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Effect of hydro-alcoholic extract of *Rhodiola imbricata* on growth performance, immunomodulation, antioxidant level and blood biochemical parameters in broiler chickens at high altitude cold desert

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ABSTRACT

Extremes of climate and hypobaric hypoxia cause poor growth performance in broiler chickens at high altitude. The current study was designed to investigate the effect of hydro-alcoholic extract of *Rhodiola imbricata* on antioxidant, cytokines, blood biochemical and growth performance of broilers at high altitude (3500 m). For *in-vivo* study, one day-old broiler chicks of average initial body weight 36.40±0.42 g were randomly assigned to seven groups in three replicates (10 chicks in each replicate) as per completely randomized design. Experimental groups included control (fed basal diet), and treatment T1, T2, T3, T4, T5, and T6 which received hydro-alcoholic extract of *Rhodiola imbricata* in drinking water @ 100, 150, 200, 300, 400, and 800 mg/kg body weight of chicken respectively, along with basal diet. Blood samples were collected at 0, 21st, and 42nd day. HPLC analysis of extract revealed the presence of salidroside and p-tyrosol. As a result of this study, birds in T5 group had significantly higher body weight as compared to other groups. Furthermore, they had significantly higher total antioxidant capacity, free radical scavenging activity, interleukin-2, total protein, globulin, HDL level and lower malondialdehyde, interleukin-6, cholesterol, triglyceride, LDL, glucose, A/G, ALT, AST level as compared to control group. Our results suggest that, *Rhodiola imbricata* extract @ 400 mg/kg body weight of chicken, exhibited beneficial effect on growth performance and therefore, can be used as a phytogenic feed additive for broiler chickens.

Key words: Broiler, Growth, High altitude, Immune responses, *Rhodiola imbricata*

Over the last twenty years in India, there has been remarkable growth in poultry industry (Ali 2015). However, in cold arid part of the Himalayas, the picture is very different as environmental conditions in this region are characterized by extreme temperature variations (from +35°C to –35°C), hypobaric hypoxia, high UV radiations, low humidity, and scarcity of fodders which affects livestock nutritional status and their health in this region (Biswas *et al*. 2011, Kalia *et al*. 2017). Hypobaric hypoxia alters electron transport mechanisms of mitochondria, leading to the increased generation of reactive oxygen species (ROS) which leads to cellular dysfunction and decline in the productiveness of antioxidant defence system (Miller *et al*. 2013). These types of adverse climatic conditions contribute to high altitude oxidative stress to poultry birds which ultimately reduce the growth rate of broiler chicks and thus, produce low return of income for poultry farmers at high altitude (Biswas *et al*. 2011, Kalia *et al*. 2017). However, the Himalayan region has the numerous spices of medicinal plants which are widely used for curing high-altitude maladies as prophylactic and therapeutics agent (Kala 2006) and incorporation of herbal medicinal plant extracts in poultry diet in the form of feed additives could be valuable alternatives for the health and nutrition of the chickens for this area.

Rhodiola imbricata is a unique medicinal plant and is adapted to grow in stressful climatic conditions of high altitude Trans-Himalaya cold desert (Ramakrishna and Ravishankar 2011). A number of metabolites like flavonoids and phenolic acids have been found in good quantity in *Rhodiola* plant extract, particularly which obtained from roots have been shown to possess antistress properties (Radomska-Lesniewska *et al*. 2015). This plant is used in local medicinal system for improvement of human work performance by reducing the effect of high altitude stress on health (Kala 2005, Ballabh and Chaurasia 2007). Various studies in different experimental animals reported that it has antioxidant, immunomodulatory, and radioprotective

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properties (Arora *et al*. 2005, Senthilkumar *et al*. 2013, Li *et al*. 2013). Most of these effects are due to presence of constituents such as salidroside, ρ-tyrosol, and flavonoids (Zhou *et al*. 2015). It was reported that supplementation of *Rhodiola crenulata* root mix in broiler diet had beneficial effect on mortality rate through reducing the effects of hypoxia (Li *et al*. 2014). However, to the best of our knowledge, no study had been reported so far that investigated the effect of *Rhodiola imbricata* roots extract on growth performance, antioxidant level, immune responses, and blood biochemical parameters in broiler birds. Therefore, the present study was undertaken to examine the effects of hydro-alcoholic extract of *Rhodiola imbricata* on growth performance, antioxidant level, immune responses, and various blood biochemical parameters of broiler chickens at high altitude cold desert.

