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From medicinal crop to poultry health booster: Immunoenhancing and antioxidant properties of *Bergenia ligulata* in chicken splenocytes as a potential commercial immunomodulator

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Abstract

Bergenia ligulate is an important ornamental medicinal plant of the Himalayan region, known for its extensive biological activities, low maintenance and wide range of pharmaceutical applications. In the present study aqueous extract of rhizomes of *Bergenia ligulata* (BLAE) was evaluated for its *in vitro* immunomodulatory and antioxidant activity through lymphocytes proliferation assay (LPA), lipid peroxidation (LPO), reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) assays in chicken lymphocytes. The extraction yield of the rhizome of *Bergenia ligulata* in water was about 8.90%. BLAE showed significant antioxidant activity in DPPH free radical scavenging assay. Maximum non-cytotoxic dose (MNCD) of BLAE for lymphocyte culture was determined to be 200 µg/mL. LPA showed that BLAE enhanced lymphocyte proliferation of B cells and T cells. Treatment with BLAE significantly increased the level of GSH, SOD, CAT and decreased the level of LPO in chicken lymphocytes, thus indicating the antioxidant potential of BLAE. The efficiency of poultry production is rising due to increased global consumption of chicken over red meat, leading to industry intensification, and the focus is shifting from antibiotic growth promoters to non-antibiotic alternatives like phytogenics to combat antibiotic resistance. The study reveals significant immunomodulatory and antioxidant properties of BLAE, thus suggesting that it could be a potential feed additive in poultry production. However, further *in vivo* research is required to evaluate its efficacy and safety for application in commercial poultry farming.

Key words: Antioxidant, *Bergenia ligulata*, chicken, DPPH assay, immunomodulation, lymphocytes, proliferation assay.

Introduction

Herbal products have gained attention from clinical perspective in recent years due to several benefits such as lower toxicity and side effects, more cost effectiveness *etc.* (Arya *et al.,* 2017; Ambwani *et al.*, 2018; 2019; Pandey and Ambwani**,** 2022). *Bergenia ligulata* (*B. ligulata*), commonly known as 'Paashanbheda' (Paashan = rockstone, bheda = piercing), is a perennial herb with short, thick, fleshy and procumbent stems, stout rootstock and oval-shaped 5-15 cm long leaves. It is distributed in the temperate Himalayan region (Kashmir to Nepal) at altitude range 2000-2700 m. It belongs to the family 'Saxifragaceae' and is considered one of the highest-valued, elite temperate medicinal herbs, particularly in the Asian continent, including India, Pakistan and Nepal. Rhizomes of plants from *Bergenia* genus have been used in folk medicine for their antiscorbutic, antipyretic, diuretic, astringent as well as ophthalmic properties (Kumar and Tyagi, 2013). It is used as a kidney stone dissolver in the indigenous system of medicine (Gurav and Gurav, 2014). Rhizomes of *B. ligulata* are potentially used in the management of renal stones (Garimella *et al.,* 2001).

In the Sindh region of Pakistan, rhizomes of *B. ligulata* along with honey, is given to children during teething. Juice of *B. ligulata* leaves is used for earaches in India and China (Chowdhary *et al.,* 2009). This plant has been listed in various ancient Indian chronicles of medicine including "*Charak Samhita*", "*Sushruta Samhita*" and "*Ashtang-Hridaya*" *etc.* (Chitme *et al.,* 2010). *Bergenia* species is known to exhibit a wide range of biological activities such as anti-inflammatory, antipyretic, antioxidant, antiviral, antibacterial, antimalarial, hepatoprotective, antiulcer, anticancer, diuretic, antidiabetic, antitussive, anti-urolithiasis *etc.* (Koul *et al.,* 2020). Some of the major phytoconstituents of *B. ligulata* include Bergenin, gallic acid, tannic acid, arbutin and many more (Ruby *et al.,* 2015). Bergenin and catechin are the two major compounds found in *B. ligulata* that are mainly responsible for the antioxidant properties of the plant (Dix *et al.,* 1989). *Bergenia* extracts can significantly stimulate the expression of CD69 on lymphocytes (Tumová *et al.,* 2018). Bergenin and methanolic extract of *B. ligulata* was found to show antioxidant activity in *in vitro* assays against 1, 1-diphenyl 2-picrylhydrazyl (DPPH) free radical and lipid peroxidation (Bashir *et al.,* 2009).

