



Indigenous herbal preparation against skin mycotic infection in goats

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ABSTRACT

An indigenous preparation consisting of *Andropogon muricatus*, *Annona squamosa*, *Argemone mexicana*, *Butea frondosa*, *Melia azadirachta* and *Cedrus deodara* was found effective against dermatomycosis in 12 goats based on changes towards normalcy with respect to skin scraping examination, total leukocyte count, total erythrocyte count, hemoglobin concentration, and changes in serum biochemistry including concentration/ activities of globulin, lipids, cholesterol, triglycerides, bilirubin, AST, ALT, serum albumin and A:G ratio. Other indices like change in body weight, milk yield, physico-chemical properties of meat and hepatic excretory function also responded positively to the treatment. All infected goats recovered within 1 month of therapy with indigenous herbal preparation.

Key words: Fungal infection, Goat, Herbs, Meat, Skin

Mycotic infections are common skin diseases (Dimri and Sharma 1998). Most of the dermatophytic zoonoses are transmitted by direct or indirect contact with infected animals. Unhygienic conditions and extreme climates influence the severity of the infection. Though, several chemical drugs are effective against skin mycosis, but with the problem of drug resistance and drug toxicity (Or *et al.* 2000). Thus, it is always desirable to test alternative effective indigenous herbal preparations. In present study, an indigenous herbal preparation was tested for its therapeutic efficacy in dermatomycotic infection in goats.

MATERIALS AND METHODS

The present study was conducted in indigenous non-descript adult goats reared in field areas of district Bareilly and Udham Singh Nagar. Goats (24) of either sex suffering from ringworm infection constituted the infected group and 12 healthy goats as the control group. From the infected group, 6 severely infected goats were used to study histopathological changes in tissues and physico-chemical changes in meat. Twelve infected goats were used for the therapeutic study. Six infected goats were left as the infected control group, which were treated after the end of the experiment. The herbal drug consisted of 125 g dried powder each of *Andropogon muricatus* (*khas*), *Annona squamosa*

(*sharifah*), *Argemone mexicana* (*pila-dhatura*) and *Butea frondosa* (*chuniagond*) mixed with 250 ml of *Melia azadirachta* (*mahanimb*) plus 250 ml of *Cedrus deodara* (*deodar*) oils. Based on our earlier experiments, the mixing of these herbal powders and oils was compatible. This preparation was applied once over the affected areas, daily for 4–10 days. The therapeutic efficacy was assessed on the basis of improvement in clinical signs like alopecia, rough skin, pruritus, erythema, hyperkeratosis, ring like lesions on various body parts; skin scraping examination results, changes in haemato-biochemical parameters, milk yield, body weight gain and histopathological/physiochemical changes of tissues/meat.

Skin scraping examination: Skin scrapings were collected on days 0, 7, 14, 21 and 28 for fungal culture and microscopic examination for mite. For inoculation, a few skin scrapings were taken with a sterile dissecting needle and implanted on Sabourauds dextrose agar (SDA- dextrose 40 g; peptone 10 g; agar 20 g; distilled water adjusted to pH 5.5 1000 ml) media at well-placed intervals. The growth patterns were regularly noted during the incubation period. In SDA, cycloheximide and chloramphenicol were added to reduce the growth of saprophytic fungi and bacteria, respectively. For each sample, 2 SDA tubes were used. Of these, one was incubated at 37°C and the other at 27°C for each sample. The fungal colonies were picked up. Dermatophyte test medium (DTM: phytone peptone, 10 g; dextrose, 10 g; agar, 20 g; distilled water, 1 000 ml; phenol red (0.5%); 40 ml, cycloheximide, 0.5 g; gentamicine sulphate, 100 000 units; sterile chlortetracycline HCl, 100 000 units; 0.8 N HCl; 6

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ml) was used as a screening medium. Change in colour from yellow to red due to alkaline metabolites was noted.

Skin scraping was tested as per standard method for mite or fungal structures (hyphae and spores) visible if present. For detailed examination, skin scrapings were kept overnight in 10% NaOH for microscopic examination. Identification of fungi was done on the basis of macroscopic and microscopic characteristics, as described by Beneke and Rogers (1980).

Haematology: Blood (3 ml approx.) samples were collected in EDTA vials and analyzed for haemoglobin (Hb), total erythrocyte count (TEC), total leukocyte count (TLC) and differential leukocyte count (DLC) as per Jain (1986).

