Hippophae rhamnoides as novel phytogenic feed additive for broiler chickens at high altitude cold desert

Sahil Kalia, Vijay K. Bharti, Arup Giri, Bhuvnesh Kumar, Achin Arora & S. S. Balaje

Extremes of climate and hypobaric hypoxia cause poor growth performance in broiler chickens at high altitude. The present study examined the potential of Hippophae rhamnoides extract as phytogenic feed additive for broilers reared at 3500 m above mean sea level (MSL). Higher content of phytomolecules were recorded during characterization of the extract. Immunomodulatory activity of extract was observed in chicken lymphocytes through in-vitro studies. Thereafter, for in vivo study, 105 day old Rhode Island Red (RIR) Cross-bred chicks were randomly distributed in to control and treatments T1, T2, T3, T4, T5, and T6 which were supplemented with H. rhamnoides aqueous extract along with basal diet, at level of 100, 150, 200, 300, 400, and 800 mg/kg body weight of chicken, respectively. Among the experimental groups, birds in the T3 group represent the highest body weight. Furthermore, treatment group birds had shown better physio-biochemical indices as compared to control group birds. Interestingly, lower mortality rate due to ascites and coccidiosis was recorded in treatment groups and therefore, higher net return was observed. Hence, present investigation demonstrated the beneficial effect of H. rhamnoides extract (@200 mg/kg) at high altitude and therefore, may be used in formulation of feed additive for poultry ration.
anti-tumor, hepato protective, and radio protective. These medicinal effects of H. rhamnoides have been attributed to presence of high antioxidant content in this plant. It has been suggested by Biswas et al. that H. rhamnoides seeds, leaves, and fruit residues are ideal source of feeding material for the poultry chickens in high altitude region of trans-Himalayan. Ma et al. reported an significant enhancement in broilers performance after supplementation of flavonoids of H. rhamnoides fruits in broilers diet. However, still so far, no previous study has ever evaluates the feeding potential of H. rhamnoides in broiler chicken diet at high altitude. Therefore, the current study was performed to determine the dietary supplemental effect of H. rhamnoides extract on broiler growth performance, survivability rate, physio-biochemical indices, and cost economics of their rearing at high altitude and evaluate its potential as a phytogenic feed additive.

Methods

Plant material and extraction. Fresh H. rhamnoides fruits were gathered from the market of Leh district through local vendors, and thereafter, washed upon arrival at the laboratory and then shed dried at room temperature for 30 days. After that, dried fruit samples were powdered and extracted with distilled water in a soxhlet apparatus for 24–48 hrs.

Extract yield was calculated on the basis of the following equation:

\[
\text{Extract Yield (\%)} = \frac{\text{Total amount of extract}}{\text{Total amount of powder sample}} + 100.
\]

Characterization of the extract. H. rhamnoides fruit extract was characterized for total polyphenols, flavonoids, and carotenoids contents, and also analysed for free radical scavenging activity and total antioxidant capacity (TAC).

TAC. TAC in extract sample was determined by ferric reducing antioxidant power (FRAP) assay as suggested by Benzie and Strain. Results were expressed as µM Fe(III)/g of extract.

Free radical scavenging capacity. The effect of H. rhamnoides fruit extract on scavenging of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical was determined using the method proposed by Brand-William et al. For this assay, 0.1 mM solution of DPPH in methanol was prepared and 600 μL of this solution was allowed to react with 30 μL of sample extract. A control was treated with 30 μL of solvent instead of the sample. Solution was centrifuged (Heal Force, Neofuge 23 R, Shanghai, China) after incubation of 30 min at room temperature and supernatant was transferred into 96 well microtiter plate and the absorbance was recorded at 517 nm using of ELISA microplate reader (Spectromax M2e, Molecular Devices, California, USA). Ascorbic acid was used as reference standard. Scavenging of DPPH radical by the sample (fruit extract and plasma) was calculated by the following equation:

\[
\text{DPPH radical scavenging activity (\%)} = \frac{(\text{Ab}_{ \text{control}} - \text{Ab}_{ \text{sample}})}{(\text{Ab}_{ \text{control}})} \times 100
\]

Where \( Ab_{\text{control}} \) is the absorbance of control, and \( Ab_{\text{sample}} \) is the absorbance of sample or standard.

2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) ABTS radical scavenging capacity. The potential of H. rhamnoides extract to scavenge ABTS radicals was determined by the method proposed by Re et al. The stock solution for this assay comprised of 7 mM ABTS solution and 2.4 mM potassium persulphate solution. Two stock solutions were mixed in equal quantities for the preparation of working solution followed by incubation of 12 h in dark at room temperature. After incubation, 1 ml of working solution was mixed with 60 μL of 96% ethanol for obtaining an initial absorbance of 0.700 ± 0.02 at 734 nm. Fruit extract (33.30 μL) was allowed to react with ABTS+ solution (266.70 μL) and the decrease in the absorbance was measured after 7 min at 734 nm. Ascorbic acid was used as reference standard. The ABTS+ scavenging capacity of fruit extract was calculated as follows:

\[
\text{ABTS radical scavenging capacity (\%)} = \frac{\left(\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}}\right)}{\text{Absorbance}_{\text{control}}} \times 100.
\]

Where \( \text{Absorbance}_{\text{control}} \) is the absorbance of control, and \( \text{Absorbance}_{\text{sample}} \) is the absorbance of sample or standard.

Total phenolic content. Total phenolic content in H. rhamnoides fruit extract was evaluated by Folin-Ciocalteu colorimetric method as suggested by Gao et al.

Total flavonoid content. Total flavonoid content in Hippophae rhamnoides fruit extract was estimated by the method as suggested by Ordonez et al. For this assay, 0.5 mL of extract was allowed to mix with 0.5 mL of 2% aluminium chloride (AlCl3) ethanolic solution. After 1 h of incubation at room temperature the absorbance was measured at 420 nm. Quercetin was used as standard and total flavonoid content was indicated as mg of quercetin equivalent.

Determination of carotenoids. These were estimated by the method as suggested by Ranjith et al. For this assay, 0.5 mL of 5% sodium chloride (NaCl) and 2 mL of hexane was mixed with fruit extract. After that, the solution was vortexed for 30 seconds followed by centrifugation for 10 minutes. Absorbance was measured at
460 nm. β-carotene was used as reference standard and total carotenoids in fruit extract were indicated as mg of β-carotene equivalent.

In vitro evaluation for dose efficacy of *H. rhamnoides* extract. To determine the efficacy of *H. rhamnoides* fruit extract, its antioxidative and cytoprotective activities were initially assessed in chicken peripheral blood lymphocytes (PBL) prior to *in vivo* studies.

Blood sampling & separation of peripheral blood mononuclear cells (PBMC). We took 3 ml of blood samples from wing vein of chickens and collected those samples into sterile plastic tubes containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. For separation of PBMC, whole blood was first diluted with phosphate buffered saline (PBS, Ca²⁺ and Mg²⁺ free, Himedia) in 1:1 ratio and thereafter smoothly overlaid on Histopaque-1077 (Sigma-Aldrich, St. Louis, MO) in falcon tube and centrifuged for 30 m at 400 × g. We recovered the PBMC from gradient interface, washed them twofold with Ca²⁺ and Mg²⁺ free PBS, and centrifuged at 200 × g for 10 m. The last washing was performed with RPMI-1640 medium (R1415, Sigma-Aldrich). The pellets were then resuspended in 10% fetal bovine serum (FBS) rich RPMI-1640 medium. Plastic adherence technique proposed by Gupta *et al.* was used to separate a non-adherent (lymphocytes) cells from the adherent (monocytes) one.

