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Research Article

HISTOCHEMICAL LOCALIZATION OF MUCOPOLYSACCHARIDES, PROTEINS AND LIPIDS IN BUFFALO SKIN

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ABSTRACT

The present study was conducted on the skin of 12 buffaloes collected from slaughterhouse and postmortem hall of GADVASU, Ludhiana. The skin samples were collected from dorsal, lateral and ventral regions of head, neck, thorax, abdomen and tail. The tissues were fixed in 10% neutral buffered formalin and processed for paraffin block preparation. The paraffin sections were stained with PAS for neutral mucopolysaccharides, alcian blue for acid mucopolysaccharides, bromphenol blue for proteins. Lipids were demonstrated on cryostat sections of fresh unfixed frozen tissues. Skin structures showed a moderate to strong reaction for neutral and acid mucopolysaccharides, proteins and lipids, which play an important role in cell regeneration, collagen synthesis and as antimicrobial agent. Sweat glands showed a strong reaction for neutral and acid mucopolysaccharides, whereas sebaceous glands showed a strong to intense reaction for total lipids.

Keywords: Buffalo skin, Lipids, Mucopolysaccharides, Proteins

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Skin is the multilayered organ comprising of epidermis, dermis (Dellmann, 1993) and is responsible for protection, thermoregulation, external sensory awareness, immunological defense, wound healing, perception, excretion and also an effective barrier which prevents desiccation of electrolytes and macromolecules from the body (Bhattacharya *et al.*, 2003). The ground substance contains substantial amounts of glycosaminoglycans, rich in acid mucopolysaccharides as well as water, salts, and glycoproteins. The acid mucopolysaccharides are mainly hyaluronic acid, dermatan sulfate, and chondroitin-6-sulfate, as well as smaller amounts of heparin sulfate (Falco *et al.*, 2000). The distribution of histochemical moieties in skin has been reported in cattle (Bhayani and Vyas, 1991), pig (Sumena *et al.*, 2010), sheep (Ahmad *et al.*, 2010), goat (Razvi *et al.*, 2013), but literature on buffalo skin is scanty, so the present work was undertaken.

MATERIALS AND METHODS

The present study was conducted on skin of 12 buffaloes collected from slaughterhouse and postmortem hall of GADVASU, Ludhiana. The skin samples were collected from dorsal, lateral and ventral regions of head, neck, thorax, abdomen and tail. The tissues were fixed in 10% neutral buffered formalin. After the fixation, the tissues were processed for paraffin block preparation by acetone benzene (Luna, 1968). The blocks were prepared and sections of 5-6 µm thickness were cut with rotary microtome. These paraffin sections were stained with periodic acid Schiff for neutral mucopolysaccharides, alcian blue for acid mucopolysaccharides and bromophenol blue for proteins. The cryostat sections of 10 µm thickness of unfixed frozen skin at -20 ºC were cut and stained with Sudan black B for lipids.

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RESULTS AND DISCUSSION

The histochemical distribution of neutral mucopolysaccharides, acid mucopolysaccharides, basic protein and total lipid in different part of epidermis and dermis has been summarized in table 1. There was no difference in localization of these histochemical moieties in skin of different body regions.

Neutral mucopolysaccharide (NMPS)

Among the different layers of epidermis, stratum basale showed moderate to strong reaction, stratum spinosum moderate, stratum granulosum a weak to moderate reaction and stratum corneum showed weak reaction for neutral mucopolysaccharides. The basement membrane was strongly positive for neutral mucopolysaccharides as reported by Baba *et al.* (1990) in sheep, Bhayani and Vyas *et al.* (1991) in Gir cattle and Archana *et al.* (2006) in porcupine and Ahmad *et al.* (2010) in skin of Madras Red Sheep. Sumena *et al.* (2010) reported PAS positive reaction in upper stratum spinosum and stratum granulosum in the epidermis of Large White Yorkshire Pigs.

