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Preclinical Safety Evaluation of the *Udaravikaranashaka Churna* for Indigestion in Murrah Buffaloes

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Abstract

Utilising herbs and minerals as a medicament for the welfare of humans and animals has become an integral part of the traditional system of medicine. Moreover, despite advancements in technology, herbal medicine forms a primary source of modern medicine. In the present study, a developed herbs-mineral formation *Udaravikaranashaka Churna* (AYUSH-V-24) for indigestion was undertaken for Phase-I clinical evaluation in healthy fistulised Murrah buffaloes. The formulated drug was administered *per os* to the animals @ 15g/100kg body weight in four healthy buffaloes for a consecutive 7 days. Rumen liquor parameters were evaluated on days 0,3, and 7. Haematological parameters and biochemical markers of liver and kidney function tests were performed on days 0 and 7. Feeding AYUSH-V-24 for the period 7 days to the animals did not alter the rumen liquor colour, consistency, odour, pH, protozoal motility, total protozoal count, TEC and TLC values, while Hb and DLC values changed significantly ($p<0.05$) after seven days. We observed that the liver function (serum bilirubin, ALT, AST, and ALKP) and kidney function (serum BUN, creatinine, and protein) remained unaltered. The study suggests that the developed formulation (AYUSH-V-24) has no adverse or toxic effect on vital parameters, indicating the health status of the animals. It has been concluded that the formulation is safe to use in clinical cases of ruminal indigestion.

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Major Findings: The herbo-mineral formulation *Udaravikaranashaka Churna* (AYUSH-V-24), when administered orally to healthy Murrah buffaloes for 7 days, did not cause adverse alterations in rumen liquor, liver, or kidney function parameters. The study concludes that AYUSH-V-24 is safe for clinical use in managing ruminal indigestion.

Keywords: AYUSH-V-24, Biochemical, Buffaloes, Haematology, Protozoal Motility and Count, Rumen Liquor

1. Introduction

In the contemporary era, there has been a significant upswing in the worldwide usage of herbal products. The growing appeal of herbal remedies does not just arise from a general tendency towards natural products in treating illnesses; it is also fuelled by substantial evidence affirming their effectiveness[1,2]. The perception influences this inclination, whether accurate or not, that natural products are associated with fewer side effects and undesirable outcomes, making them cost-effective for long-term use[3]. Consequently, these natural remedies have found application in addressing everyday healthcare issues in animals and humans[4]. Notably, in Asian and African nations, 80% of the population relies on traditional medicine as their primary source for fulfilling healthcare requirements. Thus, it contributes to 75% of globally utilised plant-based therapeutic entities[5]. The Indian Pharmacopoeia (IP) serves as an official regulatory document designed to ensure the comprehensive quality control and assurance of pharmaceutical products in the Indian market, which extends to enhancing the safety, efficacy, and affordability of medicines (Indian Pharmacopoeia Commission, 2014). In India, various pharmacopoeias, including the Ayurvedic Pharmacopoeia of India, Homoeopathic Pharmacopoeia of India, Unani Pharmacopoeia of India, Siddha Pharmacopoeia of India, and IP, hold official status under the Drugs and Cosmetics Act of 1940, emphasising quality assurance[6]. Now, it is a global need and mandatory to establish safety data for herbal drugs before being used therapeutically as a part of the clinical drug development process[7,8].

Among the gastrointestinal diseases of ruminants, simple indigestion is most common and has a nutritional basis. Feeding of grain results in pH change, anorexia and ruminal hypomotility to atony (stasis) in ruminants[9]. The faeces are usually soft to watery and foul-smelling. If it persists, it may decrease body strength/immunity, cause excessive weakness, initiate secondary bacterial infections, and the animal may become prone to various disease conditions and ultimately drop in milk production[10]. Antibiotics and other medical interventions are required to treat this condition, resulting in an unnecessary financial burden on the farmers. To address such an issue, Central Council for Research in Ayurvedic Sciences (CCRAS), New Delhi, has formulated the herb-mineral formulation AYUSH-V-24 (an *Ayurvedic* formulation) derived from a classical text, *Brihat Pashu Chikitsa*, in the management of simple indigestion (*Ajirna*) in the ruminants[11,12]. By keeping the significance of basic principles of Veterinary Ayurveda. No safety reports are available on this formulation before being used therapeutically in ruminants. Hence, to fill the research gap in the clinical development of a drug, a study has been undertaken to establish the safety data of the AYUSH-V-24 formulation in healthy ruminants through different parameters before being recommended for therapeutic purposes.