MATERIALS AND METHODS

Plant material and extraction: Rhodiola imbricata roots were collected from the trans-Himalayan region (Chang-La Top, altitude = 5330 m above mean sea level, Ladakh), washed thoroughly, cut into small pieces, shed dried at room temperature and thereafter it was powdered which was further extracted with 60% ethanol in soxhlet apparatus for 24–48 h each batch.

HPLC analysis: HPLC analysis of extract was performed using Shimadzu LC10ATvp system (Shimadzu Corp., Japan) equipped with LC10ATvp binary pump, SCL-10AVP system controller, CTO-10 AS VP column oven, SPD-10 Avp UV-Visible detector and CLASS-VP version 5.33 software. The separation was performed using symmetry C18 column (250 mm × 4.60 mm, 5 µm). The mobile phase consisted of water (A), methanol (B), and acetonitrile (C) maintained at gradient flow rate of 0.5 ml/min. The sample injection volume was 20 µl and detection wavelength was set at 248 nm for acquiring chromatograms (Saunders *et al.* 2014). The chromatographic peaks of salidroside and p-tyrosol were confirmed by comparing their retention time with reference standard (Fig. 1).

Location and experimental design for in vivo *experiment: In vivo* experiment was approved by Institutional Animal Ethics Committee of DIHAR (Protocol number: DIHAR/IAEC/27/2015) and all the methods were performed as per the guidelines of animal experimentation. One day old broiler chicks (210) of average initial body weight 36.40±0.42 g were randomly assigned to seven groups in three replicates (10 chicks in each replicate) with 30 birds in each group as per completely randomized design. Experimental groups included control (fed basal diet), and treatment T1, T2, T3, T4, T5, and T6 which received hydro-alcoholic (60% ethanol) extract of *Rhodiola imbricata* in drinking water @ 100, 150, 200, 300, 400, and 800 mg/kg body weight of chicken respectively, along with basal diet. The experiment lasted for 42 days. The birds were fed on starter diet for 21 days followed by finisher diet until 42 days of age. The ingredients and analysed composition of basal diet are presented in Table 1. Basal diet was formulated according to the standard inhouse feed formula developed by our lab (Kalia *et al*. 2017). Chickens were weighed individually at every week interval. Feed and water was provided *ad lib*. Feed conversion ratio (FCR) was calculated from the ratio between feed intake and weight gain by chick. Mortality in birds was recorded daily. Blood samples were collected at 0, 21, and 42 days for isolating plasma for analysis of antioxidant, cytokine and blood biochemical parameters.

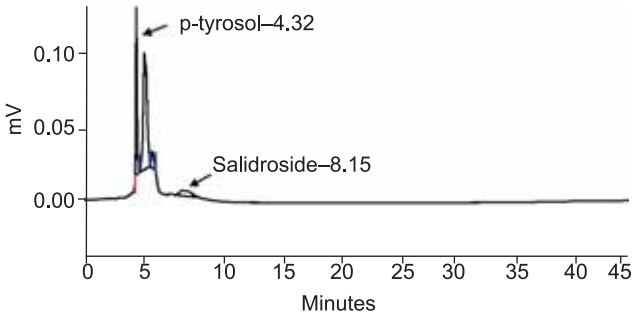

Fig. 1. HPLC chromatogram of *Rhodiola imbricata*

Table 1. Ingredients and analysed composition of basal diet

Ingredient (% diet)	Starter diet (1-21 day)	Finisher diet (22-42 day)
Maize	59.00	58.00
Soybean (Solvent extracted)	33.18	21.12
Soybean (Full fat)	-	9.58
Soybean oil	2.00	2.55
CP	2.15	-
Wheat bran	-	5.08
Salt (NaCl)	0.15	0.15
Limestone	1.50	1.50
Dicalcium phosphate	1.50	1.50
Lysine	0.13	0.13
Methionine	0.19	0.19
Vitamin and mineral premix*	0.20	0.20
Total	100	100
Analysed composition		
Protein (%)	21.56	19.31
ME (Kcal/Kg)	3100	3200
Calcium (%)	1.02	0.94
Phosphorus (%)	0.48	0.42

*Nutrition value per kg of vitamin and mineral premix contained 140,000 IU vitamin A, 70 mg vitamin E, 3,000 IU vitamin D_3, 4 mg vitamin K, 3 mg thiamine, 10 mg vitamin B_2, 8 mg vitamin B_6, 0.04 mg vitamin B_{12}, 48 mg niacin, 20 mg calcium d-pantothenate, 500 mg choline chloride, 0.20 mg biotin, 1.8 mg folic acid, 80 mg manganese, 70 mg zinc, 50 mg iron, 10 mg copper, 3 mg iodine, 0.4 mg selenium and 0.2 mg cobalt.

