Letters in Animal Biology

Journal homepage: www.liabjournal.com

Effect of Ashwagandha (*Withania somnifera*) root extract enriched shrikhand on *Salmonella* infected albino mice

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Article info
Received: 18 June 2025
Received in revised form: 19 August 2025
Accepted: 21 August 2025
Published online: 24 August 2025

Keywords
Ashwagandha root
Serum lipids
Growth performance
Immunomodulatory activity
Mice

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Reviewed by:
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Consent to publish the name of second reviewer could not be obtained

Abstract

Herbs have long been utilized as food flavor, preservatives, and medicinal ingredients. The current study investigated the effects of Ashwagandha (*Withania somnifera* (L.) root extract enriched shrikhand on growth performance, blood lipid profile, liver enzyme activities, bacterial counts, serum immunoglobulin, and spleen lymphocyte activity in albino mice infected with *Salmonella typhi*. A total of 72 albino mice (20-25 days) were fed a basal diet for one week, followed by Salmonella infection. After one week, the mice were randomly distributed into three treatment groups for 23 days: T_1 (basal diet), T_2 (basal diet + 0.7 g/kg shrikhand), and T_3 (basal diet + 0.7 g/kg Ashwagandha root extract enriched shrikhand). Compared with the other treatment groups, the T_3 group displayed the highest body weight, with no significant difference in feed intake across the treatments. However, compared to T_1 group, the blood lipid profiles in the T_2 and T_3 groups significantly improved. Lipid peroxidation was significantly ($p<0.05$) reduced in mice fed Ashwagandha root extract-enriched shrikhand accompanied by increased superoxide dismutase and catalase activities. *Salmonella* counts in the small intestine and fecal matter were significantly ($p<0.05$) lower in the T_3 group than in T_1 and T_2 groups. Serum immunoglobulin and spleen lymphocytes and immunoglobulin production was significantly enhanced in T_3 group compared to others. According to the results of the present study, the Ashwagandha root extract-enriched shrikhand has immunomodulatory, antioxidant, antibacterial, and lipid-lowering effects in Salmonella-infected mice, highlighting its potential application as a functional food for immune enhancement and infection management.

1. Introduction

Ashwagandha (*Withania somnifera* (L.) Dunal) is one of the most widely used traditional Indian medicinal herbs that have long been integral to Ayurvedic and Unani medical practices. India is among the largest producers of Ashwagandha, exporting it in a variety of forms, including dried roots, powders, dried leaves, capsules, tablets, pills, and formulated preparations (Khabiya et al. 2023). This herb exhibits several pharmacological activities, such as analgesic, anticancer, antioxidant, anti-inflammatory, antidepressant, antimicrobial, anticonvulsant, anxiolytic and hypnotic, cardioprotective, and nootropic (Langade et al. 2023). The roots of Ashwagandha are employed for their therapeutic characteristics. The plant roots of Ashwagandha contain several bioactive phytochemicals, such as phenolics, steroidal compounds, saponins, alkaloids, glycosides, and volatile oils (Naveed et al. 2022). They play several biological roles including antioxidant and anti-inflammatory roles (Mandlik et al. 2021).

Salmonella enterica serotype *typhi*, is an intracellular, facultative, gram-negative bacterium, responsible for fatal infections in both animals and humans (Mondal et al. 2023). In the food industry, *Salmonellosis* is a major cause of foodborne and waterborne diseases, causing gastroenteritis and watery diarrhea in humans and represents a major public health issue and economic burden (Ehuwa et al. 2021; Naushad et al. 2023).

Food product development and food processing are currently among the most promising and grooming sectors for nutraceuticals and functional foods. A compound annual growth rate of 8.5% is predicted between 2022 and 2030 for the global functional food market, which was estimated to be 280.7 billion USD in 2021 and is projected to reach 586.1 billion USD by 2030 (Grand View Research 2019). There are many functional foods in the market contain natural ingredients or compounds that have extraordinary protective properties such as antioxidant, anti-inflammatory, and anticancer (Sethi et al. 2016). Functional properties can be enhanced by adding probiotic bacteria or other health-promoting food ingredients to the main food matrices.

