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Anti-cold stress potential of aqueous extract of *Withania somnifera* (L.) Dunal and *Zanthoxylum armatum* DC. in goats

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Abstract

Cold stress is an important stressor for animals in high-altitude region which can negatively affect the animal's health, production, reproduction, and welfare. Stress is a reflex reaction of animals to cope with a stressor, which may cause discomfort, illness, or death. In the present study, we have investigated the anti-cold stress potential of *Withania somnifera* (L.) Dunal (Aswagandha; roots) and *Zanthoxylum armatum* DC. (Timur; fruit kernel) in Himalayan hill goats. Goats of either sex were divided into unexposed (Unex) and exposed (Ex1, Ex2, Ex3, and Ex4) groups. The animals of the unexposed group (n=6) did not receive cold stress or treatment. The animals (n=6) of the exposed group were exposed to cold stress during peak winter for a period of 4 h/day consecutively for 10 days. The Ex2 group received an aqueous extract of *W. somnifera* (roots) at a dose rate of 60 mg per kg body weight orally. Ex3 and Ex4 received an aqueous extract of *Z. armatum* orally at dose rates of 150 and 300 mg/kg, respectively. However, Ex1 acts as a control group. Blood samples were collected on the 11th day for further analysis. Total leucocyte count, BUN, and glucose concentrations were significantly ($p<0.05$) increased in Ex1 group compared to unexposed goats. *Z. armatum* treatment groups (Ex3 and Ex4) restored the blood glucose concentration within normal limits, indicating the anti stress effect. With the supplementation of *W. somnifera* in goats, oxidative stress parameters like LPO and SOD were recorded within normal limits, indicating its anti-stress efficacy. The present investigation recorded the evidence of maintaining altered oxidative, haemato-biochemical parameters in the *W. somnifera* and *Z. armatum* treatment groups as compared to the cold stress induced group. The present study revealed the cold stress potential of *W. somnifera* as well as *Z. armatum* in the goat model. The results of this study can be extrapolated to other livestock or human use with further investigations.

1. Introduction

In India, the goat husbandry sector is contributing significantly to the national economy, with 148.9 million goats, holding the second largest position in the world (20th India Livestock Census, 2019, DAHD). Goats play a crucial role in providing a subsidiary income and livelihood security to millions of small farmers, including landless labourers of rural India. In India, Goat farming is considered a highly profitable, low-investment animal husbandry venture driven by strong demand for chevon, milk, manure, and skin. Goat is considered the poor man's cow because of its immense contribution to sustaining the economy of poor farmers. Goats are adapted to various agro-climatic zones ranging from dry arid, hot and humid to cold. However, climatic stress due to a sudden rise in temperature and humidity can majorly impact the animal health, production, and welfare by altering homeostasis. Stress is a reflex reaction initiated by animals due to the inability to cope with the stressor, which may cause several side effects, from discomfort, illness, or death (Ray *et al.,* 2016). Stress arising from restraining, castration, dehorning, teeth clipping, nutrition deficiency, weaning, overcrowding, exposure to adverse climatic conditions (chilly weather, heat), excessive workload, and transportation, *etc*., can elicit behavioural as well as biological responses in animals. Stress is responsible for hormonal and oxidative changes in the body systems of animals. Cold stress is inevitable for all livestock species rearing in high-altitude areas. Stress conditions, such as hot and cold exposure, may negatively affect ruminants' health, production, reproduction, and welfare (Salama *et al.,* 2016). Physiological and behavioural changes are responsible for maintaining energy homeostasis in ruminants during cold exposure (Verbeek *et al.,* 2012). The energy requirement for maintenance increases by 20% during cold exposure and can be doubled with wet coat conditions and chilly weather environment (National Research Council, 2007). Goats are very sturdy animals that can adapt quickly to any alterations in the environment, but freezing weather during winter in the temperate region can make them elicit a stress response to acclimate to a changing environment. If, the advert or harsh conditions persist for a longer duration, it leads to stress, which may cause increased morbidity or mortality losses.