Biochemical parameters: Serum samples were harvested from 5 ml blood collected in sterilized tubes. Different biochemical parameters like blood glucose (Folin and Wu 1920), serum total protein, serum albumin (Greenburg, 1929), serum globulin (by difference of total protein and serum albumin), albumin: globulin (A:G) ratio, serum total lipids (Frings *et al.* 1972), serum total cholesterol (Wooton 1964), serum triglycerides (Gifftried and Rosenberg 1973), serum bilirubin (Ducci and Watson 1945), aspartate aminotransferase, alanine aminotransferase (Reitman and Frankles 1957), sodium and potassium (Oser 1965) were estimated as per the standard methods.

Excretory function of liver: The excretory function of liver was assessed by determination of bromosulphophthalein (BSP) retention time in 6 infected, 6 infected plus treated and recovered and 6 healthy animals which were used for the meat quality assessment during mycotic infection of the skin.

Production: The body weight of each animal was taken daily and the mean daily body weight gains were calculated up to 1 month after the start of therapy. Milk yield (liter) was noted both in the morning and evening and mean daily milk yield was calculated to observe any changes in the milk yield.

Pathological study: Gross pathological study was conducted in all the goats maintained under the present investigation. Six severely infected animals and 6 animals suffering from ringworm after 1 month of receiving therapy were sacrificed to study the microscopic changes in skin, liver, lymph node, spleen and kidneys to assess the toxicity (if any) of herbal preparation. Six animals from the healthy control group were used to study gross and histopathology of above mentioned tissues in healthy animals. The pathological studies were conducted as per Lillie and Fulmer (1976).

Physiochemical property of meat: Meat samples (400 g each) were collected from the same part of the body over thigh region from 6 infected, 6 infected- treated plus recovered and 6 healthy animals. Samples were subjected to testing for pH, water holding capacity, colour and tenderness.

pH determination: Meat sample (10 g) was homogenized with 50 ml of distilled water for 30 sec in a warring blender. The pH of the slurry was recorded by a digital pH meter.

Water holding capacity (WHC): Four replica of 1 sample were tested simultaneously. Meat (15 g) was added to 22.5 ml of salt-water solution in a centrifuge tube to make a meat homogenate. The tubes were kept in refrigerator (4°C) for 15 min and then centrifuged at a speed of 6000 rpm for 15 min. The supernatant was decanted into measuring cylinder and the volume was noted. The amount of added solution retained by the meat was recorded as the water holding capacity (ml/gm) of the meat and WHC percent was calculated as $(\text{volume of water retained}/15) \times 100$.

Colour: The colour of meat sample was tested by the tintometer.

Shear force: For evaluation of tenderness of meat, shear force required for shearing/cm thick strips of meat was measured in kg on shear press of 25 kg capacity.

The data were analyzed as per Snedecor and Cochran (1976).

RESULTS AND DISCUSSION

Alopecia is a common manifestation of skin diseases (Radostits *et al.* 2007). The associated fungi observed were *Microsporum* and *Trichophyton* sp. Clinically, the infected animals had alopecic patches, pruritus, erythema, hyperkeratosis, loss of appetite, ring like lesions on the skin, abnormal appearance and discolouration of ring structures, mainly in the head region. The clinical manifestations observed in goats with ringworm infection were reversible after the application of the prepared indigenous herbal medicine in 14 days. In literature, *Butea frondosa* and *Melia azadirachla* have been used against fungal infections, *Argemone mexicana* against chronic skin diseases, *Andropogon muricatus* against inflammation, *Annona squamosa* and *Cedrus deodara* against skin diseases (Nadkarni 1954). Thus, the preparation was predicted to have anti-fungal properties and ability to resist secondary infections.

Following medication, the skin scrapings were negative for ringworm infection after two weeks. There was a gradual improvement in the clinical lesions by days 7, 14, 21 and by day 28, the clinical lesions had improved in all the animals except one, showing slight loss of appetite, which also disappeared within next two days.