Ashwagandha root powder contains 7.45% moisture, 4.41g ash, 3.9 g protein, 0.30 g fat, 31 g crude fiber, 245 kcal, 49.9 g carbohydrates, 3.3 mg iron, 23 mg calcium, 75.7 g total carotene, and 5.8 mg vitamin C,

making it a valuable ingredient for functional foods (Joshi and Joshi 2021). Felix et al. (2025), reported that full-cream milk cheese fortified with 0.4% Ashwagandha achieved the best balance of sensory, nutritional, antioxidant, textural and microbial properties. Similarly, Ashwagandha Ghrita prepared from Desi cow milk exhibited better nutritional and antioxidant properties than cow ghee, making it a promising functional food with higher acceptance among consumers (Kumar et al. 2024). Traditional fermented milk products such as *shrikhand* are popular in western Indian regions, where cream, sugar, and other ingredients are added to chakka (strained yogurt) (Savaliya et al. 2023). Currently, dairy products are unique carriers of phytochemicals and other bioactive nutrients that have been identified as beneficial to human health (Maji et al. 2023). Since shrikhand contains relatively high levels of fat and sugar, it has the potential to incorporate higher levels of herbal extracts while maintaining or even improving sensory qualities compared to other dairy products. The main objectives of the present study were to assess the health-promoting effects of Ashwagandha supplementation on various physiological markers and to determine whether nutritional intervention can offer advantages such as improved growth, immunity, and reduce bacterial infection. In this context, the present study specifically investigated the effects of Ashwagandha root extract enriched shrikhand on salmonella infected albino mice.

2. Materials and methods

2.1 Procurement of raw material

The collection of Ashwagandha plant material for the investigation was conducted in full compliance with local and national guidelines. The roots were obtained from the Department of Rasa Shastra, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India under the supervision of Ayurvedic doctors and specialists. All necessary permissions were obtained from Institute Central Animal Ethical Committee (approval no. Dean/2015/CAEC/994) for the use of this extract in aforesaid product and in the subsequent animal study.

2.2 Extraction method

Ashwagandha roots were dried, powdered (1 mm), and were percolated four times at room temperature using various solvents (ethanol, methanol, acetone, and water), as described previously (Yadav and Rai 2018). The preparation and optimization of Ashwagandha root extract-enriched shrikhand, *i.e.*, herbal shrikhand (HS), were carried out following Yadav et al. (2024) using response surface methodology with different combinations.

2.3 Animal model and experimental design

A total of 72 male albino mice, aged 20-25 days with an average body weight 40-42 g, were procured from the Laboratory Animal Resource Section, Institute of Medical Sciences, Banaras Hindu University, India. Mice were housed in sterilized polypropylene cages with sterile rice husk bedding. Housing conditions were maintained at 24 ± 2 °C, 35–70% relative humidity, and 13–15 cubic feet/min airflow, with a 12 h light/12 h dark photoperiod. The animals were distributed among three groups with three replicates in each based on their body weights. One week before the experiment, all the mice were injected with Salmonella. None of the animals died during the 30-day study period. The experimental design followed Birla et al. (2019).

The experimental diets given in Table 1 were formulated according to the NRC (1995) feeding standards of laboratory rats ensuring adequate availability of all the essential nutrients. The treatments were as follows:

T1: Basal diet (BD) only

T2: BD + Normal shrikhand (NS)

T3: BD + Ashwagandha root extract-enriched shrikhand (0.7 g/kg body weight)

The level of Ashwagandha root extract-enriched shrikhand was within the safe range as reported for Ashwagandha leaf extract (Khan et al. 2009).

