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COMPARATIVE EVALUATION OF ANTIOXIDANT EFFECT OF HERBAL PRODUCT AND SYNTHETIC METHIONINE IN BROILERS

SEEMA. D.M, JAGADEESH, S. SANGANAL* AND R. RASHMI

Department of Veterinary Pharmacology and Toxicology, Veterinary College, KVAFSU, Hebbal, Bangalore, Karnataka, India.

ABSTRACT

Antioxidant effect of Herbal product and Synthetic Methionine was evaluated in broilers. Six groups containing 12 birds in each group. Group I served as control fed with basal diet deficient with methionine. Group II served as toxic control fed with cadmium chloride at 100 ppm. Group III and IV served as positive control fed with Methiorep® at 1.4 g/kg diet and synthetic methionine at 1.2 g/kg diet respectively. Group V and VI fed with cadmium chloride at 100 ppm upto 4 week for inducing oxidative stress, followed by Methiorep® at 1.4 g/kg diet and synthetic methionine at 1.2 g/kg diet respectively for next 2 week. Cadmium fed groups showed significantly increased level in biochemical parameters and oxidative stress marker, Thiobarbituric acid reactive substances and antioxidant enzyme i.e superoxide dismutase level and significantly decreased level in glutathione and performance parameters indicating cadmium induced oxidative damage on several organs. Histopathology of cadmium fed groups revealed the toxic effect of cadmium on liver, kidney and spleen. Supplementation of the herbal product and synthetic methionine showed improvement in all the above discussed parameters.

KEYWORDS: **Cadmium chloride, Methiorep®, Synthetic Methionine, Oxidative stress, Broilers**

*Corresponding author

JAGADEESH S. SANGANAL
Department of Veterinary Pharmacology and Toxicology, Veterinary College, KVAFSU, Hebbal, Bangalore,

INTRODUCTION

In the recent era, focus on plant research has increased all over the world and a large body of evidence show immense potential of medicinal plants used in various traditional systems. Herbal medicine is based on the premise that plants contain natural substances that can promote health and alleviate illness[1]. Many of the poultry industries are located near highways and close to the factories, which liberate Cadmium as a main pollutant. Plants readily absorb cadmium from soil and accumulate in various parts. Shell-fish such as mussels, scallops and oysters and other fish accumulate Cd and may be major source to poultry and other livestock feeding fishmeal and oyster shell grit as calcium source[2]. Methionine, a sulphur containing amino acid, which plays a vital role in protein synthesis, which acts as a precursor for glutathione, reduce generation of reactive oxygen species, regulates cell division and acts as most important methyl group donor to methylation reaction of DNA and other molecules[3]. Higher concentration of synthetic methionine in the diet gets metabolized into highly toxic compounds like methyl propionate there by adversely altering the performance of birds[4]. Currently, the quest for natural antioxidants for dietary, cosmetic and pharmaceutical uses has become a major industrial and scientific research challenges. A variety of free radical scavenging antioxidants exist within the body in which many of them are derived from dietary sources like fruits, vegetables and tea[5]. Several herbs have been reported to counter the peroxidative stress in biological system due to several stressors like pesticides, mycotoxins and heavy metals leading better performance potential in birds[6]. However, published literature on comparative evaluation of antioxidant effect between herbal products and synthetic methionine is scanty. Henceforth, present study was designed to evaluate antioxidant effect on oxidative stress induced by cadmium chloride in broilers by using an herbal product and synthetic methionine.