Cell Culture. PBL suspension (100µL/well) was cultured in microtiter plate with 100µL/well of different dose concentrations of *H. rhamnoides* extract (100, 200, 400, 800 ng/mL, and 1, 2, 4, 8, 50, 100, 200, 400 µg/mL), 1µg/mL of concanavalin A as positive control, and medium as negative control, at 41 °C in a 5% CO₂ incubator for 24 h.

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) proliferation assay. The calorimetric method of MTT assay described by Mosmann was used to assess the proliferative activity of extract in chicken PBL. Following incubation of chicken PBL with *H. rhamnoides* extract for 24 h, 50µL of MTT solution was added to each well and after 4 h of incubation, 100µL of dimethyl sulfoxide (DMSO) was added to solubilize the formazan product. By using microplate reader absorbance was taken at 570 nm.

\[ \text{% Cell viability} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Control}} \times 100 \]

Cytoprotective assay against hydrogen peroxide 

\( \text{H}_2\text{O}_2 \) induced toxicity. To analyse the cytoprotective activity of plant extract against the toxic effect of \( \text{H}_2\text{O}_2 \), PBL cells were first cultured into 96 well plates and incubated for 24 h at 41 °C. Thereafter, cells were simultaneously treated with different concentrations of *H. rhamnoides* extract and 100µM \( \text{H}_2\text{O}_2 \) (Merck, India) for 2 h. MTT assay was used to determine the cell viability.

In vivo experiment. Institutional Animal Ethics Committee of DIHAR approved the field experiment and all the methods were performed as per the guidelines of animal experimentation. The experiment was carried out under deep litter system in the solar poultry house of DIHAR having a stacking density of 0.80 square feet per bird in 2 × 2 feet of pen size (5 birds/pen). The ambient temperature of the house was maintained at 25–32 °C by using local bukhari (a heating device). A total of 105 one day old RIR cross-bred broiler chickens were randomly distributed to seven experimental groups as per completely randomized design. There were 3 replications per treatment with 5 chickens per replicate pen. Chickens in the control group were fed the basal diet whereas chickens in the six treatment groups were supplemented with aqueous extract of *H. rhamnoides* fruit in drinking water @ 100 mg/kg body weight of chicken (T1), @ 150 mg/kg body weight of chicken (T2), @ 200 mg/kg body weight of chicken (T3), @ 300 mg/kg body weight of chicken (T4), @ 400 mg/kg body weight of chicken (T5), and @ 800 mg/kg body weight of chicken (T6), respectively, in addition of basal diet. Experiment was conducted from 0 to 42 days of broiler chick age. A standard in house feed formula specific for high altitude poultry chickens developed by our laboratory was used to formulate the basal diet. All the birds were given the same basal diet, included a starter diet (21.56% protein, 12.97 MJ/Kg ME, 1.02% calcium, and 0.48% phosphorus) from day 1 to day 21 and a finisher diet (19.31% protein, 13.38 MJ/Kg ME, 0.94% calcium, and 0.42% phosphorus) from day 22 to d 42. The ingredients and analysed composition of basal diet are presented in Table 1. On 7th day, all the chickens were vaccinated for Newcastle disease. Every chicken was individually weighed at each week interval. Throughout the experiment, water and feed intake was measured. During the experimental trial autopsy inspection of dead birds was done to find out the cause of death. Economics of the experiment was also estimated based on the rearing cost of chickens.

Blood collection. For collection of blood sample we randomly picked nine chickens from each group (3 chickens from each replicate pen) at 0, 21, and 42 day and took 3 ml of blood samples from wing vein of chickens and collected those samples into sterile plastic tubes containing EDTA as an anticoagulant. EDTA tubes containing blood were centrifuged at 3500 RPM for 10 m at 4 °C to obtain clear plasma and stored at ~ 80 °C until use.

Physio-biochemical indices

Determination of plasma antioxidant parameters. Plasma TAC and free radical scavenging activity were analysed as described earlier in the section. For plasma TAC the results were indicated as FRAP value (µM Fe (II)/L of plasma).

Lipid peroxidation assay (LPO). Lipid peroxidation assay was performed by measuring malondialdehyde (MDA) concentration in plasma samples according to the previously described method. For this assay, 375 mg of thiobarbituric acid (TBA) was dissolved in 2 mL of 0.25 N hydrochloric acid (HCL) followed by 15 g of trichloroacetic acid (TCA) for a final volume of 100 mL. To properly dissolve TBA, solution was heated in water.
bath (GFL water bath, Burgwedal, Germany) at 55 °C for 15 minutes. Thereafter, 500 µL of TCA-TBA-HCL solution was mixed properly with 250 µL of plasma sample. This solution was again heated in boiling water bath for 15 minutes. After cooling, to remove flocculent precipitate solution was centrifuged and absorbance was taken at 535 nm against a blank that contained all reagents except the plasma sample.

**Determination of blood biochemical parameters.** The level of total protein, albumin, glucose, creatinine, alanine transaminase (ALT), aspartate transaminase (AST), low density lipoprotein (LDL), triglyceride, cholesterol, and high density lipoprotein (HDL) in chicken plasma samples were evaluated with commercial biochemical kits (Span Diagnostics, India) according to suggested methodology.

**Statistical analysis.** Data were analyzed by one way analysis of variance (ANOVA) using completely randomized design. Values were expressed as mean ± standard error. Statistical significant values were assumed at P < 0.05. For growth performance, 3 replicates pen per treatment (5 broiler chickens per replicate pen) served as experimental unit.

**Results**

**Free radical scavenging capacity of extract.** The details of free radical scavenging of aqueous extract of *H. rhamnoides* compared to positive control ascorbic acid are shown in Table 2. *H. rhamnoides* scavenged the DPPH and ABTS radical at concentration of 20 to 100 µg/ml and scavenging activity increases with increase in the extract concentration. Positive control ascorbic acid was also found to produce inhibition of free radicals at similar concentration.