Indigenous Traditional Knowledge (ITK) is an asset for developing new drugs or therapies for various chronic and non-infectious diseases. *Zanthoxylum armatum* DC. is an evergreen, tiny, sub-deciduous, and spiny tree commonly found in the valleys of the Himalayas, the North-east part of India, Nepal, Bhutan, Pakistan, Myanmar, and Bangladesh. The common names of the plant are:

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Indian prickly ash,Winged prickly ash, Timur, and Timru. *Withania somnifera* (L.) Dunal (Common name: Ashwagandha) is a medicinal plant that belongs to the Solanaceae family. It is popularly referred to as Indian ginseng due to its use as an adaptogen in the Indian system of medicine for a long time. Other vernacular names of the plants are: Winter cherry, Ajagandha, Queen of Ayurveda, *etc. W. somnifera* is usually referred to as Rasayana in Ayurveda due to its antistress, anticancer, antiarthritic, neuroprotective, analgesic, and anti-inflammatory activities. The fresh roots of the herb give a smell like a horse, hence called Ashwagandha, with the belief that its consumption will give endurance and power like a horse (Ray *et al.,* 2016). It is referred to as a tonic, aphrodisiac, narcotic, diuretic, anthelmintic, astringent, thermogenic, and stimulant. *W. somnifera* has a wide spectrum of activity against various diseases of the liver, gastric, cardiac, and immunological disorders (Mishra and Patnaik, 2020). Herbal medicine is making a significant contribution to reducing the health stress of humans (Meena and Indiragandhi, 2025). The presence of polyphenols in the seaweeds can act as a source of antioxidants or antibacterials, which can help to fight against the disease in humans or animals (Patel *et al.,* 2024). Herbal medicine is a good alternative for the prevention of stress in animals. Protecting animals from cold stress will be helpful for the maintenance of health, production, and reproductive performance at an optimum level. It is also vital for the welfare of animals. Adaptogens are generally used to increase body strength against pathological or physiological stresses. The reports on anti cold stress potential of *Z. armatum* and *W. somnifera* in small ruminants are not known to date. The goatery is an important sector for the livelihood support of small and marginal farmers of hilly areas. Currently, the scientific evidence on the management of cold stress using medicinal plants is not available for small ruminants. The medicinal plants are safe and cost effective, also. The development of alternative therapy to treat chronic inflammatory and stress conditions is essential due to the persistent side effects of allopathy (Aswany *et al.,* 2023). Hence, considering the above fact, in the present study, we have investigated the anti cold stress potential of aqueous extract of *W. somnifera* (root) and *Z. armatum* (fruit kernel) in the Chougarkha goat of Uttarakhand, India.

2. Materials and Methods

2.1 Collection of plant material and extraction

Fruit kernel of *Zanthoxylum armatum* DC. (Common names: Timur,Timru) was collected from Munsiyari region of Pithoragarh District of Uttarakhand, India, and the roots of *Withania somnifera* (L.) Dunal (Common name: Aswagandha) were collected from Bareilly, Uttar Pradesh, India (Figure 1). The plant specimens have been identified by Dr. Nidhi Sharma, Scientist, ICAR-Indian Veterinary Research Institute, Mukteswar, Nainital, Uttarakhand. The specimens are maintained at the Institute with Herbarium No. ZAF-20-/IVRI, and WSR-02-/IVRI. The fruit kernel and roots were cleaned, air dried, and pulverized in an electric grinder into a powder form. They were subjected to the decoction extraction method using 60 g of powdered extract in 1 litre of water and boiled at a temperature of 100°C for 1 h. The extracts were filtered and dried at 41°C for further use. The extracts were stored at 4°C until further use.

2.2 Ethical approval

The study was conducted at Experimental Goat Farm (EGF), Surmane of ICAR-Indian Veterinary Research Institute, Mukteswar, Nainital, Uttrakhand. The farm located at an altitude of 2286 (7500 feet) meters above sea level, having coordinates of 29.4722°N and 79.6479°E. The study was approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi (F.No 25/14/2017 CPCSEA dated 13/7/17).

Figure 1: Powdered form of *Withania somnifera* (L.) Dunal (left) and *Zanthoxylum armatum* DC. (Right).