2. Materials and Methods

2.1 Drug Formulation and Analytical

The AYUSH-V-24 is a herbo-mineral formulation selected from the *Karikalpalta Arthat Brihat Pashu Chikitsa* text. Moreover, now this formulation is also revealed in the Ayurvedic Formulary of India, Vol. IV as *Udaravikaranashaka Churna*. The Pharmacognostical and analytical study of the crude drugs procured was done at the Central Ayurveda Research Institute, CCRAS, Patiala. The standard operating procedure (SOP) of drug manufacturing was followed, and a prepared formulation (AYUSH-V-24) was finally provided. The drug formulation consisted of 24 ingredients mentioned in (Table 1); hence name was given AYUSH-V-24.

All chemicals of analytical grade and glassware used in the present study were purchased from well-established firms. All biochemical kits, such as BUN, Creatinine, SGPT/ALT, SGPT/AST, ALK Phosphatase, Bilirubin, Total Protein, Albumin, Chloride, LDH kit, Glucose, Calcium, Phosphorus, Total Protein, Albumin, and Creatinine Kinase, *etc*., were procured from ERBA diagnostics, Mannheim GmbH, manufactured by Transasia Bio-Medicals LTD.

Table 1. Ingredients of AYUSH-V-24

S. N.	Sanskrit name	Botanical name	Part used	Pro portion
1	*Śuṇthī*	*Zingiber officinale*	Rz.	1/3 part
2	*Pippalī*	*Piper longum*	Fr.	1/3 part
3	*Marica*	*Piper nigrum*	Fr.	1/3 part
4	*KṛṣṇaJīraka*	*Carum carvi*	Fr.	1 Part
5	ŚvetaJīraka	*Cuminum cyminum*	Fr.	1 Part
6	*Citraka*	*Plumbago zeylanica*	Rt.	1 Part
7	*Vacā*	*Acorus calamus*	Rz.	1 Part
8	*ŚuddhaHingu*	*Ferula foetida*	O.R.	1 Part
9	Śatapuṣpā	*Anethum Sowa*	Fr.	1 Part
10	*Rājikā*	*Brassica juncea*	Sd.	1 Part
11	*Śaṭī*	*Hedychium spicatum*	Rz.	1 Part
12	*Yavānī*	*Trachyspermum ammi*	Fr.	1 Part
13	*Haridrā*	*Curcuma longa*	Rz.	1 Part
14	*Āmalakī*	*Emblica officinalis*	P.	1/3 part
15	*Bibhītaka*	*Terminalia bellerica*	P.	1/3 part
16	*Harītakī*	*Terminalia chebula*	P.	1/3 part
17	*Viḍaṅga*	*Embelia ribes*	Fr.	1 Part
18	*Kaṭuka*	*Picrorhiza kurroa*	Rz.	1 Part
19	*Taṅkaṇa (Śuddha)*	Borax	-	1 Part
20	*Yavakṣāra*	Potassium Nitrate	-	1 Part
21	*Sarjikṣāra (Svarjikṣāra)*	Sodium bicarbonate	-	1 Part
22	*Sphaṭikā (Bharjita)*	Alum	-	1 Part
23	*Saiṅdhava Lavaṇa*	Rock Salt	-	1 Part
24	*Sauvarcala Lavaṇa*	Black Salt	-	1 Part

2.2 Experimental Animals and Ethical Approval

Prior to commencing the experiment, ethical permission for the inclusion of different species of ruminants in the clinical trial of the research project was obtained from Institutional Animal Ethics Committee (IAEC) and Committee for the Control and Supervision of Experiments on Animals (CCSEA), Ministry of Fisheries, Animal Husbandry and Dairying, Government of India (Approval No. V-11011 (13)/2/2020-CPCSEA-DADF dated 10/02/2020).