Table 1. Composition of experimental diets of mice (NRC 1994)

Ingredients	Diet composition (%)		
	T_1	T_2	T_3
Shrikhand	0	0.7	0
Ashwagandha root extract-enriched *Shrikhand*	0	0	0.7
Cornstarch	37	36.3	36.3
Casein	20	20	20
Dex-cornstarch	13	13	13
Sucrose	10	10	10
Fiber (cellulose)	5	5	5
Soybean oil	10	10	10
Mineral mix	3.5	3.5	3.5
Vitamin mix	1	1	1
L-Cystine	0.3	0.3	0.3
Choline bitartarate	0.20	0.20	0.20

2.4 Injection of antigen

The injections were administered subcutaneously near the head region. *Salmonella typhi* (2 mg/ml) was suspended in phosphate buffer solution (PBS) and emulsified in Freund's complete adjuvant, with slight modifications from Nurjayadi et al. (2018). Although *S. typhi* is not a natural pathogen for mice, it can induce infection easily in mice during experiments. The specific objectives of relevance to human diseases, ethical considerations, and biosafety issues all support the use of *S. typhi* rather than *S. typhimurium* in a mouse model (Mathur et al. 2012). Each mouse was injected 100 emulsion intraperitoneally followed by an additional booster dose with Freund's incomplete adjuvant after one week (Kuo-Haller et al. 2010).

2.5 Determination of growth performance and tissue weight

A digital electronic balance was used to weigh the mice, feed, and feed residue throughout the experiment. The feeds offered and the feed residues were recorded daily, whereas, the mice were weighed weekly in the morning before offering the feed. After the experimental period, all the mice were euthanized and dissected (Yan et al. 2011). The primary organs (spleen, liver, lung, kidney, heart, and adipose) were collected and recorded individually.

2.6 Preparation of mice for sampling

For euthanasia, the mice were kept in a cage connected to a chloroform solution feeding bottle. The animals were then pinned in a dissection tray and dissected. The samples of feces, small intestine, liver, and spleen lymphocytes were collected (Lin et al. 1998). The samples were stored in phosphate buffer (pH 7.4) for further analysis.

2.7 Determination of serum lipid and liver enzyme activities

During the experiment, all albino mice were fasted for 12 h before blood collection. Blood samples (2-3 mL) from all the mice were collected through cardiac puncture in anticoagulant-free tubes (Parasuraman et al. 2010) and serum was separated from the blood by centrifugation (MSE Minor, England) at 4000 rpm for 10 min. The serum samples were stored at -20°C for biochemical analysis. Serum triglyceride (TRI), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and total cholesterol (CHO) levels were estimated using commercial diagnostic kits (Qualigens India Pvt. Ltd.) following the manufacturer's instructions. Portion of liver samples collected from each animal were homogenized (Potter-Elvehjem Teflon homogenizer) in 10% w/v ice-cold phosphate buffer (0.1 m, pH 7.4) and subsequently centrifuged for 30 min at 4°C at 2,000 rpm. The collected supernatant was used for catalase (CAT), lipid peroxidation (LPO), and superoxide dismutase (SOD) assays (Mossa et al. 2015).

2.8 Determination of bacterial count

Salmonella counts were determined after 20 and 30 days by sacrificing two mice per replicate. A portion of the small intestine was removed, homogenized, and 100 mL of the homogenized fecal suspensions (1 g/mL) were serially diluted 1:10. The dilutions were plated on Luria-Bertani agar (LB) plates which were overlaid with Salmonella agar. The colony forming units (CFU) were enumerated after 16 h of incubation (Mathur et al. 2012). The data were presented as the means ± SD from triplicate samples across three separate trials.

2.9 Determination of immunoglobulin in the serum

The serum IgA, IgE, IgG, and IgM levels were assayed via sandwich enzyme-linked immunosorbent assay (ELISA) method (Ayoub et al. 2003). Pure anti-mouse IgG and IgA monoclonal antibodies were coated on 96-well ELISA plates (BD Falcon) at a concentration of 2 µg/ml in coating buffer (PBS, pH 7.2) followed by overnight incubation at 4°C. The plates were blocked with binding buffer containing PBS-5% and skim milk for two h after being washed with PBS containing Tween-20 (PBST). Standard solutions and serum (1:1000 dilution) were added to wells, incubated with biotinylated secondary anti-mouse IgA, IgE, IgG, and IgM monoclonal antibodies (2 g/ml) for 1 h, followed by streptavidin-conjugated Horseradish peroxidase (1:1000) for 1 h. After that, 100 μl of the substrate (0.4 mg/ml ortho phenylenediamine 0.02%, 30% H_2O_2 in 0.1 M citrate buffer, pH 5.2) was added to the plates and incubated for 30 min. The reaction was stopped with 50 μl of 2 M H_2SO_4 and the absorbance was read at 490 nm by an ELISA plate reader (Thermo Multi Scan, Finland) with a reference wavelength of 690 nm. The concentrations of IgA, IgE, IgG, and IgM were determined using standard curves.