2.3 Experimental animal model and sampling

The anti-cold stress potential of *W. somnifera* and *Z. armatum* was studied in Himalayan hill goats. For this purpose, animals of either sex were divided into unexposed (Unex) and exposed (Ex) groups as per following details shown in table:

Groups	Treatment	Duration
Unex	Without cold exposure/No treatment	10 day
Ex1	Exposed to cold stress/ No treatment	
Ex2	Exposed to cold stress + *W. somnifera* (roots) @ 60 mg per kg body PO	
Ex3	Exposed to cold stress + *Z. armatum* @ 150 mg per kg body weight PO	
Ex4	Exposed to cold stress + *Z. armatum* @ 300 mg per kg body weight PO	

The animals (n=6) of exposed groups were exposed to cold stress during peak winter for a period of 4 h/day consecutively for 10 days. On 11th day, blood samples were collected in heparinised collection tubes and subjected to haematological, biochemical and oxidative stress parameters estimation.

2.3.1 Parameters of study

2.3.1.1 Hematology

Various hematological parameters like, haemoglobin (Hb), packed cell volume (PCV), red blood cell (RBC) count, total leucocytic count (TLC), white blood cell (WBC) count and differential leukocyte count (DLC) were estimated as per standard procedure (Chauhan, 2003).

2.3.1.2 Biochemical parameters

The concentration of different analytes like serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST), blood urea nitrogen (BUN), serum creatinine, and serum glucose were analyzed using available commercial (Autospan diagostic limited, India) kits.

2.3.1.3 Oxidative stress indices

To assess oxidative stress, the level of lipid peroxidase (LPO), superoxide dismuatse (SOD) and catalase (CAT) was determined in serum using commercial ELISA kits (Cayman chemical company, USA).

2.4 Statistical analysis

The alpha error was set at 0.05 and p value <0.05 was considered statistically significant. The difference in the mean values among different groups was analyzed using One way ANOVA. The data is represented as mean ± SEM.

3. Results

3.1 Hematological parameters

Results revealed a significant (p<0.05) leucocytosis in cold-exposed animals (Ex1 group) as compared to the unexposed (Unex) group (16.60 ± 1.56 x 10^3/µl vis-a-vis 10.25±1.37 x 10^3/µl). However, the TLC values were comparable (p<0.05) in Unex and Ex2, Ex3, and Ex4 groups (Table 1). The TEC values were comparable (p<0.05) in unexposed and all exposed groups; although, it was slightly higher in the Ex1 group. Similarly, we could not observe a significant difference in the Hb and PCV values among all groups.

3.2 Biochemical parameters

Biochemical analysis recorded a significant (p<0.05) elevation of blood glucose concentration in the Ex1 (86.23 ± 3.26 mg/dl) group in comparison to the Unex (54.76 ± 6.71 mg/dl) group. The glucose levels in exposed groups Ex3 and Ex4 were comparable to those of the unexposed (Unex) group (Table 2). The glucose level of the Ex2 (*W. somnifera*) group was comparable to that of the Unex and Ex1 groups. The blood urea nitrogen (BUN) levels were significantly (p<0.05) elevated in comparison to Unex; however, the BUN levels of Ex2, Ex3, and Ex4 were maintained within the normal range as compared to unexposed animals. The levels of liver enzymes like alanine aminotransferase (ALT), aspartate aminotransferase (AST), and creatinine did not vary significantly across all the groups.

Table 1: Haematological parameters of unexposed and exposed group of goat

Groups	Hb (gm/dl)	TLC (10^3/µl)	TEC(10^6/µl)	PCV (%)
Unex	11.00 ± 0.40^a	10.25 ± 1.37^a	13.25 ± 0.47^{bc}	35.72 ± 1.73^a
Ex1	10.60 ± 0.24^a	16.60 ± 1.56^b	13.80 ± 0.37^c	34.74 ± 0.87^a
Ex2	10.00 ± 0.57^a	14.17 ± 1.83^{ab}	11.50 ± 0.42^{ab}	33.19 ± 1.82^a
Ex3	9.83 ± 0.60^a	13.33 ± 1.62^{ab}	11.67 ± 0.76^{ab}	32.60 ± 2.11^a
Ex4	10.80 ± 0.37^a	11.00 ± 0.44^a	11.40 ± 0.60^a	35.74 ± 1.18^a

