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Research Article

THERAPEUTIC EFFICACY OF RAJUVAS IMMUNITY BOOSTER POWDER IN HEMATOBIOCHEMICAL AND CLINICAL CIRCUMSTANCES IN CATTLE AFFECTED WITH LUMPY SKIN DISEASES

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ABSTRACT

The designed investigation was conducted on adult crossbred cattle in the Bikaner region of Rajasthan state that had the typical clinical indications of Lumpy skin disease. The animals were kept on individual holdings as well as on private dairy farms. A clinical examination and PCR were prime methods used to confirm for lumpy skin disease. In this trial 16 cattle were selected that underwent clinical inspections and analysis of their serum biochemistry and haematology aspects. Cattle were randomly divided into two groups: Group-II received symptomatic medication, which included enrofloxacin and flunixin meglumine, while Group III-received RAJUVAS immune booster powder. Group-I consisted of eight healthy adult cattle. The most common clinical signs of LSD-affected cattle comprised pyrexia, lacrimation, lethargy, lymph node enlargement, skin nodules and oedema. Rectal temperature, heart rate, and respiration rate were considerably ($P<0.05$) greater in clinical vital measures, although rumen motility was significantly ($P<0.05$) lower. The haematological data indicate that the mean values of Hb, PCV, TEC, TLC, and platelet count were considerably lower ($P<0.01$). Additionally, showed significantly more neutrophilia and lymphopenia, pre-treatment hypoproteinemia, hypoalbuminemia, and elevated levels of AST, ALT, ALP, creatinine and blood urea nitrogen (BUN) showed by LSD affected cattle when compared to the healthy cattle. Haemato-biochemical markers showed progress towards normalcy following 15 days of therapeutic care of LSD-affected cattle using a polyherbal formulation; however, the effectiveness of various treatment regimens at various intervals varied. Therapeutic effect of immunity booster powder was observed in the LSD affected cattle showed significant results in various clinical signs and haemato-biochemical parameters.

Keywords: Adult cross cattle, Lumpy skin disease, RAJUVAS Immunity Booster Powder

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Lumpy skin disease is a viral infection of cattle and triggered by Lumpy skin disease virus (LSDV). The virus together with the poxviruses of the sheep and goat belong to the genus Capripoxvirus (CaPV), subfamily Chordopoxvirinae, of the family Poxviridae (Buller et al., 2005). Capripoxvirus cause significant economic losses worldwide and the World Organization for Animal Health considered this disease (LSD) as notifiable disease (OIE, 2017). The virus replicates Intracellularly within fibroblasts, macrophages, pericytes and endothelial cells leads to vasculitis and lymphangitis in affected tissues (Coetzer, 2004).

Mechanical transmission of LSDV occurs by numerous blood-sucking insects such as mosquitoes and midges (Tuppurainen and Oura, 2012), can be transferred through blood, nasal discharge, lacrimal secretions, semen, and saliva and also transmitted through infected milk to suckling calves (Tuppurainen *et al.*, 2005). Major clinical signs of Lumpy Skin disease may be indicated towards persistent fever, widespread of skin nodules, enlarged superficial lymph nodes, conjunctivitis, keratitis, corneal opacity, oedema in the brisket and legs (Constable *et al.*, 2017).

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Ethnoveterinary medicine (EVM) is a scientific term for traditional animal health care that encompasses knowledge, skills, methods, practices, and beliefs about animal health care found among community members (McCorkle, 1986). Ethnoveterinary practice to animal health is as old as the domestication of various livestock species (Sri Balaji & Chakravarthi, 2010). The EVM provides valuable alternatives to and complements western-style veterinary medicine (Iqbal *et al.*, 2005).

The aim of this study was to describe the hematobiochemical and clinical findings associated with natural clinical infection of lumpy skin disease in cattle and therapeutic efficacy of different treatment regimen at different time intervals.

MATERIALS AND METHODS

Animals

The proposed study was carried out in Bikaner district. A total of 16 cattle were screened for clinical LSD symptoms before getting selected for the current investigation after that the presence of the Lumpy skin disease virus was verified by PCR.Each animal was monitored during the day to recording of physiological parameter and all the LSD affected cattle were further

carried out to know the body temperature, heart rate respiration rate and ruminal motility at the interval of a week.

