AETIOLOGICAL AND THERAPEUTIC INVESTIGATIONS ON LEUKODERMA IN INDIAN BUFFALOES (*BUBALUS BUBALIS*)

S.M. Gapat[1], A.U. Bhikane[2,*], P.B. Hase[3] and G.R. Gangane[4]

ABSTRACT

Leukodema like chronic skin disease in buffaloes refractive to usual treatment is encountered in Marathwada region of India. The disease is causing heavy economic losses to animal owners due to substantial reduction in market value. Hence the present study was planned to investigate etiological and therapeutic aspects of leukoderma buffaloes. Eighteen buffaloes from 11 villages of Latur District presented with depigmentation of skin formed the material for the present study. Blood samples were processed for complete blood cell count on fully automated haematology cell counter, while serum copper, iron, zinc and ceruloplasmin were estimated on automated biochemical analyzer. The skin biopsy from affected buffaloes was examined for histopathological changes. Nine affected buffaloes were treated with copper sulphate 300 mg /100 kg body weight once daily orally till recovery. An overall incidence of the disease was found to be 0.43%. The characteristic clinical finding was graying of hair and depigmentation of skin on various parts of the body. Haemo-biochemical analysis revealed highly significant ($P<0.01$) decrease in Hb, PCV, TEC, serum copper, ceruloplasmin and zinc in leukoderma affected buffaloes when compared with healthy control buffaloes. The histoarchitectural studies of leukoderma affected buffalo skin revealed total absence of melanin, melanocytes and melanophores in epidermis. All treated buffaloes clinically cured within 55 to 210 days. It is concluded that leukoderma in buffaloes is caused by copper deficiency and can be successfully treated with copper sulphate.

Keywords: leukoderma, copper deficiency, melanin, copper sulphate, buffalo

INTRODUCTION

Leukoderma means loss of normal skin pigmentation (Radostits *et al*., 2007). It may be congenital or acquired. Congenital or hereditary leukoderma develops due to lack of melanocytes or failure of melanocytes to produce melanin or failure of transfer of melanin to epidermal cells. It can also be acquired through loss of existing melanin or melanocytes (depigmentation). The exact mechanism of depigmentation of skin includes breakdown in the conversion of tyrosine to melanin because of reduced tyrosinase activity

[1]Venky (India) Pvt. Ltd. Hyderabad, India

[2]Department of Veterinary Clinical Medicine, College of Veterinary and Animal Sciences, MAFSU, Dist-Latur (Maharashtra), India, *E-mail: draubhikane@rediffmail.com

[3]Veterinary Clinical Medicine, College of Veterinary and Animal Sciences, Parbhani, India

[4]Veterinary Pathology, College of Veterinary and Animal Sciences, Parbhani, India

resulting in achromotrichia and leukoderma in copper deficiency (Underwood, 1977).

Leukoderma is a common skin disease of man in tropical countries (Manson-Bahr, 1960), however it is rare in domestic animals. The clinical cases of chronic skin disease in buffalo characterised by whitish discolouration of skin and refractive to the acaricides, antibiotics, antihistaminics and corticosteroids are being reported by field veterinarians and animal owners from Marathwada region of India. The disease is causing heavy economic losses to animal owners as it is reducing the market value of buffaloes by 50 to 60 percent due to misbelieves amongst people that the consumption of milk from affected buffaloes is hazardous for human health and few people are of strong opinion that leprosy may be contracted from drinking of milk and eating of flesh of affected buffaloes. In view of these facts, the present study was planned to investigate etiological and therapeutic aspects of leukoderma buffaloes.

MATERIALS AND METHODS

The present study was carried on 18 clinical cases of leukoderma in buffaloes reported from 11 villages of Latur District of India. The data pertaining to age, sex, breed, number of lactations, feeding regimen, appetite, water intake, milk yield, body condition, site, number and colour of skin lesions and fertility status was recorded.