MATERIALS & METHODS

Methiorep® is a polyherbal combination containing herbs like *Boerhavia diffusa, Trigonella fenum graecum, Phaseolus mungo, Glycine max* and *Eclipta alba* and pure form of synthetic methionine were obtained from Ayurvet Limited, Solan, Himachal Pradesh. Broiler chicks (Ross) of 72 day old were procured from M/s Suguna poultry farm limited, Bangalore. The Chicks were acclimatized for one week and then randomly divided into six groups containing 12 birds in each group. Vaccination was done as per routine farm practice. The approval of the Institutional Animal Ethics Committee (IAEC) with No. LPM/IAEC/106/2012 was obtained prior to start of the experiment. Group I served as negative control fed with basal diet deficient with methionine. Group II served as toxic control fed with cadmium chloride at 100 ppm. Group III and group IV served as positive control fed with Methiorep® at 1.4 g/kg diet and synthetic methionine at 1.2 g/kg diet respectively. Group V and group VI fed with cadmium chloride at 100 ppm upto 4 week for inducing oxidative stress, followed by Methiorep® at 1.4 g/kg diet and synthetic methionine at 1.2 g/kg diet respectively for next 2 week. Serum biochemical parameters like alanine amino transferase (ALT), aspartate amino transferase (AST), creatinine, blood urea nitrogen were estimated from the serum samples collected on 4^{th} and 6^{th} week of experiment using semi automatic biochemical analyser (ARTOS, Bangalore). Performance parameters like average body weight, weekly body weight gain, feed consumption and feed conversion ratio (FCR) were recorded at weekly intervals in all groups. TBARS, a oxidative stress marker and Antioxidant enzymes viz., Superoxide dismutase and GSH were evaluated in liver and kidney tissues collected at the end of the experiment.

Tissue preparation

Sample of liver and kidney were rapidly excised into ice cold normal saline and then blotted dry and stored at -20ºC for further analysis. Liver and kidney tissues were homogenised with ice cold 0.1 mol/l Tris-HCl buffer of pH 7.4 to make 10% homogenate w/v (1 g of liver or kidney crushed in 10 ml of ice cold 0.1 mol/l Tris-HCl buffer of pH 7.4). This homogenate was centrifuged at 3000 rpm for 10 min, the supernatant was collected and used for total proteins, superoxide dismutase, glutathione and Thiobarbituric acid reactive substances level estimation.

Protein estimation[7]

The homogenate (100 µl) was made up to 1.0 ml with distilled water. To this, 5 ml of freshly prepared alkaline copper sulphate solution (a mixture of 50 ml of 2% sodium carbonate in 0.1N sodium hydroxide and 1.0 ml of copper sulphate in 1% potassium sodium tartarate) was added and kept for 10min at room temperature. 0.5 ml of Folin-Ciocalteau reagent was added and allowed to stand at dark for 30 min. The resultant blue colour was read at 660 nm in spectrophotometer. The bovine serum albumin was used as standard.

Superoxide dismutase estimation[8]

To 0.5 ml of homogenate, 0.25 ml of ethanol and 0.15 ml of chloroform was added and mechanically shaken for 15 minutes and centrifuged at 13,000 x g for 15 minutes at 4˚C. The supernatant was separated and used for the test. Assay mixture consists of 2 ml of 0.1 M Tris-HCl, 0.5 ml of homogenate, 1.5 ml of distilled water and 0.5 ml of pyrogallol. OD value was taken for 3 min at 420 nm wave length. The enzyme activity was expressed in terms of unit per minute per g of protein. One unit of enzyme corresponds to the amount of enzyme that inhibits pyrogallol auto-oxidation reaction by 50 percent.

Glutathione peroxidase estimation[9]

0.2 ml of 0.6 mM DTNB in 0.2 M sodium phosphate, pH 8.0, 0.1 ml of homogenate and 0.9 ml of 0.2 M phosphate buffer was taken and read at 412 nm against blank**.**

Estimation of lipid peroxidation[10]

Tissue homogenate (500 µl) and 1ml of 10% trichloroacetic acid and 1ml of 0.67% thiobarbituric acid were taken in a tightly stoppered tube. The tube was placed in boiling water bath for 45 minutes, cooled and centrifuged at 3200 rpm for 10 minutes. Finally, the supernatant was taken for estimation. The absorbance of the chromophore was read at 532 nm against blank. The concentration of test samples was obtained using a molar extinction coefficient of Malondialdehyde (MDA). At the end of the experiment all birds were sacrificed and a necropsy was conducted on each carcass to observe any gross pathological changes. The representative tissue samples were processed for histopathology and stained with haematoxylin and eosin[11].

Statistical analysis

The data obtained from the present study were subjected to statistical analysis. The data were analyzed by using two-way ANOVA, Bonferroni post-test for growth performance and biochemical parameters, One-way ANOVA for antioxidant parameters. Mean values and standard error of mean were calculated and all the values are expressed as Mean±SE (Graph Pad Prism, Trial version 5).

