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In Vivo Effect of Medicinal Plants on Some Serum Biochemical Parameters in Laying Hens

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Abstract

Effect of medicinal plants on overall performance and serological parameters were explored in the laying birds. Forty weeks old 210-egg type birds were used as tested criteria. Birds were randomly alienated to 7 experimental groups with 3 replicates, consisted of 10 birds/replicate. Data were recorded for serology and haematology of laying birds. Serum hepatic, lipids, kidney function parameters, serum antibody titre against Newcastle disease and haematological parameters were estimated at the end of trial. Significantly lower egg yolk and serum cholesterol was recorded in group Bl^{-1}. Medicinal plants supplementation significantly lower the triglycerides level over the control group. Significantly improve/high level of high density lipoprotein was recorded in Tg^{-1}, Bl-1 and Ta-1, while low level of low density lipoprotein was recorded in Bl-1 and Ta-1. Haematological parameters significantly improved in medicinal plants treated groups except haemoglobin and red blood cells counts. Hepatic biomarkers ALT and AST were recorded non-significant in all treated groups, while serum ALP and total protein was significantly altered in groups Bl-1 and Ta-0.5. Non-significant observations were recorded for nephro-protective effect of medicinal plants in laying birds. It is concluded from the results that medicinal plants possess hypolipidemic, haematological and hepato-nephro protective effects.

Keywords: Hepato-nephro protection • Hypolipidemic • Medicinal plants

Introduction

Phytogenic have some active compounds that are responsible for medicinal value. Common and prominent active compounds of the medicinal plants are alkaloids, tannin, flavonoid and phenolic compounds. Their concentration may vary from plant to plant and from part to part. Medicinal plants have been reported for antioxidant properties and inhibit peroxidation reactions. Medicinal plants contain active ingredients such as saponins, phenolic and flavonoids compounds that possess antibacterial and antioxidant properties. Medicinal plants have hypoglycemic and hypocholesterolemic activities at insulin receptors and gastrointestinal level to improve peripheral glucose utilization and glucose tolerance. The distribution of groups based on treatments as illustrated in (Table 1).

Group	Replicates		
	R1	R2	R3
Control	10	10	10
Tg-1	10	10	10
Tg-2	10	10	10
Bl-0.5	10	10	10
Bl-1	10	10	10
Ta-0.5	10	10	10
Ta-1	10	10	10

Tg-1 and Tg-2 = Groups that were supplemented with 1 and 2%Trigonella foenum-graecumseeds powder/Kg of feed

Bl-0.5 and Bl-1 = Groups that were supplemented with 0.5 and 1%Berberis lycium root powder/Kg of feed

Ta-0.5 and Ta-1 = Groups that were supplemented with 0.5 and 1 %Terminalia arjuna seeds powder/Kg of feed

Table1: Layout for the experiment.

Liver play vital role in metabolism, excretion and maintain homeostasis regulation of the body. Exposure of liver to environmental toxins may infect the liver and lead to various ailments like hepatitis, cirrhosis and hepatic toxicity. According to a survey about 81% of poultry feed ingredients are contaminated with mycotoxin. Mycotoxin affect the vital organs such as Gastrointestinal Tract (GIT), immune system and especially liver, resulting in reduced productivity and promote mortality. Toxin affect the liver by

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decreasing hepatic gene expression of superoxide dismutase, glutathione S-transferase and increasing hydrolase activities and gene expression of Interleuken-6 and cytochrome p450 and 2H1. Toxin may affect the different sort of hepatic genes which helpful in many ways i.e. antioxidant activities, growth and development factors (Insulin), fatty acid metabolism, energy production and cell proliferation (ornithine decarboxylase). To reduce the load of mycotoxin in poultry feed different types of toxin binders are used to degrade mycotoxin.

Modern medicines have little to offer for alleviation of hepatic diseases. Serum biomarkers of liver rise up in blood stream to indicate haptic damage. Scientific reviews about the use of medicinal plants in liver toxicity revealed that medicinal plants improve the liver function. Medicinal plants normalize serum biomarkers by preventing intracellular enzymes leakage due to its membrane stabilizing activity, healing of hepatic parenchyma and regeneration of hepatocytes. Medicinal plants due to their antioxidant activates prevent hepatotoxicity and keep the liver healthy [1].

Based on the above facts, the present project was design to evaluate the hypolipidemic and hepato-nephro protective properties of common indigenous medicinal plants e.g. *Trigonella foenum-graecum, Berberis lycium* and *Terminalia arjuna* with the following main objectives:

- To evaluate the in-vive effect of medicinal plants on serum lipid profile of laying hens
- To study the positive impact of medicinal plants on hepato-nephro protective effects

Materials and Methods

Experimental birds

Forty-week-old 210 birds were used for the study. Upon arrival, the birds were placed in pens and allowed to acclimate for one week.

Experimental Layout

Two hundred ten (210) laying hens were randomly assigned into seven (7) groups with 3 replicates of 10 birds each (30 laying hens per group) (Table 2).