A detailed history was recorded for every LSD affected cattle as Breed, age, sex, approximate body weight, feeding practices, recorded for associated signs and symptoms of pyrexia, nodules formation at various body parts, anorexia, weakness etc.

Haemato-biochemical Examination:

For haematological examination 2 ml blood samples from these LSD affected cattle were collected on day 0 and then weekly interval for two weeks from jugular vein puncture in sterile vacutainers having ethylene diamine tetra acetic acid (EDTA) disodium salt as an anticoagulant added at the rate of 1 mg/ml of blood for haematology and in non-anticoagulant coated sterile vacutainers for biochemical analysis. Serum was collected from whole collected blood by centrifuge at 3000 rpm for 15 min and stored at -20º C.

Haematological examination: Blood samples were analysed for haemoglobin, packed cell volume, total erythrocyte count, total leukocyte count and differential leukocyte count as described by Feldman *et al.* (2000). Biochemical analysis of serum samples was made to ascertain liver function by estimating serum total protein, albumin and globulin, alkaline phosphatase (ALKP), serum aspartate aminotransferase (SGOT), serum alanine aminotransferase (SGPT), Blood Urea Nitrogen (BUN), Serum Creatinine and Total bilirubin. These were determined by the colorimetric method by using the IDEXX VetTest Chemistry Analyzer.

Molecular Diagnosis:

Tissue samples from all LSD affected cattle were collected in sterile tissue collection tubes containing PBS solution. These sample were kept in ice box and carried out to laboratory for further procedure. These sample were also kept at -20º C till to sample processing for molecular detection through PCR. Lumpy skin disease suspected cattle were later verified by PCR testing for the Lumpy skin disease virus. Genomic DNA was isolated from whole blood using NucleoSpin® Tissue XS kit (LOT. 2106/004 MACHEREY-NAGEL Germany) as per protocol in manufacturers manual described. Using a pair of primers with the forward primer "ATGTCTGATAAAAAATT ATCTCG" and the reverse primer "ATCCATACCATCG TCGATAG," a 570-bp amplicon of highly conserved nucleotide sequences from the ORF 103 gene was amplified.

Therapeutic Trial:

In the treatment trial experiment, all the selected 16 cattle were divided into two groups comprising eight animals in each group. In these eight animals of group-II were treated with standard symptomatic treatment *viz*. Enrofloxacin @ 5mg/kg Body weight & Flunixin @ 1.1 mg/kg Body weight for 5 days and eight animals of group-III were treated with RAJUVAS immune-booster powder@ 286 mg per kg body weight once orally daily for 15 days.

RESULT AND DISCUSSION

The clinical profile and general state of health of LSD-affected cattle from all groups were tracked at weekly intervals, with the final observation being made during the second week of the treatment regimen, as shown in the table 1. Cattle in all groups experienced 100% survival rates and while none of the animal displayed pyrexia, lymphadenitis, lacrimation or nasal discharge. Post treatment few animals show mild clinical signs of emaciation, tiny skin nodules, edema, respiratory distress, lameness and decreased milk production at the end of the treatment regime. Almost comparable clinical results have been described in lumpy skin disease by (Jalali *et al.*, 2017; Neamat-Allah & Mahmoud, 2019; Keshta *et al.*, 2020; Shilpa *et al.*, 2022; Hatzade *et al.*, 2022; Jafarsab *et al.*, 2022 and Sandeep, 2023).

LSD infected animals showed, pyrexia 40-41° C for large release and rapid clearance of pyrogens (Ismail and Yousseff, 2006). Following the first febrile state is viremia lasts for about 4 days. Subsequent skin lesions, signs develop in certain locations as a result of the virus replicating in specific cells including fibroblasts, pericytes and endothelial cells of lymphatic and blood arteries (Abdulqa *et al.*, 2016; Hailu *et al.*, 2014).The inappetence observed could be a natural sequel to fever (Constable *et al.*, 2017).The complications of LSD resulted from damage of skin or mucous membranes that were followed by secondary bacterial invasion in addition to stress induced immunosuppression, anorexia, persistent fever and severe debilitation. This finding is in close agreement with (Fayez and Ahmed, 2011; El-Neweshy *et al.*, 2013). Lameness was a result of enlargement of prescapular and prefemoral lymph nodes (Aly *et al.*, 2006).