RESULTS

Alanine aminotransferase in Group II, V and VI was significantly (P<0.01) (P<0.001) higher than the control group value (Table 1). Alanine aminotransferase in Group III, IV and V was significantly (P<0.05) (P<0.01) lower than the control group value (Table 1). Aspartate aminotransferase in Group II, V and VI was significantly (P<0.01) (P<0.001) higher than the control group value (Table 1). Aspartate aminotransferase in Group III, IV, V and VI was significantly (P<0.05) (P<0.01) lower than the control group value (Table 1). Blood urea nitrogen (BUN) in Group II, V and VI was

significantly (P<0.01) (P<0.001) higher than the control group value (Table 2). Blood urea nitrogen (BUN) in Group V was significantly (P<0.05) lower than the control group value (Table 2). Creatinine (Creat) in Group II, V and VI was significantly (P<0.001) higher than the control group value (Table 2). Creatinine (Creat) in Group III, IV and V was significantly (P<0.05) (P<0.01) lower than the control group value (Table 2). Superoxide dismutase (SOD) in Group II and VI was significantly (P<0.001) higher than the control group value in both liver and kidney tissue (Table 3 and 4). Thiobarbituric acid reactive substances (TBARS) in Group II and VI was significantly (P<0.05) (P<0.01) higher than the control group value in both liver and kidney tissue (Table 3 and 4). Glutathione (GSH) in Group II and VI was significantly (P<0.05) lower than the control group value in both liver and kidney tissue (Table 3 and 4). Body weight in Group II, V and VI was significantly (P<0.001) lower than the control group value (Table 5). Body weight in Group III, IV, V and VI was significantly (P<0.001) higher than the control group value (Table 5). Body weight gain in Group II, V and VI was significantly (P<0.001) lower than the control group value (Table 6). Body weight gain in Group V and VI was significantly (P<0.001) higher than the control group value (Table 6). Weekly feed consumption in Group II, V and VI was significantly (P<0.001) lower than the control group value (Table 7). Weekly feed consumption in Group V and VI was significantly (P<0.05) higher than the control group value (Table 7). Feed conversion ratio did not differ significantly (P>0.05) in treated (Group II, III, IV, V and VI) birds (Table 8). Microscopically liver from group II revealed focal necrosis, infiltration of inflammatory cells, mild congestion and hyperplasia of bile duct epithelium (Fig 2.1). Kidney revealed degeneration of tubular epithelium, swollen glomeruli and presence of hyaline casts (Fig 2.2). Spleen showed lymphocytosis and formation of secondary lymphoid follicle (Fig 2.3). Liver from group V revealed mild congestion of sinusoids, mild necrosis and infiltration of inflammatory cells (Fig 3.1). Kidney showed mildly swollen glomerulus (Fig 3.2). Spleen showed mild congestion (Fig 3.3). Liver from group VI revealed mild hyperplasia of bile duct epithelium (Fig 4.1). Kidney showed swollen glomerulus and tubular epithelial cells with eosinophilic cytoplasm (Fig 4.2). Spleen showed formation of secondary lymphoid follicle (Fig 4.3).

Table 1

Alanine amino transferase (U/L) and Aspartate amino transferase (U/L) level of different group broilers

Groups	ALT (U/L)		AST (U/L)	
	4th week	6th week	4th week	6th week
Group I	65.54±1.41	75.47±4.27	262.47±9.78	316.3±14.68
Group II	92.00±2.29***	94.51±1.98**	355.8±26.70**	403.3±27.93***
Group III	67.74±2.77	61.36±4.19*	239.5±7.03	254.2±11.47*
Group IV	60.90±3.63	60.04±2.58*	258.0±8.67	220±24.12**
Group V	87.26±3.28***	58.33±6.76**	350.5±26.16**	241.23±10.73*
Group VI	91.25±1.07***	83.25±7.85	356.7±26.99**	253.6±8.92*

Values are mean ± SE, n=12, ***P<0.001, **P<0.01, *P<0.05

Table 2

Blood urea nitrogen (mg/dl) and creatinine (mg/dl) of different group broilers.

