Int.J.Curr.Microbiol.App.Sci (2018) Special Issue-7: 249-254

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7692 **Special Issue-7 pp. 249-254**
Journal homepage: http://www.ijcmas.com

Original Research Article

Therapeutic Efficacy of Multimineral Injection to Treat Leukoderma in Buffaloes

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Keywords

Buffalo, leukoderma and multimineral injection

A B S T R A C T

Leukoderma or vitiligo is patchy dipigmentation of skin with premature graying of local hairs with the usual manifestation as appearance of patches of gray or white hair. Leukoderma as a pigmentry disorder of unknown cause characterized by depigmented patches due to destruction of melanocyte. Causative factors of leukoderma may include environmental insult, autoimmune response in which melanocytes are destroyed by immune system and inherited predisposition and/or copper deficiency. Treatment with injection Minshot, combined injections of copper, zinc, selenium and manganese, showed promising results and can be used in field to treat leucoderma in buffaloes.

Introduction

Leukoderma or vitiligo is common depigmenting skin disorder characterized by acquired, idiopathic, progressive, circumscribed hypomelanosis of the skin and hair. According to Radostitis *et al.*, (2007) leukoderma is the patchy dipigmentation of skin with premature graying of local hairs with the usual manifestation as appearance of patches of gray or white hair. According to Cerundolo *et al.*, (1991) in buffalo there is depigmentation that affects skin melanocytes. Im *et al.*, (1994) defined leukoderma as a pigmentry disorder of unknown cause characterized by depigmented patches due to destruction of melanocyte.

Many theories (biochemical theory, cytotoxic theory, oxidant-antioxidant theory, neural theory, viral theory, autoimmune theory, self-destruct theory, growth factor theory and convergence theory) have been proposed to explain this (Kaur Navneet *et al.*, 2012). Causative factors of leukoderma may include environmental insult (Cumming & Nordlund, 1995), autoimmune response in which melanocytes are destroyed by immune system (Fishman *et al*., 1997), and inherited predisposition and/or copper deficiency (Panduranga Rao *et al.*, 2002). Kumar *et al.*, (2007) observed distinct areas of depigmentation (Achromotrichia) and localized alopecia of skin on both left and right side of neck, thorax, medial aspect of

hind legs and inguinal region in hypocuprosis in an affected buffalo heifer. Because the disease is still not understood, there is a plethora of different treatments, controlled trials assessing efficacy of natural health products (vitamins, minerals, herbs and other supplements).

Dube *et al.,* (2014) observed that there is highly significant decrease in serum copper with non-significant changes in zinc iron and molybdenum in leukodermic buffaloes. Singh *et al.,* (2007) recorded decreased plasma copper, Zinc and sulphur levels as compared to normal and non-significantly higher Molybdenum and iron level in leukodermic buffaloes. In affected buffaloes the level of copper increased from 56.53mg/dl to 89.02 mg/dl after treatment with mineral mixture and copper sulphate (Gapat *et al.,* 2013). Gapat *et al.,* (2016) administered copper sulphate @ 300 mg/100 kg body weight once daily orally till recovery Serum copper and ceruloplasmin values significantly ($P<0.01$) increased after treatment, suggesting improvement in copper status of animal body. The serum zinc values significantly increased whereas iron values showed non-significant changes after treatment. Varun *et al.,* (2014) successfully treated leukoderma in a buffalo with ivermectin.

The present study was conducted to study the therapeutic efficacy of multi mineral injection, Minshot (Intas pharmaceuticals limited) treatment protocols in leukodermic buffaloes.

Materials and Methods

The study was conducted in and around the Narendra Deva University of Agriculture and Technology, Kumarganj, Faizabad (U.P.). The buffaloes showing white patches on various part of body were selected for the study. All buffaloes were clinically examined. Respiration, pulse rate, heart rate, rectal temperature, feed intake, rumen motility was in normal range. Urination and defecation was normal. Fecal examination and skin scrapping were performed for the detection of any parasitic eggs/larvae in the feces and detection of demodex, mange and fungal infection respectively. Blood samples were collected aseptically from jugular vein of each animal using sterilized disposable syringe and placed in two sets of sterile glass tubes, first set with anticoagulant, EDTA (@ 1 mg/ml of blood) for hematological purposes and second set without anticoagulant for serum separation. The blood samples were analyzed for Hb, PCV, TLC, TEC by using standard methods (Benjamin, 1985). The serum micro minerals such as copper, iron, zinc and manganese were estimated on atomic absorption spectrophotometer using standard diagnostic kits at Department of Soil Science, College of Agriculture, G.B.P.U.A. & T., Pantnagar U.S. Nagar Uttarakhand. Diagnosis of leucoderma in buffaloes were based on the basis of history, clinical signs, fecal examinations, and skin scrapping, blood and serum micro minerals analysis. The data generated was subjected to statistical analysis as per method described by Snedecor and Cochran (1994).

Results and Discussion

The study was carried on 08 buffaloes showing white patches. Buffaloes were administered with injection Intamin @ 10 ml (total dose) subcutaneously at weekly interval. Before the start of therapy deworming of all animal was done with Bolus Fenbendazole @5-7.5mg/kg body weight. Therapeutic efficacy of treatment was adjudged as seen visibly and on the basis of haematological and serum mineral status pre and post therapy. The animal was

thoroughly examined as per method described by Radostitis *et al.*, (2007). The location, extent, pattern and colour of lesion were recorded and the changes were observed post therapy. After 10 days of deworming observation pertaining to change in skin colour, hair colour, milk yield and fertility status were recorded at 10 days interval during course of treatment. Blood sample was collected before and after treatment (day 0 and day 60). The blood sample was analyzed for Hb, PCV, TLC, TEC, MCV, MCH, and MCHC by using standard protocol. The serum sample was used for analysis of micro minerals viz. Cu, Fe, Zn and Mn.