### Table 1. Ingredients and chemical composition of basal diet. *Vitamin and mineral premix supplied per kilogram of diet: 14000 IU of vitamin A, 70 mg of vitamin E, 3000 IU of vitamin D3, 4 mg of vitamin K, 3 mg of thiamine, 10 mg of vitamin B6, 8 mg of vitamin B12, 0.04 mg of vitamin B12, 48 mg of niacin, 20 mg of calcium, 50 mg of choline chloride, 0.20 mg of biotin, 1.8 mg of folic acid, 80 mg of manganese, 70 mg of zinc, 50 mg of iron, 10 mg of copper, 3 mg of iodine, 0.4 mg of selenium, and 0.2 mg of cobalt.

| Ingredients (% diet) | Starter diet (0–21 day) | Finisher diet (22–42 day) |
|---------------------|-------------------------|--------------------------|
| Maize               | 59.00                   | 58.00                    |
| Soyabean (Solvent extracted) | 33.18              | 21.12                    |
| Soyabean (Full fat)  | —                       | 9.58                     |
| Soyabean oil        | 2.00                    | 2.55                     |
| Fish Meal           | 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 & Mineral premix* | 0.20               | 0.20                     |
| Total               | 100                     | 100                      |

**Table 2.** Free radical scavenging activity of extract. Scavenging capacity of aqueous extract of *H. rhamnoides* was determined against DPPH and ABTS radical. Ascorbic acid was used as a reference standard. Values are given as mean ± S.E.

| Inhibition (%) | DPPH radical scavenging capacity | ABTS radical scavenging capacity |
|---------------|----------------------------------|----------------------------------|
| Concentration (µg/ml) | *H. rhamnoides* extract | Ascorbic acid | *H. rhamnoides* extract | Ascorbic acid |
| 20            | 31.85 ± 0.65 | 39.57 ± 0.76 | 15.63 ± 0.40 | 21.36 ± 1.12 |
| 40            | 32.01 ± 0.78 | 45.40 ± 0.89 | 19.27 ± 0.25 | 29.37 ± 0.45 |
| 60            | 35.89 ± 0.81 | 49.80 ± 0.63 | 24.41 ± 0.39 | 35.86 ± 0.54 |
| 80            | 38.25 ± 0.67 | 55.98 ± 0.57 | 30.40 ± 0.63 | 41.18 ± 0.71 |
| 100           | 39.47 ± 0.90 | 60.59 ± 1.08 | 35.25 ± 0.84 | 55.94 ± 0.96 |

*Vitamin and mineral premix supplied per kilogram of diet: 14000 IU of vitamin A, 70 mg of vitamin E, 3000 IU of vitamin D3, 4 mg of vitamin K, 3 mg of thiamine, 10 mg of vitamin B6, 8 mg of vitamin B12, 0.04 mg of vitamin B12, 48 mg of niacin, 20 mg of calcium, 50 mg of choline chloride, 0.20 mg of biotin, 1.8 mg of folic acid, 80 mg of manganese, 70 mg of zinc, 50 mg of iron, 10 mg of copper, 3 mg of iodine, 0.4 mg of selenium, and 0.2 mg of cobalt.
TAC. TAC of H. rhamnoides was determined by FRAP assay and was recorded to be 425.54 ± 16.14 µM Fe (II)/g of extract (data not shown).

Phytomolecules content. Total polyphenolic content in H. rhamnoides extract was recorded to be 76.28 ± 3.25 mg gallic acid (GAE)/g of extract. Flavonoid content was recorded to be 35.14 ± 2.18 mg quercetin (QE)/g of extract and carotenoid content was recorded to be 4.19 ± 0.70 mg/100 g of extract (data not shown).

In vitro efficacy. Treatment of H. rhamnoides extract with chicken lymphocytes increase the proliferation of cells at all dose concentrations in between 100 ng/mL to 400 µg/mL as compared with the untreated control cells (Fig. 1a). However, proliferation of H. rhamnoides stimulated PBL was less as compared to proliferation of Concanavalin A stimulated PBL. H. rhamnoides extract was also found to reduce the H₂O₂ induced oxidative stress in lymphocytes at similar concentration as compared with H₂O₂ stimulated control cells (Fig. 1b). The highest cytoprotective activity of H. rhamnoides was recorded at 2 µg/ml dose concentration.

Growth performance. Chickens in T3 group had significantly higher body weight as compared with control and other treatment groups at 21 days (Table 3). Whereas, no differences were observed in body weight among the control and other treatment groups at 21 days. Further, at 42 day, we observed a significantly higher body weight in all treatment groups as compared with control group and within treatment groups, birds in the T3 group represented the highest body weight. Throughout the experiment cumulative feed and water intake did not differ among the experimental groups. Feed conversion ratio (FCR) value in the T3 group was found to be significantly improved among the experimental groups (Table 3).

Mortality rate and economics. Maximum mortality rate (26.67%, 4/15) was observed in the control group (Table 4) chicken followed by T1, T2, T4, T5 and T6 (13.30%, 2/15), which was followed by T3 (6.67%, 1/15). Autopsy inspection showed 13.30%, 6.67%, 6.67%, 0%, 0%, and 6.67% mortality in chickens caused by ascites and 6.67%, 0%, 6.67%, 0%, 0%, 6.67%, and 0% mortality caused by coccidiosis in control, T1, T2, T3, T4, T5, and T6 groups, respectively.

We also estimated the economy of the experiment based on the rearing cost of chickens in each group. Extra price of the extract was added to feed price whereas other expenses stayed unchanged. H. rhamnoides decreased the mortality rate in chickens and which subsequently higher net return (Table 4).
Average weight at 21 day (gm/chick) 192.80a
Feed conversion ratio at 42 day 4.78d
Initial average body weight (gm/chick) 38.86
Cumulative water intake up to 42 day (ml/chick) 2138.76
Cumulative feed intake up to 42 day (gm/chick) 1519.20
Average weight at 42 day (gm/chick) 356.75a

| Parameters                  | Control         | T1             | T2             | T3             | T4             | T5             | T6             |
|-----------------------------|-----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Initial average body weight (gm/chick) | 38.86 ± 0.60 | 39.60 ± 0.58 | 39.93 ± 0.57 | 38.20 ± 0.52 | 38.53 ± 0.58 | 38.20 ± 0.51 | 39.33 ± 0.54 |
| Average weight at 21 day (gm/chick) | 192.80 ± 3.30 | 194.26 ± 1.97 | 192.40 ± 3.45 | 222.13 ± 5.26 | 190.26 ± 3.82 | 189.20 ± 5.99 | 191.06 ± 8.41 |
| Average weight at 42 day (gm/chick) | 356.75 ± 10.06 | 410.70 ± 10.38 | 402.53 ± 12.32 | 470.33 ± 12.68 | 394.26 ± 6.69 | 409.20 ± 6.78 | 392.14 ± 5.55 |
| Cumulative feed intake up to 42 day (gm/chick) | 1519.20 ± 9.65 | 1521.08 ± 9.54 | 1520.37 ± 8.70 | 1530.27 ± 14.59 | 1544.58 ± 16.48 | 1518.62 ± 11.63 | 1527.50 ± 11.39 |
| Feed conversion ratio at 42 day | 4.78 ± 0.08 | 4.10 ± 0.06 | 3.54 ± 0.08 | 4.34 ± 0.06 | 4.09 ± 0.05 | 4.33 ± 0.04 |
| Cumulative water intake up to 42 day (ml/chick) | 2138.76 ± 17.37 | 2140.71 ± 11.67 | 2145.69 ± 12.92 | 2140.39 ± 11.39 | 2155.02 ± 16.36 | 2150.71 ± 11.86 | 2155.43 ± 11.96 |

Table 3. Effect of aqueous extract of *H. rhamnoides* on growth performance of broiler chickens. Chickens in the control group were fed the basal diet whereas the six treatment groups, in addition of basal diet received aqueous extract of *H. rhamnoides* in drinking water @ 100 mg/kg body weight of chicken (T1), @ 150 mg/kg body weight of chicken (T2), @ 200 mg/kg body weight of chicken (T3), @ 300 mg/kg body weight of chicken (T4), @ 400 mg/kg body weight of chicken (T5), and @ 800 mg/kg body weight of chicken (T6), respectively from days 0 to 42. Results are presented as mean ± S.E. Experimental unit 3 replicates pen (5 broiler chickens per replicate pen). Means bearing the different superscripts (a, b, c, d) in a row differ significantly (*P* < 0.05).