In the dermis layer, connective tissue showed moderate to strong reaction for PAS. Within hair follicle, the internal epithelial root sheath showed moderate to strong reaction; external epithelial root sheath showed weak to moderate reaction, whereas Ahmad *et al.* (2010) reported intense

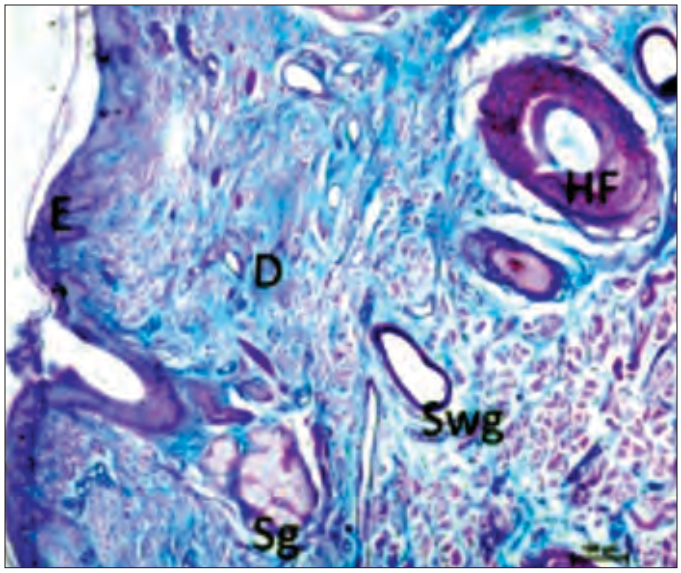

Fig. 1. Head ventral region of skin showing PAS-AB reaction in epidermis, hair follicle, dermis, sweat gland and sebaceous gland. PAS-AB × 100

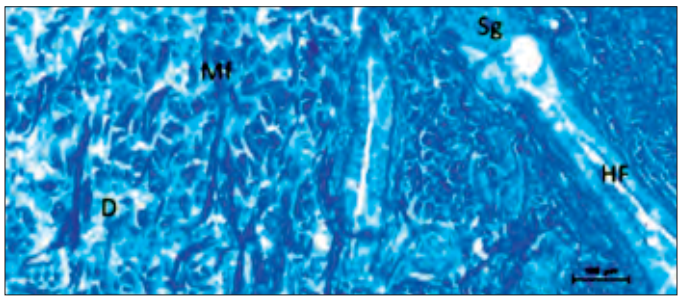

Fig. 3. Abdomen dorsal region of skin showing reaction for proteins in dermis, hair follicle, sebaceous gland and muscles fibers. Bromphenol blue × 100

reaction of PAS in the hair follicle of Madras Red Sheep. Sumena *et al.* (2010) reported that the cells of outer root sheath of hair follicle around the keratogenous zone were strongly positive for PAS. The cells of internal root sheath and dermal papilla were positive for PAS in Large white Yorkshire pigs. In the present study, sweat glands showed strong reaction for neutral mucopolysaccharides as reported earlier by Montagna and Yun (1964) in pig. Taha and Abdalla (1980) in Dromedary camel observed that the secretory cells of sweat glands were intensely PAS positive; however, the lumina of the tubules and the ducts were consistently unreactive for PAS. Ahmad *et al.* (2010) reported an intense PAS reaction in the sweat gland of sheep. Razvi *et al.* (2013) observed that the secretory epithelium of sweat glands in Bakerwali goats' skin were positive for PAS reaction. In the present study, sebaceous glands showed weak to moderate reaction, whereas Montagna and Yun (1964) observed no reaction of PAS in secretory portions and a weak reaction in ductular epithelium of sebaceous glands of pigs. Ahmad *et al.* (2010) reported a moderate reaction of PAS in sebaceous