2.10 Preparation and cultivation of spleen lymphocytes

The spleen samples were finely chopped, minced, and filtered through a sterile mesh (40 µ pore size). The splenic cell suspension was centrifuged at 190 g for 5 min and subjected to hemolysis buffer (155 mM NH_4Cl, 15 mM $NaHCO_3$, and 1 mM EDTA, pH 7.3). The cell suspension was washed with PBS followed by centrifugation for 05 min at 2000 rpm. Finally, the pellet is resuspended in Roswell Park Memorial Institute (RPMI)-1640 medium supplemented with 10% Fetal bovine serum along with 10 mM Na PB as a control and Ashwagandha root extract manufactured by SAFC Biosciences (Lenexa, KS, USA). Each 96-well culture plate was seeded with 1×10^6 cells/mL spleen lymphocyte suspension and incubated at 37°C in an incubator with 5% CO_2 for 24 h. Then the spleen cells were cultured with lipopolysaccharide (LPS) and concanavalin A (Con A and LPS 0.5 µg/mL each) for 96 h (Nijnik et al. 2009).

2.11 Determination of IgA, IgG, and IgM isotypes by ELISA

The levels of IgA, IgG, and IgM antibodies in splenocyte supernatants were determined by using sandwich ELISA kits (Bethyl Laboratories Montgomery, Texas, USA) (Malik et al. 2007). For cell culture, Con A and LPS (Biological Labs Campbell, CA, USA) were dissolved in PBS at pH of 7.4.

2.12 Statistical analysis

All the data are presented as means ± SD. The experiment was conducted in a completely randomized design with three treatments and three replications for each. The obtained data were analysed by one-way ANOVA using the post-hoc Duncan's multiple test with statistical significance level set at $p<0.05$. The IBM SPSS (version 25.0) was used for all of the statistical analysis.

3. Results and Discussion

3.1 Effects on growth performance and tissue weight

The effects of herbal shrikhand on growth performance and tissue weights of albino mice are presented in Table 2. The body weights of the mice in both normal *Shrikhand* (T_2) and herbal *Shrikhand* (T_3) groups increased significantly ($p<0.05$) compared with the basal diet control group (T_1). The highest body weight of the mice was found in T_3 (49.42±1.35 g), followed by T_2 (48.84±2.64 g) and T_1 (48.37±1.61 g). There was no significant difference in feed intake among the treatment groups. However, feed conversion ratio significantly improved in T_3 (8.75±0.49) group, followed by the T_2 (9.54±1.29), and T_1 (10.13±1.02) groups. These results are corroborated by Elsemelawy et al. (2016), who reported that supplementation with Ashwagandha root enhanced growth performance in male rats sodium arsenite toxicity. In addition, Ashwagandha root powder has been reported to improve the growth performance of broiler chicks by regulating gut microbiota that aid in the efficient feed utilization (Jyotsana et al. 2019). Moreover, the supplementation of *Withania somnifera* to the broiler chicken diets improves calcium retention, bone calcification, and tibial stiffness which ultimately supports increased growth performance in broiler chicken (Mohammadi et al. 2022; Mirakzehi et al. 2018). The organ weights, such as spleen, lungs, and heart, were not affected significantly among the groups. However, the weights of liver, kidney, and adipose tissue increased significantly ($p<0.05$) in T_2 and T_3 compared to T_1. Shimmi et al. (2012) also reported that kidney weight increased significantly with Ashwagandha root extract supplementation, which countered the gentamicin induced electrolyte imbalance in rats. This might be because the root extract possesses strong immune potentiating compounds, such as withanolide, withanolide A, withanone etc., which have been attributed to its effectiveness (Paul et al. 2021). Similarly, Azimi et al. (2020) reported that hydro-alcoholic *Withania somnifera* leaf extract significantly

Table 2. Effect of Ashwagandha root extract-enriched *shrikhand* on growth performance and tissue weight of albino mice

