted to a total of 200 ml. 10 ml of this solution was further diluted to 100 ml with distilled water. 0.2 M Hydrogen peroxide, Stock dichromate/acetic acid solution: Mixed 5% potassium dichromate with glacial acetic acid (1:3 by volume). Working dichromate/acetic acid solution: The stock was diluted to 1:5 with distilled water to make the working dichromate/acetic acid solution.

Procedure: The reaction mixture of 1.0 ml of buffer and 0.4 ml of water, 0.2 ml of the enzyme extract was added to initiate the reaction. 2.0 ml of the dichromate/acetic acid reagent was added after 0, 30, 60, 90 seconds of incubation. To the control tube the enzyme was added after the addition of the acid reagent. The tubes were then heated for 10 min. and then color developed was read at 610 nm. The activity of catalase was expressed as U/ml.

3. Results

From the results showed in table 1, it was observed that birds receiving extract @ 200 & 300 mg/kg bw alone and with L ascorbic acid (T₄, T₅, T₆ and T₇) significantly reduced ($p < 0.05$) Thiobarbituric Acid Reactive Substances (TBARS mmoles/100 g tissue) level in liver tissue as compared to lead control group (T₂). Similarly, groups of birds treated with extract @ 200 & 300 mg/kg bw with L ascorbic acid (T₅ and T₇) & extract @ 300 mg/kg bw alone (T₆) has highly significant increase ($p < 0.05$) Glutathione (GSH mmoles/100 g tissue) level than that groups of birds treated with extract @ 200 mg/kg alone (T₄) and as compared to the lead control group (T₂).

From the results of antioxidant enzymes from the blood, it was observed that, broiler birds treated with extract @ 200 & 300 mg/kg bw with L ascorbic acid (T₅ and T₇) showed highly significant increase ($p < 0.05$) in Glutathione Peroxidase (GPx μmol/L of blood) level than birds treated with extract @ 200 and 300 mg/kg bw alone (T₄ and T₆) when compared with lead control group (T₂). Similarly, the value of Superoxide Dismutase (SOD U/ml of blood) from all extract treated groups found to be significantly increased ($p < 0.05$), however, the group of birds treated with extract @ 300 mg/kg bw with L-ascorbic acid (T₇) showed highly significant increase in SOD level as compared with normal & lead control group (T₁ and T₂). Further, The value for Catalase level (U/ml of blood) from all treatment groups were found to be significantly increased ($p < 0.05$) as compared with the lead control Group (T₂), where as the birds treated with extract @ 300 mg/kg bw with L-ascorbic acid (T₇) showed highly significant increase in catalase level than the other lead induced treatment group.

Table 1: Effect of *Zingiber officinale* rhizomes extract on Lipid Peroxidation in Liver & Antioxidant Enzymes level in Blood on lead induced toxicity in broiler.

Lipid Peroxidation in Liver and Antioxidant Enzymes level in Blood	T ₁ Control Group	T ₂ Lead acetate @ 200 mg/kg feed	T ₃ Lead acetate @ 200 mg/kg feed + AA @ 200 mg/kg feed	T ₄ Lead acetate @ 200 mg/kg feed + Ext. @ 200 mg/kg b.w.	T ₅ Lead acetate @ 200 mg/kg feed + Ext. @ 200 mg/kg b.w. + AA @ 200 mg/kg feed	T ₆ Lead acetate @ 200 mg/kg feed + Ext. @ 300 mg/kg b.w.	T ₇ Lead acetate @ 200 mg/kg feed + Ext. @ 300 mg/kg b.w. + AA @ 200 mg/kg feed
TBARS (mmoles/100 g tissue)	113.51±7.07 ^b	185.86±8.41 ^a	109.96±9.96 ^{bc}	118.34±9.43 ^b	93.54±4.07 ^c	104.94±3.23 ^{bc}	90.76±2.38 ^c
GSH (mmoles/100 g tissue)	89.08±3.12 ^{bc}	53.36±3.70 ^e	86.12±4.14 ^c	76.92±2.36 ^d	94.98±3.53 ^{ab}	88.88±2.58 ^{bc}	99.21±1.02 ^a
GPx (μmol/L)	704.40±14.33 ^a	506.40±13.30 ^c	589.40±27.05 ^b	573.60±11.89 ^b	665.80±11.33 ^a	560.00±9.02 ^b	687.00±13.54 ^a
SOD (U/ml)	128.20±1.72 ^d	106.00±1.19 ^e	146.80±7.14 ^c	143.60±7.71 ^c	166.80±4.41 ^b	163.00±4.23 ^b	182.40±3.55 ^a
Catalase (U/ml)	636.38±22.56 ^b	587.26±18.35 ^c	622.58±28.19 ^b	616.63±16.74 ^b	643.40±19.25 ^b	624.90±20.06 ^b	724.83±21.37 ^a