Groups	BUN (mg/dl)		Creatinine (mg/dl)	
	4th week	6th week	4th week	6th week
Group I	4.203±0.15	5.800±0.29	0.420±0.02	0.487±0.02
Group II	5.675±0.29**	7.603±0.51***	0.852±0.04***	0.897±0.04***
Group III	3.910±0.34	3.832±0.22	0.395±0.05	0.308±0.03*
Group IV	3.670±0.20	2.322±0.35	0.440±0.04	0.285±0.01**
Group V	5.568±0.28**	4.353±0.28*	0.782±0.04***	0.350±0.02*
Group VI	5.643±0.34**	4.878±0.23	0.853±0.05***	0.367±0.03

Values are mean ± SE, n=12, ***P<0.001, **P<0.01, *P<0.05

Table 3

Enzyme profile in liver tissue of different group broilers.

Groups	SOD (U/g protein)	TBARS (Nano moles MDA/g protein)	GSH (mg/g protein)
	6th week	6th week	6th week
Group I	61.2±2.62	139.85± 2.73	71.11±0.35
Group II	181.0 ±30.25***	195.35±5.92*	33.29±0.84*
Group III	82.7±3.09	139.52± 5.78	74.81±3.60
Group IV	112.5±8.34	134.32± 3.27	45.51±3.36
Group V	102.4±2.30	136.52± 8.56	58.23±2.29
Group VI	116.1± 8.70	166.21± 11.39	33.43±0.72*

Values are mean ± SE, n=12, ***P<0.001, **P<0.05,

Table 4
Enzyme profile in kidney tissue of different group broilers.

Groups	SOD (U/g protein)	TBARS (Nano moles MDA/g protein)	GSH (mg/g protein)
	6th week	6th week	6th week
Group I	75.1±1.49	145.42±5.59	72.47±0.89
Group II	168.6 ±10.93***	207.12±9.27*	36.63±3.07*
Group III	69.7±1.69	157.37±14.29	67.81±2.584
Group IV	79.6±1.07	144.95±5.89	57.51±2.12
Group V	110.3±3.56	162.51±2.89	63.54±2.92
Group VI	173.3± 9.43***	222.08± 11.31**	50.38±6.00

Values are mean ± SE, n=12, ***P<0.001, **P<0.01, *P<0.05

Table 5
Weekly body weight (g) of different group broilers

Groups	0th day	1st week	2nd week	3rd week	4th week	5th week	6th week
Group I	44.83±0.89	120.3±4.41	288.6±8.70	576.4±22.93	926.8±32.28	1297±53.59	1144±53.67
Group II	45.17±1.25	104.8±5.95	160.9±8.64	280.8±18.338***	450.3±34.24***	763.3±69.16***	1003±78.56***
Group III	43.17±1.01	139.3±6.04	294.6±17.91	614.4±27.28	957.6±22.11	1422±49.79	1743±62.60***
Group IV	46.67±1.52	146.5±5.66	320.9±14.67	635.5±36.04	1008±35.59	1527±37.78***	1842±66.74***
Group V	45.50±1.11	114.8±7.32	196.4±13.20	332.5±22.13***	506.6±33.46***	871.5±64.43***	1577±66.74***
Group VI	45.67±1.21	108.6±7.33	180.7±9.72	334.8±18.40***	494.8±18.35***	806.7±56.92***	1463±53.67***

Values are mean ± SE, n=12, ***P<0.001

Table 6
Weekly body weight gain (g) of different group broilers.

Groups	1nd week	2rd week	3th week	4th week	5th week	6th week
Group I	74.75±4.68	171.08±8.87	287.33±22.0	345.6±18.56	270.50±20.42	325.75±33.34
Group II	59.92±6.13	56.08±9.03	119.83±12.10	167.00±17.49***	128.67±13.75***	183.6±10.64***
Group III	97.42±7.0	155.3±13.52	319.8±18.30	347.3±32.37	316..2±37.11	464.6±55.30
Group IV	99.83±5.70	174.4±11.17	314.6±37.50	372.9±44.26	354.3±57.92	518.9±27.57
Group V	69.33±7.23	96.17±5.44	136.1±12.52	174.1±14.92***	486.4±49.26***	527.0±74.24***
Group VI	62.92±7.14	67.25±7.13	148.5±13.28	183.7±15.37***	484.4±68.22***	549.2±69.07***

Values are mean ± SE, n=12, ***P<0.001,

Table 7
Weekly feed consumption (g) per bird of different group broilers.