Bergenin as well as methanolic extract of *B. ligulata* are known to exhibit marked dissolution of calculi in kidney as well as urine constituents (Satish and Umashankar, 2006). When hydro-methanolic extract of *B. ligulata* rhizomes was given to ethylene glycol-induced urolithiatic rats at the dose of 5-10 mg/kg body weight, an improvement in renal function was found and there was reduced deposition of CaC_2O_4 crystal in the renal tubules

(Bashir and Gilani, 2009). Dried rhizomes of *B. ligulata* is a known herbal antipyretic drug and is used to prevent fever (Ruby *et al.,* 2012). When ethanolic extract of roots and rhizomes of *B. ligulata* was given at the rate of 500 mg/kg body weight to yeast-induced pyretic rats, a reduction in rectal temperature was observed (Nardev *et al.,* 2009). Studies on animal models have also shown strong anti-diabetic activity of *B. ligulata* (Koul *et al.,* 2020) and the possible mechanism for this effect could be the presence of (+)-afzelechin in the plant which acts as an inhibitor of α-glucosidase enzyme as found in enzyme inhibition assay (Ruby *et al.,* 2012) and inhibition of this enzyme is related with the delayed absorption of carbohydrates in the small intestine of rats (Saijyo *et al.,* 2008). Aqueous as well as ethanolic extract of rhizomes of *Bergenia* spp. was found to have significant anti-inflammatory activity in rat model (Koul *et al.,* 2020). When aqueous and ethanolic extract of *B. ligulata* was given at the dose rate of 1 g/kg body weight to the rats, there was attenuation of inflammation as there was decreased level of succinate dehydrogenase (Sajad *et al.,* 2010) and bergenin may be responsible for the anti-inflammatory activity. *B. ligulata* extract also lead to reduction in inflammatory mediators (Singh *et al.,* 2021). *B. ligulata* when given to rats showed hepatoprotective activity as there was significant reduction in the levels of SGPT, SGOT, ALP and total bilirubin (Nardev *et al.,* 2009). Intravenous administration of *B. ligulata* extract at a dose of 50 mg/kg body weight in dogs showed hypotensive properties. Alcoholic extract of the rhizome of *Bergenia* spp. exhibit anti-bradykinin action in isolated guinea pig ileum (Gurav and Gurav, 2014). Various bioactive molecules are present in the plant that are known for their pharmacological properties such as polyphenols (bergenin, arbutin, and catechin), flavonoids and quinones (Koul *et al.,* 2020).

The poultry industry has utilized antibiotics as growth promoters for over 60 years to enhance production performance in broilers but they are no longer permissible in the production of food animals in many countries due to the emergence of antibiotic resistance which is one of the major concerns for human health. Due to this threat, there is a dire need for the addition of non-antibiotic alternatives as feed additives for improved health and performance of broilers (Maron *et al.,* 2013; Landy *et al.,* 2023). Extensive research has been conducted on the use of phytogenics as feed additives in livestock (Gadde *et al.,* 2017). One such important plant is *Bergenia ligulata,* which is a versatile perennial herb and bridges the gap between ornamental horticulture and medicinal plant applications. *B. ligulata* fits well into the category of phytogenic feed additive due to its rich phytochemical profile and biological activities. Research has shown that *B. ligulata* extracts can modulate immune responses and exhibit potent antioxidant effects, both of which are crucial for maintaining poultry health and productivity.

The present study aimed to determine the immunomodulatory and antioxidative potential of aqueous extract of rhizomes of *B. ligulata* (BLAE) by performing *in vitro* lymphocyte proliferation assay and *in vitro* antioxidant assays after exposure of BLAE in chicken lymphocytes.

Materials and methods

Collection of plant material and preparation of plant extract: The rhizomes of *B. ligulata* used in the present study 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.- GBPUH-1437). Then the aqueous rhizome extract of *B. ligulata* (BLAE) was prepared as per the method described by Fuloria *et al.,* 2023.

Phytochemical analysis of the plant extract: The plant extract was analysed for the presence of different phytochemicals *i.e.,* phenolics, flavonoids, tannins, terpenoids and phlobatannins by using qualitative assays as per the method described by Thakur *et al.* (2018) and Deb *et al.* (2018). The total phenolics and total flavonoids of the plant extract were quantified as per the method described by Mansour *et al.* (2011).

Antioxidant potential of the plant extract: To assess the antioxidant activity of BLAE, DPPH free radical scavenging assay was performed in which different concentrations of BLAE were dissolved in methanol that was then mixed with 0.1 mM methanolic solution of DPPH. This mixture was then incubated at room temperature for 30 minutes and then optical density was measured at 517 nm. Then the percentage of DPPH inhibition was determined as per Shivhare *et al.* (2010).

Collection of chicken spleens and isolation of chicken lymphocytes from spleens: In the present study, spleens were collected in sterile Dulbecco's phosphate buffer saline (DPBS) from healthy broilers (Cobb strain) of 4-6 weeks of age from a local slaughterhouse. Then these were processed to isolate the lymphocytes under sterile conditions using the standard protocol as per the method described by Ambwani *et al.* (2023).