Determination of blood biochemical parameters: All blood plasma biochemical parameters including glucose, cholesterol, triglyceride, high density lipoprotein (HDL), low density lipoprotein (LDL), total protein, albumin, creatinine, ALT and AST were analysed with commercial available biochemical kits (Span Diagnostics, India) in biochemical semi-auto analyser (BIOTRON BTR-830)

according to suggested methodology. The concentration of plasma globulin was calculated by subtracting the value of albumin from total protein.

Determination of plasma antioxidant parameters: Antioxidant parameters, viz. DPPH radical scavenging activity and total antioxidant capacity (TAC) of plasma samples were analysed by the methods described by Brand-William *et al*. (1995) and Benzie and Strain (1996) respectively.

Lipid peroxidation assay (LPO): The lipid peroxidation assay was performed by measuring malondialdehyde (MDA) level in plasma samples. The level of MDA in plasma samples were estimated as per Buege and Aust (1978).

Determination of cytokines: Commercial available ELISA kits (Biolegend, San Diego, CA) were used to determine the concentration of three cytokines: IL-1, IL-2, and IL-6 in plasma samples following the instructions in respective kit manuals.

Statistical analysis: Data were analyzed with version 17.0 SPSS software (SPSS, Chicago) using one-way ANOVA. Results were expressed as a mean ± standard error. Duncan's multiple range test was used to compare means of different experimental groups. Significant statistical difference was assumed at the level of $P<0.05$.

RESULTS AND DISCUSSION

HPLC analysis of R. imbricata *extract:* HPLC analysis of *Rhodiola imbricata* extract revealed major peaks at retention times 4.32 and 8.15 min at 248 nm which further confirmed the presence of p-tyrosol and salidroside at a concentration of 232.40 and 191.01 µg/ml, respectively, in 1 mg/ml of extract. It has been well reported that antioxidant capacity of plant is associated with phenolic content (Gengaihi *et al*. 2014) and the presence of bioactive phytomolecules, viz. p-tyrosol and salidroside in this plant root extract may possibly be responsible for its pharmacological antioxidant properties under stressful high altitude climatic conditions (Panossian *et al*. 2010).

Growth performance: At 42 day of age, live body weight of chickens in T1, T2, T3, T4, and T5 groups was significantly higher ($P<0.05$) as compared to control group and highest body weight was recorded in T5 group birds followed by T3, T4, T2, T1, and T6 (Table 2). No significant ($P>0.05$) differences were observed in mean value of cumulative feed and water intake among the groups. FCR value in T5 group was significantly improved among the groups (Table 2). Highest mortality rate was observed in control group. Our present findings of low body weight support the belief that high altitude reduces the growth performance in broiler chickens. Our results concorded with reports of Balog *et al*. (2000) and Li *et al*. (2014) who found similar reduction in body weight of broilers reared at 2900 m and 2986 m, respectively. However, final body weight of broilers in those two studies was much higher than that of our present findings. The reason behind this could be attributed to difference in altitude of experimental site. We performed our study at 3500 m above mean sea level, while in those studies experimental sites were located in between 2900 to 3000 m.

Moreover, the decrease in body weight might be due to reduction in energy intake and increase in energy expenditure at high altitude due to hypobaric-hypoxic condition. Due to disturbance in energy balance at high altitude, the rate of catabolic activities and intestinal malabsorption increases and which leads to decrease in body mass (Balog *et al*. 2000, Kayser and Verges 2013, Kalia *et al*. 2017). However, the increase in the body weight in treatment groups might be due to active components (salidroside, p-tyrosol, phenolics, flavonoids etc.) in *Rhodiola imbricata* which can stimulate increased digestion and metabolism of nutrients causing higher efficiency in

Table 2. Effect of *Rhodiola imbricata* on growth performance of broiler chickens