Parameter	T_1	T_2	T_3	SEM	P-value
Initial body weight (g)	42.04±4.1	42.12±2.1	42.10±3.2	2.04	0.78
Weight gain (g)	6.32±1.26^c	6.72±2.41^b	7.32±4.35^a	8.51	<0.01
Feed intake (g)	64.05±4.36	64.12±3.12	64.11±2.16	1.03	1.02
Final body weight (g)	48.37±1.61^c	48.84±2.64^b	49.42±1.35^a	9.54	0.02
Feed conversion ratio	10.13±1.02^a	9.54±1.29^b	8.75±0.49^c	8.42	<0.01
Tissue weight (g)					
Spleen	0.58±0.08	0.58±0.10	0.58±0.14	1.02	0.68
Liver	8.72±0.41^c	9.35±0.03^b	9.87±0.47^a	8.45	<0.01
Lung	1.41±0.05	1.41±1.02	1.42±0.07	1.10	1.02
Kidney	1.83±0.08^c	1.91±0.16^b	2.08±0.06^a	9.54	<0.01
Heart	1.10±0.05	1.11±0.04	1.10±0.05	0.01	0.41
Adipose	10.07±1.39^b	10.31±0.14^b	11.89±0.56^a	8.67	0.02

^{a,b,c} Means bearing different superscripts within the row differ significantly (p<0.05); SEM: Total standard error of the mean

increased the spleen weight of broiler chicken compared to the control birds. Such observations indicate that the bioactive chemicals in the root extract may effectively stimulate host immune responses by enhancing phagocytosis and intracellular death of microbes by peritoneal macrophages (Chandrasekaran et al. 2017).

3.2 Effect on lipid profile and liver enzyme activities

The effects of herbal shrikhand on the serum lipid profile of albino mice are presented in Table 3. Previous studies have shown two-fold increase in plasma cholesterol, phospholipids, triacylglycerol, and free fatty acids due to *salmonellosis* (Rotimi et al. 2012). In general, cholesterol and triacylglycerols increase the possibility of heart disease, fatty liver disease, diabetes, carcinogenesis, peripheral vascular disease, and atherosclerosis in humans (Ismaiel et al. 2019). In this study, the concentrations of total cholesterol, LDL cholesterol, and triglycerides were significantly lower (p<0.05) in the T_3 group mice compared to T_1 and T_2 groups, whereas inverse trend was observed in HDL-C. The antioxidant catechins present in Ashwagandha roots may explain these improvements, as catechins enhance homocysteine metabolism by stimulating cystathionine synthase activity (Hamelet et al. 2007). On similar lines Sheikh and Gallehdari (2023) reported that herbal supplementation improved the blood lipid profile of diabetic mice and Patel et al. (2019) reported that consumption of Ashwagandha root might be beneficial for improving serum homocysteine levels and lipid profiles.

Table 3. Effect of Ashwagandha root extract-enriched *shrikhand* on serum lipid profile of albino mice

Parameter	T_1	T_2	T_3	SEM	P-value
Total cholesterol	49.72±0.34^a	47.41±0.12^b	43.83±0.59^c	7.84	<0.05
HDL	31.43±1.15^c	32.81±1.05^b	34.6±1.21^b	8.69	<0.05
LDL	0.39±1.28^a	0.36±2.65^b	0.32±1.59^c	9.54	<0.05
Triglyceride	65.56±4.23^a	63.17±1.13^b	61.14±6.55^c	9.57	<0.05

^{a,b,c} Means bearing different superscripts within the row differ significantly (p<0.05); SEM: Total standard error of the mean; HDL: High density lipoprotein; LDL: Low density lipoprotein

Table 4 shows the impact of herbal shrikhand on the liver enzyme activity of albino mice. In T_3 group, lipid peroxidation significantly (p<0.05) decreased, while superoxide dismutase and catalase activities increased compared to T_2 and T_1 groups. Ashwagandha

Table 4. Effect of Ashwagandha root extract-enriched *shrikhand* on liver enzyme activity of albino mice

Parameter	T_1	T_2	T_3	SEM	P-value
LPO	0.48±0.02^a	0.47±0.02^b	0.45±0.01^c	8.75	<0.05
SOD	5.92±0.18^c	7.95±0.51^b	9.21±0.65^a	9.34	<0.05
CAT	55.19±1.86^c	59.60±2.21^b	71.32±1.56^a	8.62	<0.05