Molecular Detection:

Lumpy skin disease was confirmed by isolation and identification of lumpy skin disease virus (LSDV)genome (ORF103) through 570-bp amplicon, all sample was found positive to LSDV. These results corroborate the literature reports (Zhu *et al.*, 2013; Sohair and Gaafar, 2016; Khameis *et al.*, 2018; Hala *et al.*, 2021).

Physiological Parameters:

In the present study, the statistical analysis of

pretreatment data in LSD-affected cattle, the mean values of rectal temperature, heart rate, respiration rate and showed significant (P<0.01) higher, while rumen movement showed significant (P<0.01) lower as compared to healthy cattle (G-I) between the groups and within the groups data analysis show significant difference with time interval during the study. After treatment, data analysis showed that rectal temperature, heart rate, respiration rate and rumen movement values began to show a significant improvement on day 7th and by day 15th, in mean values of both treatment groups were approaching toward normal levels. Similar clinical findings were reported by Jafarsab *et al.* (2022), Kamer *et al.* (2022) and Sandeep (2023).

Haematological Parameters

The haematological data revealed that pre-treatment mean values of Hb, PCV, TEC, TLC, Lymphocytes and platelets in LSD affected cattle (G II and III) was significantly lower, whereas neutrophils showed significantly higher as compared to healthy cattle (G-I). Post-treatment within group weekly analysis of data revealed significant decrease in mean values of Hb, PCV, TEC, TLC, Lymphocytes and platelets of both treated groups up to 15th day. There is increase in Hb, PCV, TEC, TLC, Lymphocytes and platelets concentration rate is more in G-III as compared to G-II on 15th day. After therapeutic management of LSD affected cattle with standard symptomatic treatment and RAJUVAS immune booster powder, improvement towards normalcy in haematological parameters were observed with variation in efficacies of different treatment regimens at different time intervals.

In the present study, in LSD affected cattle there were significant alterations in pre-treatment mean values of most of the haematological parameters results agreed

Table 1. Comparison of clinical observations of pre and post- treatment in LSD affected cattle

S. No.	Clinical Signs		Days	G-II	G-III
1.	Fever		0	8	8
			15th	0	0
2.	Anorexia		0	8	7
			15th	2	0
3.	Emaciation		0	6	7
			15th	2	0
4.	Lymph node enlargement	Prescapular	0	0	1
			15th	0	0
		Prefemoral	0	3	0
			15th	0	0
		Prescapular+ Prefemoral	0	5	6
			15th	0	0
5.	Skin Nodules	Localized	0	1	3
			15th	0	1
		Generalized	0	7	5
			15th	6	4
6.	Edema		0	0	1
			15th	1	1
7.	Respiratory involvement		0	7	8
			15th	0	1
8.	Lachrymal		0	8	7
			15th	0	0
9.	Nasal secretion		0	5	3
			15th	0	0
10.	Lameness		0	3	4
			15th	1	0
11.	Reduce lactation		0	7	5
			15th	3	0
12.	Corneal opacity		0	0	0
			15th	1	0

Table 2. Mean ± SE values of Physiological parameter in healthy and LSD affected cattle at weekly interval