Parameter	Treatments						
	Control	T1	T2	T3	T4	T5	T6
Initial average body weight (g/chick)	36.40±0.42	36.06±0.55	35.66±0.39	36.86±0.33	36.06±0.45	35.93± 0.38	36.53± 0.36
Average weight at 21 day (g/chick)	193.33^{a}± 4.30	210.93^{b}± 4.58	210.00^{b}± 4.22	214.00^{bc}± 3.83	216.66^{bc}± 3.63	225.20^{c}± 4.49	190.46^{a}± 3.03
Average weight at 42 day (g/chick)	366.28^{a}± 6.44	384.26^{b}± 5.20	408.80^{c}± 5.45	420.42^{c}± 6.29	420.26^{c}± 5.61	461.71^{d}± 7.80	382.57^{ab}± 4.93
Cumulative feed intake up to 42 day (g/chick)	1525.61± 6.90	1521.87± 7.26	1518.37± 5.79	1520.01± 6.94	1520.20± 6.51	1517.83± 5.87	1518.12± 6.98
Feed conversion ratio at 42 day	4.62^{d}± 0.03	4.37^{c}± 0.06	4.07^{b}± 0.03	3.96^{b}±0.06	3.96^{b}± 0.04	3.56^{a}± 0.05	4.39^{c}± 0.07
Cumulative water intake up to 42 day (ml/chick)	2235.46± 15.17	2230.36± 14.19	2245.80± 20.49	2240.01± 18.58	2250.44± 19.24	2255.36± 16.84	2235.11± 13.96
Mortality (%)	13.33 (02/15)	6.66 (01/15)	0 (00/15)	6.66 (01/15)	0 (00/15)	6.66 (01/15)	0 (00/15)

C, T1, T2, T3, T4, T5, and T6 represent groups of chickens that received *Rhodiola imbricata* at concentration level of 0, 100, 150, 200, 300, 400, and 800 mg/kg body weight of chicken, respectively. Means bearing the different superscripts ($^{a, b, c, d}$) in a row differ significantly ($P<0.05$).

the utilization of feed which results in enhanced growth in birds (Ahmed *et al*. 2015). The increase in body weight also might be due to antioxidant (Senthilkumar *et al*. 2013) activity of *R.imbricata* that stimulates protein synthesis by bird enzymatic system and eliminates the production of free radicals which ultimately reduces the stress in poultry birds and improves their performance. Authors strongly believe that supplementation of *R.imbricata* extract enhanced the anabolic activities and reduced the catabolism of muscular proteins and body tissue in broilers.

Blood biochemical parameters: All the blood biochemical parameters are presented in Tables 3–5. A significant (P<0.05) increase in plasma total protein and globulin concentration was recorded in treatment groups at 42 day (Table 3). This elevated total protein level might be to adaptogenic activity of *R. imbricata* (Ishaque *et al.* 2012) which emphasis higher amino acid absorption in intestinal tissues, increased protein synthesis and decreased muscle protein catabolism. Globulins are the globular proteins and are produced by the immune system cells and the increase in the globulin content in this study may be due to immune stimulating activity of salidroside (Li *et al*. 2013) under stressful conditions. However, no difference was recorded in albumin concentration. A significant decrease in A/G ratio was noticed in treatment groups (Table 3) which might be due to increased globulin concentration, which also indicates improved immunity in birds (Mishra *et al*. 2012). Level of cholesterol and triglyceride were significantly (P<0.05) lower in T2 and T5 groups as compared to control group (Table 4). Similarly, the level of LDL was decreased in treatment groups whereas the HDL concentration was increased in treatment groups (Table 4). This effect may be due to the reduced activity of HMG-CoA reductase, key regulatory enzyme in cholesterol synthesis by bioactive phytomolecules (salidroside, ρ-tyrosol and polyphenols) present in *R.imbricata* extract. The results of our study were

Table 3. Effect of *Rhodiola imbricata* on total protein, albumin, globulin, and albumin/globulin level of broilers