The four adult fistulated Murrah buffalo males aged between 5-6 years were enrolled for this study from the Department of Animal Nutrition and Dairy Farm of Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana. Throughout the experiment, all the animals were provided with routine green and dry fodder and concentrate along with water *ad lib* as per the standard management practices. The study was conducted on four healthy fistulated male buffaloes (2 repeats; interval 15 days). AYUSH-V-24 formulation was administered orally @ dose of 15 gm/100 kg body weight, twice a day for consecutively for 7 days[13].

2.3 Sample Collection

From the rumen of fistulated animals, blood samples were collected in Na_2-EDTA vials for haematological and biochemical parameters on 1- and 7-days post-drug administration. Rumen liquor samples were collected on day 1, 3, and 7 post-drug administration, with the frequency of 0 h (before feeding) and 3 and 7 h (post-feeding) on the day of collection.

2.4 Evaluation of Ruminal Parameters

2.4.1 Protozoal Motility

Protozoal motility was assessed by visualising fresh ruminal liquor under the microscope (10X) and was graded from 0 to 4, depending on the progressive movement (0 is no motility, and 4 is the highest motility).

2.4.2 Protozoal Count

Approximately 5 mL of ruminal fluid was well mixed into 5 mL of formalin solution (dilution 1:1), and then, a 2.0 mL aliquot was pipetted into a test tube containing 18 mL glycerol-buffer-methyl green mixture (1:20 final dilution). The mixture was allowed to stand for 4 h before counting, and mixed well before counting. The counting chamber (Sedgewick-Rafter Cell) was filled with the fluid and was carefully covered with a coverslip. The slide was set for 10 min to allow the protozoa to settle. At least 25 microscopic fields were counted (10X). The standard formula (Protozoa/mL = Number of organisms in 50 fields x dilution x organisms per count) was used for calculation[14].

2.4.3 Ruminal pH

An approx. 5mLrumen liquor sample was taken in a beaker, and pH was measured using a digital hand pH

meter. Further, the colour, odour, and consistency of rumen liquor were also recorded.

2.5 Haemato-Biochemical Parameters

Haematological parameters were performed manually following standard laboratory protocols[15,16], which included Haemoglobin (Hb), Packed Cell Volume (PCV), Total Erythrocyte Count (TEC), Total Leucocyte Count (TLC) and Differential Leucocyte Count (DLC). The sera samples were analysed for biochemical parameters such as Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), total protein, albumin, Blood Urea Nitrogen (BUN), creatine kinase and chloride using standard commercial kits (ERBA Diagnostics Mannheim GmbH, Germany) using VITROS 350 System chemistry, Diagnostic Laboratory Facility, GADVASU, Ludhiana.

2.6 Statistical Analysis

Data analysis was performed using SPSS 2.0 and GraphPad Prism, Version 4.0 statistical software. All the data were expressed as Mean ± SEM. All the data were compared by employing a paired t-test and one-way ANOVA with Tukey's multiple comparisons as a post hoc test, depending on the data type generated. A p-value of <0.05 was considered statistically significant.

3. Results

3.1 Effect on Health and Rumen Liquor Parameters

Animals were monitored regularly in the morning and evening during the seven days of treatment. Animals were found healthy; no adverse or toxic manifestations were noticed during the observation period in any of the animals. No alteration in feed intake and water intake was noticed during the study period.

Colour, odour, and consistency of ruminal liquor were recorded on days 1, 3, and 7, with frequency of 0, 3, and 7 h on each day. The rumen liquor was brownish green. The consistency was found to be watery to slightly viscous in all the animals before and after feeding at different time intervals and on different days of post-feeding. The odour of the liquor was slightly aromatic to the typical smell; no altered odour was noticed at any of the intervals (Table 2).

