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Exploring the immunomodulatory and antioxidant capacities of *Berberis aristata* in avian lymphocytes

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ABSTRACT

India is one of the leading producers for eggs and broilers globally and thus has witnessed significant growth in the poultry sector. Different poultry stressors can negatively impact the growth, gut health, immune function and production reproductive performances of poultry. Antibiotic growth promoters (AGPs) have been used in the poultry industry as they also combat stress in poultry along with growth promotion. But the use of antimicrobials as growth promoter raises the concern of antibiotic resistance and its potential hazardous effect on human health as well as the environment. Several other alternatives are available for AGPs such as acidifiers, probiotics, prebiotics, antibacterial peptides and phytobiotics. Phytobiotics are known to possess several benefits for poultry as well as livestock such as immunomodulation, anti-inflammatory effect, antioxidant effect, maintain intestinal health *etc*. One such important plant is *Berberis aristata* belonging to the family Berberidaceae which is used in traditional system of medicine for the management of various inflammatory disorders. In the present study aqueous root extract of *Berberis aristata* (BAE) was evaluated for its immunomodulatory and antioxidant activity. The extraction yield of the roots of *Berberis aristata* in water was about 9.78%. The BAE showed presence of phenolics, flavonoids, tannins, terpenoids and contained about 104.97 mg/gm of BAE and 83.14 mg/gm of BAE of phenolics and flavonoids, respectively. BAE showed significant antioxidant activity in DPPH free radical scavenging assay. Maximum non-cytotoxic dose (MNCD) of BAE for lymphocyte culture was determined to be 600 µg/ ml. Lymphocytes proliferation assay (LPA) showed that BAE enhanced lymphocyte proliferation of B-cells stimulated by lipopolysaccharide (LPS) and T-cells stimulated by Concanavalin A and Phytohaemagglutinin M (PHA). Treatment with BAE significantly reduced lipid peroxidation (LPO) and increased the level of reduced glutathione, superoxide dismutase, and catalase in chicken lymphocytes, thus indicating antioxidant potential of BAE. The study reveals significant immunomodulatory and antioxidant properties of BAE, thus suggesting that it could be a potential alternative to AGPs in poultry production. But further *in vivo* studies are required to explore its efficacy and safety for its application in commercial poultry farming.

Keywords: *Berberis aristata*, Chicken, Lymphocytes proliferation assay, Immunomodulation, Antioxidant.

INTRODUCTION

A significant growth has been observed in poultry sector in India as a result of which India has become the third-largest producer of eggs and the eighth-largest producer of broilers in the world and around 5-6% of annual growth is observed in the sector [1]. Poultry meat has become the most consumed meat in India. Long term exposure to sub-therapeutic level of antibiotics has elevated antibiotic resistance in bacterial population which is affecting farm animals, humans as well as the environment and holds a huge risk of zoonosis from poultry sector [2,3]. Due to the risks associated with antibiotic growth promoters (AGPs) effective alternatives are required which include acidifiers, probiotics, prebiotics, antibacterial peptides and phytobiotics which are considered promising alternative to AGPs [4]. Phytoconstituents are known to serve various benefits to livestock and poultry such as immunomodulation, anti-inflammatory properties, antioxidant properties, maintain intestinal health *etc*. [5,6]. Recent reports indicate that herbal products play a significant role in boosting immune response and are in prominence due to their potential health benefits and minimal side effects [7].

Immunomodulation refers to altered activity of immune system which can either be increased that is referred to as immunostimulation/ immunopotentiation or decreased that is referred to as immunosuppression [8]. Along with boosting immunity, the usage of herbal products in diet, additionally improves gut microbiome and reduce inflammation [9]. One such important herbal plant is *Berberis aristata* belonging to the family Berberidaceae which is used in traditional system of medicine for the management of various inflammatory disorders [10]. It is a hard, spinous and glabrous yellowish evergreen herb which is majorly found growing in sub-Himalayan region and Nilgiri Hills of Southern India. The major alkaloid present in *Berberis aristata* is berberine, which possess very important

pharmacological activities and may be present either in roots, stem bark, rhizomes or leaves followed by palamatine [11]. Alcoholic and aqueous extract of *Berberis* species was found to suppress the proliferation of T-cells and enhance the expansion of B-cells along with alteration in production of different cytokines in lymphocytes [12]. Aqueous extract of roots of *Berberis aristata* are known to possess anti-inflammatory activity when tested in rats at the rate of 500-1000 mg/kg [13]. Berberine can directly benefit the immune system by modulating naïve CD4+ T cells differentiation in adjuvant-induced arthritis model of rats [14]. Hydroalcoholic extract of *Berberis aristata* showed anti-inflammatory properties in experimental rat model of carrageenan-induced paw edema and cotton pellet-induced granuloma [10]. Aqueous extract of bark of *Berberis aristata* suppressed ear thickness and increased the content of Reduced glutathione (GSH) and Superoxide dismutase (SOD) in ear homogenate in extract treated dermatic mice [15].

