DISCUSSION

In Ayurveda, cow’s urine has been described as an excellent indigenous medicine for various diseases of human and animals. In the “Sushruta Samhita” the properties of cow urine are well documented. It possesses bitter taste and act as antineoplastic, antibacterial, antifungal, antiviral, anticonvulsive, antispasmodic and non-toxic and able to cure arthritis, enteritis, hepatitis, diabetes, gastric ulcer etc... In Vedas, the sacred Hindu writing, which are said to be the oldest books in Asia, it has also been mentioned as “Amrita” (beverages of immortality), the nectar of God.

Keeping in view the enormous role of cow’s urine in medical and veterinary medicine, a scientific experiment was undertaken in goats to elucidate the efficacy of cow urine on wound healing by gross, histomorphological and histochemical examinations.

GROSS EXAMINATION

GENERAL APPEARANCE

The wound surface was moist with pinkish appearance in group I and II. No exudate could be seen oozing out from these wounds. However, the surface of wounds of group III appeared
pinkish and wet. The wounds in group III were also exudating with some offensive smell. The appearance of wounds simulated the findings of Bhargava et al., (1988). Absence of exudation in group I and II may be attributed to the antibacterial activity of cow urine employed. Achliya et al. (2004) and Sharma (2001) also established the antibacterial activities of cow urine in their experiment. The cause of oozing surface with some offensive smell in group III might be due to the presence of infection in wound because no measures were taken to control it and it is evident that breach in surface of the body exposes tissues to the danger of infection (Harding Rains and Melville Capper, 1971).

**INFLAMMATION**

The signs of severe inflammation were observed in and around the wounds of group I and II upto 3\textsuperscript{rd} post-operative days whereas in group III, it was conspicuous upto 7\textsuperscript{th} day. The attenuation of inflammation appeared after day-5 to 6 in group I and II but it prolonged for a period of 15 to 16 days in group III. The early intense inflammation in group I and II as compared to group III might be due to release of cytokines, which is considered as an important mediators of acute inflammation (Robbins and Kumar, 1987). Cow urine has been reported to increase the
secretion of cytokines like Interleukin-1 and Interleukin-2 (Yadav, 2005).

The early subsidence of inflammation in the wounds of group I and II may be correlated with the antioxidant property of cow urine (Singh et al., 2004). It might be due to urea content of cow urine which promotes hyperaemia and increase in lymph flow (Vohringer, 1994). Palkhotin (1984) reported early suppression of inflammation due to increased blood circulation. The curtailment in the period of inflammation might be also due to the antibacterial property of cow urine (Achliya et al., 2004, Sharma, 2001); phagocytic property (Yadav, 2005) and immunomodulatory effect (Chauhan et al., 2001). Early formation of granulation tissue also might have played a role in suppressing the inflammation in treated groups. Varshney and Verma (1990), Varshney et al. (1994) and Pandey et al. (1998) have reported decrease in inflammatory response with the progress of granulation tissue formation.

**APPEARANCE OF GRANULATION TISSUE**

Granulation tissue appeared on an average by day-3 in group II and by day- 4 to 5 in group I. Whereas, it could be observed on an average by day-7 in group III. Gupta (2003) observed the similar findings with respect to appearance of granulation tissue following treatment with cow urine. Early appearance of
granulation tissue in group I and II may be due to the fact that cow urine promoted fibroplasias and neovascularization in these groups as substantiated by histological findings. Its faster appearance in group II as compared to group I and III may be ascribed to the presence of various useful ingredients in the cow’s urine like vitamin C and traces of mineral enhancing the healing process when administered orally and applied topically. Vitamin C has been reported to be an important factor for neovascularization and formation of good quality collagen from the proliferating fibroblasts (Runnels et al., 1976 and Vegad, 2000). Park et al. (1994) also reported the formation of granulation tissue from 2 to 7 days in rabbits treated with yeast glucans. Yurdzh (1988) observed that during granulation tissue formation the concentration of iron was relatively found to be higher in brown cattle. Bapu (2001) analyzed the cow’s urine and found that the traces of iron being excreted in the cow’s urine. Apart from the presence of useful ingredients in the cow’s urine having vital role to play during wound healing process, the presence of iron in the cow’s urine may also be one of the contributing factors for early development of granulation tissue in urine treated wounds.
FILLING OF WOUND GAP

The two-third depth of the wound cavity was filled with the granulation tissue by day-7 to 9 in both group I and II. Complete filling of wound gap was a bit faster in group II than group I followed by group III. But in group III, about one third gap was filled by day 10 to 12 and one half by day 14 to 15. This finding was in agreement with the findings of Jadon et al. (1985) with different treatments. In the present study, in spite of iso-managemental condition, early filling of wound gap might be attributed to hygienic surroundings, sufficient outer protection as well as early development of granulation tissue.