^{a,b,c} Means bearing different superscripts within the column differ significantly (p<0.05); SEM: Total standard error of the mean; LPO: Lipid peroxidation; SOD: Superoxide dismutase; CAT: Catalase

supplementation thus reduced hepatic malondialdehyde production and enhanced enzymatic antioxidant defenses, consistent with previous reports of its strong antioxidative potential (Mikulska et al. 2023; Chandrasekhar et al. 2012). SOD activity protects against superoxide radicals by converting them to hydrogen peroxide, which is further neutralized by catalase, thereby reducing oxidative stress (Fujii et al., 2022). This mechanism prevents lipid peroxidation and necrotic changes in tissues (Spiteller 2003). These findings demonstrate that herbal shrikhand confers protection from free radical damage by enhancing natural antioxidant systems.

3.3 Effects on bacterial count

The effects of herbal shrikhand on the *Salmonella* counts in the small intestine and feces of albino mice are presented in Table 5. The *Salmonella* count in the small intestine and feces decreased significantly ($p<0.05$) in T_2 group which further got decreased significantly ($p<0.05$) in T_3 compared to T_1. Since dissection was performed only after 20 days of feeding, the *Salmonella* count in the small intestine could not be determined on first day. The *Salmonella* count in feces was 74.80±0.67 in the T_3 group on the first day, but after 30 days of feeding, it significantly ($p<0.05$) reduced to 32.34±0.33. The antimicrobial properties of Ashwagandha root extract may explain the decline of *Salmonella* counts. Rizwana (2012) reported strong antibacterial action of methanolic leaf extracts of Ashwagandha against methicillin-resistant *Staphylococcus aureus*, surpassing vancomycin. Similarly, herbal extract mixture improved gut microbiota by decreasing *coliform* count and increasing *lactobacilli* count (Attia et al. 2017). The immune upregulation and increased nutrient digestibility have been reported with Ashwagandha root/leaf extract which might have resulted in decrease of *Salmonella* counts (Azimi et al. 2020; Khan et al. 2009).

Table 5. Effect of Ashwagandha root extract-enriched *shrikhand* on *Salmonella* count in small intestine and feces of albino mice

Parameter	T_1	T_2	T_3	SEM	P-value
Intestine					
0 days	-	-	-	-	-
20 days	76.7±0.67^a	65.7±0.67^b	45.55±0.67^c	6.56	<0.01
30 days	96.6±0.33^a	54.5±0.33^b	28.53±0.33^c	8.24	<0.01
Fecal matter					
0 days	74.3±0.67	75.7±0.67	74.80±0.67	1.02	1.52
20 days	83.3±0.33^a	63.3±0.33^b	49.60±0.33^c	7.65	<0.01
30 days	98.3±0.33^a	54.8±0.33^b	32.34±0.33^c	4.37	<0.01

^{a,b,c} Means bearing different superscripts within the row differ significantly ($p<0.05$); SEM: Total standard error of the mean

3.4 Effect on serum and spleen lymphocyte immunoglobulin

The effects of herbal shrikhand on the serum immunoglobulin profiles of albino mice are presented in Table 6. Serum IgA levels significantly differed among the groups, with the lowest IgA concentration in T_3 group (24.1±2.14) and the highest in T_1 group (27.7±0.69). Conversely, IgG concentration increased significantly ($p<0.05$) in T_3 group, while no significant differences were observed in serum IgM and IgE levels. The immunoglobulin production by splenic lymphocyte culture in response to LPS or Con A stimulation revealed higher concentrations of IgA, IgG and IgM in T_3 group compared to othet two groups which did not differ significantly from each other (Table 7). An increase in IgA relative to IgG indicates the mouse mucosal immune response to *Salmonella* infection, emphasizing the role of mucosal immunity against gastrointestinal infections and specific role of IgA in gut-associated immunity (He et al. 2020; Lorenz et al. 2015). These results are corroborated by Mirakzehi et al. (2017) and Yamada et al. (2017), who reported enhanced antibody production in chicken against SRBCs and in mice splenic lymphocytes, respectively, as a result of Ashwagandha extracts. Collectively, the immunomodulatory role of HS appears to be mediated through increased humoral immunity, free radical scavenging, and upregulated IgA, IgG, and IgM production. The immune system plays a crucial defensive role in defending the human body against invaders, including viruses, bacteria, and allergies (Shao et al. 2023). Although it is believed that IgG and IgM concentrations control allergies by preventing allergens from attaching to cells, the IgA

