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Cutaneous Toxicity of Sodium Lauryl Sulphate, Nickel, and Their Combination in Guinea Pigs: Biochemical and Histopathological Observations

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Sodium lauryl sulphate (SLS)-an anionic surfactant is commonly used in the preparation of many cosmetic and detergent products. Considerable evidence has accumulated about dermal reactions varying from cellular degeneration, necrosis and release of enzymes to increase in skin fold thickness consequent to SLS treatment (Gibson and Teall 1983; Elder 1983; Wahlberg 1983; Mathur and Gupta 1985; 1986; Mathur et al. 1988). The heavy metals such as nickel(Ni) (22 mg/Kg) and other materials present in detergents may come in direct contact of skin (Cronin 1980; Sundermann 1980; Mathur et al 1987). Studies by Nilzen and Wikstrom (1985) earlier demonstrated increased sensitivity of skin of guinea pigs to nickel and chrome when these agents were mixed with SLS. In the present study different doses were taken to investigate the effects of SLS, Ni and their combination on the skin of guinea pigs since reports on biochemical and histopathological aspects of cutaneous toxicity of these chemicals are scarce.

MATERIALS AND METHODS

Forty eight male albino guinea pigs (250 gm average body weight) obtained from ITRC's Animal Facility were divided into four groups. They were maintained on a pellet diet (Hind Lever, India) and water *ad libitum*. The hair on the back of each animal was removed with an electric clipper. Animals of first three groups were treated daily with 50 mg/Kg SLS (Sisco Research Lab. India), 50 mg/Kg Ni as nickel sulphate hexahydrate (E. Merck, Germany) and SLS+Ni (each 50 mg/Kg) suspended in 1 ml of distilled water, applied to a clipped skin area of 2 x 2 cm respectively for 7 and 14 days. The control animals received a local application of 15 mg/Kg sodium sulphate in identical manner.

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After 7 or 14 days of treatment the animals of control and treated groups were sacrificed and the test skin areas excised and collected for various biochemical and histological studies.

For the latter the representative skin portions were fixed in 10% buffered formalin and embedded in paraffin after processing. Serial sections of 5u thickness were cut and stained with hematoxylin and eosin. However, for various biochemical estimations the skin homogenates were prepared in 0.15 M KCl for lipid peroxidation (Ohkawa et al 1979; Utley et al 1967) in 0.4 N perchloric acid for histamine estimation (Shore 1959), in ice cold 0.25 M sucrose solution for superoxide dismutase activity utilising its ability to inhibit the autoxidation of pyrogallol (Marklund and Marklund 1974) and glutathione content (Ellman 1959). The protein content was determined according to the method of Lowry et al (1951) using bovine serum albumin as standard. Ni estimations in skin were carried out using an atomic absorption spectrophotometer. All the results are exprssed as mean and their standard errors. Comparisons were made with their respective control groups using Student's `t' test.

The above experiments were again conducted with another set of animals using 5 mg/Kg and 0.5 mg/kg doses of Ni and SLS and the same parameters were determined.

RESULTS AND DISCUSSION

The biochemical effects observed in the skin of guinea pigs exposed to 50 mg/Kg dose of SLS, Ni or their combination for 7 or 14 days are summarised in Table 1. Ni alone or in combination with SLS caused significantly enhanced lipid peroxidation in the skin both at 7 and 14 days of treatment. In addition, there was significant increase in the histamine in skin at both intervals following SLS or SLS+Ni treatment. The enzymatic activity of SOD in skin did not change following either Ni or SLS treatment but their combination produced significant inhibition in SOD both at 7 and 14 days treatment. Also there was concomitant increase in the content of Ni in skin at 7 and 14 days following treatment with Ni alone or Ni+SLS.

The biochemical changes in skin of guinea pigs observed in 5 mg/kg exposure group showed some alterations in lipid peroxidation, histamine and glutathione contents in Ni+SLS group. The Ni also showed accumulation tendency in Ni+SLS group (Data not shown). The 0.5 mg/kg dose group showed no significant changes in any group.

Table 1. Effect of nickel (Ni), sodium lauryl sulphate (SLS) or their combination on biochemical alterations in the skin of guinea pigs

		Ni		SLS		Ni+SLS	
Parameters	Control	Day 7	Day 14	Day 7	Day 14	Day 7	Day 14
Lipid peroxidation (n moles Malondialdehyde formed/mg protein/hr)	0.72±0.10	1.34±0.20 c	1.51±0.08 b	0.40±0.29	0.77±0.13	1.11±0.04 c	1.61±0.35 b
Histamine (ug/mg protein)	0.64±0.07	0.66±0.05	0.39±0.07	0.91±0.04 a	0.93±0.04 a	0.98±0.05 a	1.13±0.08 a
Superoxide dismutase (units/mg protein)	2.25±0.20	2.53±0.14	2.81±0.48	2.52±0.23	2.48±0.36	1.31±0.20 b	1.18±0.33 b
Glutathione (u mole/mg protein)	1.04±0.20	0.92±0.13	2.30±0.05 a	0.93±0.28	2.29±0.03 a	1.49±0.43	2.24±0.22 a
Nickel(ug/g)	0.38±0.09	0.66±0.08	1.07±0.10 b	0.30±0.12	0.36±0.05	1.04±0.14 b	2.34±0.04 a

Each value represent Mean +S.E. for 6 animals.
a=p <0.001; b=p <0.01; c=p <0.05, when compared to control (Student's`t'test)