| Description                  | Control         | T1             | T2             | T3             | T4             | T5             | T6             |
|------------------------------|-----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Total mortality (%)          | 26.67           | 13.30          | 13.30          | 6.67           | 13.30          | 13.30          | 13.30          |
| Mortality by ascites (%)      | 13.30           | 6.67           | 6.67           | 0.00           | 0.00           | 0.00           | 6.67           |
| Mortality by coccidiosis (%)  | 6.67            | 0.00           | 6.67           | 0.00           | 0.00           | 0.00           | 6.67           |
| Mortality by other reasons (%)| 6.67            | 6.67           | 0.00           | 6.67           | 6.67           | 6.67           | 6.67           |
| Cost of extract/chicken (Rs.) | Nil             | 0.69           | 1.02           | 1.58           | 1.96           | 2.74           | 5.40           |
| Cost of feed/chicken (@25/Kg Rs.) | 37.98         | 38.02          | 38.00          | 38.26          | 38.61          | 37.97          | 38.18          |
| Total feed cost/ (Kg)        | 37.98           | 38.71          | 39.02          | 39.84          | 40.57          | 40.71          | 43.58          |
| Sale of chicken at 42 day (@Rs. 200/Kg live weight) | 71.35 | 82.14 | 80.50 | 94.06 | 78.85 | 81.84 | 78.43 |
| Loss due to mortality (Rs.)*  | 71.35           | 82.14          | 80.50          | 94.06          | 78.85          | 81.84          | 78.43          |
| Total benefit per group (Rs.) | —               | 121.12         | 124.40         | 191.34         | 127.70         | 121.70         | 128.53         |

Table 4. Economics and mortality rate (%) in chickens supplemented with *H. rhamnoides* extract. *Due to limited availability of fresh chickens at high altitude the rates are very high. *Loss due to mortality = Sale cost per chicken × total mortality. *Total benefit per group = Loss from mortality in control – loss from mortality in treatment.

**Plasma blood biochemical status in chickens.** The supplementation of *H. rhamnoides* extract significantly increased the concentration of total protein, albumin, and globulin in treatment group birds (Table 5). Moreover, birds in the T3 group represent the highest level of total protein and globulin at 42 day of age. A significant decrease in albumin to globulin (A/G) ratio was noticed in treatment group birds at both 21 and 42 days of age. Within the treatment groups, significantly lowest A/G ratio was noticed in T3 group birds at 42 day of age.

Mean concentrations of plasma cholesterol and LDL were significantly reduced in treatment group birds as compared to control group at 21 and 42 days. Among the treatment groups, lowest concentration of cholesterol and LDL was recorded in plasma of T3 and T4 group birds (Table 6). Furthermore, significant higher level of HDL was recorded in treatment group birds and T3 group represents maximum HDL concentration. Moreover, no differences were observed in plasma triglyceride level among the experimental groups. A significant lower glucose level was recorded in treatment group birds as compared to control group at 21 day (Table 7). No differences were observed in the mean values of creatinine and ALT among the experimental groups whereas, AST level was reduced in T3 and T4 group birds as compared to control group.

**Plasma antioxidant status in chickens TAC.** We observed a significant increase in TAC in treatment groups that were supplemented with *H. rhamnoides* extract compared with the control group at both 21 and 42 day (Table 8). Birds in the T3 group represent the maximum TAC throughout the experiment.

**DPPH scavenging activity.** DPPH scavenging activity in treatment group birds was increased as compared to the birds in the control group at both 21 and 42 day. Within the treatment groups, birds in the T3 group represent the maximum scavenging activity (Table 8).

**LPO.** The concentration of MDA was decreased in treatment groups as compared with control group and lowest MDA concentration was observed in T3 group birds at 42 day of age (Table 8).
and due to the previous reports that polyphenolic compounds are the prime phytomolecules in *H. rhamnoides* these constituents, that polyphenolic content in plant herbs are associated with their antioxidant capacity\(^3^2\) and in this study, capacity, which might be due to the occurrence of diverse range of phytomolecules. It has been highly described \(^1^,^1\)-diphenyl-2-picryl hydrazine in the presence of hydrogen donating antioxidant\(^3^1\). Whereas, ABTS assay is extracts. The DPPH assay is very simple and sensitive and has been widely used to test the ability of compounds reduced by hydrogen donating antioxidants\(^2^3\).

Results in the antioxidant content of this plant\(^3^0\). In this study, DPPH and ABTS radical scavenging capacity of the *H. rhamnoides* extract was increased in a dose dependent manner, similar to positive control ascorbic acid. DPPH and ABTS\(^+\) are stable free radical and are widely used to measure antioxidant capacity of plant extracts. The DPPH assay is very simple and sensitive and has been widely used to test the ability of compounds as free-radical scavengers or hydrogen donors. The assay is based on the reduction of purple DPPH radical to 1,1-diphenyl-2-picryl hydrazine in the presence of hydrogen donating antioxidant\(^3^1\). Whereas, ABTS assay is based on the generation of a blue/green ABTS\(^+\) by oxidation of ABTS with potassium persulfate that can be reduced by hydrogen donating antioxidants\(^2^5\). *H. rhamnoides* extract was also found to be rich in total antioxidant capacity, which might be due to the occurrence of diverse range of phytomolecules. It has been highly described that polyphenolic content in plant herbs are associated with their antioxidant capacity\(^3^2\) and in this study, *H. rhamnoides* extract was found rich in total phenolics, flavonoids and carotenoids content. These results confirmed the previous reports that polyphenolic compounds are the prime phytomolecules in *H. rhamnoides* and due to these constituents, *H. rhamnoides* exhibit its pharmacological antioxidant properties\(^4^1\) which are important for the scavenging of free radicals under stressful conditions of high altitude.

The capability of a plant extract to activate lymphocyte proliferation and enhance cytoprotection against free radicals is mostly ascribed to its higher polyphenolic and carotenoids content\(^3^3^\,^4^4\). Since the phenolics, flavonoids