Table 6. Effect of Ashwagandha root extract-enriched *shrikhand* on immunoglobulins A, G, M, and E of serum levels of albino mice

Parameter	T_1	T_2	T_3	SEM	P-value
IgA (μg/ml)	27.7±0.69^a	26.51±0.12^a	24.1±2.14^b	7.54	0.01
IgG (mg/ml)	0.75±0.13^b	0.77±0.57^b	0.81±0.17^a	8.69	0.02
Ig M (μg/ml)	343.1±17.11	345±14.36	344.2±74.86	1.21	4.12
Ig E (ng/ml)	12.5±0.93	12.4±0.69	11.98±1.03	0.21	1.25

^{a,b,c} Means bearing different superscripts within the row differ significantly ($p<0.05$); SEM: Total standard error of the mean; HDL: High density lipoprotein; LDL: Low density lipoprotein

Table 7. Effect of Ashwagandha root extract-enriched *shrikhand* on Immunoglobulin production by spleen lymphocytes of albino mice

Dietary groups	Mitogen (10 µg/ml)		Ig Concentration (mg/ml)		
	LPS	Con A	IgA	IgG	IgM
T_1	-	-	20.91±0.41^b	9.6±0.85^b	160.7±2.49^b
T_2	-	-	32.36±1.35^b	12.5±0.32^b	184±0.57^b
T_3	-	-	108.14±2.49^a	44.6±0.98^a	786.5±22.01^a
SEM			7.34	6.12	7.59
P-value			0.02	<0.01	<0.01
T_1	+	-	18.01±1.16^b	9.2±0.50^b	171.1±8.24^b
T_2	+	-	20.32±0.41^b	9.8±0.68^b	174±0.41^b
T_3	+	-	204.2±3.94^a	41.9±1.06^a	211.7±32.64^b
SEM			5.68	4.65	6.54
P-value			<0.01	<0.01	<0.01
T_1	-	+	9.9±0.76^b	7.2±0.45^b	79.3±2.89^b
T_2	-	+	10.5±0.57^b	8.1±0.41^b	85.71±0.71^b
T_3	-	+	80.2±1.51^a	10.4±0.41^a	525.4±11.86^a
SEM			6.68	7.74	5.74
P-value			0.01	<0.01	<0.01

^{a,b,c} Means bearing different superscripts within the column differ significantly (p<0.05); SEM: Total standard error of the mean; LPS: Lipopolysaccharide; Con A: Concanavalin A

concentration is particularly important in regulating allergies (Shamji et al. 2021). Similar response patterns were observed when Con A and LPS mitogens were added to the cell culture.

4. Conclusions

The present study revealed that *shrikhand* enriched Ashwagandha root extract exerts a potent immune-modulating effect due its phytochemical components. It enhanced growth performance, improved serum lipid profiles, increased antioxidant enzyme activities, and reduced intestinal load of *Salmonella*. In addition enhanced humoral immune response was observed in terms of elevated IgG, IgM, IgA production. Hence, the enrichment of dairy products with herbal extracts such as Ashwagandha offers promising health-promoting benefits, particularly in protecting against gastrointestinal infections.

Declarations

Funding: None

Conflict of interest: The authors declare no competing interests

Ethics approval and consent of participation: All protocols set by the Institutional Animal Care and Use Central Animal Ethical Committee, Institute of Medical Sciences at Banaras Hindu University, Varanasi, India were followed (Approval No. Dean/2015/CAEC/994)

Acknowledgements: The authors acknowledge the facilities provided by the Department of Dairy Science and Food Technology, Banaras Hindu University and the Institute of Medical Science, Banaras Hindu University, Varanasi, India for providing Albino mice

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Citation

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