Group (Inclusion % of feed)	Means ± SE				
	Egg yolk Cholesterol (mg/g)	Serum			
		Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Control	15.90a ± 0.30	193.38a ± 1.98	222.11a ± 2.12	42.41b ± 0.69	106.54b ± 2.57
Tg-1	15.60a ± 0.11	197.30a ± 2.05	205.69b ± 2.03	42.90b ± 0.34	113.27a ± 2.06
Tg-2	15.36a ± 0.12	188.30b ± 1.49	199.19c ± 1.81	44.03a ± 0.27	104.43ba ± 1.15
Bl-0.5	15.43a ± 0.08	190.45ab ± 3.19	209.08b ± 4.10	42.16b ± 0.30	106.47b ± 3.82
Bl-1	14.66b ± 0.12	182.11c ± 1.69	197.82c ± 0.73	44.35a ± 0.41	98.18c ± 2.06
Ta-0.5	15.56a ± 0.20	191.97ab ± 1.77	199.74c ± 0.76	42.86b ± 0.25	109.16b ± 1.63
Ta-1	15.40a ± 0.11	182.09c ± 1.86	197.49c ± 0.64	44.28a ± 0.37	98.30dc ± 2.10
P-Value	0.0035	0.0001	0.0071	0.0001	0.0226

Means having different superscript in column are significantly different at α=0.05

Tg-1 and Tg-2=Groups that were supplemented with 1 and 2% Trigonella foenum-graecumseeds powder/Kg of feed

Bl-0.5 and Bl-1=Groups that were supplemented with 0.5 and 1% Berberis lycium root powder/Kg of feed

Ta-0.5 and Ta-1=Groups that were supplemented with 0.5 and 1% Terminalia arjuna seeds powder/Kg of feed

Table2: Hypolipidemic effect of medicinal plants in egg type birds.

Group 1 was served as control and was fed commercial layer diet without additives, group Tg-1 and Tg-2 were fed commercial layer diet supplemented with 1 and 2% *Trigonella foenum-graecum* seed powder respectively. Groups Bl-0.5 and Bl-1 were fed with diet contains 0.5 and 1% *B. lycium* root powder respectively, while groupsTa-0.5 and Ta^{-1} were fed ration containing 0.5 and 1% *T. arjuna* seed powder respectively. Supplementation of medicinal plants in egg type bird's ration was continued for a period of 5 weeks.

Biochemical indicators

Lipid profile of the birds feeding different level of medicinal plants: Blood was collected from three birds per replicate at the end of experiment. Serum was separated through centrifugation process.

Biochemical analysis for determination of lipid profile: Lipid profile include total cholesterol, triglycerides, high density lipoprotein (HDL) and low density lipoprotein (LDL). Total cholesterol determination tests was carried out by enzymatic colorimetric method using chemistry analyser (Map lab plus Japan). Triglyceride was determine by the standard protocol developed by Buccolo and David (1973). Low density lipoprotein (LDL) was calculated using the following formula:

LDL= [Total Cholesterol]-[HDL]-[Tg]/5

Preparation of egg yolk for cholesterol analyses: Egg shell and albumen were carefully removed and egg yolk was weighed and crumbled. Based on W/V, 1 gram of yolk sample was homogenized with 15 ml of chloroform-methanol at ratio of 2:1, then filtered and filtrates was used as egg yolk sample [2].

Analyses: By using RANDOX® cholesterol kit, egg yolk cholesterol was analysed from egg yolk.

Effect of medicinal plants on liver enzymatic activities and kidney function: To evaluate the liver (AST, ALP, ALT and protein) and kidney function (BUN and creatinine) tests, blood was collected from two birds/replicate. Then blood was centrifuge to collect serum for determination of these enzyme and biochemical indicators through the protocol developed by International Federation of Clinical Chemistry and Laboratory Medicine (IFCC).

Haematological aspect of medicinal plants: Blood was collected from randomly selected two birds/replicate. Blood was collected in EDTA (2 mg) containing test tubes. The blood samples

were subjected to haematological analysis for Haemoglobin (Hb) concentration, packed cell volume (PCV), white blood count (WBC) and deferential leukocytes count (DLC)using standard protocol of Sanderson and Philips (1981) haematological analyser.

Statistical analysis of the data: Data was analysed through statistical package Scientific Analysis System (SAS) by using Complete Randomize Design (CRD) as described by Steel and Torrie (1981).

Results

The present research trial was planned to study the effect of medicinal plants (*Trigonella foenum-graecum, Berberis lycium* and *Termenelis arjuna*) on the hypolipidemic, hepatoprotective, haematology and nephron protective in egg type birds. The results obtained are as under:

Hypolipidemic activities of medicinal plants in egg type birds

Mean egg yolk cholesterol and serum lipid profile of laying birds is presented in Table3.