Unex- Unexposed; Ex1-Exposed 1; Ex2-Exposed 2; Ex3-Exposed 3; Ex4-Exposed 4.
Values are represented as mean ± SEM
Values with different superscript differ significantly

Table 2: Biochemical parameters of unexposed and exposed group of goats

Groups	ALT (IU/L)	AST (IU/L)	BUN (mg/dl)	Creatinine(mg/dl)	Glucose(mg/dl)
Unex	30.03 ± 3.05^a	48.06 ± 3.20^a	12.40 ± 0.36^a	0.93 ± 0.20^a	54.76 ± 6.71^a
Ex1	29.56 ± 2.90^a	50.25 ± 2.21^a	17.66 ± 0.95^b	1.13 ± 0.17^a	86.23 ± 3.26^b
Ex2	25.40 ± 4.05^a	48.03 ± 3.15^a	11.03 ± 0.75^a	0.84 ± 0.15^a	67.09 ± 3.33^{ab}
Ex3	37.56 ± 2.67^a	40.61 ± 5.71^a	13.29 ± 2.97^{ab}	1.28 ± 0.28^a	58.39 ± 3.45^a
Ex4	31.38 ± 5.84^a	51.87 ± 9.04^a	12.86 ± 1.12^{ab}	1.45 ± 0.22^a	59.80 ± 7.24^a

Unex-Unexposed; Ex1-Exposed 1; Ex2-Exposed 2; Ex3-Exposed 3; Ex4-Exposed 4.
Values are represented as mean ± SEM
Values with different superscript differ significantly

Table 3: Oxidative stress indices of unexposed and exposed group of goats

Group	LPO	SOD	CAT
Unex	14.47 ± 0.82^a	8.24 ± 0.91^a	166.13 ± 5.97^b
Ex1	41.64 ± 2.96^b	6.09 ± 0.45^a	107.93 ± 6.74^a
Ex2	22.03 ± 1.50^a	7.71 ± 0.30^a	156.53 ± 6.36^b
Ex3	22.19 ± 2.21^a	33.73 ± 26.63^a	142.33 ± 8.77^b
Ex4	24.29 ± 2.93^a	7.22 ± 0.67^a	158.56 ± 6.43^b

Unex- Unexposed; Ex1-Exposed 1; Ex2-Exposed 2; Ex3-Exposed 3; Ex4-Exposed 4
Values are represented as Mean ± SEM

3.3 Oxidative stress parameters

The results of oxidative stress indices are shown in Table 3. Results revealed a significantly (p<0.05) elevated lipid peroxidase (LPO) (41.64 ± 2.96 nmol/ml) and significantly reduced catalase (107.93 ± 6.74 U/ml/min) levels (p<0.05) in Ex1 in comparison to Unex. However, levels of LPO (22.03 ± 1.50 nmol/ml) and SOD (7.71 ± 0.30 U/ml) in Ex2 (*W. somnifera*) group were similar to the value of the Unex group, indicating its anti-stress effect. The LPO levels (22.19 ± 2.21 nmol/ml and 24.29 ± 2.93 nmol/ml) in *Z. armatum* treatment groups (Ex3 and Ex4) were comparable to the Unex group. The levels of catalase in all extract supplemented groups, Ex2, Ex3, and Ex4 (156.53 ± 6.63 U/ml; 142.33 ± 8.77 U/ml; 158.56 ± 6.43 U/ ml) were similar to the value of catalase in the unexposed group (166.13 ± 5.97 U/ml). The levels of superoxide dismutase (SOD) were reduced only non-significantly in Ex1 compared to the Unex. The SOD levels of all the groups did not reveal any significant changes. These results suggest that aqueous extracts of *W. somnifera* (root) and *Z. armatum* (fruit kernel) possess an anti cold stress potential in goats.