WOUND CONTRACTION

The percentage of wound contraction in group I and II was found to be extensive and almost the entire gap was minimized by contraction in group II. However, in group III the wound contraction was not extensive to that extent. The early appearance and maturation of granulation tissue might be responsible to the extensive wound contraction in urine treated groups. Singh and Singh (1993) reported that the progress of wound contraction centres around the development of granulation tissue. Most extensive wound contraction in group II might be due to nutrient
content of cow urine, like proteins, minerals and vitamins, which might have added effect in wound healing after combined uropathy. It might be also due to quality of cow urine to synthesize cytokines like IL-1 and IL-2. Vegad (2000) reported that proteins, vitamin C and zinc play an important role in wound healing. He also reported that IL-1 stimulates the synthesis of both collagen and collagenase by fibroblasts.

**COMPLETION OF EPITHELIALIZATION**

Epithelialization was found completed by day-17 to 20 in group II and by day- 18 to 21 in group I. However, epithelialization got completed by day 30 to 35 in group III, leaving a marked scar. Bhargava *et al.* (1988) observed somewhat similar finding with *Annona squamosa* treated wounds, which healed completely by epithelialization in 21 day, whereas control wounds did not healed completely upto 30 days. Earliest shedding of scab occurred in group II followed by group I and III. Early completion of epithelialization in group I and II as compared to Group III might be due to early shedding of scab. Jennings and Florey (1970) suggested that scab can delay the epithelialization process, since the epithelialization must circumvent or digest its way through the dense material that it encounters for it, does not attach itself to the surface of scab. The diminution in size of scar in group I and II as
compared to group III might be due to extensive wound contraction. Mayers et al. (1980) also held the similar viewpoint. Gupta (2003) also reported wound healing without apparent scar mark with cow urine treatment in rat.

**HISTOMORPHOLOGICAL OBSERVATIONS**

**EPITHELIALIZATION**

Histological studies of collected tissue revealed that the process of epithelialization started earlier and was more conspicuous in group I and II as compared to group III. Certain important ingredients of cow urine like minerals and vitamins might be responsible for early and better epithelialization in group I and II, as it has been reported that vitamin A is required for epithelial formation and cellular differentiation (Douglas Mackay et al., 2003). Bhargava et al. (1986) reported early epithelialization in treated wound with *Adhatoda vasica* as compared to control one. Bhargava et al. (1988) also reported more distinct epithelialization in treated wounds with *Annona squamosa* than control. Earlier epithelialization might also be due to vitamin C and copper content of cow urine which favours early formation of granulation tissue. Formation of granulation tissue provides a surface for the epithelial
cells to migrate over and confers resistance to infection (Katiyar, 1999).

FIBROPLASIA

Microscopic examination of tissues exhibited that the intensity of fibroblast proliferation was more in group II, followed by group I and was least in group III in early phase of healing. But in later stage of observation, all the groups manifested fibroplasia that did not alter significantly. Gupta (2003) also reported that the cow urine treated rats did not require any supportive therapy and showed early development of granulation tissue and regeneration of epidermis with fibroplasia. Intensive fibroblast proliferation in group I and II might be a sequel to marked infiltration of macrophages and neutrophils in early phase of healing. Fibroblast and angioblast invade the area cleaned by macrophages and neutrophils. Macrophages and blood monocytes together with the mesenchymal cells are the precursors of fibroblast and angioblasts (Damjanov, 1976). Yadav (2005) reported that the cow urine increases the phagocytic activity of macrophages and also increases the secretion of IL-1 and IL-2. Intense fibroplasias might also have been resulted due to vitamin content of cow urine. Increased fibroblastic proliferation after administration of vitamin A was observed by Al Sadi (1976). From observation on day-14 and -21 it
was explicit that in group I and II, the process of fibroplasia had been plateaued, heralding the initiation of maturation phase. Maturation of wound begins with diminution in number and size of fibroblasts (Heinze, 1976; Johnston, 1981).