The blood samples collected from jugular vein in sterile citrated vials from 18 leukoderma affected and 9 healthy control buffaloes before and after completion of trial were processed for complete blood cell count on fully automated haematology cell counter (Model: Abacus Junior Vet, Diatron GMBH, Austria), while serum copper, iron, zinc and ceruloplasmin (Cp) were estimated on automated biochemical analyzer (Chemistry Analyzer C A -2005) using standard diagnostic kits. The skin biopsy was also collected in 10% neutral buffered formalin from apparently healthy as well as affected buffaloes before and after treatment. Each skin piece was processed as per the standard procedures and then stained with haematoxylin and eosin (H and E). The stained sections were examined for histopathological changes (Cullings, 1974).Nine affected buffaloes were treated with copper sulphate 300 mg/100 kg body weight once daily orally till recovery. The efficacy of treatment was judged on the basis of pigmentation of skin and hair coat, improvement in haematological parameters, histopathological findings, restoration of serum copper and ceruloplasmin levels.

Statistical analysis was carried out as per the methods described by Snedecor and Cochran (1994). The student ‘t’ test for unequal number of observations was used for comparison of physiological and haemato-biochemical values in leukoderma affected buffaloes (n=18) with healthy control group (n=9), where as student ‘t’ test for equal number of observations was used for comparison of values in leukoderma affected buffaloes before and after treatment.

RESULTS AND DISCUSSION

Out of 4229 buffaloes screened for depigmentation of skin from 11 villages in Latur District, eighteen cases were found clinically positive for leukoderma indicating an overall incidence of 0.43%. The present incidence rate was comparable to that reported by Randhawa (1993) in buffaloes from Punjab (0.84%). Recently Ahmed *et al*. (2009) reported clinical

signs of copper deficiency manifested by hair discolouration in 19.12% buffaloes from Egypt. Season wise distribution of clinical cases of leukoderma revealed higher occurrence in summer season (83.33%) followed by winter (11.11%) and monsoon (5.56%).

On the contrary, higher occurrence during winter (Randhawa, 1993) and rainy season (Panduranga Rao *et al*., 2002) has earlier been reported in Indian buffaloes. In the present study out of 18 buffaloes affected with leukoderma, 4 buffaloes (22.22%) were young i.e. below 4 years and 14 (77.78%) were adult i.e. above 4 years. The present findings are in general in agreement with Panduranga Rao *et al*. (2002). Higher incidence in adult buffaloes may be attributed to lower rate of absorption of copper in adult animals (5 to 10%) as compared to young (15 to 30%) animals (McDowell, 1992) and increase in the requirement of copper for synthesis of milk and development of foetus in adult lactating and pregnant animals, respectively. In the current investigation, sex-wise distribution revealed occurrence of leukoderma in female buffaloes only. The present findings are in agreement with Randhawa (1993).

Physiological status wise distribution of clinical cases was found to be maximum in pregnant buffaloes(50.00%) followed by lactating (33.33%) and growing (16.67%) animals. The present findings corroborates with Panduranga Rao *et al.* (2002) However, Randhawa (1993) reported maximum occurrence of leukoderma in heifers followed by dry and lactating buffaloes. In the present study, most of the leukoderma affected buffaloes i.e. 14 (76.98) were maintained either exclusively on dry fodder i.e. *Kadbi*/*Sorghum* straws or dry fodder *plus* concentrate about 1 kg. Only 4 buffaloes (22.22%) were receiving green fodder. The present observations supports the views expressed by Randhawa (1999) who mentioned that copper deficiency is primarily seen in ruminants thriving exclusively on forage. In affected buffaloes, the duration of illness varied from 30 to 240 days indicating chronic nature of disorder. The initial characteristic clinical finding was graying of hair (achromotrichia) on various parts of the body followed by depigmentation of skin. The fresh lesions were brownish-white/rosy white in colour which turned into milky white on aging. In the beginning the depigmentation was of pin head size which extended peripherally and coalesced with other lesions of the adjoining area to form white patches of irregular sizes (Figure 1 and Figure 2).

Similar observations were earlier recorded in leukoderma affected buffaloes by Sinha *et al*. (1976) and Panduranga Rao *et al.* (2002). The depigmentation of skin and hairs in affected buffaloes was attributed to deficient melanin production due to reduced activity of copper containing enzyme tyrosinase which is required for the formation of melanin (Sastry, 1983). The current research showed maximum distribution of lesions on neck, abdomen, followed by tail, chest, udder and head. The data reveals that the first site of lesion was more commonly on abdomen (38.88%) followed by neck (27.78%), chest (11.11%) and udder (11.11%). The typical pattern of depigmentation might be due to less exposure of ventral area of the body to direct sunlight as compared to dorsal region as opined by Bhayani and Vyas (1991).Moreover Chandra and Bhardwaj (1969) on histopathological examination noticed maximum melanin pigmentation in the dorsal region, intermediate pigmentation in lateral region and less pigmentation in ventral region in buffaloes. The temperature, heart rate, respiration rate and ruminal motility were found