4. Discussion

Stress has become an important issue in animal welfare, production, and health management systems. The goat is thought to be suitable for every climate due to better thermoadaptability capacity (Serradilla *et al.,* 2018), drought tolerance (Stone *et al.,* 2020), and its capacity to thrive on unconventional and limited pasture (Chebli *et al.,* 2020), along with disease resistance capacity compared to other domestic livestock species (Pal and Chakravarty, 2020). Goat husbandry practices in the temperate hilly region receive an unperceived threat of cold stress during extreme winter. If, the normal adaptability mechanism fails to acclimate to a changing environment, the animals will suffer from mild to severe stress. It can affect the behaviour, production, and reproductive performance, along with the welfare of animals. To prevent the animals from cold stress, herbal supplementation is common in tribal farmers of Uttarakhand in India. Goats are commonly described as rustic and adaptable animals with a wide range of thermal tolerance (Salama *et al.,* 2014). Several studies argue that goats can have different extents of thermotolerance and maybe get affected by severe cold, which depends upon the breed of goats (Boe and Ehrlenbruch, 2013). Meager data is available on the impact of cold stress in goats as compared to other species like cattle. Previous studies conducted to evaluate the effect of cold exposure in Saanen (Thompson and Thompson,1972; Faulkner *et al.,* 1980) and Angora goats (Wentzel *et al.,* 1979) are 3 to 4 decades old. These research studies are constrained to a limited period (2-3 days) for cold stress induction. Very little is known regarding the long term effects of cold exposure on metabolic and production indicators under a controlled environment. Insufficient data is available on the effect of cold stress in goats and its amelioration strategies. Our study recorded significantly elevated total leucocyte count (TLC) in goats exposed to cold stress (Ex1); however, treatment with *Z. armatum* (300 mg per kg body weight) restores the TLC count to a level comparable to healthy goats. A study conducted by Al Musawi *et. al.* (2017) also revealed the significantly higher total white blood cell (WBC) count in goats exposed to cold climates. Our report is in accordance with the results of Etim *et. al*. (2014), who also recorded the significantly elevated WBC count in cold-stress induced goats. There were no significant changes in haemoglobin and PCV value of the control and cold-induced stress group of cattle (Berian *et al.,* 2019). A study on the effect of cold-stress on different broiler strains indicated that cold-stress significantly ($p \leq 0.05$) reduced blood haemoglobin (9.77 vs 10.09 %), glucose (213.07 vs 249.11 mg/dl), total protein (4.46 vs 4.00 mg/dl), and total lipids (449.20 vs 487.88 mg/dl) levels (Mahmoud, 2020). Contrary to our reports, Sundaresan *et. al.* (1990) experienced a significant decrease in total WBC count ($p \leq 0.05$) in cold-induced sub-acute stress in Wistar albino rats. The increased levels of corticosteroids during stress response may be responsible for immune modulation or suppression (Comsa *et al.,* 1982). Increased WBC count in the current study may be due to a response to elevated stress hormone- corticosterone. Chronic stress can cause depletion of total leukocyte count due to the prolonged effect of corticosterone.

The present experiment revealed significant hyperglycaemia in goats exposed to cold stress (Ex1) as compared to all other groups. The goats that have received *Z. armatum* extract at 300 and 150 mg per kg body weight dose (Ex3 and Ex 4) rates restore the blood glucose concentrations similar to those of the healthy goats (Unex). These results revealed the anti stress effect of *Z. armatum* in goats. Coloma García *et al.* (2017) recorded the elevated blood glucose level in cold-exposed goats compared to goats maintained under a thermoneutral zone without any major changes in insulin and cholesterol levels. A metabolomics study on the effect of cold exposure in goats revealed the higher α - and β-glucose concentration in the plasma of cold-exposed goats (Mehaba *et al.,* 2017). An experiment conducted by Coloma Garcia *et al.* (2017) showed that blood glucose and NEFA levels were increased, and BHBA and triglyceride values were decreased significantly ($p \leq 0.05$) in stress-affected animals. Exposure to a cold environment increases glucose output from the liver (Thompson *et al.,* 1978) and, consequently, glucose in the circulation might be increased (Faulkner *et al.,* 1980). Our results are according to these reports. In contrast to our results, Al Musawi *et al.* (2017) reported non-significant changes in blood glucose concentration between different groups exposed to cold; however, blood urea concentrations were significantly elevated in rams and ducks compared to the younger population. Cold stress is thought to alter the metabolic rate of an individual to maintain a homoeothermic state, which may cause altered carbohydrate, protein, and lipid metabolism. The altered metabolism may be responsible for enhanced blood glucose, NEFA, BUN, and creatinine concentrations. A study in equines by Priyanka *et al.* (2020) on the adaptogenic potential of *W. somnifera* in different stress modules, revealed a significant elevation in serum glucose, creatinine, and AST ($p \leq 0.05$), however, other parameters like total protein, albumin, cholesterol, ALT, and BUN did not vary significantly. They also observed a significant reduction in serum glucose, creatinine, and AST levels in *W. somnifera* treated horses (Priyanka *et al.,* 2020). Previous studies have revealed that flavonoids concentration of the roots of *W. somnifera* was responsible for the reduction in elevated blood glucose levels in experimental animals. *W. somnifera* and its glycol- withanolides present in it are reported to induce the transportation of glucose in cells *via* insulin secretion and increase the GLUT receptor activity (Sarangi *et al.,* 2013). Stress response hormones like epinephrine, triiodothyronine, thyroxine, and cortisol can enhance glucose production and synergistically increase blood glucose during cold exposure.