Maximum non-cytotoxic dose determination (MNCD): MNCD of BLAE was calculated as per the method described by Ambwani *et al.* (2023). The percent cell viability was calculated by comparing the absorbance of the treated cells with that of the control cells.

Lymphocytes proliferation assay (LPA): LPA was performed as per the method described by Ambwani *et al.* (2023). In this assay, lymphocytes were stimulated using various cell culture-tested mitogens namely, Concanavalin A (Con A), Phytohaemagglutinin (PHA) and *Escherichia coli* (serotype 0111:B4) derived lipopolysaccharide (LPS) each at a concentration of 5 µg/mL in RPMI-1640 medium. A 200 µl volume of lymphocytes suspension at a concentration of 1×10^6 cells/mL was added to each well of 96-well flat bottom tissue culture plate, after which the cells were exposed to MNCD of BLAE in the presence of various mitogens in triplicate.

Detection of antioxidant status: After 68 hours of incubation, the control and BLAE-treated chicken lymphocytes were harvested and cell lysates were prepared which were stored at -80°C to determine the antioxidant potential of BLAE. Ascorbic acid was used as a positive control (Ambwani *et al.,* 2023).

Membrane lipid peroxidation (LPO): The LPO was calculated by measuring malondialdehyde (MDA) (a biochemical marker for LPO). MDA reacts with thiobarbituric acid (TBA) to form a coloured product and the extent of the reaction was quantified by measuring absorbance at 532 nm. The results were expressed in nmol MDA/mg protein (Li *et al.,* 2014).

Reduced glutathione (GSH): Estimation of levels of GSH in the cell lysate was done based on the principle of Ellman's

reaction in which DTNB (5,5′-dithiobis-2-nitrobenzoic acid) when added to the compounds containing sulfhydryl groups produce a yellow-coloured product which can be quantified by measuring absorbance at 412 nm. The results were expressed in mM of GSH/mL (Nanto-Hara *et al.,* 2021).

Superoxide dismutase (SOD): The activity of SOD was measured by a reaction in which autooxidation of pyrogallol takes place which leads to the production of superoxide and the inhibition of superoxide-dependent reduction of MTT dye takes place and then the optical density of the reaction mixture was taken at 570 nm. DMSO is added to stop the reaction and help in the solubilization of the formazan crystals. The SOD activity was expressed in terms of SOD units where 1 unit SOD represents mg of protein required to inhibit the reduction of MTT by 50% (Lee *et al.,* 2019).

Catalase (CAT): It was based on the principle that the catalase enzyme present in the sample can decompose 2 H_2O_2 molecules to 2 molecules of H_2O and O_2 and this reaction leads to a decrease in absorbance which is measured at 240 nm. The CAT activity is measured as a change in absorbance over time (ΔA_{240}) (Orta-Zavalza *et al.,* 2014).

Statistical analysis: The statistical analysis of the data was performed with the help of Origin Software and the results found in the study were presented as Mean ± standard deviation. Any significant differences between treatment and control groups were analysed using one-way analysis of variance and Tukey's post-hoc test.

Results

Collection of the plant material and extract preparation: The aqueous rhizomes extract of *B. ligulata* (BLAE) was prepared with the percent yield of 8.90% (Fig. 1; Table 1).

Table 1. Percent yield of aqueous extract of *B. ligulata* (BLAE)

Weight of dried plant powder (g)	Weight of extract obtained (g)	Percent yield (%)
100	8.90	8.90

Phytochemical analysis of the plant extract: Biochemical qualitative tests on BLAE showed the presence of phenolics, flavonoids, tannins and terpenoids (Table 2). The total phenolics and flavonoid content of BLAE was found to be 33.144±1.43 mg/gm and 12.777±1.32 mg/gm of the extract, respectively (Table 3).

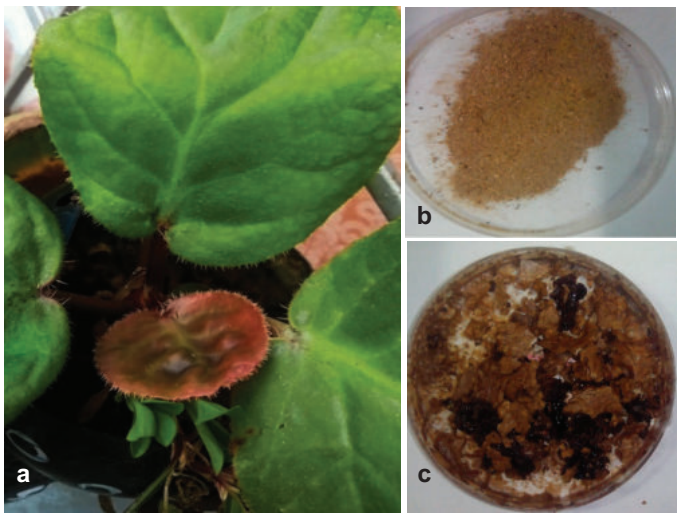

Fig. 1. a) *Bergenia ligulata* plant, b) Dried rhizome powder of *Bergenia ligulata*, c) Aqueous extract of *Bergenia ligulata* (BLAE)