Parameters	Groups						
	Control	Tg-1	Tg-2	Bl-0.5	Bl-1	Ta-0.5	Ta-1
Haemoglobin (g/dl)	13.63 ± 0.06	14.50 ± 0.30	14.50 ± 0.17	14.20 ± 0.17	14.73 ± 0.18	14.53 ± 0.06	14.26 ± 0.12
PCV (%)	26.90c ± 0.05	36.26a ± 0.12	33.10b ± 0.26	36.26a ± 0.37	37.26a ± 0.18	33.46b ± 0.29	34.63b ± 0.26
WBCs (103/µl)	88.56a ± 0.54	85.43ab ± 0.29	87.17a ± 0.40	87.96a ± 0.41	86.96ab ± 0.29	83.56b ± 0.08	87.73a ± 0.18
PLTs (103/µl)	17.66a ± 0.33	11.66c ± 0.33	12.66ba ± 0.33	13.00ba ± 0.57	19.66a ± 0.33	14.66b ± 0.33	17.66a ± 0.33
RBCs (103/µl)	2.91 ± 0.02	2.13 ± 0.14	2.33 ± 0.14	2.71 ± 0.08	2.63 ± 0.08	2.76 ± 0.05	2.65 ± 0.04
Granulocytes (103/µl)	5.50ba ± 0.05	6.73b ± 0.12	9.60a ± 0.15	7.36b ± 0.08	8.36a ± 0.14	8.66a ± 0.33	4.70c ± 0.05

Means in rows having different superscript are significantly different at α=0.05

Tg-1 and Tg-2 = Groups that were supplemented with 1 and 2% Trigonella foenum-graecumseeds powder/Kg of feed

Bl-0.5 and Bl-1 = Groups that were supplemented with 0.5 and 1% Berberis lycium root powder/Kg of feed

Ta-0.5 and Ta-1 = Groups that were supplemented with 0.5 and 1% Terminalia arjuna seeds powder/Kg of feed

Table3: Effect of medicinal plants on haematological parameters in egg type birds.

None of the medicinal plants affect egg yolk cholesterol except B. lycium when used at the level of 1% in the feed. Significantly lower egg yolk cholesterol was recorded in group Bl-1than other experimental groups.

Serum cholesterol was significantly altered by inclusion of medicinal plants as feed additive in laying ration. T. arjuna and B. lycium inclusion at the rate of 1 % brought positive changes in serum cholesterol. Serum cholesterol was significantly lower in group Ta-1 and Bl-1, while highest serum cholesterol was recorded in group Tg-1 (197.30 mg/dl) and the control (193.38 mg/dl).

Supplementation of commercial layer ration with medicinal plants as feed additive significantly altered triglyceride level in the serum of laying hens. Serum triglyceride was significantly lower in groups Tg-2 (199.19 mg/dl), Bl-1 (197.82 mg/dl), Ta-0.5 (199.74 mg/dl) and Ta-1 (197.49 mg/dl) as compared to the control. Highest triglyceride value (222.11 mg/dl) was recorded in the control group.

Supplementation of laying ration with hypolipidemic agent (medicinal plants) significantly affected serum High Density Lipoprotein (HDL). High density lipoprotein was significantly higher and the same in groups Tg-2 (42.90 mg/dl), Bl-1 (42.16 mg/dl) and Ta-1 (42.82 mg/dl) as compared to other groups. Similarly, supplementation of commercial layer ration with medicinal plants as hypolipidemic agent significantly affected Low Density Lipoprotein (LDL). Low Density Lipoprotein (LDL) significantly lowered in Bl-1 and Ta-1 groups, while significantly highest LDL value (113.27 mg/dl) was observed in Tg-1group.

Haematological aspect of Medicinal plants in egg-type birds

Effect of medicinal plants inclusion in commercial layer ration as feed additive on haematology of egg type birds is listed in (Table 4).

Group(Inclusion % of feed)	Means ± SE			
	ALT	AST	ALP	S. Protein
	(IU/L)	(IU/L)	(IU/L)	(g/dl)
Control	21.66 ±0.88	26.00 ±0.57	2184.00a ± 107.85	6.72c ± 0.09
Tg-1	22.66 ±0.88	26.33 ±0.88	1247.33ba ± 6.17	8.21a ±0.36
Tg-2	22.33 ±0.88	24.66 ±1.76	1336.67b ± 21.27	7.71b ± 0.08
Bl-0.5	22.00 ±0.58	25.00 ±0.57	1304.67b ± 50.02	5.83d ± 0.11
Bl-1	20.66 ±1.20	27.00 ±1.15	1031.67c ± 7.26	6.67c ± 0.08
Ta-0.5	21.66 ±1.45	25.66 ±0.88	1157.00c ± 8.74	6.78c ± 0.05
Ta-1	21.33 ±0.88	25.66 ±1.20	1461.00b ± 21.07	6.73c ± 0.11
P-Value	0.606	0.706	0.0001	0.0001

Means having different superscript in column are significantly different at α=0.05

Tg-1 and Tg-2=Groups that were supplemented with 1 and 2% Trigonella foenum-graecumseeds powder/Kg of feed

Bl-0.5 and Bl-1=Groups that were supplemented with 0.5 and 1% Berberis lycium root powder/Kg of feed

Ta-0.5 and Ta-1=Groups that were supplemented with 0.5 and 1% Terminalia arjuna seeds powder/Kg of feed

Table4: Hepatoprotective role of medicinal plants in egg type birds.