| Groups | 0-day | 21st day | 42nd day |
|--------|-------|----------|----------|
| Total protein (g/dL) |       |          |          |
| Control | 3.36 ± 0.18 | 3.51 ± 0.07 | 3.57 ± 0.13 |
| T1     | 3.31 ± 0.15 | 4.72 ± 0.09 | 5.64 ± 0.08 |
| T2     | 3.35 ± 0.13 | 4.76 ± 0.14 | 5.23 ± 0.09 |
| T3     | 3.33 ± 0.13 | 4.89 ± 0.12 | 5.77 ± 0.11 |
| T4     | 3.40 ± 0.14 | 4.65 ± 0.13 | 5.30 ± 0.27 |
| T5     | 3.37 ± 0.17 | 4.74 ± 0.10 | 5.12 ± 0.21 |
| T6     | 3.34 ± 0.14 | 4.60 ± 0.14 | 5.51 ± 0.13 |
| Albumin (g/dL) |     |          |          |
| Control | 2.08 ± 0.12 | 2.18 ± 0.08 | 2.23 ± 0.15 |
| T1     | 2.05 ± 0.12 | 2.88 ± 0.10 | 3.10 ± 0.08 |
| T2     | 2.04 ± 0.11 | 2.87 ± 0.11 | 2.98 ± 0.06 |
| T3     | 2.03 ± 0.16 | 2.91 ± 0.21 | 3.05 ± 0.19 |
| T4     | 2.06 ± 0.15 | 2.78 ± 0.17 | 3.02 ± 0.06 |
| T5     | 2.07 ± 0.12 | 2.80 ± 0.11 | 2.89 ± 0.08 |
| T6     | 2.06 ± 0.13 | 2.79 ± 0.15 | 3.00 ± 0.14 |
| Globulin (g/dL) |     |          |          |
| Control | 1.28 ± 0.08 | 1.33 ± 0.15 | 1.34 ± 0.20 |
| T1     | 1.26 ± 0.10 | 1.84 ± 0.18 | 2.16 ± 0.19 |
| T2     | 1.31 ± 0.14 | 1.89 ± 0.16 | 2.25 ± 0.13 |
| T3     | 1.30 ± 0.19 | 1.98 ± 0.23 | 2.72 ± 0.28 |
| T4     | 1.34 ± 0.11 | 1.87 ± 0.21 | 2.28 ± 0.23 |
| T5     | 1.30 ± 0.09 | 1.94 ± 0.15 | 2.23 ± 0.23 |
| T6     | 1.28 ± 0.09 | 1.81 ± 0.18 | 2.51 ± 0.16 |
| A/G ratio (g/dL) |    |          |          |
| Control | 1.63 ± 0.10 | 1.64 ± 0.12 | 1.66 ± 0.14 |
| T1     | 1.63 ± 0.08 | 1.57 ± 0.09 | 1.22 ± 0.10 |
| T2     | 1.56 ± 0.07 | 1.52 ± 0.11 | 1.32 ± 0.08 |
| T3     | 1.56 ± 0.09 | 1.47 ± 0.10 | 1.12 ± 0.11 |
| T4     | 1.54 ± 0.08 | 1.49 ± 0.09 | 1.32 ± 0.09 |
| T5     | 1.59 ± 0.11 | 1.44 ± 0.11 | 1.30 ± 0.10 |
| T6     | 1.61 ± 0.13 | 1.54 ± 0.12 | 1.20 ± 0.08 |

Table 5. Effect of *H. rhamnoides* extract on total protein, albumin, globulin, and A/G ratio in broiler chickens. Chickens in the control group were fed the basal diet whereas the six treatment groups, in addition of basal diet received aqueous extract of *H. rhamnoides* in drinking water @ 100 mg/kg body weight of chicken (T1), @ 150 mg/kg body weight of chicken (T2), @ 200 mg/kg body weight of chicken (T3), @ 300 mg/kg body weight of chicken (T4), @ 400 mg/kg body weight of chicken (T5), and @ 800 mg/kg body weight of chicken (T6), respectively from days 0 to 42. Results are presented as mean ± S.E. Experimental unit 3 replicates pen (3 broiler chickens per replicate pen). Means bearing the different superscripts (a, b, c, d) in a columns differ significantly (P < 0.05).

Discussion

*H. rhamnoides* plant is habituated to cultivate in stressful surroundings of high altitude and is tolerated to abiotic stresses and such stressful conditions could upregulate the pathway of synthesis of secondary metabolites which results increase in the antioxidant content of this plant\(^3^6\). In this study, DPPH and ABTS radical scavenging capacity of the *H. rhamnoides* extract was increased in a dose dependent manner, similar to positive control ascorbic acid. DPPH and ABTS\(^+\) are stable free radical and are widely used to measure antioxidant capacity of plant extracts. The DPPH assay is very simple and sensitive and has been widely used to test the ability of compounds as free-radical scavengers or hydrogen donors. The assay is based on the reduction of purple DPPH radical to 1,1-diphenyl-2-picryl hydrazine in the presence of hydrogen donating antioxidant\(^3^1\). Whereas, ABTS assay is based on the generation of a blue/green ABTS\(^+\) by oxidation of ABTS with potassium persulfate that can be reduced by hydrogen donating antioxidants\(^2^5\). *H. rhamnoides* extract was also found to be rich in total antioxidant capacity, which might be due to the occurrence of diverse range of phytomolecules. It has been highly described that polyphenolic content in plant herbs are associated with their antioxidant capacity\(^3^2\) and in this study, *H. rhamnoides* extract was found rich in total phenolics, flavonoids and carotenoids content. These results confirmed the previous reports that polyphenolic compounds are the prime phytomolecules in *H. rhamnoides* and due to these constituents, *H. rhamnoides* exhibit its pharmacological antioxidant properties\(^4^1\) which are important for the scavenging of free radicals under stressful conditions of high altitude.

The capability of a plant extract to activate lymphocyte proliferation and enhance cytoprotection against free radicals is mostly ascribed to its higher polyphenolic and carotenoids content\(^3^3^\,^4^4\). Since the phenolics, flavonoids
and carotenoids content was recorded in a higher amount in our extract and might be due to their synergistic effect these phytomolecules could have been stimulated the proliferation of T lymphocytes and boost the immune system. It also indicated the mitogenic activity of *H. rhamnoides* in chicken lymphocytic cells. Our results are in agreement with the reports of Geetha et al. and Dorhoi et al. where the *H. rhamnoides* extract modulated the lymphocytes proliferation in laboratory animals, respectively.

Adverse effects of high altitude hypoxia reduce the growth performance in broiler chickens and our present findings of low body weight in broilers also support this hypothesis. Our results are in agreement with the reports of our previous work from our laboratory where we found similar reduction in the body weight of RIR cross-bred broilers at high altitude. The reduction in the growth performance might be because of altitude of the experimental site which lies under high altitude and coupled with low PO\(_2\). Moreover, the decrease in the body weight might be due to the reduction in energy intake and increase in energy expenditure at high altitude. This misbalance in energy utilization leads to decrease in body mass through poor intestinal malabsorption and increase in the catabolism which ultimately reduces the overall growth.

Moreover, improvement in the birds that were supplemented with *H. rhamnoides* extract could have been due to the synergistic effect of phenolics, flavonoids, and carotenoids present in *H. rhamnoides* fruit extract which could help in the higher utilization of feed by stimulating increased digestion of nutrients in gastrointestinal tract of chickens. Also due to its higher antioxidant property, *H. rhamnoides* may eliminate the production of free radicals which ultimately reduces the oxidative stress in poultry chickens and improves their growth performance. The net economic return also disclosed higher profit in the treatment groups chickens with lesser