The present study was based on the hypothesis that aqueous extract of *Berberis aristata* (BAE) will exhibit immunomodulatory and anti-oxidant effect. BAE was subjected to various biochemical assays to detect presence of various phytoconstituents. Lympocytes proliferation assay and *in vitro* antioxidant assays were conducted to evaluate the immunomodulatory and antioxidant potential due to *in vitro* exposure of BAE in chicken lymphocytes.

MATERIALS AND METHODS

Collection of the Plant Material and Preparation of Plant Extract

The roots of *Berberis aristata* were collected from the Berinag (Distt. Pithoragarh) of Uttarakhand, India (29.80°N, 80.07°E), which were then authenticated by Dr. D.S. Rawat, Assistant Professor, Department of Biological Sciences, College of Basic Sciences and Humanities, G.B. Pant University of Agriculture & Technology, Pantnagar, Uttarakhand, India (Voucher specimen No.- 1207). Then the aqueous root extract of *Berberis aristata* (BAE) was prepared [16]. The study employed usage of cell culture grade chemicals.

Phytochemical Analysis of the Plant Extract

Various qualitative and quantitative analyses were performed in the plant extract. Qualitative tests were carried out to detect the presence of phenolics, flavonoids, tannins and terpenoids in the plant extract [17]. For quantitative analysis of the plant extract total phenolics and total flavonoids of the plant extract was measured [18].

Antioxidant potential of the Plant Extract

Anti-oxidant potential of the plant extract was determined by using DPPH radical scavenging assay [19]. As per the method, different concentrations of BAE were dissolved in methanol which were then mixed with 0.1mM methanolic DPPH solution. After incubating the mixture for 30 minutes at room temperature, the absorbance was taken at 517 nm and the inhibition percentage of DPPH was calculated.

Collection of chicken spleens

Spleens used in the present study were collected from healthy Cobb strain broiler chicken aged 4-6 weeks from the local slaughterhouse in sterile Dulbecco's phosphate buffer saline (DPBS). Then using the standard protocol and under sterile conditions, the spleens were processed promptly to isolate the lymphocytes.

Isolation of chicken lymphocytes from spleens

Lymphocytes were isolated from chicken spleens. In brief, spleens were minced using sterile scissors after being places in a sterile Petri dish containing RPMI-1640 media. Density gradient centrifugation was used to separate the lymphocytes and the cell suspension was then layered upon lymphocytes separation media (LSM) at a density of 1.077 g/ml, and then centrifuged at 400 x g for 30 minutes at room temperature. After being carefully separated from the interface, the lymphocytes were washed twice with DPBS at 250 x g for 5 min at room temperature after which they were suspended in RPMI-1640 medium. The trypan blue dye exclusion assay showed that more than 95% of the cells were viable [20].

Maximum non-cytotoxic dose determination

Maximum non-cytotoxic dose determination (MNCD) of BAE was determined. Briefly, chicken lymphocytes were placed in a 96-well flat bottom plate at a concentration of 1×10^6 cells per ml which were then exposed to different concentration of BAE (15-3000 µg/ml) in triplicate and kept in a CO_2 incubator (at 40°C with 5% CO_2) for 68 hours. Then, the cells were examined under an inverted microscope to observe for any signs of cytotoxicity caused by BAE exposure. After 68 hours of incubation period, the media from each well was removed carefully, and then 20 µl of MTT solution (5 mg/ml stock) diluted in 200 µl of media was added to each well and the plate was further incubated for 4 hours in the dark at 40°C in the CO_2 incubator. After completion of the incubation, the media was removed and the dark blue formazan crystals were dissolved by adding 200 µl of dimethyl sulfoxide (DMSO) to each well and then the absorbance was measured at 570 nm using an ELISA Reader. The cell survival rate was calculated by comparing the absorbance of the treated cells with that of the control cells and then expressed as percentage [20].

Lymphocytes proliferation assay

Lymphocytes proliferation assay (LPA) was performed [20]. Mitogenic stimulation of the lymphocytes was performed using different cell culture tested mitogens *i.e.*, Concanavalin A (ConA), Phytohaemagglutinin M form (PHA-M) and *Escherichia coli* (serotype 0111:B4) derived lipopolysaccharide (LPS) each at a concentration of 5 µg/ml in RPMI-1640 medium. 200 µl of lymphocytes suspension (1×10^6 cells per ml) was added to flat bottom 96-well tissue culture plate, which were then exposed to MNCD of BAE in presence of different mitogens in triplicate.

Evaluation of antioxidant status of BAE exposed cells

After treating chicken lymphocytes with MNCD of BAE followed by incubation of 68 hrs in CO_2 incubator, the control and BAE treated cells were harvested and cell lysate was prepared which was stored at -80°C to determine the antioxidant status. Ascorbic acid was used as positive control [20].