Incorporation of medicinal plants as feed additive in layer ration did not altered mean Haemoglobin (Hb) value. However, medicinal plants treated groups showed numerically high Hb values as compared to the control. Haematocrit value/packed cell volume (PCV) is significantly improved by the medicinal plants supplementation as feed additive in layer ration. Significantly higher PCV was recorded for the groups fed with *B. lycium* at both levels and *Trigonella foenum-graecumat* 1%, while control group showed lowest haematocrit value (26.90 %) [3].

Medicinal plants as feed additive in layer ration significantly altered the white blood cell (WBCs) count. Significantly highest count for WBCs was recorded for groups Bl-0.5, Ta-1, Tg-2 and control. Lowest WBCs count was found in Ta-0.5 (83.56*103/µl). Similarly, feed added medicinal plants significantly affected platelets cell count. Significantly highest platelets cells count was recorded for groups Bl-1, Ta-1 and control, while lower platelets cell count (11.66*103/µl) was recorded in Tg-1 group.

Supplementation of medicinal plants didn't altered red blood cells (RBCs) count in all treatment groups. Commercial layer ration supplemented with medicinal plants as feed additive significantly affected granulocytes count. Significantly highest count for granulocytes was recorded in groups Tg-2 (9.60*103/µl), Bl-1 (8.36*103/µl) and Ta-0.5 (8.66*103/µl). While lowest (4.26*103/µl) granulocytes count was found in group Ta-1 that was supplemented with 1% *T. arjuna* seed powder.

Hepatoprotective effect of medicinal plants

Feed added medicinal plants were evaluated as hepatoprotective agent in egg type birds. Hepatoprotective activities of medicinal plants are listed in (Table 4).

The evaluation was carried out on the basis of measuring different liver biomarkers/enzymes including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and serum total protein.

Supplementation of medicinal plants as feed additive in laying ration didn't significantly altered mean ALT and AST values for all the treatment groups. However, numerically lower ALT and AST values were recorded for the group fed with *B. lycium* at the rate of 1 % and Trigonella foenum-graecumat the rate of 2% respectively.

Significant hepatoprotective activity of medicinal plants was observed in term of Alkaline Phosphatase (ALP) in laying hens. Inclusion of *B. lycium* at the rate of 1 % and T. arjunaat 0.5 percent in the feed of egg type birds resulted in significantly lower serum ALP level as compared to all other groups. Control group showed highest (2184.00 IU/L) ALP level. Alkaline phosphatase recorded for the group Tg-1 was not different from the control. Medicinal plants significantly affected serum total protein. Incorporation of Trigonella foenum-graecumseed powder at the rate of 1% resulted in significantly higher (8.21 g/dl) serum total protein and was followed by groups Tg-2, Bl-1, Ta-0.5, Ta-1 and the control. Lowest value for total serum protein was recorded in group Bl-0.5 (5.83 g/dl).

Nephro-protective role of medicinal plants

Medicinal plants (Fenugreek, Berberis lycium and Termenelis arjuna) were evaluated by measuring Blood Urea Nitrogen (BUN) and serum creatinine for kidney function (Table 5).

Group(Inclusion % of feed)	Means ± SE	
	BUN (mg/dl)	Creatinine (mg/dl)
Control	7.33 ± 0.33	0.52 ± 0.02
Tg-1	8.66 ± 0.33	0.55 ± 0.01
Tg-2	7.00 ± 1.15	0.44 ± 0.01
Bl-0.5	7.33 ± 0.33	0.62 ± 0.01
Bl-1	7.33 ± 0.33	0.53 ± 0.01
Ta-0.5	8.66 ± 0.88	0.52 ± 0.02
Ta-1	8.00 ± 0.57	0.48 ± 0.01
P-Value	0.3782	0.1024
Tg-1 and Tg-2=Groups that were supplemented with 1 and 2% Trigonella foenum-graecumseeds powder/Kg of feed		
Bl-0.5 and Bl-1=Groups that were supplemented with 0.5 and 1% Berberis lycium root powder/Kg of feed		
Ta-0.5 and Ta-1=Groups that were supplemented with 0.5 and 1% Terminalia arjuna seeds powder/Kg of feed		

Table5: Nephro-protective role of medicinal plants in egg type birds.

The present findings revealed that the studied medicinal plants did not affect kidney function. Non-significantly results were recorded for BUN and serum creatinine.