Groups	1nd week	2rd week	3th week	4th week	5th week	6th week
Group I	94.8±5.56	234.3±8.80	437.0±36.19	752.0±51.36	625.5±25.15	726.8±16.77
Group II	70.04±4.48	225.4±24.83	430.5±5.13	431.2±1.16***	272.3±21.83***	443.3±9.93***
Group III	95.4±4.89	285.6±15.26	433.1±2.60	826.9±26.15	730.3±30.21	822.8±22.79
Group IV	92.09±4.55	257.4±6.90	470.7±70.02	825.5±24.74	725.4±25.40	740.9±15.39
Group V	86.13±6.40	235.7±2.51	416.3±1.99	405.4±24.07***	750.5±39.89	895.1±5.44*
Group VI	75.05±0.49	238.1±6.32	417.6±3.01	354.3±57.91***	486.4±49.26	875.7±25.05*

Values are mean ± SE, n=12, ***P<0.001, *P<0.05

Table 8
Average feed conversion ratio (FCR) of different groups broilers.

Groups	1nd week	2rd week	3th week	4th week	5th week	6th week
Group I	1.21±0.05	1.48±0.01	1.79±0.00	2.080±0.02	2.16±0.01	2.25±0.05
Group II	1.29±0.03	1.65±0.10	2.37±0.05	2.70±0.05	2.85±0.05	2.90±0.20
Group III	1.16±0.15	1.40±0.09	1.67±0.02	2.02±0.02	2.11±0.11	2.15±0.05
Group IV	1.16±0.06	1.45±0.05	1.72±0.07	2.10±0.10	2.16±0.01	2.15±0.05
Group V	1.26±0.01	1.74±0.02	2.57±0.07	2.64±0.01	2.53±0.01	1.88±0.22
Group VI	1.30±0.03	1.75±0.01	2.43±0.12	2.67±0.01	2.54±0.01	1.84±0.04

Values are mean ± SE, n=12

Figure 1
Sections of 1) liver, 2) kidney and 3) spleen of Group I broiler Revealing normal architecture (H&E 50X, 100 X).