Table 2. Qualitative phytochemical analysis of BLAE

Phytochemicals	BLAE
Phenolics	+
Flavonoids	+
Tannins	+
Terpenoids	+
Phlobatannins	-

Table 3. Quantitative phytochemical analysis of BLAE

Quantitative Analysis	BLAE
Total phenolics content (mg/gm extract)	33.144±1.43
Total flavonoids content (mg/gm extract)	12.777±1.32

Determination of antioxidative potential of BLAE by DPPH free radical scavenging assay: In the DPPH free radical scavenging assay, it was observed that increasing the concentration of BLAE led to an increase in percent scavenging of DPPH free radicals which was comparable with that of Ascorbic acid as shown in Fig. 2.

Determination of maximum non-cytotoxic dose of BLAE in chicken lymphocytes: The MNCD of BLAE was found to be 200 µg/mL by using MTT assay as shown in Fig. 3.

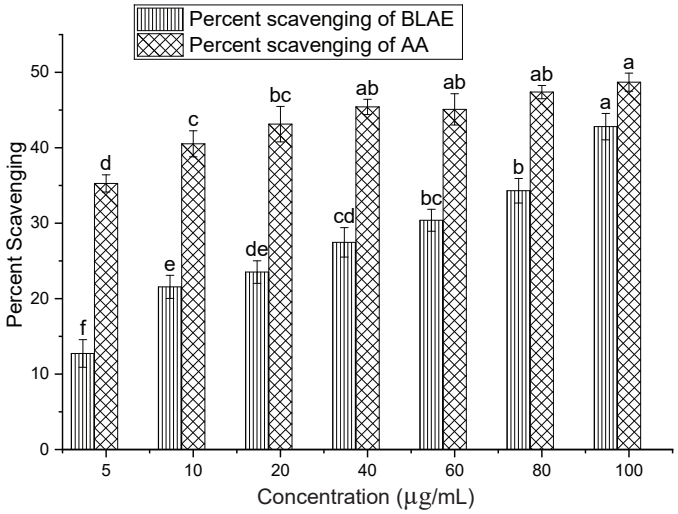

Fig. 2. Percent scavenging of BLAE and AA in DPPH assay

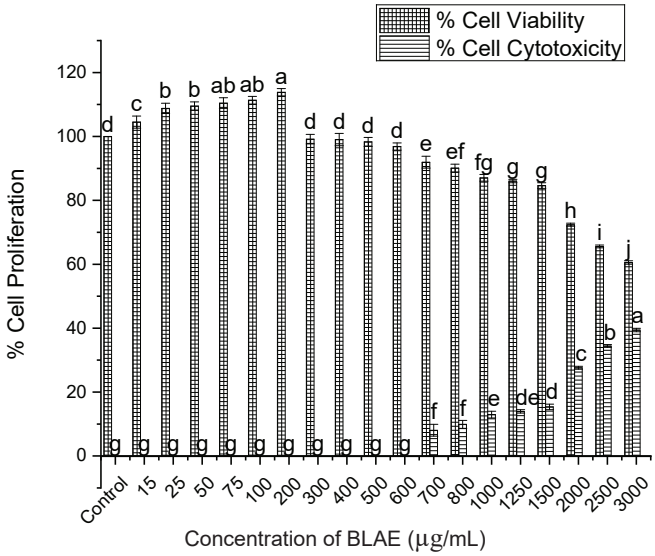

Fig. 3. Percent cell viability of chicken lymphocytes in presence of different concentrations of BLAE