Discussion

The present research trial was planned to study the effect of medicinal plants (*Trigonella foenum-graecum, Berberis lycium and Termenelis arjuna*) on the lipid profile, liver function, haematology and kidney function in egg type birds. The results obtained are discussed as under:

Hypolipidemic activities of Medicinal plants against standard drug Atorvastatin in egg type birds

The present research findings revealed that supplementation of *B. lycium* as feed additive in egg type birds ration at the rate of 1%significantly lower (14.43 mg/g) egg yolk cholesterol contents. Serum cholesterol was significantly lower in group Ta-1 and Bl-1, while highest serum cholesterol was recorded in group Tg-1 (197.30 mg/dl) and the control (193.38 mg/dl). Low yolk and serum cholesterol in *B. lycium* and *T. arjuna* treated group might be due to high saponin and fibre contents. The active ingredient of medicinal plants "Saponin" compete with cholesterol at binding site or interfere with cholesterol biosynthesis in liver, while some fibre possess the gel forming activities which decrease the cholesterol intake by mucosal cell through altering the chyme pH that will lead to cholesterol ester formation [4].

The findings of present that supplementation of *T. arjuna* in egg type bird's ration significantly lowered the serum total cholesterol are supported by results which stated that *T. arjuna* supplementation resulted in significantly lower total cholesterol contents in human. Similarly, it was found that *T. arjuna* significant reduced total cholesterol in rabbits. The current investigation are allied with the findings which reported that *Berberis lycium* and *Silybum marianum* significantly reduced serum cholesterol in broiler chickens. Previous researchers reported that water based infusion of herbal mixture in broiler chicks had significantly reduced the mean serum cholesterol level. The findings of present study on supplementation of medicinal plants in egg type birds ration as hypocholesteromic agent are allied with findings which documented that feed added *Berberis lycium* in broiler ration significantly reduced mean serum cholesterol level. In favour of present findings, Lansky, 1993 and Chen, 1986 reported that fiber portion like gums, pectin's and mucilage's of plants reduced the absorption of cholesterol at intestinal level that lead to decrease in serum cholesterol. Contrary to the present findings, it was reported that inclusion of medicinal plant in broiler chicken ration did not change the mean serum cholesterol level. The contradiction in findings of both experimental trials might be due to difference in medicinal plants, its level of supplementation and bird's species.

Mean serum triglyceride level was significantly reduced by supplementation of commercial layer ration with medicinal plants in the present study. Serum triglyceride was significantly lower and the same in groups Tg-2 (199.19 mg/dl), Bl-1 (197.82 mg/dl), Ta-0.5 (199.74 mg/dl) and Ta-1 (197.49 mg/dl) as compared to the control. Highest triglyceride value (222.11 mg/dl) was recorded in the control group. The hypolipidemic activity of natural products (medicinal plants) can be correlated to the presence of flavonoids and seponin, due to their properties of inhibiting cholesterol biosynthesis, absorption and modifying the activity of lipogenic and lipolytic enzymes which leads to reduced lipid metabolism. It was reported that extracted exo-biopolymer from plants kingdom had inhibitory effect on lipid deposition at hepatocyte level by inhibiting or decrease the HMG-CoA reductase (lipogenic enzymes) activity in liver. The low triglyceride level in *T. arjuna* supplemented group may be due the increase in serum lipoprotein lipase and plasma LCAT by *T. arjuna that* cause haptic bile acid synthesis and increase the degradation of lipids to natural sterols.

Significant effects of T. arjuna on serum triglycerides in current study allied with work/findings that stated that administration of arjuna (Terminalia arjuna) bark powder in broiler ration significantly reduced the serum triglyceride level. Patel and Pundarikakshudu (2016) reported similar findings that ayurvedic preparation of medicinal plants had significant effect on triglycerides level in experimental animals which support the present findings. Similarly, Incorporation of T. arjuna at level of 1% as feed additive in broiler ration significantly reduced triglyceride level. It was reported that supplementation of Trigonella foenum-graecumin prediabetic patients diet significantly reduced the serum triglycerides level. It was reported that poly herbal (cinnamon, Arjuna and Amla) product in laying ration of white leghorn birds had significantly reduced the triglyceride level. It was also reported that herbs as hypolipidemic agent in broiler ration significantly reduced serum triglyceride level in broilers.

Mean serum High Density Lipoprotein (HDL) and Low Density Lipoprotein (LDL) levels were significantly affected by supplementation of commercial layer ration with medicinal plants in the present study. High density lipoprotein was significantly higher in Tg-2 (42.90 mg/dl), Bl-1 (42.16 mg/dl) and Ta-1 (42.82 mg/dl) groups as compared to other groups. Significantly lower and the same LDL value was recorded in groups Bl-1 and Ta-1, while significantly highest LDL value (113.27 mg/dl) was observed in Tg-1 group. The increased level of HDL and decrease level of LDL in medicinal plants treated groups could be attributed to the presence of saponins, flavonoids and polyphenolic compounds that lowered the total cholesterol and its fractions in lipoproteins. These active ingredients interfering with metabolism or biosynthesis of lipids, increase fecal bile acid secretion and stimulate receptor-mediated catabolism of LDL-cholesterol and increase uptake of LDL from blood by liver.