Group	0 day	21 day	42 day
	Total protein (g/dl)		
Control	3.95 ± 0.10	4.62^{a} ± 0.12	4.55^{a} ± 0.17
T1	3.91 ± 0.06	4.66^{a} ± 0.17	5.28^{b} ± 0.21
T2	3.96 ± 0.11	4.36^{a} ± 0.18	5.17^{b} ± 0.27
T3	3.95 ± 0.07	5.20^{c} ± 0.26	6.06^{c} ± 0.25
T4	3.94 ± 0.10	5.17^{c} ± 0.21	5.92^{c} ± 0.19
T5	3.96 ± 0.07	5.59^{d} ± 0.24	6.31^{d} ± 0.30
T6	3.90 ± 0.09	4.87^{b} ± 0.19	5.67^{c} ± 0.21
	Albumin (g/dl)		
Control	2.44 ± 0.07	2.71 ± 0.14	2.90 ± 0.13
T1	2.44 ± 0.05	2.67 ± 0.09	3.11 ± 0.15
T2	2.42 ± 0.05	2.58 ± 0.13	3.10 ± 0.10
T3	2.43 ± 0.09	2.59 ± 0.17	2.97 ± 0.18
T4	2.42 ± 0.06	2.63 ± 0.11	3.01 ± 0.15
T5	2.41 ± 0.10	2.60 ± 0.14	2.94 ± 0.15
T6	2.40 ± 0.08	2.69 ± 0.16	3.12 ± 0.21
	Globulin (g/dl)		
Control	1.51 ± 0.08	1.91^{a} ± 0.06	1.65^{a} ± 0.12
T1	1.47 ± 0.06	1.99^{ab}± 0.08	2.17^{b} ± 0.11
T2	1.54 ± 0.06	1.78^{a} ± 0.09	2.07^{b} ± 0.09
T3	1.50 ± 0.09	2.61^{c} ± 0.08	3.09^{c} ± 0.17
T4	1.52 ± 0.07	2.54^{c} ± 0.13	2.91^{c} ± 0.20
T5	1.55 ± 0.11	2.99^{d} ± 0.11	3.37^{d} ± 0.21
T6	1.50 ± 0.09	2.18^{b} ± 0.08	2.55^{b} ± 0.17
	A/G ratio (g/dl)		
Control	1.61 ± 0.07	1.42^{c} ± 0.04	1.76^{d} ± 0.12
T1	1.66 ± 0.08	1.34^{b} ± 0.06	1.43^{c} ± 0.10
T2	1.57 ± 0.08	1.45^{c} ± 0.09	1.50^{c} ± 0.10
T3	1.62 ± 0.10	0.99^{a} ± 0.07	0.96^{a} ± 0.08
T4	1.59 ± 0.10	1.04^{a} ± 0.05	1.03^{ab} ± 0.14
T5	1.55 ± 0.11	0.87^{a} ± 0.06	0.87^{a} ± 0.09
T6	1.61 ± 0.10	1.23^{b} ± 0.05	1.22^{b} ± 0.13

C, T1, T2, T3, T4, T5, and T6 represent groups of chickens that received *Rhodiola imbricata* at concentration level of 0, 100, 150, 200, 300, 400, and 800 mg/kg body weight of chicken, respectively. Means bearing the different superscripts ($^{a, b, c, d}$) in a column differ significantly (P<0.05).

Table 4. Effect of *Rhodiola imbricata* on cholesterol, triglyceride, HDL, and LDL level of broiler

Group	0 day	21 day	42 day
	Cholesterol (mg/dl)		
Control	215.75 ± 2.98	212.00^{b} ± 3.62	208.75^{b} ± 3.96
T1	218.50 ± 3.66	207.25^{b} ± 4.32	206.25^{b} ± 3.70
T2	214.25 ± 2.95	190.00^{a} ± 4.60	181.25^{a} ± 5.17
T3	212.75 ± 3.68	207.75^{b} ± 4.80	205.00^{b} ± 5.30
T4	216.50 ± 4.92	209.25^{b} ± 6.28	201.00^{b} ± 3.67
T5	212.50 ± 3.37	184.00^{a} ± 4.14	176.25^{a} ± 3.30
T6	216.00 ± 3.65	212.25^{b} ± 5.10	207.50^{b} ± 5.05
	Triglyceride (mg/dl)		
Control	108.82 ± 1.91	101.24^{c} ± 1.06	99.54^{c} ± 0.67
T1	109.44 ± 1.76	100.37^{c} ± 1.63	98.71^{c} ± 0.64
T2	107.48 ± 1.71	092.64^{b} ± 1.30	85.61^{b} ± 1.24
T3	108.75 ± 1.79	099.70^{c} ± 1.16	93.36^{b}± 0.72
T4	109.35 ± 1.31	098.44^{c} ± 1.00	91.69^{b} ± 0.89
T5	107.65 ± 1.33	088.52^{a} ± 1.26	78.24^{a} ± 0.98
T6	108.17 ± 1.24	100.55^{c} ± 1.01	97.47^{c} ± 0.88
	HDL (mg/dl)		
Control	16.54 ± 0.36	24.71^{a} ± 0.34	23.24^{a} ± 0.44
T1	17.16 ± 0.36	25.44^{b} ± 0.24	39.04^{b} ± 0.40
T2	16.32 ± 0.35	29.58^{d} ± 0.25	48.26^{d} ± 0.48
T3	16.29 ± 0.24	27.52^{c} ± 0.28	42.34^{b} ± 0.51
T4	17.04 ± 0.21	27.12^{c} ± 0.32	44.19^{c} ± 0.61
T5	16.50 ± 0.22	32.16^{e} ± 0.32	54.42^{e} ± 0.52
T6	16.81 ± 0.21	24.38^{a} ± 0.23	39.38^{b} ± 0.41
	LDL (mg/dl)		
Control	48.16 ± 1.68	44.31^{c} ± 1.62	43.59^{d} ± 1.68
T1	48.55 ± 1.40	42.90^{b} ± 1.74	35.24^{c} ± 1.63
T2	49.04 ± 1.76	40.63^{b} ± 1.93	31.16^{b} ± 1.92
T3	48.62 ± 1.66	41.86^{b} ± 1.79	33.46^{b} ± 1.58
T4	48.10 ± 1.55	41.46^{b} ± 1.75	32.77^{b} ± 1.98
T5	49.12 ± 1.76	38.35^{a} ± 1.85	29.18^{a} ± 1.88
T6	48.21 ± 1.77	44.29^{c} ± 1.82	35.18^{c} ± 1.75