| Groups | 0 day | 21st day | 42nd day |
|--------|-------|---------|---------|
| **Cholesterol (mg/dL)** |       |         |         |
| Control | 178.25 ± 0.23 | 169.67 ± 0.92 | 165.32 ± 0.38 |
| T1 | 177.50 ± 0.42 | 158.00 ± 0.30 | 138.50 ± 0.39 |
| T2 | 179.50 ± 10.96 | 155.17 ± 0.78 | 139.67 ± 0.19 |
| T3 | 177.50 ± 13.24 | 142.50 ± 0.98 | 128.50 ± 0.30 |
| T4 | 180.00 ± 0.76 | 147.57 ± 0.16 | 131.67 ± 0.24 |
| T5 | 175.25 ± 0.72 | 158.67 ± 0.47 | 137.00 ± 0.26 |
| T6 | 178.00 ± 0.79 | 158.00 ± 0.80 | 143.67 ± 0.71 |
| **Triglyceride (mg/dL)** |       |         |         |
| Control | 138.87 ± 0.49 | 130.25 ± 0.71 | 123.22 ± 0.96 |
| T1 | 136.63 ± 0.16 | 127.38 ± 0.68 | 120.50 ± 0.48 |
| T2 | 131.93 ± 0.94 | 123.97 ± 0.50 | 120.00 ± 0.96 |
| T3 | 135.21 ± 0.83 | 128.17 ± 0.93 | 121.00 ± 0.22 |
| T4 | 132.48 ± 0.81 | 121.33 ± 0.86 | 118.75 ± 0.11 |
| T5 | 134.87 ± 0.19 | 123.94 ± 0.71 | 119.25 ± 0.06 |
| T6 | 135.92 ± 0.94 | 127.16 ± 0.63 | 123.00 ± 0.49 |
| **HDL (mg/dL)** |       |         |         |
| Control | 19.81 ± 0.80 | 20.06 ± 0.77 | 20.40 ± 0.84 |
| T1 | 19.70 ± 0.73 | 27.16 ± 0.80 | 31.64 ± 1.04 |
| T2 | 20.04 ± 0.69 | 26.19 ± 0.75 | 31.50 ± 0.82 |
| T3 | 19.50 ± 0.86 | 30.56 ± 0.85 | 41.17 ± 1.02 |
| T4 | 20.16 ± 0.71 | 27.49 ± 0.81 | 38.12 ± 0.90 |
| T5 | 19.71 ± 0.81 | 26.83 ± 0.85 | 37.89 ± 0.91 |
| T6 | 19.39 ± 0.71 | 27.07 ± 0.80 | 32.23 ± 0.85 |
| **LDL (mg/dL)** |       |         |         |
| Control | 53.35 ± 1.07 | 51.86 ± 0.96 | 50.43 ± 0.88 |
| T1 | 53.91 ± 0.90 | 44.80 ± 1.01 | 41.05 ± 0.92 |
| T2 | 54.12 ± 1.15 | 46.04 ± 1.12 | 40.59 ± 1.10 |
| T3 | 53.80 ± 1.01 | 42.70 ± 0.89 | 36.24 ± 0.95 |
| T4 | 53.26 ± 0.91 | 42.91 ± 1.03 | 37.12 ± 1.07 |
| T5 | 54.07 ± 1.10 | 45.77 ± 1.04 | 40.25 ± 0.90 |
| T6 | 53.90 ± 0.97 | 45.90 ± 0.90 | 41.18 ± 0.83 |

Table 6. Effect of *H. rhamnoides* extract on cholesterol, triglyceride, HDL and LDL level in broiler chickens. Chickens in the control group were fed the basal diet whereas the six treatment groups, in addition of basal diet received aqueous extract of *H. rhamnoides* in drinking water @ 100 mg/kg body weight of chicken (T1), @ 150 mg/kg body weight of chicken (T2), @ 200 mg/kg body weight of chicken (T3), @ 300 mg/kg body weight of chicken (T4), @ 400 mg/kg body weight of chicken (T5), and @ 800 mg/kg body weight of chicken (T6), respectively from days 0 to 42. Results are presented as mean ± S.E. Experimental unit 3 replicates pen (3 broiler chickens per replicate pen). Means bearing the different superscripts (a, b, c, d) in a columns differ significantly (P < 0.05).
respectively. The reduced level of cholesterol might be due to the inhibitory effect of flavonoids of *H. rhamnoides* on the activity of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase and acetyl coenzyme A. Transferase, key regulatory enzymes in cholesterol biosynthesis. Reduced level of LDL and the level of HDL in treatment group birds. HDL are referred as good cholesterol as it transport excess cholesterol to liver. In the present study, *H. rhamnoides* extract reduced the level of plasma cholesterol and LDL whereas, increased level of HDL in treatment group birds. Enhanced level of HDL in treatment group birds might be due to the ability of polyphenolic compounds of *H. rhamnoides* to transport the cholesterol from liver to peripheral tissues. Protein synthesis. Albumin protein is a useful marker of inflammation and elevated level of albumin protein in treatment group chickens could have been due to more nutritional content of *H. rhamnoides* extract under stressful conditions. Ratio of A/G was decreased in treatment groups and this might be due to increase globulin concentration, which also indicates improved immunity in birds. Table 7. Effect of *H. rhamnoides* extract on glucose, creatinine, AST and ALT level in broiler chickens. Chickens in the control group were fed the basal diet whereas the six treatment groups, in addition of basal diet received aqueous extract of *H. rhamnoides* in drinking water @ 100 mg/kg body weight of chicken (T1), @ 150 mg/kg body weight of chicken (T2), @ 200 mg/kg body weight of chicken (T3), @ 300 mg/kg body weight of chicken (T4), @ 400 mg/kg body weight of chicken (T5), and @ 800 mg/kg body weight of chicken (T6), respectively from days 0 to 42. Results are presented as mean ± S.E. Experimental unit 3 replicates pen (3 broiler chickens per replicate pen). Means bearing the different superscripts (a, b, c, d) in a column differ significantly (P < 0.05).
Similarly, *H. rhamnoides* extract decreased the glucose level in birds and this might be due to reduce gluco-
neogenesis with decrease in the glucocorticoid secretion by *H. rhamnoides* flavonoids45. Plasma creatinine level
did not differ among the groups which exhibit non-toxic and non-pathological effect of *H. rhamnoides*
extract. Hence, *H. rhamnoides* extract provides protection from oxidative stress due to its higher
radical scavenging activity in broiler chickens at high altitude.

In this study, *H. rhamnoides* extract was supplemented to broiler birds as a source of antioxidant and the
extracts was found to possess higher TAC and free radical scavenging capacity, as determined by reduced DPPH
and ABTS activity. Additionally, antioxidant parameters such as MDA, FRAP, and DPPH were analysed too in
blood plasma samples of chickens for determining the effect of *H. rhamnoides* extract on antioxidant enhance-
ment. Results indicated that supplementation of *H. rhamnoides* in broilers enhanced the level of TAC and free
radical scavenging activity while reduced the level of MDA in plasma samples. In free radical scavenging process
the antioxidants present in *H. rhamnoides* may prevent oxidation of biological molecules by reducing the rate of
free radical chain initiation e.g., either by scavenging initiating free radicals or by stabilizing transition metal rad-
icals such as copper and iron5. Moreover, increased in the antioxidant defense level and decreased in the oxidative
stress marker MDA might probably due to the synergistic effect of phenolics, flavonoids, and carotenoids that
were present in *H. rhamnoides* fruit extract. Earlier reports of Purushothaman et al.44 and Zhou et al.46 revealed a
marvellous depletion in MDA concentration and higher antioxidant defense level in animals with the supplementa-
tion of *H. rhamnoides* extract. *H. rhamnoides* extract provides protection from oxidative stress due to its higher
antioxidative activity with its ability to scavenge free radicals47 and its ability to reduce the activation of caspase-3,
downregulating the expression of pro-apoptotic genes Bax, and upregulating the expression of anti-apoptotic
genes Bcl-248. Better growth performance in broilers in this study could also be connected with potent antioxidia-
tive properties of the *H. rhamnoides* extracts. Hence, *H. rhamnoides* has beneficial effect on nutrient digestibility
and scavenging of free radical and therefore, could be applicable as a broilers feed additive at high altitude.