to be non significant with each other in affected and healthy (control) buffaloes. The appetite, water intake, rumination, conjunctivae, defecation and urination were apparently normal. There was reduction in milk yield and delay in pubertal and post partum oestrus in affected animals. On haemoanalysis, highly significant ($P<0.01$) decrease in haemoglobin (Hb), packed cell volume (PCV) and total erythrocyte count (TEC) along with insignificant changes in total leucocyte count (TLC), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) in leukoderma affected buffaloes as compared to healthy were noted (Table 1). Similar findings were recorded by Soodan *et al.* (2007) and Ahmed *et al.* (2009) in hypocuprosis in buffaloes. Significantly lower values of Hb, PCV and TEC may be attributed to copper deficiency noticed in affected buffaloes in this study. It is well known that copper is required for absorption, mobilization and utilization of Iron for haemoglobin synthesis. In copper deficiency there is an impairment of iron release from reticuloendothelial cells due to decreased activity of ceruloplasmin resulting in decreased erythropoesis (Fisher, 1975; Pankaj Kumar *et al*., 2003).

Biochemically, highly significant ($P<0.01$) decrease in serum copper, ceruloplasmin and zinc was noticed in leukoderma affected buffaloes when compared with healthy control buffaloes. Serum iron values showed non significant variation between leukoderma affected and healthy buffaloes (Table 1). Hypocupraemia associated with leukoderma in buffaloes could be attributed to low copper intake due to prevailing feeding practices such as exclusive feeding of dry roughages viz. *straw*/*kadbi* which are naturally poor in copper (<5 ppm), feeding of very less quantity of concentrates which are generally optimum to rich in copper (>10 ppm) and non-supplementation of diet with mineral mixture. Earlier Gajbe *et al*., (1976) found reduced availability of copper to plants due to excess of molybdenum and alkaline pH of soils of Latur District. Subsequently, Kawitkar (2004) observed deficiency of copper in dry (82.38%) and green (64.28%) forages from the disease prone area. It can further be attributed to increased copper requirement in lactating buffaloes for milk production or in pregnant animals for foetal development or in heifers for growth.The histoarchitectural studies of skin in four leukoderma affected buffaloes revealed total absence of melanocytes and melanophores in epidermis. All the layers of epidermis were devoid of melanin pigment. The important lesion noticed was proliferation of large number of blood vessels in the papillary layer of dermis. The sebaceous and sweat glands did not show any significant histopathological changes. The sections of skin also showed focal thickening of stratum basale, mild to marked loss of stratum corneum and epidermal vacuolization (Figure 3 and Figure 4). The histopathological changes noticed were attributed to reduced synthesis of melanin owing to decreased activity of tyrosinase as a result of deficiency of copper. The histopathological findings noted in present study are in close approximation with Sinha *et al.* (1976) and Randhawa (1993). All 9 cases treated with copper sulphate alone 300 mg/100 BW orally once daily through *jaggary* till recovery completely cured indicating 100% clinical cure rate. The recovery period ranged from 55-210 days with an average value of 104.18±10.82 days in recovered animals (Figure 5 and Figure 6). Gill and Gill (1975) treated a case of vitiligo in buffalo bull with 0.5 gm of copper sulphate for 14 days and found regaining of black colour of skin in about 9 months. Sinha *et al.* (1976) treated a case of leukoderma with 18 injections of acetylarsan

Table 1. Mean haemato-biochemical values in leukoderma affected and healthy (Control) buffaloes.