C, T1, T2, T3, T4, T5, and T6 represent groups of chickens that received *Rhodiola imbricata* at concentration level of 0, 100, 150, 200, 300, 400, and 800 mg/kg body weight of chicken, respectively. Means bearing the different superscripts ($^{a, b, c, d, e}$) in a column differ significantly (P<0.05).

Table 5. Effect of *Rhodiola imbricata* on glucose, creatinine, ALT, and AST level of broilers.

Group	0 day	21 day	42 day
	Glucose (mg/dl)		
Control	288.75 ± 6.71	287.25^{c} ± 8.98	278.00^{d} ± 6.64
T1	287.25 ± 5.57	240.75^{a} ± 8.17	267.00^{cd} ± 5.52
T2	292.50 ± 5.86	262.75^{b} ± 8.56	269.25^{d} ± 5.12
T3	287.00 ± 6.98	263.75^{b} ± 7.58	226.00^{b} ± 9.06
T4	287.50 ± 7.50	240.00^{a} ± 9.54	266.00^{cd} ± 7.56
T5	290.50 ± 5.36	265.00^{b} ± 6.55	197.75^{a} ± 6.60
T6	287.50 ± 7.41	249.70^{ab} ± 7.32	246.00^{bc} ± 6.27
	Creatinine (mg/dl)		
Control	0.18 ± 0.04	0.14 ± 0.02	0.20 ± 0.04
T1	0.20 ± 0.04	0.13 ± 0.05	0.13 ± 0.03
T2	0.22 ± 0.06	0.16 ± 0.03	0.16 ± 0.02
T3	0.21 ± 0.03	0.17 ± 0.03	0.16 ± 0.02
T4	0.18 ± 0.05	0.16 ± 0.04	0.17 ± 0.04
T5	0.22 ± 0.06	0.13 ± 0.02	0.19 ± 0.04
T6	0.17 ± 0.04	0.13 ± 0.02	0.13 ± 0.02
	AST (IU/l)		
Control	88.27 ± 2.43	85.00^{c} ± 2.97	82.00^{d} ± 2.73
T1	88.50 ± 1.95	61.50^{a} ± 2.02	56.25^{c} ± 2.01
T2	86.00 ± 2.35	59.00^{a} ± 1.47	53.25^{c} ± 2.75
T3	85.75 ± 2.25	57.25^{a} ± 2.05	46.77^{a} ± 2.04
T4	87.75 ± 2.50	65.00^{b} ± 2.61	54.50^{c} ± 2.75
T5	88.00 ± 2.00	60.25^{a} ± 2.01	45.00^{a} ± 2.12
T6	88.25 ± 2.54	61.00^{a} ± 2.04	48.45^{b} ± 1.89
	ALT (IU/l)		
Control	16.00 ± 1.75	15.00^{c} ± 0.81	14.00^{c} ± 0.48
T1	15.25 ± 1.43	10.00^{b} ± 0.40	11.32^{b} ± 0.77
T2	16.00 ± 0.91	12.25^{b} ± 0.62	10.00^{b} ± 0.40
T3	16.25 ± 1.43	10.25^{b} ± 0.85	11.00^{b} ± 0.81
T4	16.25 ± 0.62	11.75^{b} ± 1.03	10.00^{b} ± 0.81
T5	15.50 ± 1.65	08.75^{a} ± 0.62	08.32^{a} ± 0.63
T6	16.25 ± 1.54	11.00^{b} ± 0.70	11.75^{b} ± 0.75

C, T1, T2, T3, T4, T5, and T6 represent groups of chickens that received *Rhodiola imbricata* at concentration level of 0, 100, 150, 200, 300, 400, and 800 mg/kg body weight of chicken, respectively. Means bearing the different superscripts ($^{a, b, c, d}$) in a column differ significantly (P<0.05).

consistent with the findings of Zhang *et al*. (2012).