The levels of Hb, TEC and neutrophils were significantly ($P < 0.05$) lower in the infected group (Table 1) as compared to control group on day 0, which was normalized by days 7, 14, 21 and 28 of treatment. On the other hand, the levels of TLC and lymphocytes were significantly ($P < 0.05$) higher in the infected group as compared to their levels in the control group on day 0, which became comparable to their respective control group values on days 7, 14, 21 and 28. The reduction in the hemoglobin concentration may be due to low erythrocyte counts (Jain 1986). The decreased TEC may be due to severity of skin lesions, which reduce the respiratory function of the skin producing hypoxia and toxemia as

Table 1. Haemato-biochemical changes in goats

Parameter	Group	0 day	7th day	14th day	21st day	28th day
Hb (g/dl)	Infected	9.41±0.65 ^{Ba}	9.79±0.19 ^{Ba}	10.09±0.29 ^{Ba}	11.21±0.31 ^{Ab}	12.12±0.27 ^{Ab}
	Control	12.23±0.78 ^{Aa}	12.29±0.51 ^{Aa}	12.23±0.87 ^{Aa}	12.32±0.59 ^{Aa}	12.24±0.59 ^{Aa}
TEC (× 10 ⁶ cmm)	Infected	8.21±0.51 ^{Ba}	8.81±0.61 ^{Ba}	10.29±0.47 ^{Ab}	10.89±0.43 ^{Ab}	11.29±0.59 ^{Ab}
	Control	10.59±0.39 ^{Aa}	11.01±0.74 ^{Aa}	11.31±0.57 ^{Aa}	11.21±0.67 ^{Aa}	11.51±0.56 ^{Aa}
TLC (× 10 ⁶ cmm)	Infected	13.81±2.53 ^{Ba}	13.51±2.61 ^{Ba}	11.81±2.19 ^{Ab}	11.79±2.49 ^{Ab}	11.01±2.32 ^{Ab}
	Control	10.19±3.52 ^{Aa}	11.81±3.20 ^{Aa}	11.12±3.51 ^{Aa}	11.83±2.83 ^{Aa}	10.29±2.19 ^{Aa}
DLC (%)	Infected	20.17±3.61 ^{Ab}	24.08±3.72 ^{Bb}	27.19±3.59 ^{Ab}	27.48±3.72 ^{Ab}	27.89±3.81 ^{Ab}
(a) Neutrophil	Control	29.51±3.12 ^{Aa}	29.61±4.10 ^{Aa}	31.00±3.27 ^{Aa}	30.17±3.61 ^{Aa}	29.16±4.17 ^{Aa}
(b) Lymphocyte	Infected	65.71±5.29 ^{Da}	63.01±4.32 ^{Ba}	60.56±5.71 ^{Ab}	60.39±5.53 ^{Ab}	58.11±5.37 ^{Ab}
	Control	57.61±5.49 ^{Aa}	57.50±4.71 ^{Aa}	57.18±4.71 ^{Aa}	57.07±5.29 ^{Aa}	57.81±5.47 ^{Aa}
Protein (g/dl)	Infected	5.82±0.47 ^{Aa}	6.11±0.71 ^{Aa}	6.27±0.59 ^{Aa}	6.58±0.29 ^{Aa}	6.80±0.41 ^{Aa}
	Control	6.31±0.32 ^{Aa}	6.56±0.39 ^{Aa}	6.58±0.51 ^{Aa}	6.48±0.61 ^{Aa}	6.81±0.51 ^{Aa}
Albumin (g/dl)	Infected	2.36±0.52 ^{Ba}	2.82±0.71 ^{Aa}	2.86±0.61 ^{Ab}	3.21±0.25 ^{Ab}	3.41±0.67 ^{Ab}
	Control	3.18±0.61 ^{Aa}	3.34±0.62 ^{Aa}	3.35±0.51 ^{Aa}	3.34±0.41 ^{Aa}	3.56±0.51 ^{Aa}
Globulin (g/dl)	Infected	3.46±0.41 ^{Ba}	3.29±0.40 ^{Aa}	3.41±0.41 ^{Aa}	3.37±0.31 ^{Aa}	3.29±0.81 ^{Aa}
	Control	3.13±0.21 ^{Aa}	3.21±0.22 ^{Aa}	3.23±0.31 ^{Aa}	3.14±0.62 ^{Aa}	3.35±0.51 ^{Aa}
A:G ratio	Infected	0.68±0.43 ^{Ba}	0.85±0.52 ^{Aa}	0.83±0.47 ^{Aa}	0.95±0.71 ^{Aa}	1.03±0.51 ^{Ab}