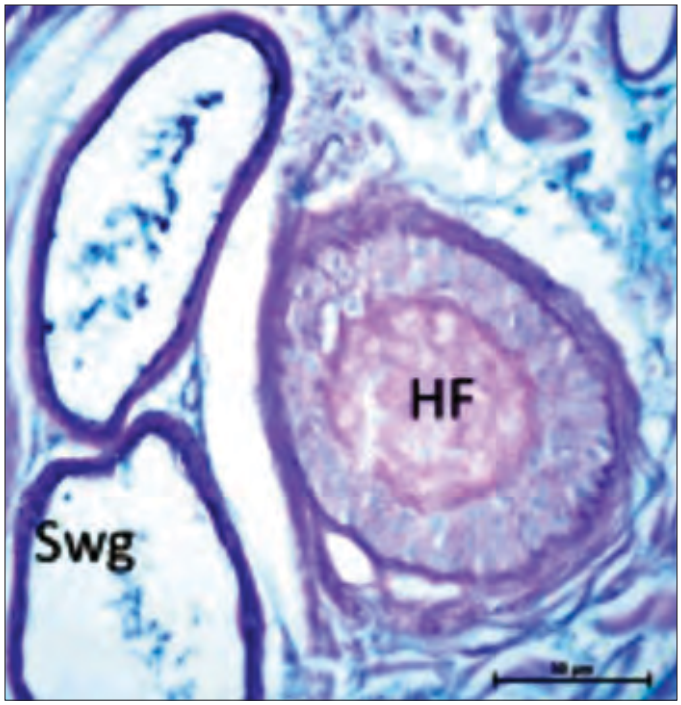

Fig. 2. Abdomen dorsal region of skin showing PAS–AB reaction for neutral and acid mucopolysaccharides in sweat gland and Hair follicle. PAS-AB × 400

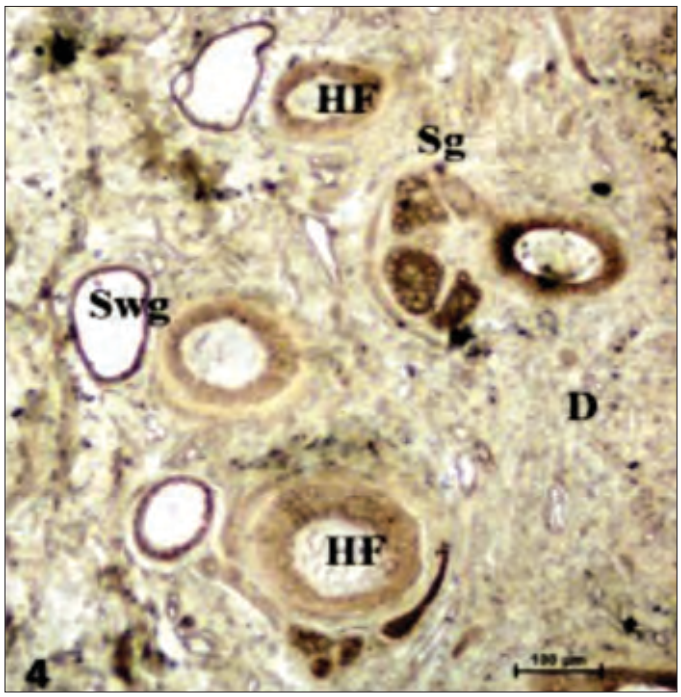

Fig. 4. Abdomen dorsal region of skin showing Sudanophilic reaction for lipids in hair follicle, sebaceous gland, sweat gland and dermis. Sudan Black B × 100

glands of sheep. Blood vessels and nerve fibres showed a moderate to strong reaction and muscles were moderately positive.

One of the most characteristic features of regeneration of cutaneous wound is the process of cell differentiation, through which an array of new cell types arise, each of them distinguish by synthesis of a specific repertoire of differentiation

products. Mucopolysaccharides are complex polysaccharides that have a role in the regeneration of cell proliferation, cell-matrixinteraction, cell-cell communications, and control of exchange of metabolites, ions, fluids and water (Mech *et al.*, 2014).

Acid mucopolysaccharide (AMPS)

Stratum basale showed a moderate to strong reaction, stratum spinosum showed moderate reaction, stratum granulosum weak to moderate reaction and stratum corneum showed weak reaction. Sumena *et al.* (2010) reported alcian blue positive areas in the upper stratum spinosum and stratum granulosum in the skin of Large White Yorkshire pigs.

In the dermis layer, connective tissue showed a moderate reaction for acid mucopolysaccharides. Within hair follicle, the internal epithelial root sheath showed a weak to moderate reaction, external epithelial root sheath showed moderate to strong reaction. Tsukise and Meyer (1983) observed an intense reaction for acid mucopolysaccharides in hair follicles of domestic pigs. Later on, Urmacher (1990) also observed an intense Alcian blue reaction in outer root sheath of hair follicle in pigs. Sumena *et al.* (2010) reported that cells of outer and inner root sheath of hair follicle around the keratogenous zone showed an intense AB positive activity.