Membrane lipid peroxidation

The lipid peroxidation (LPO) was estimated in which malondialdehyde (MDA) served as a biochemical indicator for LPO which reacts with thiobarbituric acid (TBA) to produce a coloured product and the reaction can be quantified by taking absorbance at 532 nm. The results were expressed in nmol MDA/mg protein [21].

Reduced glutathione

The level of reduced glutathione (GSH) in cell lysate was measured using Ellman's reaction. In this reaction DTNB (5,5′-dithiobis-2-nitrobenzoic acid) when added to the compounds containing sulfhydryl groups produced a yellow-coloured product which was quantified by measuring absorbance at 412 nm and the final results were expressed as mM of GSH per ml [22].

Superoxide dismutase

The activity of superoxide dismutase (SOD) was based on the reaction involving production of superoxide via autooxidation of pyrogallol and then measuring the inhibition of superoxide-dependent reduction of the tetrazolium dye MTT, and the absorbance was measured at 570 nm. DMSO was added to the reaction mixture to cease the reaction and assist the solubilization of the formed formazan during the

reaction. The activity of SOD was expressed in the SOD units, where 1 unit of SOD represented the amount of protein (in mg) which is required to inhibit the MTT reduction by 50% [23].

Catalase

The activity of catalase (CAT) in the cell lysate was assessed depending on the principle that enzyme CAT present on the sample decomposes two molecules of H_2O_2 to two molecules of H_2O and O_2. There is decrease in absorbance at 240 nm after decomposition of H_2O_2 and the change in absorbance over time (ΔA_{240}) is taken as the measure of CAT activity [24].

Statistical analysis

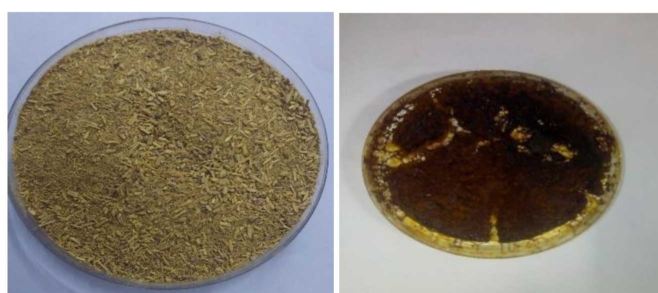
The data analysis was conducted using Origin Software and the findings were presented as Means ± standard deviation. One-way analysis of variance (ANOVA) was used to find any statistically significant difference between treated and control groups. $p<0.05$ was considered as statistically significant.

RESULTS

Phytochemical Analysis of the Plant Extract

The authenticated plant material was subjected to aqueous extract preparation with the percent yield of BAE obtained as 9.78%. (Figure 1, Table 1). The extract showed presence of phenolics, flavonoids, tannins and terpenoids as presented in Table 2. The total phenolic and flavonoid content of BAE was found to be 104.96±1.706 mg Gallic acid equivalent (GAE) /gm extract and 83.15±1.481 mg Gallic acid equivalent (GAE) /gm extract, respectively (Table 3).

a) *Berberis aristata* plant **b)** *Berberis aristata* roots



c) *Berberis aristata* dried roots powder **d)** Aqueous extract of root *Berberis aristate*

Figure 1: Berberis aristata

Table 1: Percent yield of aqueous extract of BAE

S. No.	Plant name	Weight of dried plant material powder (g)	Weight of extract obtained (g)	Percent yield
1.	*Berberis aristata*	100	9.78	9.78

Table 2: Qualitative test of BAE

S. No.	Phytochemical Analysis	Roots extract of *Berberis aristata* (BAE)
1.	Phenolics	+
2.	Flavonoids	+
3.	Tannins	+
4.	Terpenoids	+

Table 3: Quantitative test of BAE for presence of phenolics and flavonoids

S. No.	Quantitative Analysis	BAE
1.	Total phenolics content (mg GAE /g extract) ±SD	104.96±1.706
2.	Total flavonoids content (mg GAE /g extract) ±SD	83.15±1.481

Determination of Antioxidative Potential of BAE by DPPH free radical scavenging assay

In DPPH free radical scavenging assay, with the increase in concentration of BAE there was increase in percent scavenging of DPPH free radicals and the ascorbic acid (AA) was kept as control as shown in Figure 2.