	Control	1.01±0.51 ^{Aa}	1.04±0.57 ^{Aa}	1.03±0.32 ^{Aa}	1.06±0.62 ^{Aa}	1.06±0.44 ^{Aa}
Glucose (mg/dl)	Infected	62.19±4.16 ^{Aa}	62.17±4.19 ^{Aa}	61.31±4.12 ^{Aa}	60.81±5.10 ^{Aa}	60.35±4.07 ^{Aa}
	Control	59.51±4.08 ^{**}	59.15±4.17 ^{**}	59.23±4.18 ^{Aa}	59.73±4.79 ^{Aa}	50.07±4.63 ^{Aa}
Lipids (mg/dl)	Infected	199.5±4.32 ^{Ba}	192.17±4.01 ^{Ba}	186.10±2.13 ^{Ba}	180.14±2.12 ^{Ba}	177.91±2.32 ^{Ab}
	Control	166.31±2.75 ^{Aa}	165.12±2.61 ^{Aa}	169.61±3.01 ^{Aa}	161.45±3.25 ^{Aa}	169.57±2.09 ^{Aa}
Cholesterol (mg/dl)	Infected	91.12±4.17 ^{**}	84.07±5.16 ^{Aa}	83.71±5.26 ^{Aa}	83.51±4.51 ^{Aa}	83.16±4.57 ^{Aa}
	Control	88.16±4.61 ^{Aa}	78.19±4.53 ^{Aa}	79.15±4.62 ^{Aa}	81.36±4.10 ^{Aa}	85.15±4.32 ^{Aa}
Triglycerides (mg/dl)	Infected	44.11±1.51 ^{**}	42.61±1.61 ^{**}	42.41±1.55 ^{Aa}	42.16±1.71 ^{Aa}	41.61±1.61 ^{Aa}
	Control	41.17±1.61 ^{**}	41.41±1.76 ^{**}	41.15±1.21 ^{**}	41.51±1.86 ^{Aa}	41.31±1.31 ^{Aa}
Bilirubin (mg/dl)	Infected	0.22±0.060 ^{Aa}	0.19±0.06 ^{Aa}	0.18±0.03 [^]	0.17±0.05 ^{Ab}	0.16±0.04 ^{Aa}
	Control	0.15±0.03 ^{Aa}	0.14±0.02 ^{Aa}	0.13±0.01 ^{Aa}	0.14±0.04 ^{Aa}	0.14±0.09 ^{Aa}
ALT (GPT) (RFU/ml)	Infected	78.11±1.91 ^{*1}	67.31±2.61 ^{Ba}	57.19±1.83 ^{1*}	50.82±1.19 ^{Bb}	49.61±2.15 ^{Ab}
	Control	46.31±2.17 ^{**}	45.12±1.16 ^{**}	44.31±2.29 ^{**}	46.32±1.22 ^{Aa}	46.37±1.79 ^{Aa}
AST (GOT) (RFU/ml)	Infected	170.12±2.14 ^{Aa}	171.14±4.72 ^{**}	168.15±4.17 ^{Aa}	172.15±5.10 ^{Aa}	168.21±5.14 ^{Aa}
	Control	171.62±3.15 ^{**}	172.16±3.79 ^{Aa}	169.12±4.68 ^{Aa}	173.17±5.14 ^{Aa}	169.14±4.79 ^{Aa}
Sodium (m. eq./litre)	Infected	144.51±5.71 ^{**}	142.71±6.15 ^{Aa}	145.16±9.71 ^{Aa}	144.68±7.62 ^{Aa}	146.08±6.02 ^{Aa}
	Control	144.01±5.62 ^{A3}	142.10±9.68 ^{Aa}	143.71±8.61 ^{Aa}	143.17±6.97 ^{Aa}	145.71±6.98 ^{Aa}
Potassium (m. eq./litre)	Infected	4.50±0.19 ^{Aa}	4.51±0.21 ^{Aa}	4.50±0.12 ^{Aa}	4.53±0.27 ^{Aa}	4.57±0.98 ^{Aa}
	Control	4.51±0.67 ^{Aa}	4.52±0.32 ^{Aa}	4.51±0.61 ^{Aa}	4.49±0.32 ^{Aa}	4.51±0.67 ^{Aa}

Values with different small letters (within group) and capital letters (between groups) differ significantly (P<0.05).

described by (Pasko and Chotchaev 1974). The increased leukocyte levels may be explained due to inflammatory reactions produced as a result of fungal invasion or due to the allergic reactions produced in the skin by fungus. The increase in lymphocytes and a neutropenia in goats suffering from fungal skin infection was also observed (Sidhu 1991).