Sweat glands showed strong reaction for AMPS (Fig. 1 & 2); whereas Montagna and Yun (1964) observed that ductular epithelium was weakly positive for acid mucopolysaccharides in pigs and Singh *et al.* (1976) reported that the lining cells of sweat glands in the paralumbar region of Indian buffalo calves were negative for acid mucopolysaccharides. Sebaceous glands showed weak to moderate reaction. Blood vessels and nerve fibres showed moderate to strong reaction, whereas muscle fibers were weak to moderately positive for acid mucopolysaccharides.

The exact composition of the acid mucopolysaccharides in the skin varies from one region to another and also with age and sex. Wharton's substance in the umbilical cord contains a preponderance of hyaluronic acid and relatively little sulfated mucopolysaccharides. In contrast, the skin in an adult contains little hyaluronic acid but more sulfated acid mucopolysaccharides of the type of dermatan sulfate and chondroitin-6-sulfate (Falco *et al.,* 2000). The acid mucopolysaccharides in the ground substance are important to balance the water and salt metabolism within the skin. Probably, the salt-extractable hyaluronic acid is particularly important for the water-binding capacity of the connective tissue ground substance, while the sulfated mucopolysaccharides play a special role in the production of the collagen and elastic fibers (Falco *et al.*, 2000).

Basic protein

Stratum basale showed moderate to strong reaction,

Table 1

Histochemical distribution of neutral and acid mucopolysaccharides, basic proteins and total lipids in different structures of buffalo skin

Structure	Neutral mucopoly-saccharides	Acid mucopoly-saccharides	Basic proteins	Total Lipids
Epidermis				
Stratum basale	++/+++	++/+++	++/+++	+/++
Stratum spinosum	++	++	+/++	+
Stratum Granulosum	+/++	+/++	+/++	+
Stratum corneum	+	+	+/++	0
Dermis				
Connective tissue	++/+++	++	++/+++	+/++
Hair follicle				
Internal epithelial root sheath	++/+++	+/++	++/+++	++/+++
External epithelial root sheath	+/++	++/+++	++/+++	++
Sweat gland	+++	+++	+/++	+/++
Sebaceous gland	+/++	+/++	+/++	+++/++++
Blood vessels	++/+++	++/+++	+++	+/++
Nerves	++/+++	++/+++	+/++	+/++
Muscles	++	+/++	+++	+/++

0 negative, + weak, ++ moderate, +++ strong, ++++ intense

whereas stratum spinosum, stratum granulosum and stratum corneum were weak to moderately positive for proteins. However, Ogava *et al.* (1979) in human and Ahmad *et al.* (2010) in sheep reported a positive reaction for proteins in outer layer of skin.

In the dermis layer, connective tissue showed moderate to strong reaction. In hair follicle, the internal epithelial root sheath and external epithelial root sheath showed moderate to strong reaction (Fig. 3), similar to observations of Ogava *et al.* (1979) in human and Ahmad *et al.* (2010) in sheep. Sweat glands and sebaceous glands showed weak to moderate reaction for basic protein. Blood vessels and muscle fibres were strongly positive and nerve fibers showed a weak to moderate reaction for proteins.

Total lipid

Stratum basale was weak to moderately positive, spinosum and granulosum were weakly positive and stratum corneum showed no reaction for sudanophilic lipids. Urmacher (1990) observed that epidermal cells of stratum corneum and stratum spinosum showed a positive reaction for lipid in pigs. Ahmad *et al.* (2010) reported that the stratum corneum of Madras Red Sheep was mildly positive for lipids.

In the dermis layer, connective tissue showed a weak to moderate reaction. In hair follicle, the internal epithelial root sheath showed moderate to strong reaction and external epithelial root sheath was moderately positive. Sweat glands showed weak to moderate reaction for lipids (Fig. 4). Sebaceous glands showed a strong to intense reaction as observed by Britt *et al.* (1985) and Bhayani *et al.* (2004) in sheep and Urmacher (1990) in pigs. Blood vessels, nerve fibres and muscles were weak to moderately positive for lipids.

Lipids play an essential role in the formation and maintenance of both the permeability and antimicrobial barriers. A hydrophobic extracellular lipid matrix in the stratum corneum composed primarily of ceramides, cholesterol, and free fatty acids provides the barrier to the movement of water and electrolytes. A variety of lipids, such as fatty alcohols, monoglycerides, sphingolipids, phospholipids, and in particular free fatty acids, have antimicrobial activity and contribute to the antimicrobial barrier (Feingold, 2009).

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