In conclusion, this is a first report that demonstrated the feed additive potential of *H. rhamnoides* fruit extract
in broiler chickens at high altitude. *H. rhamnoides* extract containing phenolics, flavonoids, carotenoids content
ameliorate the hypobaric hypoxia induced reduced growth performance in broiler chickens through attenuating the

| Groups | 0 day | 1st day | 2nd day | 3rd day |
|--------|-------|---------|---------|---------|
| MDA (nmol/mL) |       |         |         |         |
| Control | 8.61 ± 0.58 | 8.31 ± 0.30 | 8.06 ± 0.18 |
| T1     | 8.58 ± 0.68 | 6.05 ± 0.26 | 5.81 ± 0.12 |
| T2     | 8.59 ± 0.64 | 6.32 ± 0.24 | 6.57 ± 0.14 |
| T3     | 8.61 ± 0.67 | 5.91 ± 0.13 | 4.04 ± 0.32 |
| T4     | 8.63 ± 0.63 | 6.47 ± 0.16 | 5.49 ± 0.18 |
| T5     | 8.59 ± 0.70 | 6.94 ± 0.14 | 5.47 ± 0.13 |
| T6     | 8.97 ± 0.48 | 6.93 ± 0.16 | 5.50 ± 0.08 |

Table 8. Effect of *H. rhamnoides* extract on MDA, TAC, and DPPH free radical-scavenging activity in broiler
chickens. Chickens in the control group were fed the basal diet whereas the six treatment groups, in addition
of basal diet received aqueous extract of *H. rhamnoides* in drinking water @ 100 mg/kg body weight of chicken (T1), @ 150 mg/kg body weight of chicken (T2), @ 200 mg/kg body weight of chicken (T3), @ 300 mg/kg body
weight of chicken (T4), @ 400 mg/kg body weight of chicken (T5), and @ 800 mg/kg body weight of chicken
(T6), respectively from days 0 to 42. Results are presented as mean ± S.E. Experimental unit 3 replicates pen
(3 broiler chickens per replicate pen). Means bearing the different superscripts ( a, b, c, d) in a columns differ
significantly (P < 0.05).
effect of oxidative stress. *H. rhamnoides* extract improves the antioxidant defense level in broilers which also contributes to increased growth. Net economic return revealed the higher profit in *H. rhamnoides* supplemented groups because of lesser mortality. Moreover, *H. rhamnoides* extract at dose concentration of 200mg/kg body weight of chicken has shown better effect as compared to other dose regime. Therefore, considering the beneficial effects of *H. rhamnoides*, a new feed additive may be prepared/formulated using the same extract in certain ration or preparation.