In the present study, a significant elevation in lipid peroxidase levels (LPO) and a significant reduction in catalase level was recorded in the cold exposed goats compared to all other groups, whereas superoxide dismutase (SOD) levels experienced only a non-significant reduction. However, levels of LPO and SOD were maintained within normal limits in goats receiving an aqueous extract of *W. somnifera* (Ex2), indicating its antistress effect. An experiment in horses subjected to different stress modules exhibited a higher mean thiobarbituric acid reacting substance (TBARs) concentration on days 15^{th}, 18^{th}, and 21^{st} of the experiment than the average mean values in the control group. Researchers recorded that the elevated TBAR concentrations were diminished significantly in *W. somnifera* supplemented horses (Priyanka *et al.,* 2020). A study on the effect of cold stress in different broiler strains revealed significantly elevated levels of malondialdehyde (MDA; 2.80 vs. 1.74 nmol/ml) and superoxide dismutase (SOD; 79.70 vs. 47.32 U/g Hb) in stress induced groups (Mahmoud, 2020). Our study also evidenced the reduction in LPO levels in *W. somnifera* treated goats, indicating the anti stress potential. The current study evidenced reduced catalase levels in the cold-exposed group (Ex1) compared to the healthy group (Unex). The catalase levels in goats receiving *Z. armatum* (Ex3 and Ex4) and *W. Somnifera* (Ex2) were equivalent to those of the healthy goats (Unex). A study in transition dairy goats associated with subclinical hypocalcemia has revealed a significant reduction in superoxide dismutase (SOD) activity at 2 weeks before kidding ($p \leq 0.01$); however, catalase (CAT) values were significantly reduced in the subclinical hypocalcaemic and hyperketonemic goats at 2^{nd} and 3^{rd} week postpartum, respectively ($p \leq 0.05$) (Huang *et al.,* 2021). Similar results are recorded in our study, irrespective of the stress conditions like cold stress or transition stress. One of the studies showed that black cumin, fenugreek, garlic, and their mixture helped in diabetes management along with the amelioration of oxidative stress parameters (Sheikh *et al.,* 2024). *W. somnifera* have several scientific evidences for its adaptogenic activity, which mainly acts through regulation of the body's stress response systems. The key bioactive constituents, particularly withanolides and sitoindosides, modulate the hypothalamic-pituitary-adrenal (HPA) axis, leading to reduced cortisol/corticosterone levels under stress conditions. Standardized *W. somnifera* extract trials in humans also reported significant reductions in stress, anxiety scores, and serum cortisol levels, confirming its role as a true adaptogen that modulates stress resilience capacity and maintains homeostasis. The present investigation is the primary report on the adaptogenic potential of *Z. armatum* in animal models. In our earlier study, aqueous extract of the fruit kernel of *Z. armatum* at a 500 mg dose rate exhibited anti- cold stress activity in a rat model. Oral supplementation of *Z. armatum* extract restored the normal activity of oxidative stress parameters and stress hormones in rats (Gurav *et al.,* 2024). The fruit kernel of *Z. armatum* is reported to have higher concentrations of compounds like flavonoids, saponins, and phytosterols, which may be responsible for the prevention of oxidative stress and enhancement of non-specific resistance in goats.