Sr. No.	Parameter	Affected (n=18)	Healthy (n=9)	't' values
1	Hb (g/dl)	11.12±0.26	12.84±0.38	3.379 **
2	PCV (%)	35.70±1.02	41.23±1.07	3.426 **
3	TEC (× 10^6/µl)	6.37±0.15	7.53±0.23	3.811 **
4	TLC (× 10^3/ µl)	11.23±0.57	11.85±1.04	0.517 NS
5	MCV (fl)	55.94±0.59	54.89±1.02	0.890 NS
6	MCH (pg)	17.85±0.21	17.71±0.34	0.193 NS
7	MCHC (g/L)	31.28±0.20	31.10±0.41	0.208 NS
8	Cu (µg/dl)	56.53±0.70	75.61±1.11	14.490 **
9	Zn (µg/dl)	74.83±0.80	86.68±1.55	6.779 **
10	Fe (µg/dl)	103.68±0.78	105.80±0.80	1.825 NS
11	Cp (mg/L)	123.59±0.41	131.27±1.27	5.671 **

NS - Non significant ** - Highly significant (P<0.01)

Table 2. Mean haemato-biochemical values observed in leukoderma affected buffaloes supplemented with copper sulphate (n=9).

Sr. No.	Parameter	Before Treatment	After Treatment	't' values
1	Hb (g/dl)	11.28±0.61	12.06±0.21	1.291 NS
2	PCV (%)	37.30±1.97	41.06±1.48	2.633 *
3	TEC (× 10^6 / µl)	6.57±0.31	7.37±0.38	2.469 *
4	TLC (× 10^3/ µl)	10.18±1.37	10.94±1.27	0.410 NS
5	MCV (fl)	56.56±0.65	56.67±1.43	0.066 NS
6	MCH (pg)	17.57±0.31	17.94±0.61	0.631 NS
7	MCHC (g/l)	30.29±0.48	31.91±0.46	2.490 *
8	Cu (µg/dl)	55.90±1.08	78.55±1.14	13.038 **
9	Zn (µg/dl)	74.98±1.21	87.85±0.51	11.080 **
10	Fe (µg/dl)	104.03±1.11	105.07±0.92	0.872 NS
11	Cp (mg/L)	124.11±0.57	131.29±0.62	9.421 **

NS - Non significant * - Significant (P<0.05) ** - Highly significant (P<0.01)

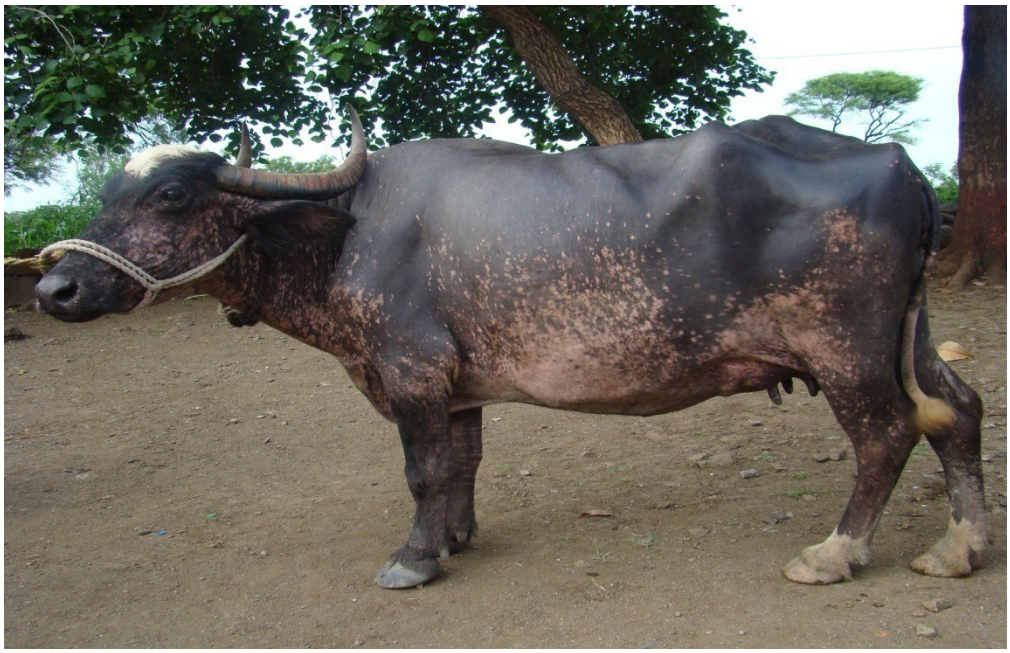

Figure 1. Note widespread reddish brown patches on most parts of the body in a eight year old lactating buffalo suffering from leukoderma.