A significant decrease in plasma glucose level was observed in treatment group birds (Table 5) which might be due to reduced glucocorticoid secretion with *R.imbricata* supplementation (Panossian and Wikman 2010). No difference (P>0.05) was observed in creatinine concentration among the experimental groups which exhibit non-pathological effect of *R.imbricata* on kidney. Moreover, AST and ALT levels were significantly reduced in treatment groups (Table 5) which indicate hepatoprotective activity (Senthilkumar *et al*. 2014) and the non-toxic effect of *R.imbricata* extract within birds liver cells.

Antioxidant parameters: TAC and DPPH scavenging activity of chicken plasma samples is shown in Table 6. A significant (P<0.05) higher level of TAC and DPPH scavenging activity was measured in treatment groups and birds in T5 groups represent highest scavenging activity at

Table 6. Effect of *Rhodiola imbricata* on malondialdehyde, TAC, and DPPH radical-scavenging activity in broilers

Group	0 day	21 day	42 day
	MDA (nmol/ml)		
Control	8.55 ± 0.33	7.98^{b} ± 0.22	7.76^{c} ± 0.12
T1	8.62 ± 0.34	7.69^{ab}± 0.22	6.32^{b} ± 0.16
T2	8.50 ± 0.17	7.91^{b} ± 0.18	6.39^{b} ± 0.09
T3	8.44 ± 0.24	7.69^{ab} ± 0.15	6.41^{b} ± 0.07
T4	8.51 ± 0.23	7.77^{ab} ± 0.19	6.62^{b} ± 0.11
T5	8.45 ± 0.20	7.46^{a} ± 0.22	5.67^{a} ± 0.13
T6	8.60 ± 0.13	7.73^{ab} ± 0.26	6.67^{b} ± 0.12
	FRAP value (µmol/l)		
Control	572.25 ± 23.94	676.00^{a} ± 29.53	629.50^{a} ± 33.10
T1	568.00 ± 22.58	761.50^{b} ± 44.52	781.00^{b} ± 48.88
T2	569.00 ± 20.29	691.00^{ab} ± 42.45	778.00^{b} ± 50.00
T3	569.25 ± 22.45	691.75^{ab} ± 35.96	765.00^{b} ± 43.77
T4	570.50 ± 23.48	773.00^{b} ± 32.78	791.00^{b} ± 42.68
T5	571.00 ± 20.12	808.25^{c} ± 38.60	856.00^{c} ± 46.58
T6	570.00 ± 23.46	780.50^{b} ± 37.95	784.00^{b} ± 48.38
	DPPH radical-scavenging activity (%)		
Control	42.32 ± 2.55	45.06^{a} ± 1.82	43.70^{a} ± 2.21
T1	42.08 ± 3.21	51.35^{b} ± 2.48	58.64^{b} ± 2.10
T2	42.70 ± 3.45	50.61^{ab} ± 3.30	58.20^{b} ± 2.93
T3	42.77 ± 3.88	53.80^{b} ± 3.10	56.13^{b} ± 1.66
T4	42.83 ± 2.57	55.74^{b} ± 2.80	56.08^{b} ± 2.65
T5	41.80 ± 2.36	57.25^{b} ± 2.71	65.23^{c} ± 1.90
T6	41.99 ± 2.59	56.09^{b} ± 3.28	57.08^{b} ± 2.70

C, T1, T2, T3, T4, T5, and T6 represent groups of chickens that received *Rhodiola imbricata* at concentration level of 0, 100, 150, 200, 300, 400, and 800 mg/kg body weight of chicken, respectively. Means bearing the different superscripts ($^{a, b, c, d}$) in a column differ significantly (P<0.05).

42 day. Furthermore, MDA level was significantly decreased in treatment groups and lowest MDA level was recorded in T5 group (Table 6). The increase in the antioxidant system and decease in oxidative stress marker MDA in this study may possibly be due to higher content of active phytomolecules such as salidroside, p-tyrosol and polyphenols in *Rhodiola root* extract. Previous reports of Calcabrini *et al*. (2010) and Xu and Li (2012) in laboratory animals indicated reduction in oxidative stress by eliminating ROS production on administration of *Rhodiola* extract. These phytomolecules protects the cells from oxidative stress through H_2O_2 scavenging, ferric reducing, ferrous chelating, and hypochlorite scavenging activities (Qian *et al*. 2012, Zhou *et al*. 2014, Chiang *et al*. 2015). This could be due to its higher antioxidative property and its ability to inhibit the caspase-3 activation, and intercellular Ca^{2+} production, upregulating the antiapoptotic genes Bcl-X_L and Bcl-2 and by downregulating the proapoptotic genes Bax. In the present study, total antioxidant capacity of plasma samples in treatment group birds was positively associated with free radical scavenging activity and both of these activities were negatively correlated with MDA level. Effective antioxidative properties of *R.imbricata* can also be implicated with improved growth performance observed in broilers. Hence,