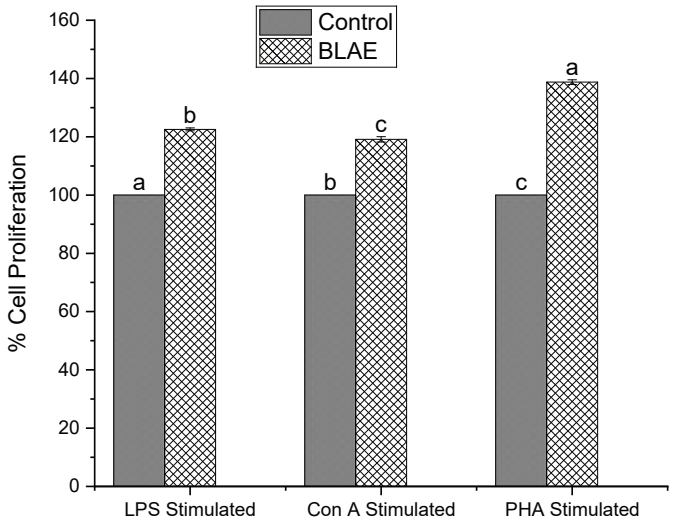

Fig. 4. Effect of BLAE exposure on mitogens stimulated lymphocytes

Effect of BLAE on lymphocytes proliferation: There was significant increase in B and T cell proliferation when BLAE treated lymphocytes were stimulated using different mitogens (LPS, Con A, PHA) (Table 4 and Fig. 4).

Table 4. Effect of BLAE on mitogens stimulated lymphocytes proliferation

Treatment		Control	BLAE
LPS stimulated	Proliferation (%)	100	122.53
	Change in proliferation (%)	-	22.53
Con A stimulated	Proliferation (%)	100	119.10
	Change in proliferation (%)	-	19.10
PHA stimulated	Proliferation (%)	100	138.76
	Change in proliferation (%)	-	38.76

Determination of antioxidative potential of BLAE using *in vitro* antioxidant assays: BLAE was found to decrease lipid peroxidation and increase levels of reduced glutathione, activity of SOD and catalase thus showing improved antioxidant levels in chicken lymphocytes in various *in vitro* antioxidant assays which are shown in Table 5.

Table 5. Antioxidative status of chicken lymphocytes treated with BLAE

Treatment	Lipid peroxidation (nM MDA/gm)	Reduced glutathione (mM/mL)	Superoxide dismutase (SOD units/mg of protein)	Catalase (H_2O_2 utilized mM/min/mg of protein)
Control	146.667 ± 1.777^{A}	0.062 ± 0.006^{C}	33.76 ± 1.449^{C}	149.934 ± 1.286^{C}
BLAE	123.077 ± 1.539^{B}	0.125 ± 0.004^{B}	42.411 ± 1.399^{B}	155.494 ± 1.059^{B}
AA	91.795 ± 2.351^{C}	0.197 ± 0.011^{A}	58.996 ± 1.193^{A}	161.64 ± 1.293^{A}
SEM	1.567	0.006	1.103	0.994

The values (Mean ± SD) having different superscripts in columns differ significantly ($P< 0.05$) as determined by one-way ANOVA and Tukey's post-hoc test.

Discussion

The present study demonstrated that the aqueous rhizome extract of Bergenia ligulata (BLAE) had a percent yield of 8.90% and contained phenolics, flavonoids, tannins, and terpenoids, with quantitative estimates of 33.144±1.43 mg/gm and 12.777±1.32 mg/gm for total phenolics and flavonoids, respectively. These findings align with earlier reports that B. ligulata is rich in alkaloids, tannins, flavonoids, and phenols (Vaishali *et al.*, 2008), and that ethanolic extracts contain alkaloids, flavonoids, saponins, triterpenes, polysterols, phenols, tannins, carbohydrates, and glycosides (Kaur and Kaur, 2018). Other active phytoconstituents such as bergenin, pashaanolactone, β-sitosterol, stigmesterol, tannic acid, gallic acid, parasorbic acid, isovaleric acid, and 1,8-cineole have been previously identified (Ruby *et al.*, 2012).

The antioxidant activity of BLAE, confirmed by DPPH free radical scavenging assay in the current study, showed a concentration-dependent increase in radical scavenging comparable to ascorbic acid. This corroborates prior reports of substantial antioxidant potential in B. ligulata (Vaishali *et al.*, 2008; Agnihotri *et al.*, 2015). Bergenin, a bioactive compound, has been reported to exhibit antioxidant activity in hydrogen peroxide radical scavenging with an IC50 of 32.54 µg/mL (Salimo *et al.*, 2023), as well as free radical scavenging, increased SOD and GSH levels, and reduced lipid peroxidation in animal models (Sajad *et al.*, 2010; Qi *et al.*, 2018). In vitro, 11-O-galloylbergenin demonstrated strong antioxidant activities, with EC50 values of 7.45±0.2 µg/mL in DPPH test and 5.39±0.28 µg/mL in reducing power assay, outperforming α-tocopherol in the phosphomolybdate assay (Uddin *et al.*, 2014).