Present investigation are allied with the results documented that 3-days schedule administration of T. arjuna in broiler ration significantly increased the level of HDL and reduced the serum LDL. It was documented that feed supplementation of T. arjuna in deit significantly improved the level of HDL and reduced the level of LDL. Poly-herbal water based infusion at different concentration has resulted in decrease serum LDL level in meat-type birds which support findings of the present study. It was stated that supplementation of arjuna in angina patients diet resulted in significantly increased serum HDL and decreased LDL levels. It was stated that laying ration supplemented with medicinal plants significantly altered the serum LDL values. Findings of experts supported the present results that arjuna significantly increase serum HDL and lower LDL in oxidative stressed patients. Schedule administration of water based infusion of medicinal plants in broiler production resulted in significant reduction in serum LDL and improved HDL level. It was reported that ethanol extract of the bark of T. arjuna in induced hypercholesteromic rats had significantly affected serum HDL and LDL levels. The current investigation are inline with findings/results that supplementation of T. arjuna in the laying ration of birds significantly decreased the serum LDL value. Administration of B. lycium in broiler ration up to 2% resulted in significant decline in serum LDL value. It was concluded that supplementation of T. arjuna in rabbit ration significantly decrease the level of LDL [5].

Effect of medicinal plants on haematology of egg type birds

Incorporation of medicinal plants as feed additive in layer ration did not altered mean Haemoglobin (Hb) value in laying hens. The values obtained in the current study for Hb seem to be within the normal range which suggest that animals/birds were not in an anaemic condition. Besides, the medicinal plants treated groups had numerically higher value for Hb than the control, which indicates that a more efficient erythropoiesis occur following administration of medicinal plants. Plants have some phytochemicals associated with erythropoietin promoting activity, immunostimulatory activities and thrombopoietin stimulation that cause increase in haemoglobin.

Present research findings are allied with findings reported that medicinal plants (Terminalia chebula Reitz., Aloe vera Linn., and Tamarindus indica Linn) extract showed non significant effects on Haemoglobin (Hb) in lab animals. It was documented the non-significant effect of methanolic extract of leaf of Maerua crassifolia on Hb contents in rats, these results are allied to the present findings. The current research investigation are in-line with the findings of who

documented that oral administration of mistletoe extract did not altered mean Haemoglobin (Hb) value in lab animals. It was reported that oral administration of aqueous extract of *Aspilia africana* leaf had non-significant effect on mean Hb level of lab animals. Crude extract of Hibiscus rosa did not effect significantly Hb contents of mice, these findings are in favour of the present study. The current findings are allied with the work/findings that supplementation of *Aspilia africana* in rabbit's ration did not change the Hb contents of rabbits. In contrast to the present investigation regarding medicinal plants effect on Hb content in egg type birds, it was stated that bird's ration supplemented with medicinal plants had significant impact on mean haemoglobin value. Medicinal plants as feed additive in bird's ration significantly improved Hb level which are in-contrast to present investigation.

Haematocrit value/Packed Cell Volume (PCV) was significantly improved by supplementation of medicinal plants in the current study. Significantly higher PCV was recorded for the groups fed with *B. lycium* at both levels and Trigonella foenum-graecumat 1%. Lowest haematocrit value (26.90 %) was recorded in the control group. Increase level of PCV in egg type birds treated with medicinal plants might be due to some phytochemicals that are associated with erythropoietin promoting activity and thrombopoietin stimulation that cause increase in red blood cells.

Present findings are in line with results that reported that Trigonella foenum-graecumseed extract significantly improved the PCV value in lab animals during induced gastric ulcer. The current findings that medicinal plants significantly improved the PCV value in birds are supported by the findings stated that water based infusion of different medicinal plants including *B. lycium* significantly improve PCV value in meat type birds. Trigonella foenum-graecum supplemented feed resulted in significantly improved PCV in rabbits which support the present findings. Significant effect of medicinal plants supplementation in egg type bird's ration on PCV value are supported by the work/results that feed added Trigonella foenum-graecumseed powder in broiler ration significantly boost up the PCV value in broiler chickens. In contrast to the present findings, demonstrated that *T. arjuna* significantly decrease the hematocrit value for catfish. Reduced PCV value was observed in village chickens by feeding Aloe ferox, Agave *sisalana* and *Gunnera perpensa* (medicinal plants). Non-significant impact of aqueous extract of *Telfairia occidentalis* leaf on mean hematocrit value for broiler chickens. The contradiction in results might be due to change in bird's strains, medicinal plants and level of administration.

Medicinal plants as feed additive in layer ration significantly altered the white blood cell (WBCs) count in the present study. Significantly higher count for WBCs was recorded for groups Bl-0.5, Ta-1, Tg-2 and control, while lowest WBCs count was found in Ta-0.5 (83.56*103/µl), however white blood cell count remain within the normal range for all treatments in the current study. The crucial role of WBC in defending the body against infection and tissue damage is well known. Increase in WBCs count is the result of infection, reaction against drug and bone marrow and immune system disorder.