Levels of albumin and the A: G ratio were significantly (P<0.05) lower in the infected group as compared to their levels in the control group on day 0. On days 7, 14 and 21, these levels showed improvement in the infected group and by day 28, approached nearly normal levels of the control group. The serum globulins were significantly higher (P<0.05) in the infected group on day 0 as compared to control group. The level in the infected treated group

on days 7, 14, 21 and 28 moved towards the values of control group.

Blood glucose in different groups was comparable to the untreated control group. The serum sodium and potassium levels also did not vary significantly between the control and the infected groups at various periods. The serum lipids, bilirubin, ALT, AST, triglycerides and cholesterol levels were higher in the infected groups as compared to the control group on day 0, whereas by the end of the study, the levels of these parameters approached near the levels of the control group indicating positive effects of the therapy. The increased levels of bilirubin, AST and ALT in the infected group may be related to mild abnormalities in the liver.

The protein profile indicated a decreased albumin level, an increased globulin level and decreased A: G ratio in all

Table 2. Changes in average daily milk yield, weight gain and BSP per cent retention time in goats

Parameter	Group		
	Infected control (No treatment)	Infected and treated	Healthy
Milk yield (liters/ day)	0.712±0.41	1.516±0.46*	1.628±0.57*
Daily weight gain (g)	17.10±1.10	30.21±2.31	34.68±3.42*
BSP per cent retention	4.92±0.46	4.11±0.27	4.07±0.31*

* Value differ significantly ($P < 0.05$) from infected control group.

the animals infected with the fungi. Higher levels of globulin and lowered albumin and A: G ratio in skin infections were reported (Shah 1994, Kaneko *et al.* 1997). Here, when infection is chronic, goats suffer from loss of appetite and malnutrition. The levels of blood glucose, cholesterol, lipids, sodium, potassium and triglycerides did not show any significant alteration in the present study. The increased levels of AST, ALT and bilirubin indicated an adverse effect on the liver, which was reversible after therapy. The biochemical alterations observed due to fungal skin infection were reversible, values approaching towards normalcy after the treatment with the herbal drug.

Histopathologically, infected animals revealed hyperkeratosis, thinning of dermis with lack of dense appearance due to atrophy of sebaceous glands and hair follicles in the skin. Inflammatory cells invasion with a tendency to form foci was observed in superficial skin surface. Degeneration of hair follicles was also apparent. Spleen was grossly of normal size and microscopically, there was hyperplasia with prominence of lymphoid follicles. The liver grossly had areas of congestion; correspondingly the focal areas of congestion and haemorrhages were discerned microscopically. In addition, dismantling of hepatic cords and cytoplasmic vacuolization were strikingly increased in the hepatocytes. The kidneys and lymph nodes did not reveal any appreciable change. In animals with infection and later recovery after therapy, none of these organs showed any pathological changes. There was an improvement in the body weights of the infected group after 28 days of treatment, though comparatively lesser than that of the controls (Table 2). The milk yield also showed improvement in treated group. The muscle pH, mean water holding capacity and tenderness were significantly ($P < 0.05$) altered in the infected animals as compared with the healthy and recovered animals. The mean ($\pm$ SE) pH values of muscle from infected and recovered (infected plus treated) animals were 6.14 ± 0.04 and 6.01 ± 0.06 , respectively. The mean water-holding capacity (percentage) of muscles from infected and recovered animals was 34.12 ± 6.32 and 34.13 ± 6.15 respectively. The tenderness values (kg/cm^3) from infected and recovered animals were

0.115 ± 0.004 and 0.101 ± 0.002 respectably. The color of muscles from recovered animals was more bright red than the infected animals.

The higher pH values in meat of infected animals may be due to fast depletion of glycogen. The high shear force value in infected animals indicated a tougher meat in comparison to recovered animals. The infected animals had dull meat colour due to structural changes in the collagen fibers of muscle and due to decreased water-holding capacity, which might have affected reflectance power of the muscles. Thus, adverse effect of dermatomycotic infections is also reflected on physiochemical properties and quality of meat. The bromo-sulphophthalein retention time also indicated recovery of liver from stress following therapy with the herbal preparation.

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