Values are mean S.E. for 20 birds in each group. Values not sharing a common superscript in a column differ significantly ($p < 0.05$)

4. Discussions

Lead toxicity is mainly due to the induction of oxidative stress by increasing the levels of reactive oxygen species (ROS) such as superoxide radicals, hydrogen peroxide

(H₂O₂), Hydroperoxides (HO₂), hydroxyl radicals and lipid peroxides (Mannem, 2014; Gurer and Ercal 2000; Wang *et al.*, 2001) [33, 21, 53], also produces the imbalance between the production of free radicals and the biological system's

capacity to readily detoxify them or to repair the resulting damage (Flora, 2011) ^[16].

Lipid peroxidation is another biomarker to study oxidative stress. Thiobarbituric Acid Reactive Substances (TBARS) which measures Malondialdehyde (MDA) a major endogenous lipid peroxidation product; further the extent of lipid peroxidation by ROS can be determined by MDA levels (Jiang *et al.*, 2007 and Sumida *et al.*, 1989) ^[27, 50]. Glutathione is an essential bio-factor synthesized in all living cells and forms an important substrate for GPX, GST and several other enzymes, which are actively involved in free radical scavenging. It also play a role as non-enzymatic antioxidant by direct interaction of the SH groups with ROS or by involving in the mechanism of enzymatic detoxification reactions for ROS as a cofactor (Ding *et al.*, 2000) ^[12]. Glutathione peroxidase (GPX) is an antioxidant enzyme that catalyzes the hydrogen peroxide-reduced glutathione reaction also play important role in enzymatic defence system against the increased free radicals (Crack *et al.*, 2001) ^[9]. Superoxide dismutase (SOD) is an enzyme that alternately catalyzes the dismutation or partitioning of the superoxide (O_2^-) radical into either ordinary molecular oxygen (O_2) or hydrogen peroxide (H_2O_2) (Hayyan *et al.*, 2016) ^[22]. Catalase (CAT) is a metalloprotein that protects the tissues from highly reactive hydroxyl radicals by reducing hydrogen peroxide which is formed during various biochemical and metabolic reaction (Ahamed and Siddiqui, 2007) ^[1].

In the present investigation, it was observed that TBARS level in liver tissue was significantly increased, whereas the concentration of GSH in liver was reduced in lead treated birds as compared to normal which signifying the ongoing oxidative stress due to lead toxicity. Further, lead decreased the levels of GPX, SOD & Catalase in blood of lead treated birds significantly when compared to the normal group may be due to the impaired oxidant/antioxidant balance caused by adverse effects of lead. The findings confirm the lead intoxication produced oxidative stress by inhibiting enzymatic defence mechanism. Previous study also noticed the oxidative stress in lead toxicity as Gurer and Ercal, (2000) ^[21] who reported generation of reactive oxygen species (ROS) in lead toxicity reduces the antioxidant protection system of cells through depleting glutathione, inhibiting sulfhydryl-dependent enzymes, or increasing susceptibility of cells to oxidative attack by altering the membrane integrity. Lead inactivates glutathione by binding to sulfhydryl groups present in it which results in synthesis of GSH from cysteine via the γ -glutamyl cycle, which is usually not effective in replenishing the supply of GSH (Hultberg *et al.*, 2001) ^[24].

Flora *et al.* (2007) ^[18] reported the inactivation of Glutathione peroxidase (GPX), super oxide dismutase (SOD) and catalase (CAT) enzymes in lead toxicity. Decrease in SOD concentration reduces the disposal of superoxide radical, whereas reduction in CAT impairs scavenging of superoxide radical (Flora *et al.*, 2007) ^[18]. The decrease in CAT levels might be due to the inhibition of haem biosynthesis of catalase by lead (Patil *et al.*, 2006) ^[41]. Similarly, lead inactivates enzymes like δ -amino levulinic acid dehydratase (ALAD), glutathione reductase (GR), glutathione peroxidase (GPX) and glutathione-S-transferase, which further depresses the glutathione levels (Ahamed & Siddiqui, 2007) ^[1].