Prameters	Group	G-I			G-II			G-III		
	Time	0 day	7th day	15th day	0 day	7th day	15th day	0 day	7th day	15th day
Rectal Temperature		100.63± 0.1820^{bA}	100.53± 0.2624^{bA}	100.61± 0.2787^{bA}	103.61± 0.1370^{aA}	102.91± 0.1503^{bB}	101.38± 0.1524^{aC}	103.78± 0.2758^{aA}	104.01± 0.2415^{aA}	101.09± 0.3291^{abB}
Heart Rate		57.88± 0.4407^{bA}	58.88± 0.4407^{bA}	59.00± 0.3273^{bA}	80.13± 0.3407^{aA}	80.63± 0.4330^{aA}	70.50± 0.4880^{aB}	80.88± 0.4597^{aA}	78.88± 0.4597^{aB}	67.88± 0.3407^{bC}
Respiration Rate		39.25± 0.3660^{bA}	39.75± 0.3660^{bA}	41.50± 0.3780^{bA}	63.50± 0.6547^{aB}	69.75± 0.7792^{bA}	51.50± 0.7868^{bC}	62.88± 0.6339^{abB}	70.63± 0.5748^{abA}	59.50± 0.6547^{aC}
Rumen Movement		2.75± 0.1637^{aA}	2.63± 0.1830^{aA}	2.88± 0.1250^{aA}	1.63± 0.3740^{bA}	1.50± 0.3780^{bA}	2.13± 0.3407^{aA}	1.88± 0.3407^{bAB}	1.38± 0.3037^{bB}	2.50± 0.2182^{aA}

Mean± SE bearing different superscript (a, b, c) on between treated and control group and (A, B, C) within the group at different time period differ significantly.

Table 3. Mean ± SE values of Haematological parameter in healthy and LSD affected cattle at weekly interval

Prameters	Group	G-I			G-II			G-III		
	Time	0 day	7th day	15th day	0 day	7th day	15th day	0 day	7th day	15th day
Hb		10.15± 0.3111^{aA}	10.38± 0.1980^{aA}	10.05± 0.3134^{aA}	5.60± 0.2420^{cC}	6.73± 0.2750^{bB}	7.93± 0.3145^{cA}	5.85± 0.2130^{bB}	6.13± 0.2297^{bB}	8.98± 0.4241^{bA}
TEC		6.81± 0.1747^{aA}	6.95± 0.1307^{aA}	6.50± 0.1416^{bA}	4.71± 0.2616^{cA}	5.41± 0.2515^{cA}	4.71± 0.3021^{cA}	5.64± 0.1776^{bB}	6.04± 0.0907^{bB}	6.86± 0.3227^{aA}
PCV		28.24± 0.8507^{aA}	28.87± 0.5659^{aA}	27.97± 0.9230^{aA}	15.58± 0.6740^{bC}	18.71± 0.7444^{bB}	22.06± 0.9225^{cA}	16.17± 0.6068^{bB}	16.92± 0.5940^{bB}	24.86± 1.1748^{bA}
TLC		9.65± 0.3498^{aA}	9.79± 0.3453^{aA}	9.84± 0.4976^{aA}	6.49± 0.4339^{bC}	8.36± 0.3055^{bB}	9.26± 0.5241^{bA}	6.41± 0.4126^{bB}	7.98± 0.3122^{bA}	8.76± 0.4624^{bA}
Platelets count		491.50± 6.4890^{aB}	496.63± 7.4569^{aB}	521.13± 7.8477^{aA}	369.13± 6.6344^{bB}	328.88± 6.8281^{cC}	394.25± 6.9430^{bA}	349.38± 6.9973^{bB}	350.13± 7.6938^{bB}	410.63± 7.3515^{bA}
DLC	N	32.50± 0.8452^{bA}	32.38± 0.6797^{bA}	29.75± 0.4119^{bA}	38.00± 0.5000^{aB}	40.25± 0.6478^{aA}	37.25± 0.4226^{aB}	38.13± 0.7892^{aA}	39.75± 0.5261^{aA}	32.38± 0.6524^{abB}
	L	64.63± 0.7545^{aB}	65.13± 0.6105^{aB}	67.88± 0.5489^{aA}	57.38± 0.5957^{bB}	54.63± 0.5957^{cC}	59.25± 0.7480^{cA}	58.00± 0.7319^{bB}	56.63± 0.4978^{bB}	64.13± 0.5537^{bA}
	M	0.88± 0.2950^{aA}	0.88± 0.2950^{aA}	0.63± 0.2631^{aA}	1.25± 0.2500^{aA}	1.63± 0.2631^{aA}	0.88± 0.3407^{aA}	0.88± 0.3273^{aA}	1.00± 0.2673^{aA}	0.50± 0.2182^{aA}
	E	2.00± 0.2673^{aA}	1.50± 0.3273^{bA}	1.88± 0.2950^{bA}	3.38± 0.6797^{aA}	3.50± 0.5000^{aA}	2.63± 0.3037^{aA}	3.00± 0.2266^{aA}	2.63± 0.2631^{aA}	3.25± 0.2887^{aA}

Mean± SE bearing different superscript (a, b, c) on between treated and control group and (A, B, C) within the group at different time period differ significantly.