Table 7. Effect of *Rhodiola imbricata* on IL-1, IL-2, and IL-6 level in broilers

Group	0 day	21 day	42 day
		IL-1 (pg/ml)	
Control	5.21 ± 0.22	5.23 ± 0.19	5.22 ± 0.22
T1	5.20 ± 0.17	5.24 ± 0.24	5.27 ± 0.31
T2	5.23 ± 0.30	5.24 ± 0.26	5.27 ± 0.26
T3	5.21 ± 0.29	5.26 ± 0.35	5.29 ± 0.38
T4	5.22 ± 0.21	5.26 ± 0.30	5.31 ± 0.28
T5	5.20 ± 0.24	5.29 ± 0.27	5.30 ± 0.23
T6	5.21 ± 0.24	5.25 ± 0.21	5.29 ± 0.31
		IL-2 (pg/ml)	
Control	8.72 ± 0.37	9.04^{a} ± 0.43	9.26^{a} ± 0.48
T1	8.71 ± 0.42	9.23^{a} ± 0.55	9.39^{a} ± 0.52
T2	8.72 ± 0.34	9.19^{a} ± 0.40	9.27^{a} ± 0.41
T3	8.70 ± 0.29	10.30^{b} ± 0.53	10.32^{c} ± 0.57
T4	8.69 ± 0.33	10.26^{b} ± 0.47	10.42^{c} ± 0.43
T5	8.72 ± 0.40	10.58^{c} ± 0.51	10.60^{d} ± 0.55
T6	8.70 ± 0.37	10.14^{b} ± 0.54	9.92^{b} ± 0.49
		IL-6 (pg/ml)	
Control	7.08 ± 0.43	7.27^{b} ± 0.34	7.11^{b} ± 0.27
T1	7.11 ± 0.52	7.30^{b} ± 0.41	7.09^{b} ± 0.35
T2	7.10 ± 0.42	7.27^{b} ± 0.44	7.04^{b} ± 0.29
T3	7.07 ± 0.36	7.00^{a} ± 0.47	6.81^{a} ± 0.38
T4	7.08 ± 0.39	7.01^{a} ± 0.29	6.84^{a} ± 0.32
T5	7.09 ± 0.49	6.98^{a} ± 0.35	6.77^{a} ± 0.40
T6	7.08 ± 0.50	7.04^{a} ± 0.46	6.81^{a} ± 0.39

C, T1, T2, T3, T4, T5, and T6 represent groups of chickens that received *Rhodiola imbricata* at concentration level of 0, 100, 150, 200, 300, 400, and 800 mg/kg body weight of chicken, respectively. Means bearing the different superscripts ($^{a, b, c, d}$) in a column differ significantly (P<0.05).

this root extract has beneficial effect in nutrient digestibility and scavenging of free radicals under high altitude stress condition. Therefore, extract of this plant could be useful as broilers feed additive at high altitude for better growth rate and to reduce mortality.

Cytokine level: The levels of IL-1, IL-2, and IL-6 in chicken plasma samples are shown in Table 7. The level of IL-1 did not change among the groups whereas concentration of IL-2 was higher in T3, T4, T5 and T6 groups throughout the experiment. The concentration of IL-6 was significantly decreased in T3, T4, T5, and T6 groups as compared to control group at 21 and 42 days. The decrease in proinflammatory cytokine IL-6 may be due to potential of salidroside in downregulating NF-κB signaling pathway through decreased phosphorylation of NF-κB, a transcriptional factor controlling IL-6, TNF-α production (Li *et al*. 2013). IL-2 is produced by activated T helper cells 1 (Th1) to mediate cell mediated immunity (Boyman and Sprent 2012). In the present study, *R.imbricata* extract stimulated the production of IL-2 in chickens and most pronounced effect was experienced @ 400 mg/kg supplemented group birds. This suggests that, *R.imbricata* supplementation at particular dose (400 mg/kg body weight) concentration exerts immunomodulatory effects in broilers by mediating both cellular and humoral immunity.

In conclusion, *R.imbricata* at dose concentration of 400 mg/kg body weight of chicken provided better effect as compared to other treatments level. Hence, from the results of our study, it can be concluded that hydro-alcoholic extract of *R.imbricata* (@400 mg/kg body weight of chicken) has potential health benefits in broilers and it can definitely be used as source of phytogenic feed additive for broilers at high altitude where there is marked deficiency of nutritious poultry feeds and fodder.

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