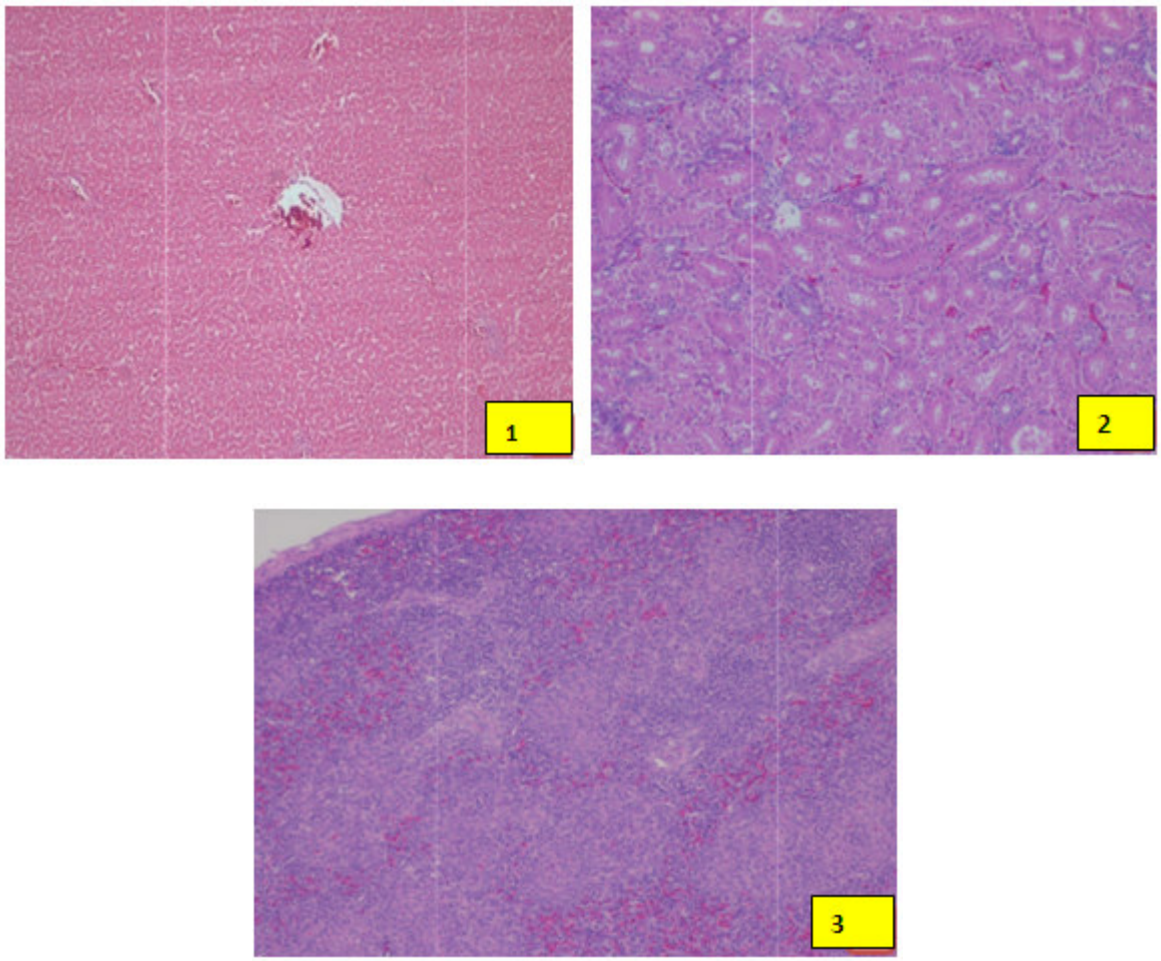
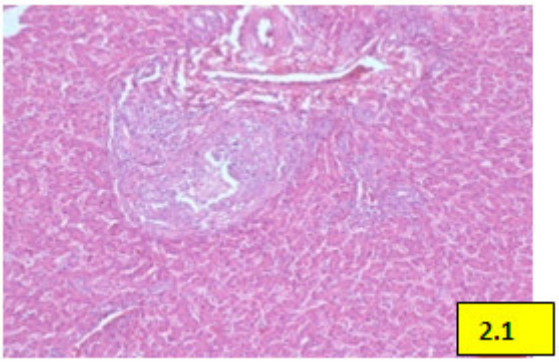
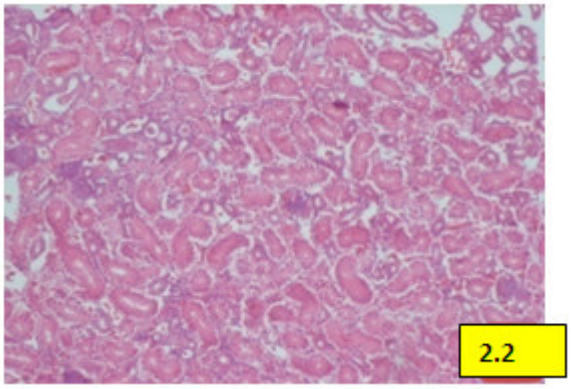


Figure 2
Sections of 1) liver, 2) kidney and 3) spleen of Group II broiler (H&E 100 X).