Table 4. Mean ± SE values of Biochemical parameter in healthy and LSD affected cattle at weekly interval

Prameters	Group	G-I			G-II			G-III		
	Time	0 day	7th day	15th day	0 day	7th day	15th day	0 day	7th day	15th day
Total protein		6.93± 0.0818^{aA}	7.10± 0.0741^{aA}	6.97± 0.054^{aA}	6.09± 0.0543^{bA}	5.20± 0.0791^{cB}	4.93± 0.0553^{cC}	6.06± 0.0663^{bA}	5.79± 0.0532^{bB}	6.22± 0.0669^{bA}
Albumin		3.05± 0.0463^{aA}	3.14± 0.0600^{aA}	3.09± 0.0455^{aA}	2.13± 0.0799^{bA}	2.20± 0.0343^{bA}	1.96± 0.0444^{cB}	2.17± 0.0347^{bC}	2.29± 0.0442^{bB}	2.74± 0.0343^{bA}
Globulin		3.89± 0.1032^{aA}	3.96± 0.0666^{aA}	3.88± 0.0496^{aA}	3.96± 0.0840^{aA}	3.00± 0.0777^{cB}	2.97± 0.0438^{cB}	3.90± 0.0711^{aA}	3.51± 0.0672^{bB}	3.48± 0.0627^{bB}
A:G		0.79± 0.0283^{aA}	0.79± 0.0242^{aA}	0.79± 0.0192^{aA}	0.54± 0.0304^{bC}	0.74± 0.0242^{aA}	0.66± 0.0196^{bB}	0.56± 0.0154^{bC}	0.65± 0.0235^{bB}	0.79± 0.0189^{aA}
SGOT		30.93± 0.2092^{bA}	31.07± 0.19^{bA}	31.00± 0.1967^{cA}	49.74± 0.3254^{aC}	68.96± 0.362^{aA}	51.18± 0.2834^{aB}	47.5± 0.3177^{aB}	69.14± 0.412^{aA}	39.25± 0.2652^{bC}
SGPT		80.43± 0.2464^{bA}	80.54± 0.2865^{bA}	80.65± 0.2585^{cA}	89.01± 0.2055^{aC}	101.13± 0.1515^{aA}	99.5± 0.1968^{aB}	88.85± 0.3077^{aC}	99.06± 0.2462^{aA}	90.95± 0.2187^{bB}
ALP		32.38± 0.375^{bA}	31.88± 0.295^{bA}	32.38± 0.4199^{cA}	41.13± 0.295^{aB}	61.75± 0.366^{aA}	60.88± 0.295^{aA}	41.25± 0.366^{aC}	61.88± 0.3981^{aA}	51.25± 0.4532^{bB}
Total bilirubin		3.95± 0.0648^{aA}	4.03± 0.0504^{cA}	3.95± 0.0412^{bA}	4.06± 0.0384^{aC}	6.23± 0.0533^{bA}	5.69± 0.1368^{aB}	4.07± 0.0478^{aC}	6.13± 0.0752^{aA}	5.13± 0.0795^{aB}
Creatinine		0.97± 0.0435^{bA}	1.02± 0.049^{bA}	1.01± 0.0508^{bA}	1.18± 0.0548^{aC}	1.60± 0.0494^{aA}	1.34± 0.0538^{aB}	1.18± 0.0564^{aB}	1.69± 0.048^{aA}	1.16± 0.0587^{abB}
BUN		20.10± 0.1616^{cA}	21.07± 0.1082^{bA}	21.73± 0.1599^{bA}	36.15± 0.1342^{bA}	34.00± 0.0896^{aB}	32.10± 0.0909^{aC}	38.51± 0.1707^{aA}	34.89± 0.0846^{aB}	22.94± 0.1164^{abC}