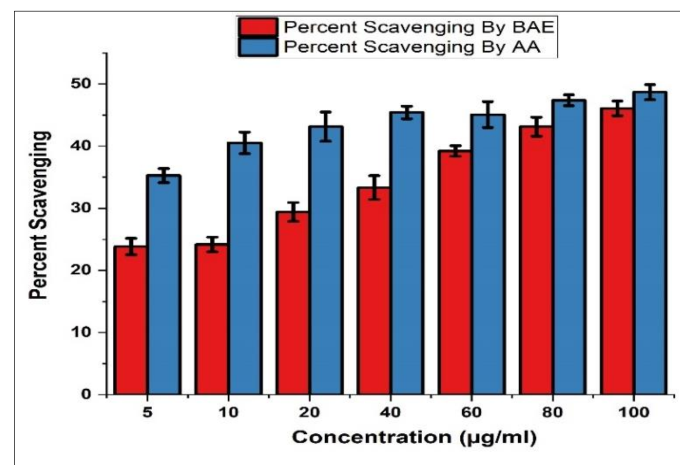


Figure 2: Percent scavenging of BAE and AA in DPPH assay

Maximum non-cytotoxic dose determination of BAE

The MNCD of BAE in chicken lymphocytes was found to be 600 µg/ml through MTT assay (Figure 3). This dose level of BAE was used for further *in vitro* experimentation to explore its immunopotentiating and antioxidant potential.

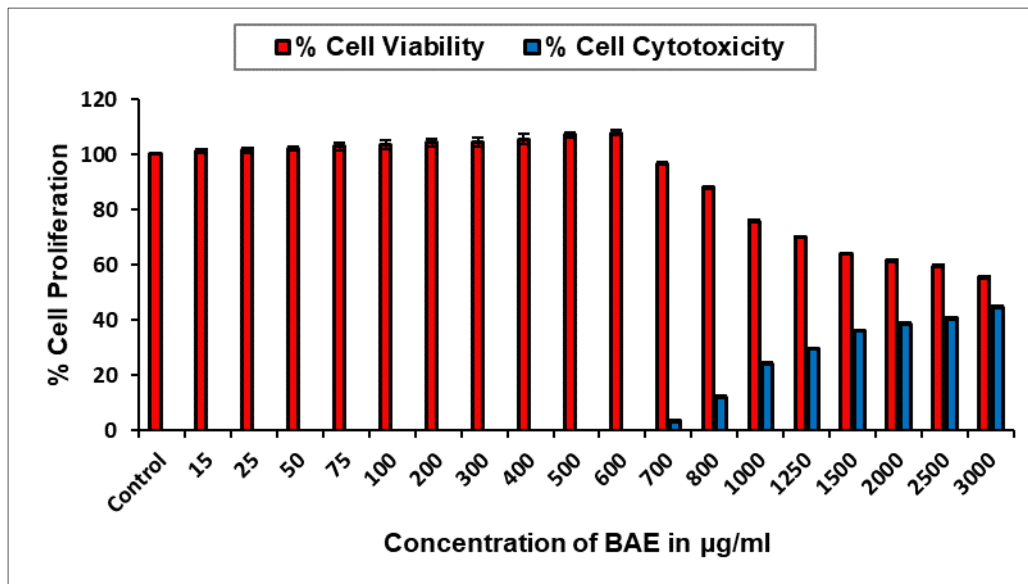


Figure 3: Percent cell viability of chicken lymphocytes in presence of different concentrations of BAE

Effect of BAE on Lymphocytes Proliferation under mitogenic stimulation

There was marked increase in B and T cells proliferation when mitogen stimulated (LPS, Con A, PHA) cells were co-treated with BAE as compared to the respective controls (Table 4, Figure 4).

Table 4: Effect of BAE on mitogen stimulated lymphocytes proliferation

S. No.	Treatment	LPS stimulated		Con A stimulated		PHA stimulated	
		% Proliferation	% Change in proliferation	% Proliferation	% Change in proliferation	% Proliferation	% Change in proliferation
1.	**Control**	100	-	100	-	100	-
2.	**BAE**	107.36	7.36	127.41	27.41	138.24	38.24

Table 5: Antioxidative status of chicken lymphocytes treated with BAE

S. No.	Treatment	Lipid Peroxidation (nM MDA/g)	Reduced Glutathione (mM/ml)	Superoxide Dismutase (SOD units/mg of protein)	Catalase (H_2O_2 utilized mM/min/mg of protein)
1.	**Control**	140±3.077^{A}	0.068±0.007^{C}	38.125±1.49^{C}	158.334±0.921^{C}
2.	**BAE**	111.283±2.351^{B}	0.168±0.005^{B}	47.124±1.21^{B}	167.227±1.397^{B}
3.	**AA**	72.308±3.077^{C}	0.259±0.004^{A}	60.708±1.553^{A}	175.567±1.19^{A}
SEM		2.33131	0.00425	1.16338	0.96762

The values (Mean ± SD) having different superscripts in columns differ significantly ($p< 0.05$)