In the current investigation, BLAE administration dramatically reduced lipid peroxidation, increased reduced glutathione levels, and elevated SOD and catalase activity in chicken lymphocytes, indicating improved antioxidant status in vitro. These results are consistent with bergenin's documented ability to increase overall antioxidant capacity and enzymatic activity of SOD, CAT, and glutathione peroxidase (Qi *et al.*, 2018).

The immunostimulatory action of BLAE, as seen by enhanced proliferation of B and T lymphocytes in response to mitogen stimulation, supports bergenin's immunomodulatory potential. Bergenin has been shown in the literature to improve both humoral and cell-mediated immunity by boosting IgM and IgG levels, improving macrophage function, NK and CTL activity, and altering the Th1/Th2 balance (Qi *et al.*, 2018). Our MNCD measurement showed a safe dosage of 200 µg/mL in chicken lymphocytes, promoting proliferation without cytotoxicity.

Furthermore, the cytoprotective potential of ethanolic B. ligulata extract, which includes reduced ROS generation, apoptosis, and inflammatory markers in oxalate-injured HK2 cells (Singh *et al.*, 2022), is consistent with the protective antioxidant and immunomodulatory responses elicited by BLAE in the current study. These combined findings indicate BLAE's considerable antioxidative and immune-enhancing characteristics, which are relevant for therapeutic applications in oxidative stress and immunosuppressive settings.In the present study, it was found that the aqueous extract of rhizomes of *B. ligulata* (BLAE) showed significant antioxidant and immunomodulatory activity in different *in vitro* assays in chicken lymphocytes culture system. BLAE was found to be rich in different phytoconstituents. BLAE-treated lymphocytes showed significant proliferation when they were stimulated with different mitogens thus showing immune-potentiating response. BLAE also exhibited improved antioxidant status of BLAE-treated chicken lymphocytes as observed through a reduction in LPO and increased in the levels of GSH, SOD and CAT. This improved antioxidant level suggests that the plant helps in reducing oxidative stress which is one of the major factors behind feeble immune status.

Thus, *B. ligulata* is a multifaceted plant that offers numerous benefits in horticulture as well. Whether used as ground cover, in rock gardens, or as an ornamental plant, *B. ligulata* enhances garden beauty and sustainability. *B. ligulata* exemplifies the integration of horticulture and medicinal plant applications across various industries. Its integration into agricultural practices can enhance sustainability, biodiversity, and economic returns for farmers. By adopting advanced cultivation techniques like tissue culture and micropropagation, the consistent and sustainable production of *B. ligulata* can be ensured, meeting the growing demand for this valuable horticultural crop. Its multifaceted benefits, particularly in the poultry industry, highlight its potential as a valuable crop for enhancing health, sustainability, and economic viability in agricultural and industrial practices. The promising results from *in vitro* studies suggest that *B. ligulata* could be integrated into poultry diets as a natural feed additive. *B. ligulata* can offer several benefits in poultry farming such as enhanced immune response, reduced use of antibiotics as growth promoters, natural growth promotion and stress reduction. Phytogenics also provide environmental safety, increase consumer preference and are cost-effective. So, integration of phytogenics in poultry farming can lead to better health and productivity of chickens along with sustainable farming practices. To incorporate *B. ligulata* as feed additive in poultry ration, further *in vivo* studies are required to fully understand the mechanism of immunomodulation and antioxidant activity of the plant.

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Conflict of interest statement: The author reports no conflicts of interest in this work.

References

Agnihotri, V., P. Sati, A. Jantwal and A. Pandey, 2015. Antimicrobial and antioxidant phytochemicals in leaf extracts of *Bergenia ligulata*: A Himalayan herb of medicinal value. *Nat. Prod. Res.*, 29(11): 1074-1077.

Ambwani, S., R. Tandon and T.K. Ambwani, 2019. Metal nano delivery systems for improved efficacy of herbal drugs. *Biosci. Biotechnol. Res. Asia*, 16(2): 251-261.

Ambwani, S., R. Tandon, T.K. Ambwani and Y.S. Malik, 2018. Current knowledge on nanodelivery systems and their beneficial applications in enhancing the efficacy of herbal drugs. *J. Exp. Biol. Agric. Sci.*, 6(1): 87-107.

Ambwani, S., R. Dolma, R. Sharma, A. Kaur, H. Singh, A. Ruj and T.K. Ambwani, 2023. Modulation of inflammatory and oxidative stress biomarkers due to dexamethasone exposure in chicken splenocytes. *Vet. Immunol. Immunopathol.*, 262: 110632.

Arya, P., S. Pandey and V. Verma, 2017. Kidney stone formation and use of medicinal plants as antiurolithiatic agents. *Univ. J. Pharm. Res.*, 2: 43-48.