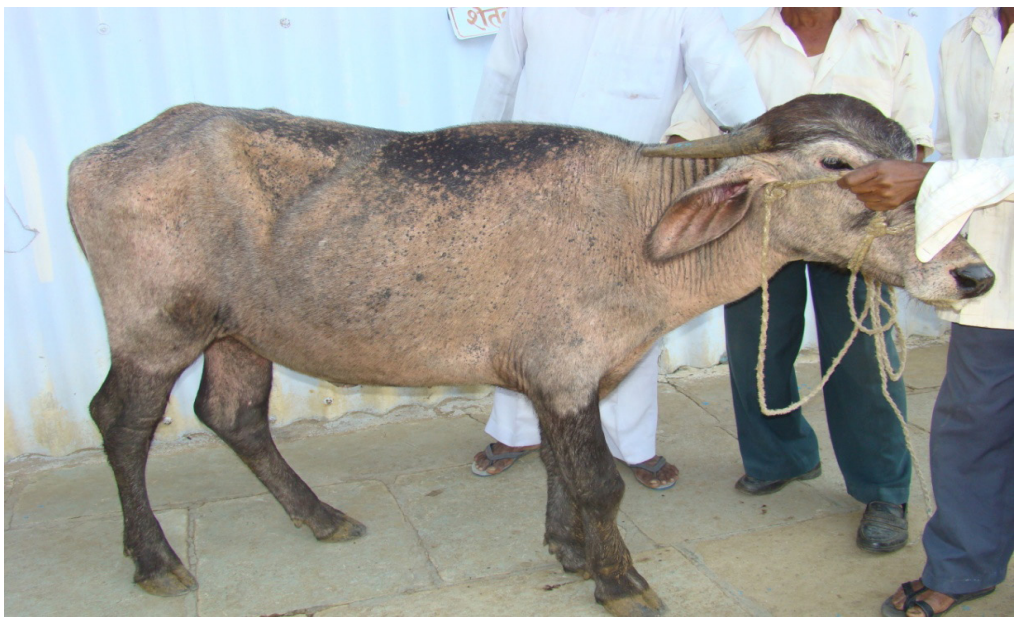

Figure 2. A case of diffuse leukoderma in a two year old buffalo heifer.

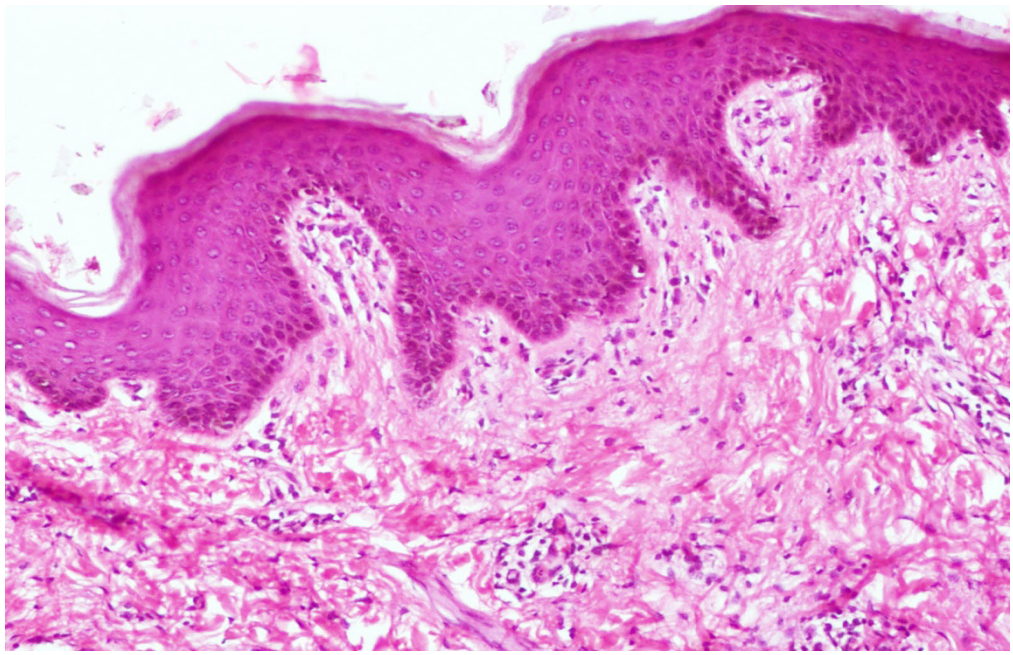

Figure 3. Note section of skin showing leukoderma evidenced by disappearance of melanin, melanocytes and focal thickening of stratum basale in Group B (H. and E. X 100).