References

1. Biswas, A., Bharti, V. K., Deshmukh, P. B., Venkatesan, G. & Srivastava, R. B. Commercial poultry farming in cold arid region of Leh-Ladakh. In: Innovatives in Agro Animal Technologies (eds. Srivastava, R. B. & Selvamurthy, W.) 216–233 (New Delhi, 2011).
2. Kalia, S., Bharti, V. K., Gogoi, D., Giri, A. & Kumar, B. Studies on the growth performance of different broiler strains at high altitude and evaluation of probiotic effect on their survivability. *Sci. Rep.* 7, 46074, https://doi.org/10.1038/srep46074 (2017).
3. Kalia, S., Bharti, V. K., Giri, A. & Kumar, B. Effect of *Prunus armeniaca* seed extract on health, survivability, antioxidant, blood biochemical and immune status of broiler chickens at high altitude cold desert. *J. Adv. Res.* 8, 677–686 (2017).
4. Miller, L. E. *et al.* Blood oxidative-stress markers during a high-altitude trek. *Int. J. Sport. Nutr. Exerc. Metab.* 23, 65–72 (2013).
5. Papadopoulo, A. *et al.* Enhancement of antioxidant mechanisms and reduction of oxidative stress in chickens after the administration of drinking water enriched with polyphenolic powder from olive mill waste waters. *Oxid. Med. Cell. Longev.* 2017, 8273160, https://doi.org/10.1155/2017/8273160 (2017).
6. Young, J. & Woodside, J. Antioxidants in health and disease. *Clin. Pathol.* 54, 176–186 (2001).
7. Kala, C. P. Medicinal plants of the high altitude cold desert in India: diversity, distribution and traditional uses. *Int. J. Biodivers. Sci. Manage.* 2, 43–56 (2006).
8. Smania, T. & Millard, C. The preservation and development of *Amchh* medicine in Ladakh. *East Asian Sci. Technol. Soc. Int. J.* 7, 487–504 (2013).
9. Ballabh, B., Chaurasia, O. P., Ahmed, Z. & Singh, S. B. Traditional medicinal plants of cold desert Ladakh-used against kidney and urinary disorders. *J. Ethnopharmacol.* 118, 331–339 (2008).
10. Sagg, S. *et al.* Adaptogenic and safety evaluation of seabuckthorn leaf extract: A dose dependent study. *Food. Chem. Toxicol.* 45, 609–617 (2007).
11. Beveridge, T., Li, T. S. C. & Oomah, B. D. Seabuckthorn products: manufacture and composition. *J. Agric. Food. Chem.* 47, 3480–3488 (1999).
12. Christkai, E. *Hippophae rhamnoides* L. (sea buckthorn): a potential source of nutraceuticals. *Food. Public. Health.* 2, 69–72 (2012).
13. Maheshwari, D. T., Kumar Yogendra, M. S., Verma, S. K., Singh, V. K. & Singh, S. N. Antioxidant and hepatoprotective activities of phenolic rich fraction of seabuckthorn (*Hippophae rhamnoides*) leaves. *Food. Chem. Toxicol.* 49, 2422–2428 (2011).
14. Geetha, S., Sai Ram, M., Singh, V., Ilavazhahan, G. & Sawnhney, R. C. Antioxidant and immunomodulatory properties of seabuckthorn (*Hippophae rhamnoides*)- an in-vitro study. *Ethnopharmacol.* 79, 373–378 (2002).
15. Tulsawani, R. Ninety days repeated gavage administration of *Hippophae rhamnoides* extract in rats. *Food. Chem. Toxicol.* 48, 2483–2489 (2010).
16. Yasukawa, K., Kitakawa, K., Kawata, K. & Goto, K. Anti-tumor promoters phenolics and triterpenoid from *Hippophae rhamnoides*. *Fitoterapia.* 80, 164–167 (2009).
17. Goel, H. C., Prasad, I., Singh, S., Sagar, R. & Sinha, A. K. Radioprotection by a herbal preparation of *Hippophae rhamnoides* RH-3, against whole body lethal irradiation in mice. *Phytomedicine.* 9, 15–25 (2002).
18. Eccleston, C. *et al.* Effect of antioxidant-rich juice (seabuckthorn) on risk factors for coronary heart disease in humans. *Nutr. Biochem.* 13, 346–354 (2002).
19. Biswas, A., Bharti, V. K., Acharya, S., Pawar, D. D. & Singh, S. B. Seabuckthorn: new feed opportunity for poultry at cold arid Ladakh region of India. *World Poult. Sci. J.* 66, 707–714 (2010).
20. Ma, J. S. *et al.* Effects of flavonoids of seabuckthorn fruits on growth performance, carcass quality, fat deposition and lipometabolism for broilers. *Poult. Sci.* 94, 2641–2649 (2015).
21. Benzie, I. F. & Strain, J. J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power". The FRAP assay. *Anal. Biochem.* 239, 70–76 (1996).
22. Brand-Williams, W., Cuvelier, M. E. & Berset, C. Use of a free radical method to evaluate antioxidant activity. *LWT- Food. Sci. Technol.* 28, 25–30 (1995).
23. Re, R. *et al.* Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free. Radic. Biol. Med.* 26, 1231–1237 (1999).
24. Gao, X., Ohlander, M., Jeppsson, N., Bjork, L. & Trukovski, V. Changes in antioxidant effects and their relationship to phytonutrients in fruits of seabuckthorn (*Hippophae rhamnoides*) during maturation. *J. Agric. Food. Chem.* 48, 1485–1499 (2000).
25. Ordonez, E. A., Gomez, J. D., Vattuone, M. A. & Isla, M. I. Antioxidant activities of *Sechium edule* Swart extracts. *Food. Chem.* 97, 452–458 (2006).
26. Ranjith, A. *et al.* Fatty acids, tocids, and carotenoids in pulp oil of three seabuckthorn species (*Hippophae rhamnoides*, *H. salicifolia*, and *H. tibetana*) grown in the Indian Himalayas. *J. Am. Oil. Chem. Soc.* 83, 359–364 (2006).
27. Gupta, S., Aggarwal, S., Lee, D. & Starr, A. Cytokine production by adherent and non-adherent mononuclear cells in chronic fatigue syndrome. *J. Psychiatr. Res.* 31, 49–56 (1997).
28. Mosmann, T. Rapid calorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods.* 65, 55–63 (1983).
29. Buege, J. A. & Aust, S. D. The thiobarbituric acid assay. *Methods. Enzymol.* 52, 306–307 (1978).
30. Kanayama, Y. *et al.* Research progress on the medicinal and nutritional properties of sea buckthorn (*Hippophae rhamnoides*) – a review. *J. Hort. Sci. Biotechnol.* 87, 203–210 (2012).
31. Flogel, A., Kim, D. O., Chung, S. I., Koo, S. I. & Chun, O. K. Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. *J. Food. Compos. Anal.* 24, 1043–1048 (2011).
32. Chen, C. *et al.* Identification, quantification, and antioxidant activity of acetylated flavonol glycosides from sea buckthorn (*Hippophae rhamnoides* spp. *sinesis*). *Food. Chem.* 141, 1573–1579 (2013).
33. Dorhoi, A., Dobren, V., Zaham, M. & Virag, P. Modulatory effects of several herbal extracts on avian peripheral blood cell immune responses. *Phytother. Res.* 20, 352–356 (2006).
34. Balog, I. M. *et al.* Ascsitez syndrome and related pathologies in feed restricted broilers raised in a hypobaric chamber. *Poult. Sci.* 79, 518–523 (2000).
35. Kalia, S., Bharti, V. K., Giri, A. & Kumar, B. 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. *Indian. J. Anim. Sci.* 87, 1200–1206 (2017).
36. Suryakumar, G. & Gupta, A. Medicinal and therapeutic potential of sea buckthorn (*Hippophae rhamnoides* L.). *Ethnopharmacol.* 138, 268–278 (2011).
37. Ritchie, R. F. *et al.* Reference distributions for the negative acute phase serum proteins, albumin, transferrin, and transthyretin: a practical, simple and clinically relevant approach in a large cohort. *J. Clin. Lab. Anal.* 13, 273–279 (1999).
38. Mediavilla, V. G. et al. The anti-inflammatory flavones quercetin and kaempferol cause inhibition of inducible nitric oxide synthase, cyclooxygenase-2 and reactive C-protein, and down-regulation of the nuclear factor kappaB pathway in Chang Liver cells. *Eur. J. Pharmacol.* 557, 221–229 (2007).

39. Shali, P. K., Kaul, S., Nilsson, J. & Cercek, B. Exploiting the vascular protective effects of high-density lipoprotein and its apolipoproteins: an idea whose time for testing in coming. *Circulation*. 104, 2376–2383 (2001).

40. Theriault, A. et al. Modulation of hepatic lipoprotein synthesis and secretion by taxifolin, a plant flavonoid. *J. Lipid. Res.* 41, 1969–1979 (2000).

41. Lien, T. F., Yeh, H. S. & Su, W. T. Effect of adding extracted hesperetin, naringenin and pectin on egg cholesterol, serum traits and antioxidant activity in laying hens. *Arch. Anim. Nutr.* 62, 33–43 (2008).

42. Cao, Q. et al. Effect of flavonoids from the seed and fruit residues of *Hippophae rhamnoides* L. on glycometabolism in mice. *J. Chin. Med. Mater.* 26, 735–737 (2003).

43. Nyhlm, H., Bjorsson, E., Aldenborg, F., Almer, S. & Olsson, R. The AST/ALT ratio as an indicator of cirrhosis in patients with PBC. *Liver. Int.* 26, 840–845 (2006).

44. Purushothaman, J. et al. Modulatory effects of seabuckthorn (*Hippophae rhamnoides* L.) in hypobaric hypoxia induced cerebral vascular injury. *Brain Res. Bull.* 77, 246–252 (2008).

45. Zhou, J., Zhou, S., Gao, Y. & Zeng, S. Modulatory effects of quercitin on hypobaric hypoxia rats. *Eur. J. Pharmacol.* 674, 450–454 (2012).

46. Rop, O., Ercisli, S., Milcek, J., Jurikova, T. & Hoza, I. Antioxidant and radical scavenging activities in fruits of 6 sea buckthorn (*Hippophae rhamnoides* L.) cultivars. *Turk. J. Agric. For.* 38, 224–232 (2014).

47. Wang, Y. et al. Protective effect of proanthocyanidins from sea buckthorn (*Hippophae rhamnoides*) seed against visible light-induced retinal degeneration *in vivo*. *Nutrients.* 8, 245 (2016).

Acknowledgements
The present study was fully supported by Defence Research and Development Organisation (DRDO), Ministry of Defence, Government of India. The authors would particularly like to thank Dr R S Chauhan and Dr Udayabanu Malairaman for providing in vitro facility at JUIT, Solan. Authors would like to thank Mr. Arun Sharma for helping in in vitro studies and all the staff of the DIHAR poultry division for the care of chicken and for their assistance during the blood sampling. Authors would also like to acknowledge Dr Vineeth Ravindran T. for his technical assistance.

Author Contributions
S.K. performed the experiments. A.G. and A.A. helps in collection of data. V.K.B., B.K. and S.S.B. conceived the project idea, designed the study and supervised the project. S.K. and V.K.B. analyzed the data and wrote the manuscript. All authors have read and approved the manuscript.

Additional Information
Competing Interests: The authors declare no competing interests.

Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2018