Bashir, S. and A.H. Gilani, 2009. Antiurolithic effect of *Bergenia ligulata* rhizome: An explanation of the underlying mechanisms. *J. Ethnopharmacol.*, 122: 106-116.

Chitme, H.R., S. Alok, S. Jain and M. Sabharwal, 2010. Herbal treatment for urinary stones. *Int. J. Pharm. Sci. Res.*, 1: 24-31.

Chowdhary, S., D. Verma and H. Kumar, 2009. Biodiversity and traditional knowledge of *Bergenia* spp. in Kumaun Himalaya. *Sci. New York*, 2: 105-108.

Deb, K., A. Kaur, S. Ambwani and T.K. Ambwani, 2018. Preliminary phytochemical analyses of hydromethanolic leaf extract of *Melia azedarach* L. *J. Med. Plants Stud.*, 6(3): 4-8.

Dix, B.S. and S.N. Srivastava, 1989. Tannin constituents of *Bergenia ligulata* roots. *Ind. J. Nat. Prod.*, 5: 24-25.

Fuloria, N., R. Goswami, S. Ambwani, R. Tandon and T.K. Ambwani, 2023. Exploring *in vitro* efficacy of roots of *Bergenia ligulata* for urolithiasis management. *IJMFM & AP*, 9(2): 1-13.

Gadde, U., W. Kim, S. Oh and H.S. Lillehoj, 2017. Alternatives to antibiotics for maximizing growth performance and feed efficiency in poultry: A review. *Anim. Health Res. Rev.*, 18(1): 26-45.

Garimella, T.S., C.I. Jolly and S. Narayanan, 2001. *In vitro* studies on antilithiatic activity of seeds of *Dolichos biflorus* Linn. and rhizomes of *Bergenia ligulata* Wall. *Phytother. Res.*, 15: 351-355.

Gurav, S.S. and N.S. Gurav, 2014. A comprehensive review: *Bergenia ligulata* Wall-A controversial clinical candidate. *Int. J. Pharm. Sci. Rev. Res.*, 5: 1630-1642.

Kaur, R. and S. Kaur, 2018. Evaluation of *in vitro* and *in vivo* antileishmanial potential of bergenin-rich *Bergenia ligulata* (Wall.) Engl. root extract against visceral leishmaniasis in inbred BALB/c mice through immunomodulation. *J. Tradit. Complement. Med.*, 8(1): 251-260.

Koul, B., A. Kumar, D. Yadav and J.O. Jin, 2020. *Bergenia* genus: Traditional uses, phytochemistry and pharmacology. *Molecules*, 25: 5555.

Kumar, V. and D. Tyagi, 2013. Review on phytochemical, ethnomedical and biological studies of medically useful genus *Bergenia*. *Int. J. Curr. Microbiol. Appl. Sci.*, 2: 328-334.

Landy, N. and F. Kheiri, 2023. Effects of hydrolyzed cottonseed protein on growth performance, carcass traits, immunity, microbial and morphological responses of the small intestine and total antioxidant capacity of serum and small intestine in broiler chickens. *Iran J. Appl. Anim. Sci.*, 13(1): 121-132.

Lee, S.A., D. Nagalakshmi, M.V. Raju, S.V. Rao, M.R. Bedford and C.L. Walk, 2019. Phytase as an alleviator of high-temperature stress in broilers fed adequate and low dietary calcium. *Poult. Sci.*, 98(5): 2122-2132.

Li, Y., Q.G. Ma, L.H. Zhao, H. Wei, G.X. Duan, J.Y. Zhang and C. Ji, 2014. Effects of lipoic acid on immune function, the antioxidant defense system and inflammation-related gene expression of broiler chickens fed aflatoxin-contaminated diets. *Int. J. Mol. Sci.*, 15(4): 5649-5662.

Mansour, R.B., B. Gargouri, M. Bouaziz, N. Elloumi, I. Belhadj Jilani, Z. Ghrabi and S. Lassoued, 2011. Antioxidant activity of ethanolic extract of inflorescence of *Ormenis africana in vitro* and in cell cultures. *Lipids Health Dis.*, 10: 1-7.

Maron, D.F., T.J.S. Smith and K.E. Nachman, 2013. Restrictions on antimicrobial use in food animal production: An international regulatory and economic survey. *Glob. Health*, 9: 48-58.

Nanto-Hara, F., H. Ohtsu, M. Yamazaki, T. Hirakawa, K. Sato and H. Murakami, 2021. Effects of dietary brown rice on the growth performance, systemic oxidative status and splenic inflammatory responses of broiler chickens under chronic heat stress. *J. Poult. Sci.*, 58(3): 154-162.