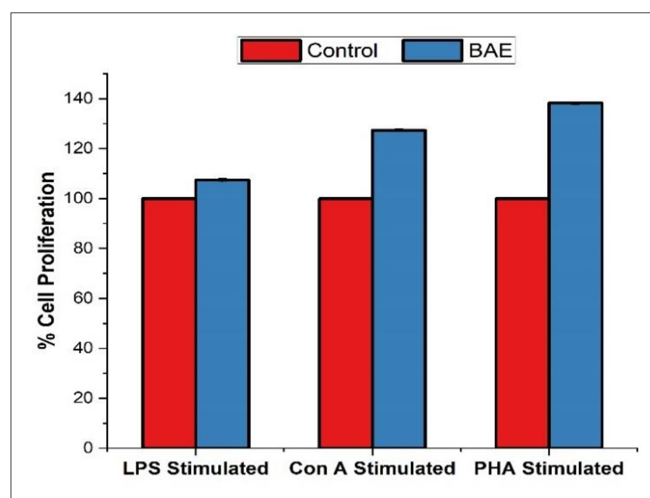


Figure 4: Effect of BAE exposure on mitogen stimulated lymphocytes proliferation

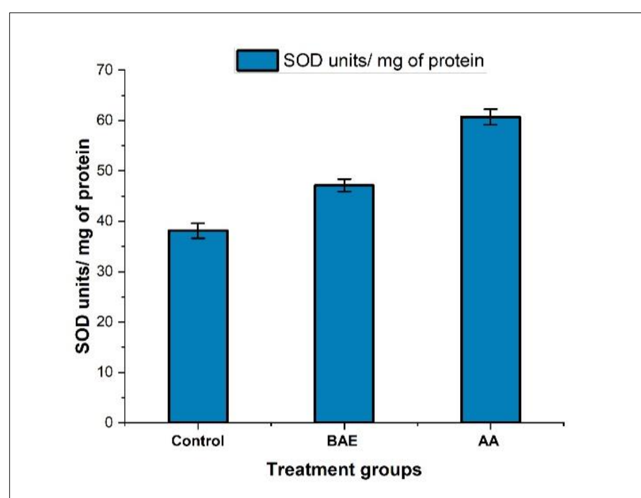


Figure 7: SOD Content in chicken lymphocytes treated with BAE

Evaluation of antioxidant status of BAE exposed cells

BAE was found to improve the antioxidant status of the chicken lymphocytes due to its *in vitro* exposure as revealed through various antioxidant assays. There was decrease in lipid peroxidation and increase in reduced glutathione, SOD and catalase activity as shown in Table 5 and Figure 5,6,7 and 8 respectively.