**NEOVASCULARIZATION**

All groups exhibited intensive neovascularization from day - 7 to day-14 but intensity was less on day-21. Group III showed minimum neovascularization as compared to group I and II at any period of observation. Early intense neovascularization in treated groups might be due to more reticular fibres formation in these groups as they serve as a scaffold to support newly formed blood vessels (Van Sickle *et al.*, 1993). The greater degree of neovascularization till day-14 in group I and II might be attributed to the intensive infiltration of macrophages and neutrophils as suggested by Damjanov, (1976). In group II the overwhelming angiogenesis might be due to Vitamin C content of the cow’s urine which promotes neovascularization. Sastry (2001) and Runnels *et al.* (1976) reported the role of Vitamin C in the neovascularization.

The nutrient content of cow urine might have contributed in the growth of granulation tissue in group II. The regression of vascularity appeared on day- 21 in group I and II, which marked the beginning of maturation phase (Heinze, 1976).
INFILTRATIVE CHANGES

Infiltrative changes were pronounced till day-7 and macrophages outnumbered neutrophils in both group I and II as compared to group III. On day-14 the infiltration of these cells appeared to be decreased and it further decreased on day-21. Comparatively there was less infiltration of these cells in group III at any period of observation. From the study, it is apparent that the urine treatments, somehow mediate the inflammatory process. It has been described that cytokines are considered as an important mediators of acute inflammation (Robbins and Kumar, 1987) and it has been reported that cow urine increases the secretion of IL-1 and IL-2 (Yadav, 2005). Bisht et al. (1999) and Kumar et al. (1998) have similar findings, where they noticed more infiltrative changes in early phase and it decreased significantly in later phase of healing in treated wounds as compared to control.

HISTOCHEMICAL OBSERVATION

COLLAGEN FIBRES

The wounds of all groups exhibited increasing trend in the quantum of collagen fibres with maximum intensity could be recorded on day-21. Group II excelled in collagenation followed by group I and III in that order. The intensity of collagen fibres in
group I and II was found to be more which may be due to intense neovascularization and inflammatory response in these groups during early period of observation. This process results in stimulation of fibroblasts for synthesizing collagen fibres (Kumar et al., 1998). Excellency of group II over group I might be due to certain valuable ingredients of cow urine received orally, like vitamin A, C, copper etc. Al Sadi (1976) observed the role of vitamin A in fibroblastic proliferation and formation of collagen in wound healing. Vegad (2000) and Runnels et al. (1976) also reported the importance of Vitamin C in collagen formation. Excellency of group II over other groups is also in agreement with Gupta (2003) who observed vertically arranged fibroblast with production of thin and better quality of collagen fibres due to systemic effect of cow urine along with topical application on surgical wound in rat.

**ELASTIC FIBRES**

All groups exhibited a gradual increase in elastic fibre content from day-7 to day-21. The quantum of collagen fibres were relatively higher in group II followed by group I and III. This observation corroborates with the findings of Bhargava *et al.* (1988), Zama *et al.* (1988) and Ansari *et al.* (1997) but with different treatments. More elastic fibres observed in group I and II
might be due to intense fibroplasias in these groups. Elastic fibres are not providing structural strength but their function is complementary to that of collagen (Robbins and Kumar, 1987). Levenson et al. (1965) reported that elastin plays no definite role in wound healing. More intense elastic fibres in group II might be due to copper content of cow urine. Katiyar (1999) reported the role of copper to maintain the proper framework of elastic and collagen.

**RETICULAR FIBRES**

The quantum of reticular fibres increased with the increase in observation time and the maximum fibres could be recorded on day-21. Reticular fibres were more in group I and II as compared to group III at any period of observations. More reticular fibres in group I and II may be ascribed to greater degree of fibroplasias in them. Reticular fibres are actually individual collagen fibrils (type III collagen) coated by proteoglycans and glycoproteins (Ham, 1957, Van Sickle et al., 1993). Biochemically, it also appears that reticulin and collagen have the same composition (Damjanov, 1976).