Enhanced oxidative stress contributes to lead-induced toxicity, where restoration of a cell's antioxidant capacity appears to provide a partial remedy. The earlier research findings has suggested that various toxic effects produced by the lead and generation of oxidative stress can be subdued by administration of various antioxidants (Gurer & Ercal, 2000) ^[21]. SOD, CAT, GPX are enzymatic antioxidants produced endogenously in the cells, whereas non enzymatic antioxidants like, flavonoids, vitamins, minerals, *etc.* are constituents of medicinal plants (Flora, 2009) ^[17]. The quantity of antioxidants present under normal physiological conditions are just adequate to quench the free radicals that are generated at a normal physiological rate but any further increase in the concentration of free radicals due to metal toxicity or environmental cause can causes an imbalance between the free radicals and antioxidants, leading to oxidative stress (Blokhina *et al.*, 2003) ^[5]. Thus the role of exogenous antioxidants becomes important if they are taken through the diet or in the form of supplements to maintain the homeostasis between free radicals and antioxidants which can prevent various deleterious effects due to heavy metal toxicity (Willcox *et al.*, 2004; Pietta, 2000) ^[54, 42].

Therefore, the present study was conducted to study ameliorative effect of 50% ethnaolic extract of rhizomes of plant *Zingiber officinale* (Ginger) on induce lead toxicity in broiler birds. It was found that extract @ 200 & 300 mg/kg alone with L-ascorbic acid significantly reduced TBARS level, similarly the reduced levels of Glutathione were significantly increased towards the normal by all treatments groups. Reduced levels of antioxidant enzyme i.e. GPX and SOD and catalase were significantly increased by extract alone and with L- ascorbic acid, as compared to lead control group.

Ginger, the rhizomes of the plant *Zingiber officinale* is world widely being used as spice having phytochemicals and bioactive compounds with antioxidant properties. Similarly, Ascorbic acid is a known eminent antioxidant vitamin involved in several biochemical processes in biological systems. Kikuzaki and Nakatani, (1996) ^[29], Fuhrman *et al.* (2000) ^[19] reported that compounds like gingerol, shogaols, gingerdiol, gingerdione, and some relating phenolic ketone derivatives found in ginger possess antioxidant activity. Youdim *et al.* (2002) ^[55] investigated the anti-oxidative nature of flavonoids which are naturally occurring polyphenolic compounds present in the ginger.

Ferrous ions play an important role as catalysts in oxidative process, leading to the formation of hydroxyl radicals and hydroperoxide decomposition by Fenton reaction. The chelating ability assayed is the ability of extracts to inhibit the complex formation of ferrozine with ferrous ions which was confirmed by studying protective effect of red and white ginger through their antioxidant activity, Fe2p chelating and OH scavenging ability and prevented oxidative stress (Obboh *et al.*, 2012) ^[39].

Kikuzaki and Nakatani, (1996) ^[29], Fuhrman *et al.* (2000) ^[19] studied enhanced antioxidant status by ginger powder supplementation and were due to the antioxidant compounds such as gingerols, shogaols, gingerdiol, gingerdione, and some relating phenolic ketone derivatives in ginger. Nile and Park, (2015) ^[37] reported *in vitro* antioxidant activity against DPPH, FRAP, and H_2O_2 radicals scavenging activity by components like 6-gingerol, 6-shogaol, and 6-paradol present in the ginger extract. Stoilova *et al.* (2007) ^[48] also reported the antioxidant effect of phenolic compounds in

alcoholic extract of ginger by scavenging were 2,2-Diphenyl-1-picryl hydrazyl radical (DPPH) and inhibiting hydroxyl radicals.

The phytochemical flavonoids found in the extract of plant *Z. officinale* is anti-oxidative in nature and prevent oxidative stress by chelating redox active metal ions and also by terminating the free radical chain reaction (Terao, 2009; Rice-Evans, 2001) [52, 44]. The capacity of flavonoids to act as antioxidants depends upon their molecular structure includes a diphenylpropane moiety which composed of two or more aromatic rings (A and B), each having at least one aromatic hydroxyl group connected via carbon chain. The chain consists of three carbons that combine with an oxygen and two carbons of one of the aromatic rings (A ring) to form a third 6-member ring (C ring) known as the pyran ring (Larson *et al.* 2012) [32]. The metal chelating ability of flavonoids arises from the appropriate positioning of the functional groups which contains both the hydroxyl groups of ring-B and the 5-hydroxy group of ring-A. The electron donating capability of flavonoid molecules to scavenge ROS rests with the 3',4'-catechol (dihydroxy) structure (B-ring). Another structural feature that contributes to the anti-oxidative nature is the presence of 2, 3 double bond in conjugation with a 4-oxo group in the C-ring (Heim *et al.*, 2002; Dugas *et al.*, 2000) [23, 13].