Our results are allied with the findings that reported that Terminalia chebula in infected mice significantly reduced WBCs count which reveal that water based extraction of medicinal plant had significantly reduced the infection and normalized the haematology of mice. The present findings conform the results that reported that water extraction of *T. arjuna* significantly altered WBCs count in rabbits. Allied to the present findings are reported that ethanolic extract of *T. arjuna* significantly reduced WBCs count in Dalton's Lymphoma Ascites (DLA-Tumour) in mice, which reflect the positive effect of *T. arjuna* in DLA tumour cells in mice by reducing the infection.

High platelets cells count was recorded for groups Bl-1, Ta-1 and control, while lower platelets cell count (11.66*103/µl) was recorded in Tg-1 group in the present study. High platelets count in experimental birds has been reported to indicate beneficial effect of medicinal plants on the oxygen carrying capacity of the blood, erythropiorosis and as well as thrombopoietin.

No relevant literature is available on the effect of *B. lycium*, Trigonella foenum-graecum and *T. arjuna* on platelets cells count in poultry, however Trigonella foenum-graecumh as been reported to inhibit the platelets aggregation in type-2 diabetes. Non-significant changes in platelets counts was reported in rats by feeding medicinal plants.

Medicinal plants effect on Red Blood Cells (RBCs) was in-significance in present study. Present findings are supported that water based extraction of Viscum album did not change the mean RBCs value in albino rats. In-significant effect of medicinal plants on RBCs in current research trial who reported non-significant effect of Acalypha wilkesiana on RBC in rats. Significant effect of medicinal plants (Trigonella foenum-graecum and garlic paste) on RBCs has been reported, which is contrast to the current study results. Significant effects on medicinal plant (Trigonella foenum-graecum) has been documented that Trigonella foenum-graecumsignificantly improve the RBCs count and also inhibit the hemolysis by inhibiting the free ridicules which destroy the Hb content of the cell (antioxidant property). Contrary to the present findings, reported significantly improved red blood count in broiler chickens by supplementation of medicinal plants. Previous studies revealed that Trigonella foenum-graecumseeds possess antioxidant activity which increase the stability of RBCmembranes through the formation of fatty complexes and increase RBCs count [6].

The present results indicates varying levels of granulocytes in various treatment groups. Significantly high and the same count for granulocytes was recorded in groups Tg-2 (9.60*103/µl), Bl-1 (8.36*103/µl) and Ta-0.5 (8.66*103/µl), while lowest (4.26*103/µl) granulocytes count was found in group Ta-1 that was supplemented with 1%T. arjuna seed powder. All the granulocytes count was at normal range. Normal value of granulocytes indicated the strong antioxidant, anti-inflammatory, anti-carcinogenic and gastro-protective effect of medicinal plants at high level of inclusion.

Very limited literature is available on the effect of T. arjuna on granulocytes count. High level of supplementation of T. arjuna in broiler ration significantly reduced the leukocytes counts (granulocytes) during infection. The present findings are supported by the results that saponin from T. arjuna significantly reduced/inhabit monocytes and T-cell (Granulocytes) in human.

Liver is known as chemical processing plants. Per minute it receive 30% of circulating blood and performing different chemical reactions (metabolism) for detoxification of harmful substances and also store essential nutrients. Fortunately, the liver is extremely resilient but any complication or inflammation of liver lead to malfunction. This malfunction results in different metabolic disorders

like impaired digestion, impaired immune system, hepatitis which leads to abnormal secretion of liver enzymes. In order to detect liver damages, serum level of Alkaline Phosphatase (ALP), Alanine Transaminase (ALT), and Aspartate Transaminase(AST) enzymes are used extensively. If there is any damage or injury to liver the levels of AST and ALT may raise.

The present findings reveal that supplementation of medicinal plants in egg type bird's ration did not altered the mean ALT and AST values. All the values were in the reference range. The non-significant effect on these enzymes mean that there was no detrimental effects due to *Trigonella foenum-graecum*, *B.lycium* or *T. arjuna* supplementation.

Present findings are allied with results that broiler ration supplementation with anacardate (calcium) as anacardatic acid showed non-significant effect on liver biomarkers (ALT and AST). The present findings are supported that supplementation of Trigonella foenum-graecumas growth promoter in rabbit ration did not affect the liver biomarkers. Present findings are supported that egg type birds ration supplemented with medicinal plant did not alter the liver biomarkers in egg type birds. The present results are inline that Propolis supplemented ration of meat type birds did not alter serum AST and ALT. Contrary to the present findings that supplementation of *T. arjuna* in albino rats diets significantly reduced the mean ALT and AST value in paracetamol induced toxicity and the plant showed hepato-protective properties. Contradiction among the results with present findings might be due to induced hepato-toxicity. It is reported that *T. arjuna* showed hepatoprotective and antioxidant activities in cadmium induced toxicity in albino rats. Non-significant effect of medicinal plants on serum ALT and AST value in egg type birds in current study are in-contrast to the findings that that water based infusion of *Azadirachta indica* showed significant effect on ALT and AST in broiler production. Contrary to the present study documented that periodic supplementation of herbs in broiler ration had good impact on serum ALT and AST. Administration of *Terminalia arjuna* at the rate of 250-500 mg/kg body weight brought significant alteration in antioxidant enzymes (ALT and AST).