2.1. Section of liver showing focal necrosis, infiltration of inflammatory cells, congestion, bile duct hyperplasia



2.2. Section of kidney showing degeneration of tubular epithelium, swollen glomeruli and presence of hyaline casts



2.3. Section of spleen showing degeneration of lymphocytosis And formation of secondary lymphoid follicle

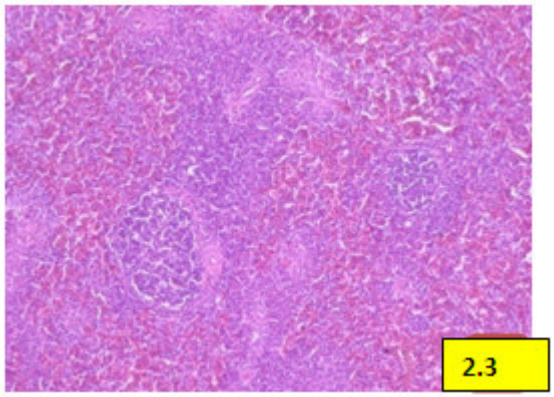
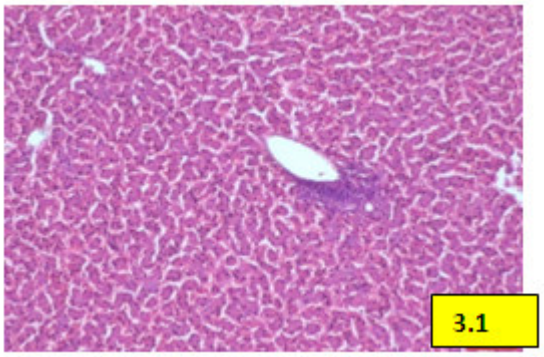
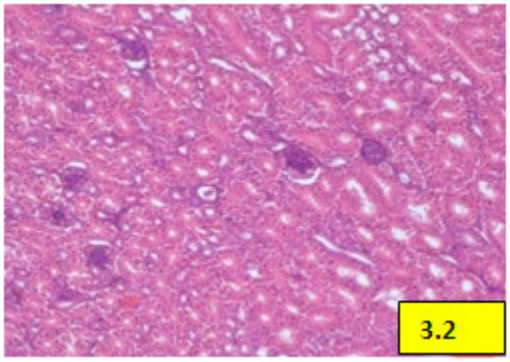


Figure 3
Sections of liver, kidney and spleen of Group V broilers (H&E 100 X)

3.1. Section of liver showing mild congestion of sinusoids, mild necrosis and infiltration of inflammatory cells



3.2. ***Section of kidney showing mildly swollen glomerulus***



3.3. Section of spleen showing mild congestion

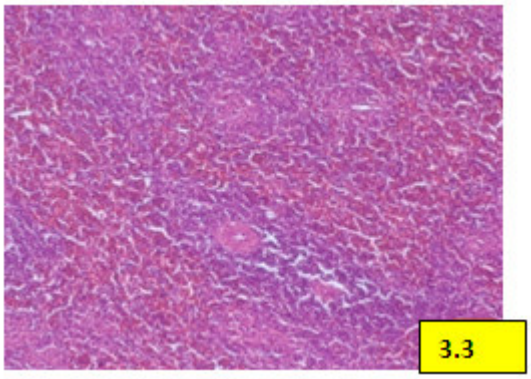
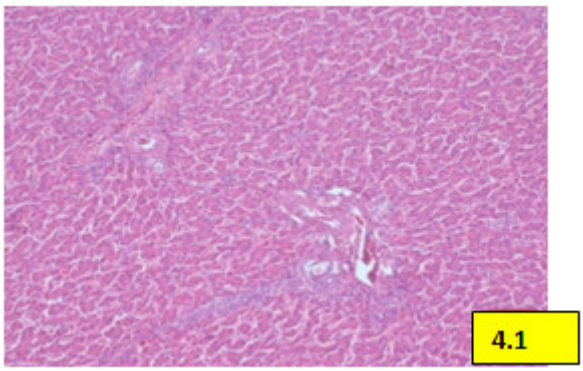
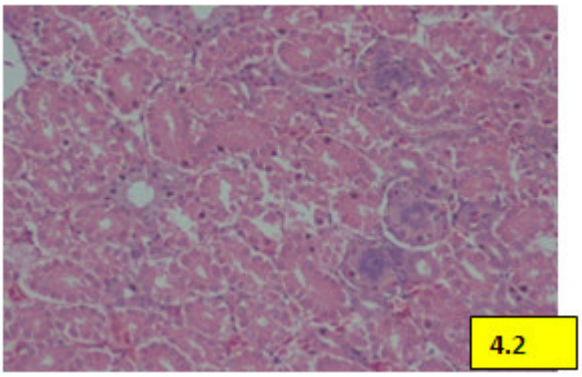


Figure 4
Sections of liver, kidney and spleen of Group VI broilers (H&E 100 X)