Cold climate at high altitude is a potent stressor for animals and humans during winter. Goats rearing at high altitudes facing cold stress may have an imbalance in redox potential with evident oxidative stress. Oxidative indices in the present study revealed a stress response in goats exposed to cold climates for a consecutive 10-day period. A dietary supplement in the form of nutraceuticals with antioxidant properties is an important strategy in controlling oxidative stress in ruminants (Colitti *et al.,* 2019). Previous studies have reported that dietary polyphenol supplementation increased the superoxide dismutase (SOD) levels in cows (Stefanon *et al.,* 2005) and goats through supplementation of grape skins and juniper oil, respectively (Yesilbag *et al.,* 2017). Our previous study revealed the strong positive reaction for the presence of flavonoids, saponins, and phytosterols in aqueous extract of the fruit kernel of *Z. armatum*. The total phenol and flavonoid content of aqueous extract of the fruits of *Z. armatum* were 33.24 ± 1.98 TPC (mg GAE/g extract) and 5.43 ± 0.46 TFC (mg CE/g extract), respectively. Due to the greater percentage of total phenol and flavonoid content in the fruit kernel extract of *Z. armatum*, it can be considered to have a better antioxidant potential. A better antioxidant potential of the *Z. armatum* (fruit kernel) can prevent the imbalance in redox potential due to cold exposure in the goats. The present study emphasized the efficacy of aqueous extract of the fruit kernel of *Z. armatum* in cold stress alleviation along with *W. somnifera*. It is a well-known adaptogen due to the presence of various phytocompunds in it. It has been used as an adaptogen in humans as well as in animals for years. A decoction of the *W. somnifera* roots is being used for colds and chills. Several Indian medicinal plants possess the immunostimulant property, which make it a good alternative for the effective management of various chronic diseases, including HIV and other stress or immunocompromised disorders (Verma *et al.,* 2022).

The presence of phenolic compounds, flavonoids, and free radical scavenging activity of *W. somnifera* roots is responsible for the antioxidant potential of the herb (Senthil *et al.,* 2015). A pretreatment with an aqueous suspension of *W. somnifera* root powder at a 100 mg/kg/oral dose rate resulted in increased corticosterone, phagocytic index, and avidity index in rats exposed to cold swimming stress. *W. Somnifera* supplementation in humans decreased cortisol levels in chronic stress by restoring the adrenal function and sympathetic nervous system (Kaushik *et al.,* 2017). The current study recorded the effect of *W. somnifera* and *Z. armatum* extracts on oxidative, haematological, and biochemical parameters of cold induced stress in local Chaougarkha goats. *W. somnifera* and *Z. armatum* extract administration restored all physiological and biochemical alterations to normal levels in treatment groups. Restoring the altered redox potential to the normal range exhibits the anti cold stress potential of both *W. somnifera* and *Z. armatum* extracts in goats. Current research findings can be beneficial in evaluating their antistress effect in human subjects in order to enhance the nonspecific resistance against many viral or immunosuppressive diseases. The limitations of the study are the small sample size, the small duration of exposure, lack of hormonal analysis.

5. Conclusion

The present study evaluated the anti-cold stress activity of aqueous extract of *W. somnifera* (root) and *Z. armatum* (fruit kernel) in Himalayan hill goats. The supplementation of these extracts prevented the alterations in the hemato-biochemical and oxidative stress markers in cold-exposed goats. The anti stress activity of *W. somnifera* may be attributed to the presence of glycolwithanolides, sitoindoside, and withaferin-A phytocompounds, whereas the fruit kernel of *Z. armatum* is reported to have a presence of flavonoids, saponins and phytosterols in higher concentrations. *W. somnifera* (root) and *Z. armatum* (fruit kernel) powder can be used for the management of cold stress in small ruminants subjected to dose optimization. The results of this study can be implicated for human or other livestock species use with further investigations.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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