Present revealed that supplementation of B. lycium at the rate of 1% and T. arjuna at 0.5 % in egg type bird ration resulted in significantly lower serum ALP level, while high (2184.00 IU/L) ALP level was recorded in the control group. Low level of ALP in medicinal plants treated groups could be attributed to its high content of phenolic compounds that act as hepatoprotective. Increased ALP serum level indicate the cellular leakage and loss of functional integrity of hepatic cell membrane (Parenchyma). Phytogenic compound (Phenol) can cause healing and regeneration of hepatocytes.

Significant effects of medicinal plants on serum ALP value has been that daily gavage of B. vulgaris leaf extract resulted in significantly decrease hepatic enzymes level in CCl4 induced hepato toxicity in lab animals. Present findings are allied with the results T. arjuna significantly recovered/improved the hepatosomatic index in lab animals. Our results are inline with the results that periodic supplementation of herbal infusion significantly lower the alkaline phosphatase level in broilers. Present findings are supported that supplementation of Terminalia in rabbit ration significantly improved liver biomarkers in induced aflatoxin toxicity in rabbits. Findings supported the present results that T. arjuna cause inhibition of lipid-per-oxidation by induced drug and maintain antioxidant enzymes level. Inline to the present study, it is concluded that *Terminalia arjuna* significantly reduced the serum ALP in Alloxan induced diabetic rats as compared to control group.

Supplementation of Trigonella foenum-graecumseed powder at the rate of 1% resulted in significantly higher (8.21 g/dl) serum total protein while lowest value for total serum protein was recorded in group Bl-0.5 (5.83 g/dl), however level of total protein remain within reference range of 6-8.3 g/dl in all groups. The high level of serum protein in Trigonella foenum-graecum treated group could be attributed to the unique free amino acid 4-OH Ile in Trigonella foenum-graecumseeds which phosphorylates a number of proteins (PI3-K, 3T3-L1 and HepG2) and antioxidant properties of Trigonella foenum-graecum which improves organ (Liver) functions. The increase level of serum protein helps in infection and also maintains the osmatic pressure at cellular level.

Significant effects of medicinal plants on serum protein has been reported that water base Trigonella foenum-graecum extract significantly improve serum protein in CCl4 induce lab animals which support the current investigation. The present findings are inline who reported that feed supplementation with *T. arjuna* resulted in significantly increase serum protein in lab animals. Present findings are supported by the findings that periodic administration of Polyherbal extract showed significant increase in the level of serum protein in broiler chicks. It was also reported that *Terminalia arjuna* significantly increased the level of serum protein in lab animals. No significant alteration in mean serum protein value was observed in albino rats by administration of medicinal plant. Contrary findings are reported of Trigonella foenum-graecumseed powder in chick ration significantly decrease the level serum protein in chicken. In contrast to the present findings reported that Trigonella foenum-graecumsignificantly reduce the serum protein level in diabetic lab animals.

Nephro-protective activities

Results obtained from present study showed that supplementation of medicinal plants as feed additive in laying ration did not altered Blood Urea Nitrogen (BUN) and serum creatinine in egg type birds. Nonprotein nitrogenous substances such as BUN and serum creatinine are increased only when renal function is below 30% of its original capacity. An increase in BUN reflects an accelerated rate of protein catabolism. Medicinal plants have antioxidant (redox) activities which would be the cause of nephron-protection by scavenging of free radicals in renal infection. Free radicals cause several major health problems. A number of free radicals are formed during biological processes. The free radicals react with cell membrane lipids causing oxidative destruction, initiate lipid peroxidation in renal tissues and ultimately cause nephrotoxicity. The antioxidant enzymes protect the cells from risky effects of free radicals. The increased activities of antioxidant enzymes are known to provide protective responses by scavenging free radicals in renal tissues.

In cyclosporine-induced renal disorders, water based extraction of Terminalia arjuna has been reported to restore renal dysfunction, which support findings of the present study. Similarly medicinal plants has good impact on kidney function during induced renal toxicity by scavenging free radicals and protect renal disorder from toxic agents

(CCl4) in lab animals. It is also reported the beneficial effect of *T. arjuna* in induced CCl4 renal toxicity in mince.

Conclusion

It is concluded from the present study that supplementation of phytogenic (*Terminalia arjuna* and *B. lycium*) in laying ration significantly improved the serology (lipid profile and liver biomarkers) and haematology, while non-significant effects of feed added phytogenic were recorded for kidney function.

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Authors Contributions

Muhammad Mushtaq: The PhD scholar, who conducted the research work.

Naila Chand: Supervisor of the current study.

Umer Sadique: Providing the lab facilities.

Sarzamin Khan: Supervise the study and provide the poultry house.

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