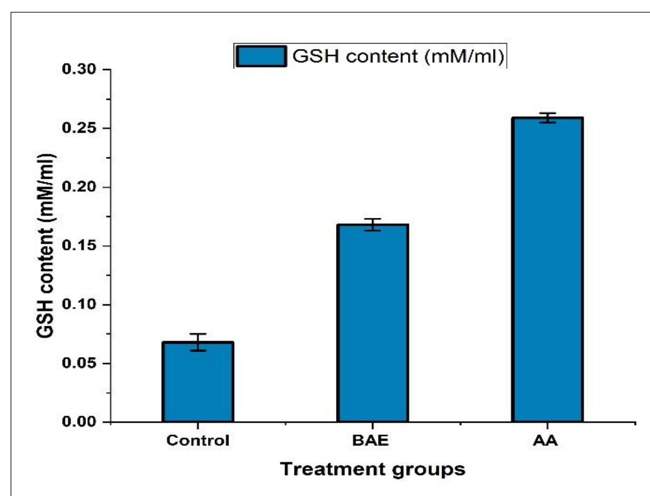


Figure 5: Reduced glutathione content in chicken lymphocytes treated with BAE

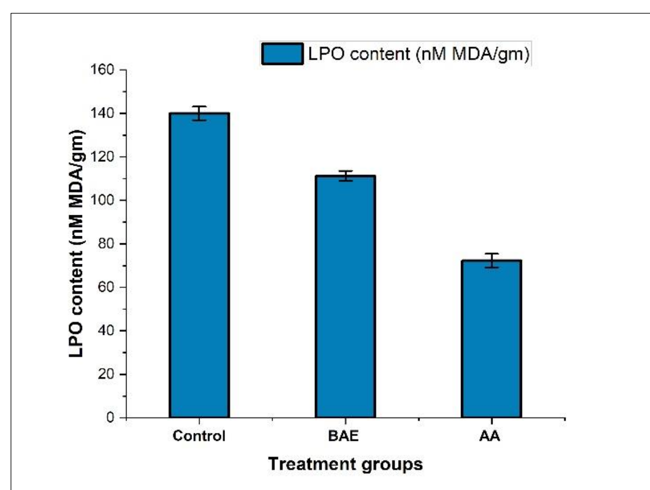


Figure 6: Lipid peroxidation in chicken lymphocytes treated with BAE

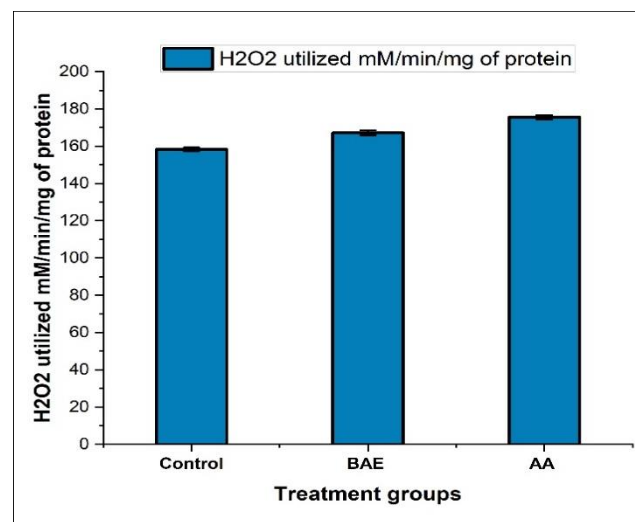


Figure 8: Catalase activity in chicken lymphocytes treated with BAE

DISCUSSION

Different classes of secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, sterols, *etc*. are present in the plant [25]. Several phytoconstituents have been reported from the plant *Berberis aristata* majority of which constitute alkaloids such as berberine, oxyberberine, berbamine, aromoline, karachine, palmatine, oxyacanthine, taxilamine, protoberberine and bis isoquinoline [26]. Methanolic extract of *Berberis aristata* stem was found to be rich in different alkaloids, phenols, flavonoids such as berberine, palmatine, benzoic acid, hydroquinone, *etc*. [27]. 0.11% of total phenol in response to standard gallic acid and 2.8% of total flavonoids in response to standard rutin have been reported from the plant [28]. The methanolic extract of stem of *Berberis aristata* showed the presence of alkaloids, coumarin, flavonoids, glycosides, polyphenols, reducing sugars, saponins, steroids, tannins and triterpenoids and the extract showed a DPPH free radical scavenging activity with an IC_{50} value of 33.31μg/ml [29]. Leaf, stem and bark extract of *Berberis aristata* showed an average of 89.14% DPPH radical scavenging activity [30]. Methanolic extracts of the stems of *Berberis aristata* exhibited high antioxidant activity as it scavenged DPPH, superoxide and nitric oxide radical and chelated metal ion in a dose dependent manner [27].

The immunomodulatory action of berberine which is a major alkaloid of *Berberis aristata* is due to its interaction with different immune cells like macrophages, T cells, B cells, mast cells, dendritic cells,

epithelial cells, keratinocytes *etc*. [31]. Splenocytes obtained from Berberine treated mice express enhanced proliferative response to the T dependent mitogens (ConA and PHA) [32]. Pre-treatment with berberine was able to inhibit the LPS (*Escherichia coli* O55:B5) induced activation of NF-KB/MAPK signalling pathway and thus inhibiting the production of inflammatory factors [33]. Splenocytes of mice immunized with *Berberis vulgaris* treated vaccine showed 197% increase in CD16+ population, 33% increase in $MHCII^+$ population, and 43% decrease in $CD3^+$ population [34]. *Berberis* spp. is known to possess antioxidant properties as it decreased TBARS production, decreased NO levels, inhibited DPPH oxidation and increased the activity of Glutathione peroxidase (GPx) and SOD [35]. When the mitogen stimulated lymphocytes were treated with alcoholic and aqueous extract of *Berberis* species there was decreased T cell proliferation and increased B cell proliferation [12]. 50% aqueous ethanolic root extract of *Berberis aristata* decreased oxidative stress in alloxan induced diabetic rats as there was increase CAT, SOD, GPx, glutathione reductase (GR) activity and reduced LPO (41.6%) and protein carbonylation (30.15%) [36]. It was also reported that the acute oral toxicity dose of the plant is very high and hence the plant can be considered biosafe and thus after further study they can be added to the list of novel metabolites [37].

CONCLUSION

In the present study it was found that the aqueous extract of roots of *Berberis aristata* (BAE) showed significant antioxidant and immunomodulatory activity in different *in vitro* assays in chicken lymphocytes culture system. BAE was found to be rich in different bioactive compounds. BAE also showed significant increase in lymphocyte proliferation when they were stimulated with different mitogens thus showing great potential to enhance immune response. BAE also exhibited improved antioxidant status of exposed chicken lymphocytes as observed through reduction in LPO and increase in the levels of GSH, SOD and CAT. This improved antioxidant activity states that the plant mitigates oxidative stress which is a common issue in poultry farming due to several stressors. *Berberis aristata* can serve as an effective alternative to antibiotic growth promoter as there is increasing need for sustainable farming. However, further *in vivo* research is necessary in order to fully understand the effects of *Berberis aristata* in poultry for its effective utilization as a phytogenic feed additive to mitigate several stressors in poultry.