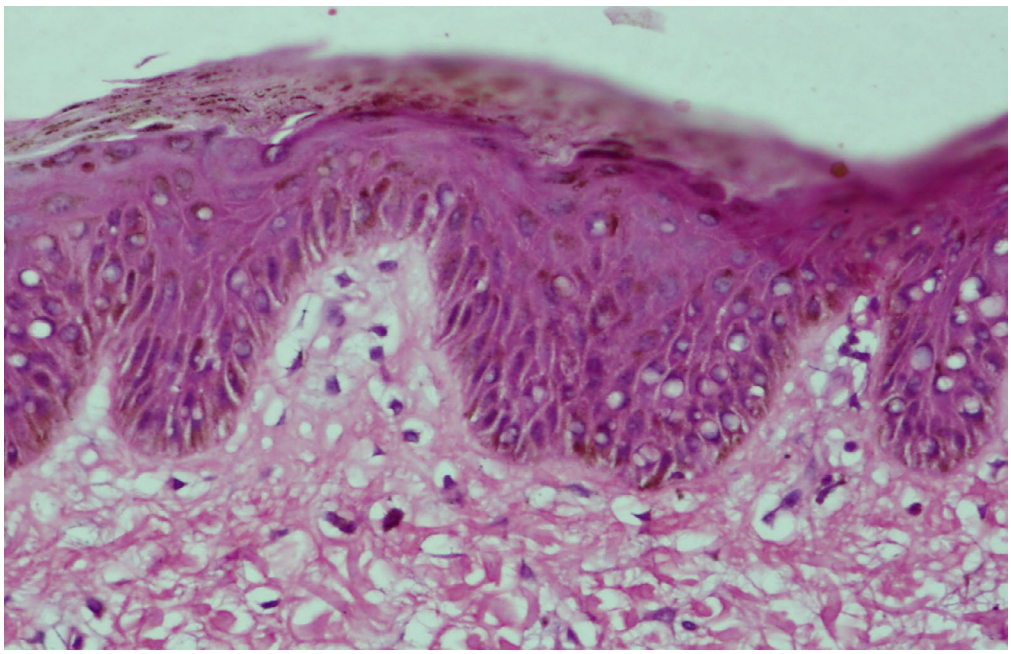

Figure 4. Marked melanosis characterized by great presence of melanin and melanocytes at its places along with epidermal vacuolization in Group B after therapy (H and E X 200).

Figure 5. A seven old leucoderma affected buffalo showing lesions over the neck region before treatment.

Figure 6. The buffalo showing complete recovery from leukoderma after treatment with copper sulphate 300 mg/100 kg body weight.

and 10 grains of copper sulphate for 2½ months and found disappearance of 90% white patches of skin. Panduranga Rao *et al.* (2002) treated clinical cases of suspected copper deficiency in buffaloes with copper sulphate 500 mg/day as drench in three spells of 15 days with a gap of one week and observed pigmentation in white patches of skin in 2 months after initiation of treatment.

The physiological values such as body temperature, heart rate, respiratory rate and ruminal motility did not vary significantly before and after treatment of leukoderma in buffaloes. The milk yield gradually increased from 3.11±0.54 to 3.44±0.56 lit/day after treatment suggesting net gain of 0.33 lit/day.Further among treated buffaloes 71.42% animals exhibited oestrus indicating improvement in fertility. A close look at data revealed significant increase in PCV, TEC and MCHC values when before treatment values were compared with after treatment values. Non-significant changes in Hb, MCV, MCH and TLC were noticed (Table 2). The improvement in PCV and TEC values may be attributed to increased erythropoeisis owing to copper supplementation. 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 (Table 2). In treated buffaloes, histopathological examination of skin indicated positive response to therapy evidenced by melanosis (Figure 4). All the cases studied responded well to the treatment given in present study. Radostits *et al.* (2007) and Chakrabarti (1994) stated that the copper is necessary for activation of tyrosinase which is required for synthesis of melanin from tyrosine. In present study a therapy given also acted in similar way there by producing melanin pigment in leukoderma treated cases.Thus it is concluded that leukoderma Indian buffaloes is caused by copper deficiency and can be successfully treated with copper sulphate.

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