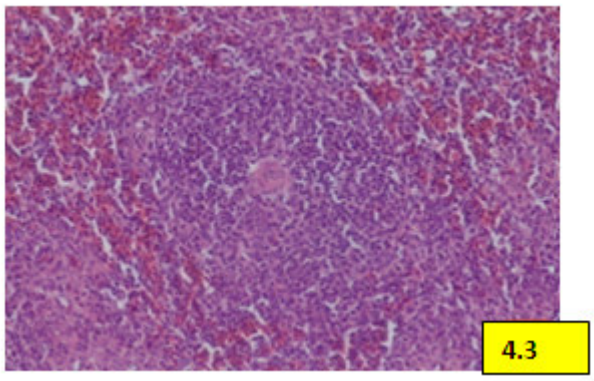
4.1. Section of liver showing mild hyperplasia Of bile duct epithelium



4.2. Section of kidney showing swollen glomerulus and tubular epithelial cells with eosinophilic cytoplasm



4.3. Section of spleen showing formation of secondary Lympho follicle



DISCUSSION

The biochemical parameters showed significantly increased level in treated groups (Group II, V and VI) compared to their respective control group (Group I) up to four week. This can be attributed to oxidative damage to liver and kidney. After four weeks, cessation of cadmium administration followed by subsequent herbal product and synthetic

methionine administration for next two week showed significantly decreased level of biochemical parameters in treated groups (Group V and VI).. This was in accordance with the findings of [12], reported similar results in biochemical parameters in broilers fed with herbal adaptogens at the level of 0.1 and 0.5 % in feed in ameliorating cadmium induced oxidative stress. Oxidative stress marker enzymes like Thiobarbituric acid reactive substances (TBARS) and antioxidant enzyme like Superoxide dismutase showed significantly increased level in both liver and kidney tissues of cadmium treated groups (Group II, V, VI) compared to their respective control group (Group I). But the enzyme level in Group V and VI treated with herbal product and synthetic mehionine was less than the cadmium control (Group II). This was in concomitance with the finding of[13], reported that the addition of cadmium chloride to the diet induced the formation of lipid peroxides in chicken. Glutathione (GSH) showed significantly decreased level in both liver and kidney tissues of cadmium treated groups (Group II, V, VI) compared to their respective control group (Group I). This can be attributed to the binding of cadmium to GSH for its excretion in bile as Cd-GSH complexes and exhaustion of GSH during the process of reduction of HOOH that was elevated by the presence of cadmium intracelluarly. But the enzyme level in Group V and VI treated with herbal product combination and synthetic methionine was more than the cadmium control (Group II). These was in accordance with the findings of[14], reported that herbal antioxidants scavenged the oxygen free radicals and averted GSH exhaustion during the process of cadmium detoxification and herbal antioxidants chelated cadmium and facilitated its elimination, thereby sparing –SH group of enzymes, GSH and proteins. There was a significant decrease in performance parameters like average body weight, body weight gain, weekly feed consumption in cadmium treated groups (Group II, V, VI) compared to their respective control group (Group I). Feed conversion ratio did not differ significantly in treated (Group II, III, IV, V and VI) birds. This can be attributed to the cadmium induced oxidative damage to several organs which in turn decreases the performance parameters in broilers[15]. After four week, cessation of cadmium administration followed by subsequent herbal product and synthetic methionine administration for next two week showed significantly increased improvement in performance parameters in treated groups (Group V and VI). This was in accordance with the findings of[12], reported similar results in performance parameters in broilers fed with 100 ppm cadmium followed by 0.1% herbal adaptogens in the feed. Histopathology of cadmium fed group revealed the toxic effect of cadmium on liver, kidney and spleen. Cadmium effect on liver was in accordance with the findings of[16, 17], reported focal necrosis, infiltration of inflammatory cells, mild congestion and hyperplasia of bile duct epithelium. Cadmium effect on kidney was in accordance with the findings of[16], reported degeneration of tubular epithelium, swollen glomeruli and presence of hyaline casts.

CONCLUSION

In the present study, cadmium fed groups showed significantly increased level in biochemical parameters and oxidative stress marker like TBARS and antioxidant enzyme i.e superoxide dismutase and significantly decreased level in GSH and performance parameters indicating oxidative damage caused by cadmium on several organs. Histopathology of cadmium fed group revealed toxic effect of cadmium on liver, kidney and spleen. Supplementation of herbal product and synthetic methionine showed improvement in all the above discussed parameters. This indicates the antioxidant effect of herbal product and synthetic methionine on oxidative stress induced by cadmium.

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