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Conflict of interest

The authors declared no conflict of interest.

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REFERENCES

1. Ministry of Fisheries (Government of India), Basic Animal Husbandry Statistics 2022, 2023.
2. Jimenez CEP, Keestra S, Tandon P, Cumming O, Pickering AJ, Moodley A et al. Biosecurity and water, sanitation, and hygiene (WASH) interventions in animal agricultural settings for reducing infection burden, antibiotic use, and antibiotic resistance: a One Health systematic review. Lancet Planet Health. 2023;7:e418-34.
3. Choudhary A, Midha T, Gulati I, Baranwal S. Isolation, Genomic Characterization of Shigella prophage fPSFA that effectively infects multi-drug resistant Shigella isolates from the Indian Poultry Sector. Microbial Pathogenesis. 2024;188:106538.
4. Biswas S, Ahn JM, Kim IH. Assessing the potential of phytogenic feed additives: A comprehensive review on their effectiveness as a potent dietary enhancement for nonruminant in swine and poultry. J Anim Physiol Anim Nutr (Berl). 2024;1-13.
5. Liu J, Wang H, Luo J, Chen T, Xi Q, Sun J et al. Synergism of fermented feed and ginseng polysaccharide on growth performance, intestinal development, and immunity of Xuefeng black-bone chickens. BMC Veterinary Research. 2024;20(1):13.
6. Zhang D, Liu J, Cheng H, Wang H, Tan Y, Feng W, Peng C. Interactions between polysaccharides and gut microbiota: A metabolomic and microbial review. Food Research International. 2022; 160:111653.
7. Arshad MS, Khan U, Sadiq A, Khalid W, Hussain M, Yasmeen A, Asghar Z, Rehana H. Coronavirus disease (COVID-19) and immunity booster green foods: A mini review. Food Science & Nutrition. 2020;8(8):3971-6.
8. Chauhan RS, Malik YS, Saminathan M, Tripathi BN. Immunomodulation. In Essentials of Veterinary Immunology and Immunopathology 2024, 143-168. Singapore: Springer Nature Singapore.
9. Dong A, Yu J, Chen X, Wang LS. Potential of dietary supplementation with berries to enhance immunity in humans. Journal of Food Bioactives. 2021;16.
10. Kumar R, Gupta YK, Singh S. Anti-inflammatory and anti-granuloma activity of *Berberis aristata* DC. in experimental models of inflammation. Indian journal of pharmacology. 2016;48(2):155-61.
11. Andola HC, Rawal RS, Rawat MS, Bhatt ID, Purohit W. Analysis of berberine content using HPTLC fingerprinting of root and bark of three Himalayan Berberis species. Asian Journal of Biotechnology. 2010;2(4):239-45.
12. Fateh S, Dibazar SP, Daneshmandi S. Barberry's (*Berberis integerrima*) ingredients suppress T-cell response and shift immune responses toward Th2: an *in vitro* study. Future Science OA. 2015;1(4):FSO49.
13. Chaudhari VM, Kokate KK. A comprehensive review of daruharidra. International Journal of Research in Ayurveda and Medical Sciences. 2018;1:153-9.
14. Dinesh P, Rasool M. Berberine mitigates IL-21/IL-21R mediated autophagic influx in fibroblast-like synoviocytes and regulates Th17/Treg imbalance in rheumatoid arthritis. Apoptosis. 2019;24(7):644-61.
15. Shinde SD, Cheke RS, Tathe PR, Jain PG, Narkhede RR. The *Berberis aristata* ameliorates oxazolone induced contact dermatitis: In-vivo and in silico evidences. Advances in Traditional Medicine. 2021; 21:685-92.
16. Ambwani S, Goswami R, Fuloria N, Tandon R, Ambwani TK. Assessment of *in vitro* anti-urolithiasis potential of *Berberis aristata* DC. 2023; 9(2):340-348.
17. Shabbir M, Khan MR, Saeed N. Assessment of phytochemicals, antioxidant, anti-lipid peroxidation and anti-hemolytic activity of extract and various fractions of Maytenus royleanus leaves. BMC complementary and alternative medicine. 2013; 13:1-3.
18. Ben Mansour R, Gargouri B, Bouaziz M, Elloumi N, Belhadj Jilani I, Ghrabi Z etc. Antioxidant activity of ethanolic extract of inflorescence of Ormenis Africana *in vitro* and in cell cultures. Lipids in health and disease. 2011;10:1-7.
19. Shivhare Y, Singh P, Upadhyay U, Sharma S, Shukla S, Singhai AK et al. Determination of Physicochemical parameters and DPPH radical scavenging activity of Chenopodium album Linn. Pharmacognosy Journal. 2010;2(14):7-10.
20. Ambwani S, Dolma R, Sharma R, Kaur A, Singh H, Ruj A, Ambwani TK. Modulation of inflammatory and oxidative stress biomarkers due to dexamethasone exposure in