Buffaloes in this group that were administered with injection Intamin @ 10 ml (total dose) subcutaneously at weekly interval, showed 83.33% recovery in 60 days. Recovery was noticed in the remaining one animal with only few leukodermic patches were left at brisket region extending up to axilla. A significant ($p<0.05$) increase in Hemoglobin and PCV from 10.67±0.24 to 13.37±0.28 gm/dl and 35.40±1.03 to 42.90±1.25% respectively was recorded post therapy respectively in comparison to pretreatment value after 60 days. The changes in TEC, TLC, MCV, MCH and MCHC were statistically non-significant (Table 1). A significant increase in level of copper was noticed (48.99±1.29 to 93.83±1.00 µg/dl) after 60 day of treatment. The iron, Zinc and Manganese level increased from 100.50±0.96 to106.32 ±1.441 µg/dl, 72.92±2.44 to 92.45±1.35µg/dl and 51.41±1.16 to 65.56±1.44 µg/dl which was statistically not significant ($p<0.05$) (Table 2).

The increase in hemoglobin level is suggestive of increased absorption and utilization of iron for RBC synthesis owing to improvement in serum copper level. In order for haemoglobin synthesis to occur, iron must be converted to ferric form before being incorporated in to the haemoglobin molecule. In copper deficiency there is an impairment of iron release from reticuloendothelial cells due to decreased activity of ceruloplasmin resulting in decreased erythropoesis (Pankaj Kumar *et al.*, 2003). Copper, zinc, cobalt, manganese, and cadmium, because of their similarity in physiochemical characteristics to iron, are able to interfere with normal iron metabolism and consequently, heme and hemoglobin synthesis (Hill, 1976).

The increase in the PCV value might be due to increase in the total RBC count and increase in Hb value. Increased TEC value can be attributed to supplementation of iron, copper and zinc those play vital role in the synthesis of RBC.

The blood sample was analyzed for Hb, PCV, TLC, TEC, MCV, MCH, and MCHC by using standard protocol

Group	No. of Animals	Treatment Protocol	Therapeutic Regimen
Group	8	Multi –mineral Injection (INTAMIN, INTAS PHARMACEUTICAL LTD.) Zinc-60 mg/ml Manganese-10 mg/ml Selenium-5 mg/ml Copper-15 mg/ml	10 ml s/c repeated at 7 days interval

Table.1 Hematology of leukodermic buffaloes pre and after the treatment

	Pre treatment	Post treatment
Hb (gm/dl)	10.67±0.24	13.37±0.28[a]
PCV (%)	35.40±1.03	42.90±1.25[a]
TEC (×10^6 /mm^3)	6.70±0.16	7.74±0.28
TLC (×10^3 /mm^3)	10.98±0.44	11.8±0.19
MCV (fl)	52.78±0.64	55.54±0.73
MCH (pg)	15.93±0.36	17.39±0.69
MCHC (%)	30.18±0.65	31.29±1.11

[a] significant difference ($p<0.05$)

Table.2 Serum Mineral status of leukodermic buffaloes pre and after the treatment

	Pre treatment	Post treatment
Copper (µg/dl)	48.99 ±1.29	93.83 [a] ±1.00
Iron (µg/dl)	100.50±0.96	106.32 ±1.44
Manganese (µg/dl)	51.41±1.16	65.56 ±1.44
Zinc (µg/dl)	72.92±2.44	92.45 [a] ±1.35

[a] significant difference ($p<0.05$)

The higher percentage increase in serum copper value resulted in better response of the protocol. Production of melanin depends upon tyrosinase, which is a copper containing enzyme. Copper ions are needed for activating enzyme tyrosinase, which is responsible for melanin synthesis (Sastry, 2012). Serum iron level increased non-significantly by 5.8%. Melanin is an iron and sulphur containing brownish pigment so increased serum iron level resulted in best treatment protocol of leukoderma. Animals showed increased milk yield and better conception.

Post therapy zinc level showed a non-significant ($p<0.05$) increase with percentage increase of 26.8%. A non-significant change in serum zinc level was also recorded by Singh *et al.,* (2008). Gapat *et al.,* (2013) also found non-significant variation in serum zinc level after treatment of leukodermic buffaloes. Zinc is involved in keratinization of epithelial tissue (Underwood and Suttle, 1999) and appears to modify cell membrane by stabilizing the membrane structure thus reducing the peroxidation damage to the cell (Nockles and Blair, 1996).

Selenium, Zinc and Copper function to maintain low tissue concentration of reactive oxygen species and lipid hydroperoxide (Bettger *et al.,* 1979). This protective benefit results in improved immune response. Selenium is important to disease resistance because it is an integral component of the enzyme glutathione peroxidase (Arthur, 1985) which functions in the cytosol of the cell (Rice and Kennedy, 1986). This enzyme catalyzes the conversion of hydrogen peroxide to water and converts lipid peroxides to lipid alcohol and thus maintains low tissue concentrations of peroxides which if allowed to amass in cell can severely damage the cells and tissues (Smith, 1988).

Treatment with injection Minshot, combined injections of copper, zinc, selenium and

manganese, showed promising results and can be used in field to treat leucoderma in buffaloes.

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