The protective effect of extract of *Z. officinale* & L-ascorbic acid against lead toxicity attributed may be due to their antioxidant mechanism like inactivating the generated ROS at molecular level, thereby terminating the radical chain reaction (chain breaking). By chelating the lead ion and preventing further formation of ROS. Lastly by chelating lead and maintaining it in a redox state, this causes incompetency to reduce molecular oxygen (Garcia & Gonzalez, 2008) [20]. These findings are mostly correlated with findings of Zhang *et al.* (2009) [56] who reported that supplementation of ginger powder increases the activities of SOD, GSH-Px but decreased the MDA content in the serum of laying hens.

McCord, (1979) [57] reported increasing activities of SOD and GSH-Px would subsequently enhance the capacity of broilers to clear out the oxygen free radicals. Zhang *et al.* (2009) [56] reported supplementation of broiler chickens diet with ginger increased total superoxide dismutase and glutathione peroxidase (GSH) reducing malondialdehyde (MDA) in serum of chickens. Kota *et al.* (2008) [30] also noticed that supplementation of ginger at the levels of 5, 10, and 50 g/kg significantly enhanced SOD and GSHPx activity (liver) and lowered MDA (liver and kidney) in rat. Farmand *et al.* (2005) [15] reported that the decrease in GPx activity due to lead toxicity was significantly increased in ginger extract treated groups. Earlier studies also showed that significant increase in CAT activity due to ginger treatment (Misra *et al.*, 2005) [34]

Reddy *et al.* (2014) [43] indicated that ginger extract at a dose of 150 mg/kg bw has increased the levels of GSH, GPX, GST and CAT in lead treated rats. Addition of ginger in ewes diets was significantly decreases MDA and increases GSH, SOD, CAT, GR-ase and GSH-Px. (Skrovankova *et al.*, 2012 and Nwozo *et al.*, 2014) [47, 38] and its antioxidant properties contribute to its radical scavenging activities (Sultana *et al.*, 2010) [49]. Ginger extract improved antioxidant enzymes activity and reduced tHcy and MDA level in ethanol treated rats (Akbari, 2017) [3]. Ginger extract modulates Pb-induced hepatic oxidative stress and

expression of antioxidant gene transcripts in rat liver (Mohamed *et al.*, 2016) [35]. Ahmed *et al.* (2008) [2] reported the protective effect of *Z. officinale* on lindane induced oxidative stress in rats by monitoring the extent of lipid peroxidation, activities of the oxygen free radicals (OFR) scavenging enzyme SOD and CAT & Glutathion redox cycle and stated that, ginger attenuated lindane induced lipid peroxidation, accompanied by modulation of OFR scavenging enzymes.

Ascorbic acid is probably the most widely studied vitamin when it comes to the prevention of lead induced oxidative stress and quenching ROS along with metal chelation which makes it a potential detoxifying agent for lead (Das & Saha, 2010; Tariq, 2007) [10, 51]. A study done by Chang *et al.* (2012) [8] showed the defensive effect of ascorbic acid on oxidative stress, developed in the hippocampus of lead exposed suckling rats. This vitamin breaks the chain of lipid peroxidation in cell membranes and scavenges free radicals ROS (Carr & Frei, 1999; Kucuk *et al.*, 2003) [7, 31].

The findings of our investigations could be attributed to the antioxidant and scavenging activities of ginger due to the high content of polyphenolic composites such flavonoids, tannins and gingerol and could be function as preventive agents against oxidative damage. Due to the antioxidants activity and the free radical scavenging properties of *Z. officinale* extract, the endogenous status of treated birds was enhanced by the elevation of GSH content, as well as GPx, SOD and CAT activities and consequently caused brought down of TBARS level in the present study.

4. Conclusion

Form this investigation it can be concluded that, extract of *Zingiber officinale* rhizomes @ 200 and 300 mg/kg body weight alone and with L-ascorbic acid has possesses remarkable antioxidant effects against lead induced toxicity in broiler birds by reducing the lipid peroxidation and oxidative stress caused due to lead toxicity.

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