chicken splenocytes. Veterinary Immunology and Immunopathology. 2023; 262:110632.
21. Li Y, Ma QG, Zhao LH, Wei H, Duan GX, Zhang JY et al. Effects of lipoic acid on immune function, the antioxidant defense system, and inflammation-related genes expression of broiler chickens fed aflatoxin contaminated diets. International journal of molecular sciences. 2014; 15(4):5649-62.
22. Nanto-Hara F, Ohtsu H, Yamazaki M, Hirakawa T, Sato K, Murakami H. Effects of dietary brown rice on the growth performance, systemic oxidative status, and splenic inflammatory responses of broiler chickens under chronic heat stress. The Journal of Poultry Science. 2021; 58(3):154-62.
23. Lee SA, Nagalakshmi D, Raju MV, Rao SV, Bedford MR, Walk CL. Phytase as an alleviator of high-temperature stress in broilers fed adequate and low dietary calcium. Poultry science. 2019; 98(5):2122-32.
24. Orta-Zavalza E, Briones-Martin-del-Campo M, Castano I, De Las Penas A. Catalase activity assay in Candida glabrata. Bio-protocol. 2014; 4(6):e1072.
25. Shahid M, Shahzad A, Malik A, Anis M. Antibacterial activity of aerial parts as well as *in vitro* raised calli of the medicinal plant Saraca asoca (Roxb.) de Wilde. Canadian journal of microbiology. 2007; 53(1):75-81.
26. Ambastha SP. The Wealth of India. Publication and Information Directorate, New Delhi, CSIR 1988; 2:118.
27. Nag G, De Pradhan I, Gupta P, De B. Analysis of Antioxidant, Acetylcholinesterase Inhibitory Properties and Chemical Composition of Some Indian Himalayan Species of Berberis: A Comparative Study. Free Radicals and Antioxidants. 2022; 12(1):22-6.
28. Patel DK, Patel K, Dhanabal SP. Standardization of *Berberis aristata* extract through conventional and modern HPTLC techniques. Asian Pacific Journal of Tropical Disease. 2012;2:136-40.
29. Lamichhane B, Adhikari S, Shrestha P, Shrestha BG. Study of phytochemical, antioxidant, antimicrobial and anticancer activity of Berberis Aristata. Journal of Tropical Life Science. 2014; 4(1):01-7.
30. Bhatt LR, Wagle B, Adhikari M, Bhusal S, Giri A, Bhattarai S. Antioxidant activity, total phenolic and flavonoid content of *Berberis aristata* DC. and Berberis thomsoniana CK Schneid. from Sagarmatha National Park, Nepal. Pharmacognosy Journal. 2018; 10(6s).
31. Ehteshamfar SM, Akhbari M, Afshari JT, Seyedi M, Nikfar B, Shapouri-Moghaddam A et al. Anti-inflammatory and immune-modulatory impacts of berberine on activation of autoreactive T cells in autoimmune inflammation. Journal of cellular and molecular medicine. 2020; (23):13573-88.
32. Sharma PC, Yelne MB, Dennis TJ. Database on medicinal plants used in Ayurveda. Vol. 1. New Delhi: Central Council for Research in Ayurveda & Siddha, 2000, 120-123.
33. Zhu Z, Xueying L, Chunlin L, Wen X, Rongrong Z, Jing H et al. Effect of berberine on LPS-induced expression of NF-κ B/MAPK signalling pathway and related inflammatory cytokines in porcine intestinal epithelial cells. Innate immunity. 2020;26(7):627-34.
34. Ghareeb DA, Elwakeel EH, Khalil R, Aziz MS, El Demellawy MA. Investigation of the Immunomodulatory effect of Berberis vulgaris on core-pulsed dendritic cell vaccine. BMC complementary and alternative medicine. 2016;16:1-1.
35. Abd El-Wahab AE, Ghareeb DA, Sarhan EE, Abu-Serie MM, El Demellawy MA. *In vitro* biological assessment of Berberis vulgaris and its active constituent, berberine: antioxidants, anti-acetylcholinesterase, anti-diabetic and anticancer effects. BMC complementary and alternative medicine. 2013; 13:1-2.
36. Singh J, Kakkar P. Antihyperglycemic and antioxidant effect of *Berberis aristata* root extract and its role in regulating carbohydrate metabolism in diabetic rats. Journal of ethnopharmacology. 2009; 123(1):22-6.
37. Sood H, Kumar Y, Gupta VK, Arora DS. Scientific validation of the antimicrobial and antiproliferative potential of *Berberis aristata* DC root bark, its phytoconstituents and their biosafety. AMB Express. 2019;9:1-6.

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