No apparent change in the protozoal motility was recorded when comparing day-wise samples and samples within the day at different time intervals, indicating no net effect of AYUSH-V-24 formulation on protozoal motility (Table 2). Ruminal pH was found to be statistically non-significant ($p>0.05$) between pre- and post-treatment during each (day 1, 3 and 7) or between days (Table 2).

The effect of AYUSH-V-24 formulation feeding in buffaloes on the first day of the experiment was found to be statistically non-effective ($p>0.05$) for the change in ruminal total protozoa count when comparing samples of 0, 3, and 7 h. AYUSH-V-24 formulation treatment repetitively for 3 days and even for 7 days on the total protozoa count of rumen was found to be statistically non-significant when compared between time intervals of 0, 3, and 7 h (Table 2).

3.2 Effect on Haematological Indicators

The AYUSH-V-24 formulation was fed to the fistulated animals for a period of 7 days, and there was no significant ($p>0.05$) change in Hb concentration was noticed, indicating that treatment does not affect haemoglobin synthesis (Table 3) A non-significant difference in TEC and TLC was observed when compared between before and after treatment within seven days of treatment of AYUSH formulation. However, a significant alteration

Table 2. Effect of AYUSH-V-24 on overall ruminal liquor parameters on day 1, 3 and 7 in Murrah buffalo males

Parameters	Day 1	Day 3	Day 7
Colour	Brown green	Brown green	Brown green
Consistency	Slightest viscous	Slight viscoid	Slight viscoid
Odor	Aromatic	Normal	Normal
Protozoal Motility	+1 to +2	+1 to +2	+1 to +2
Ruminal pH	7.05±0.03	7.14±0.08	7.11±0.03
Total Protozoa Count (x10^5)	1.69±0.09	2.00±0.08	1.85±0.06

Values are Mean±SEM (n=4x2) derived from cumulative values of 0, 3, 7 hrs reading at each time interval. Statistical significance in ANOVA with multiple comparison post hoc test at $p<0.05$ level of significance.

Table 3. Effect of AYUSH-V-24 formulation on haematological parameters in Murrah buffalo males

Parameters	Day 1 (before treatment)	Day 7 (after treatment)
Hb	16.97±0.25	16.22±0.38
TLC x 10^3/µL	1.07±6.1	1.06±3.96
TEC x 10^6/µL	10.01±0.22	9.25±0.35
PCV %	53.50±0.30*	49.25±0.35*
Neutrophils %	57.75±1.55*	60.5±1.26*
Lymphocytes %	39.00± 1.73	37.50±1.55
Eosinophil %	3.25±0.48	2.25±0.85

Values are Mean±SEM (n=4x2) with comparison of before and after treatment in a paired t-test at p<0.05 level of significance. * Indicates: significant difference.

Table 4. Effect of AYUSH-V-24 formulation on biochemical parameters in Murrah buffalo males

Parameters	Day 1 (before treatment)	Day 7 (after treatment)
Total bilirubin mg/dl	0.62±0.13	0.87±0.22
AST (IU/L)	2.10±15.03	2.53±20.79
ALT (IU/L)	1.09±4.76	1.05±6.06
ALKP (IU/L)	1.17±19.21	1.20±16.10
Total protein (g/dl)	8.42±0.21	8.17±0.07
Serum albumin (g/dl)	3.40±0.04	3.30±0.13
BUN mg/dl	14.25±1.49	13.50±0.50
Creatinine (mg/dl)	1.90±0.12	1.82±0.10
Chloride (Mmol/l)	95.75±0.47	96.00±2.12

Values are Mean±SEM (n=4x2) with comparison of before and after treatment in a paired t-test at p<0.05 level of significance.

(p<0.05) in PCV value was noticed when the AYUSH-V-24 formulation was treated in a fistulated animal for seven days compared to before treatment. It also caused a significant change (p<0.05) in the neutrophil count compared to before treatment and had a non-significant (p>0.05) effect on lymphocyte and eosinophil count.

3.3 Effect on Liver and Kidney Function Markers

The effect of AYUSH-V-24 formulation treatment on serum AST, ALT, ALP, total protein, albumin, bilirubin, BUN and creatinine levels was found to be statistically non-significant (p>0.05) when compared between values before and after seven days of treatment (Table 4).