Mean± SE bearing different superscript (a, b, c) on between treated and control group and (A, B, C) within the group at different time period differ significantly.

with (Neamat-Allah (2015), Nashwa *et al.* (2017), El-Mandrawy *et al.* (2018), Yanni *et al.* (2021), Kamr *et al.* (2022), Shilpa *et al.* (2022), Ahmad *et al.* (2023), which might be attributed to pyrexia, dehydration, haemo-concentration, hypovolaemia, absolute erythrocytosis secondary bacterial infections and host response to LSD virus. Regarding to hematological results, (Table 3) showed a significant decrease in the number of total erythrocytic count and hemoglobin concentration in diseased groups which may be due to anemia and hemosidrosis of the lymph nodes and spleen (Jain, 2000), Lymphopenia may be due to release of endogenous corticosteroid from viral infection. Increased tissue demand, neutrophil margination and leukopenia seen in the early stages of acute infectious disease in ruminants were ascribed to the decline in TLC, (Jalali *et al.*, 2017 and Shilpa *et al.*, 2022) also identified leukopenia and lymphopenia seen in the early stages of the disease.

Biochemical Parameters:

In the present study, the statistical analysis of biochemical data revealed that pre-treatment mean values of total protein, albumin and A:G ratio in LSD affected cattle (G II and III) was significantly lower, whereas SGOT, SGPT, alkaline phosphatase, serum creatinine and blood urea nitrogen show significantly higher as compared to healthy cattle (G-I). Post-treatment within group weekly analysis of data revealed significant increase in mean values of total protein, albumin and A:G ratio and decrease in value of globulin of both treated groups up to 15th day, whereas SGOT, SGPT, alkaline phosphatase, serum creatinine and blood urea nitrogen show significantly increase up to 7th day and decrease on 15th day. After therapeutic management of LSD affected cattle with polyherbal formulation for 15 days, improvement towards normalcy in serum biochemical parameters were observed with variation in efficacies of different treatment regimens at different time intervals.

Pre-treatment hypoproteinaemia, hypoalbuminemia, hyperglobulinemia and increased concentration of total bilirubin, creatinine, SGOT, SGPT and ALP and increased level of blood urea nitrogen (BUN) in LSD affected cattle as compared to healthy control group in the present study were might be attributed to decreased protein synthesis and increased catabolic rate in affected animals, altered liver metabolism due to hepatic damage resulted from viremia and muscle damage occurring due to diseased pathogenesis (Sevik *et al.*, 2016 and El-Mandrawy *et al.*, 2018). Decreased the level of total protein and albumin, likely due to increased protein catabolism ordecreased protein synthesis as well as hepatic damage (Hassan *et al.*, 2011). Serum ALP was elevated in infected cattle, this phenomenon may have been caused by the effects ofinflammation on cells lining and surrounding the biliary ducts;related to the presence of hepatic cholestasis (Abutarbush, 2015; Stockham and Scott, 2008). It has been reported thatabsolutemusclemass and level of physical activitymay influencethe rate of creatinine production and thus the serumconcentration (Smith *et al.*, 2015). Hyperbilirubinemia was seen in some affected cases and it was likely secondary to the systemic disease process (Carlson, 2002).

CONCLUSION

In the present investigation, pre-treatment vales of physiological parameters, hematological and biochemical levels were significant altered in LSD affected cattle as compared to healthy control group. After therapeutic management of LSD affected cattle with polyherbal formulations for 15 days, hematobiochemical parameter show significant difference but their values improved towards normalcy in serum heamto-biochemical parameters, were observed with variation in efficacies of different treatment regimens at different time intervals, which might be attributed to effects of constituents present in polyherbal powder. The standard symptomatic treatment was helpful in early controlling of pyrexia and secondary bacterial infection while RAJUVAS immunity booster powder show better results were found in various clinical signs, haemato-biochemical parameters.

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