Nardev, S., J. Vijay, A.K. Gupta and G. Manoj, 2009. Evaluation of ethanolic extract of root of *Bergenia ligulata* for hepatoprotective, diuretic and antipyretic activities. *J. Pharm. Res.*, 2: 958-960.

Orta-Zavalza, E., M. Briones-Martin-del-Campo, I. Castano and A. De Las Penas, 2014. Catalase activity assay in *Candida glabrata*. *Bio Protoc.*, 4(6): e1072.

Pandey, Y. and S. Ambwani, 2022. Nano metal-based herbal theranostics for cancer management: Coalescing nature's boon with nanotechnological advancement. *Curr. Pharm. Biotechnol.*, 23(1): 30-46.

Qi, Q., Z. Dong, Y. Sun, S. Li and Z. Zhao, 2018. Protective effect of bergenin against cyclophosphamide-induced immunosuppression by immunomodulatory effect and antioxidation in BALB/c mice. *Molecules*, 23: 2668.

Ruby, K., S. Sharma, R. Chauhan and J. Dwivedi, 2015. *In vitro* antioxidant and hemorrhoidal potential of hydroethanolic leaf extracts of *Bergenia ciliata*, *Bergenia ligulata* and *Bergenia stracheyi*. *Asian J. Plant Sci. Res.*, 5: 34-46.

Ruby, K.M., J. Dwivedi and R. Chouhan, 2012. *Pashanbheda*: A golden herb of Himalaya: A review. *Int. J. Pharm. Rev. Res.*, 2(2): 97-105.

Saijyo, J., Y. Suzuki, Y. Okuno, H. Yamaki, T. Suzuki and M. Miyazawa, 2008. α-Glucosidase inhibitor from *Bergenia ligulata*. *J. Oleo Sci.*, 57: 431-435.

Sajad, T., A. Zargar, T. Ahmad, G.N. Bader, M. Naime and S. Ali, 2010. Antibacterial and anti-inflammatory potential of *Bergenia ligulata*. *Am. J. Biomed. Sci.*, 2(4): 313-321.

Salimo, Z.M., M.N. Yakubu, E.L. da Silva, A.C.G. de Almeida, Y.O. Chaves, E.V. Costa, F.M.A. da Silva, J.F. Tavares, W.M. Monteiro, G.C. de Melo and H.H. Koolen, 2023. Chemistry and pharmacology of bergenin or its derivatives: A promising molecule. *Biomolecules*, 13: 403.

Satish, H. and D. Umashankar, 2006. Comparative study of methanolic extract of *Bergenia ligulata* Yeo., with isolated constituent bergenin in urolithiatic rats. *BioMed.*, 1: 80-86.

Shivhare, Y., P. Singh, U. Upadhyay, S. Sharma, S. Shukla, A.K. Singhai and P. Soni, 2010. Determination of physicochemical parameters and DPPH radical scavenging activity of *Chenopodium album* Linn. *Pharmacogn. Mag.*, 2(14): 7-10.

Singh, A., S. Tandon, D. Kumar, T. Kaur, K.K. Kesari and C. Tandon, 2022. Insights into the cytoprotective potential of *Bergenia ligulata* against oxalate-induced oxidative stress and epithelial–mesenchymal transition (EMT) via TGFβ1/p38MAPK pathway in human renal epithelial cells. *Urolithiasis*, 50(3): 259-278.

Singh, A., S. Tandon, S.P. Nandi, T. Kaur and C. Tandon, 2021. Downregulation of inflammatory mediators by ethanolic extract of *Bergenia ligulata* (Wall.) in oxalate-injured renal epithelial cells. *J. Ethnopharmacol.*, 275: 114104.

Thakur, A.V., S. Ambwani and T.K. Ambwani, 2018. Preliminary phytochemical screening and GC-MS analysis of leaf extract of *Acacia catechu* (Lf) Wild. *Int. J. Herb. Med.*, 6(2): 81-85.

Tumová, L., H. Hendrychová and D. Vokurková, 2018. Immunostimulant activity of *Bergenia* extracts. *Pharmacogn. Mag.*, 14: 328-332.

Uddin, G., A. Sadat and B.S. Siddiqui, 2014. Comparative antioxidant and antiplasmodial activities of 11-O-galloylbergenin and bergenin isolated from *Bergenia ligulata*. *Trop. Biomed.*, 31(1): 143-148.

Vaishali, A.S., M.D. Vikas, M. Krishnapriya and G. Sanjeevani, 2008. Identification of potential antioxidants by *in vitro* activity-guided fractionation of *Bergenia ligulata*. *Pharmacogn. Mag.*, 4: 79-84.

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