Haematological tests are valuable diagnostic tools for anaemia, infection, haemophilia, blood clotting disorders, and leukaemia[17,18]. In the current study, the administration of AYUSH-V-24 did not result in any alterations in all the parameters, except PCV and neutrophil. However, that is within the physiological limit[19], suggesting a lack of significant impact on major vital organs and blood components.

4. Discussion

Biochemical markers of liver dysfunction encompass a range of indicators such as serum bilirubin, ALT, AST, ALP, GGT, 5′ nucleotidase, ceruloplasmin, and α-fetoprotein, among others. Liver tests play a crucial role in revealing alterations, providing valuable insights into the health status of the liver[20]. While renal function markers include BUN and creatinine, they span from acute emergencies to chronic conditions. These tests can be instrumental in evaluating overall renal function through direct measurement or estimating the glomerular filtration rate[21,22]. In the present study, liver and kidney function parameters were within normal range, indicating AYUSH-24 formulation does not have a direct or indirect toxic effect on vital organs of the body.

In the line drug development process and the stages of the present study, the AYUSH-V-24 formulation based on the classical test of *Pashu-Ayurveda*, which has 24 ingredients, was undertaken. It is an herbal-mineral combination comprised of 18 herbs and six minerals. As per the Ayurvedic principles, the present formulation has *Rogadhikara* as *Ajirna* (indigestion), *Aganimandhya* (loss of appetite), and other diseases related to *Annavaha Srotasa* (gastrointestinal system). The cumulative properties of ingredients depict their action as *Deepaniya* (digestion and metabolism enhancer), *Pachaniya* (enhancing digestion), and *Vatanulomaka* (regularising the physiological movement of *Vatadosha*). This formulation is proposed for the management of indigestion. In the series of drug development processes, the first stage is safety. The treatment of AYUSH-V-24 formulation for seven days in fistulated animals did not alter the overall ruminal microflora, pH, biochemical, and haematological parameters, except for a slight alteration in PCV and differential leucocyte count. The minor variation in DLC may be attributed to a seasonal, mild infestation commonly observed in ruminants. However, this did not affect digestibility, as evidenced by

consistent feed intake and water consumption. Ruminal parameters remained within the normal range before and after continuous administration of the AYUSH-V-24 formulation. Notably, there was no observable impact on kidney and liver biomarkers; they remained unchanged, indicating that the ingredients present in the formulation are generally safe under normal circumstances for ruminants. Hence, formulation can be used in the treatment of indigestion in ruminants without any side effects. Further, it is recommended that the clinical safety be tested at the time of ruminal indigestion treatment under field conditions, and clinical safety data can be generated in support of the formulation.

Simple indigestion is usually associated with a sudden change in the pH of the ruminal contents, hypomotility, putrefaction of ingested feed, and impaired rumen function. Simple indigestion has a nutritional basis, although the severity of the clinical signs can vary among the animals[9]. Further, there will be production losses, requiring antibiotics and other medical interventions, an unnecessary financial burden to the farmers[23]. The effectiveness of conventional medications in addressing gastrointestinal diseases in ruminants has shown variability in reported cases. However, the escalating concerns of overuse and misuse of these chemicals, coupled with the rising prevalence of resistance and the associated high treatment costs, underscore the imperative need for the exploration and development of alternative therapies for the treatment and management of gastrointestinal diseases in ruminants[24]. Thus, in recent years, the international community has recognised the modernisation of the herbal medicine industry as a pivotal element in the broader acceptance of alternative therapies. In ruminants, the safety of new drugs may be evaluated through the ruminal parameters, liver function tests, kidney function tests, and haematological profiling, as these animals are not allowed to be sacrificed for research purposes to collect the organs as per the rules of the animal ethical approval process.

5. Conclusion

The current findings suggest that the AYUSH-V-24 formulation, developed for managing ruminant indigestion, is safe for healthy animals. The study recommends that the formulation be safely utilised in the clinical management of indigestion in ruminants.

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