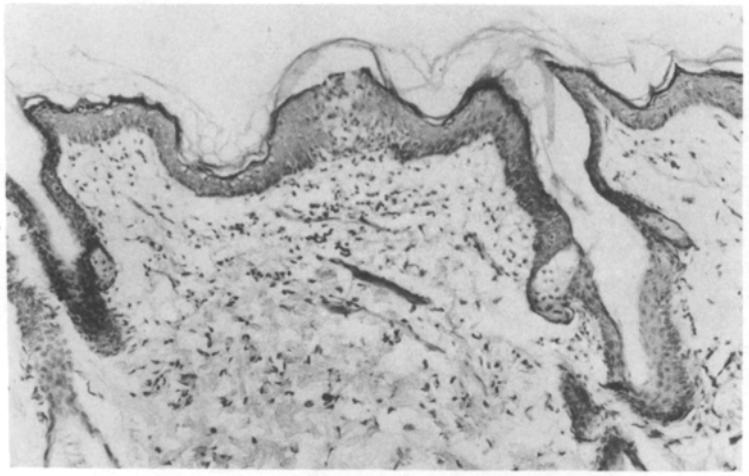

Figure 1. Skin of control guinea pig at 14 days post treatment with sodium sulphate showing slight damage to epidermis and moderate inflammatory reaction in the dermis. H & E, x 110

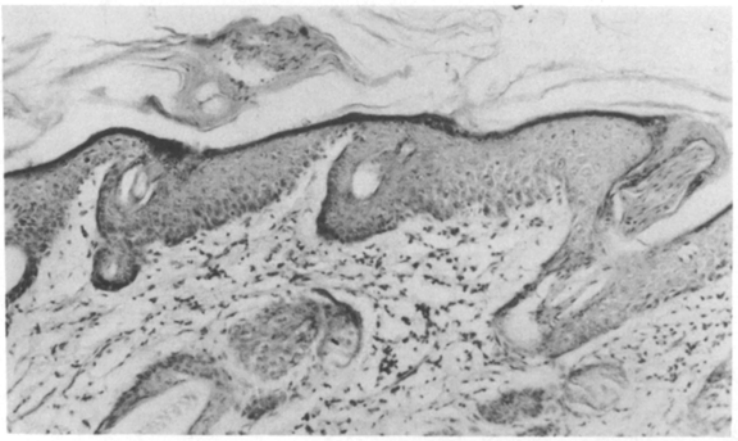

Figure 2. Skin of guinea pig at 14 days post treatment with Ni alone. Sloughing of epidermal layer together with infiltration of cells in dermis and oedema. H & E, x 110

The skin of guinea pigs exposed to 50 mg/Kg Ni alone demonstrated hyperkeratinization of epidermal layer at 7 days. There was also moderate degree of oedema in the dermis together with the presence of a few inflammatory cells. However, 14 days exposure resulted in further thickening of stratum corneum along with oedema in the epiderms. At places there occurred sloughing of the epidermis besides marked oedema of dermis and infiltration of leucocytes (Fig. 1 & 2).

Exposure to 50 mg/Kg SLS alone caused moderate thickening of skin together with oedema of epidermis

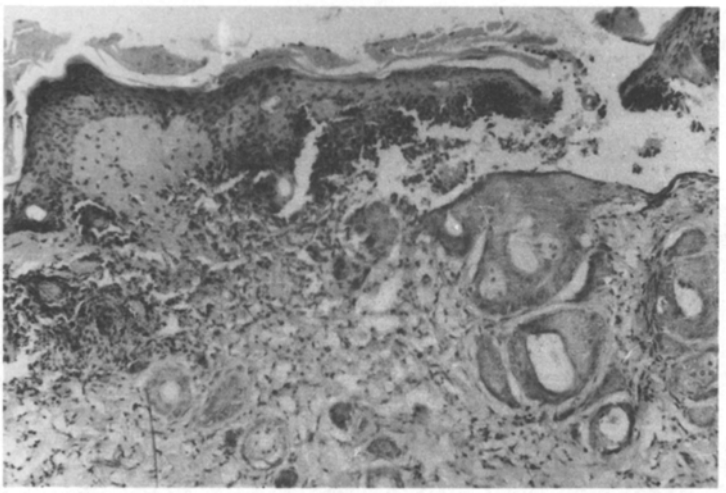

Figure 3. Skin of guinea pig at 14 days post treatment with SLS+Ni. Extensive sloughing of epidermis and intense inflammatory reactions. H & E, X 110

and dermis after 7 dyas of treatment. Dermis additionally revealed infiltration of leucocytes in its upper part. When the treatment was continued for 14 days marked sloughing of the epidermal layers took place. Basal layer of epidermis contained lot of RBC's throughout. Dermis however, continued to exhibit oedema and infiltration of leucocytes, as seen with exposure to Ni at 14 days.

Following combined exposure to Ni and SLS (50 mg/kg each) there was moderate sloughing of the epidermal layer specially the stratum corneum together with small to large haemorrhagic foci in the epidermis as well as in dermis. Marked congestion and oedema could, however, be discernible in dermis. When the dermal application was extended in dermis to 14 days there occurred quite severe epidermal sloughing. At places the epidermis became thickened due to marked infiltration of polymorphonuclear leucocytes,cellular debris and oedematous fluid (Fig. 3). Besides, there was epidermal repair, which also revealed presence of many red blood cells. Marked oedema and infiltration of cells however, coninued to persist in the dermis.

Surfactant belong to a clas of compounds that cause primary cutaneous inflammation specially when applied in high concentration to the skin of rats (Prottey and Ferguson 1975). During indiscriminate use and production of detergents industrial workers or populations in developing countries may be exposed to higher concentrations. In our earlier studies treatment with

SLS or Ni alone resulted in enhanced activity of certain marker enzymes and increase in the content of sulphydryl groups and aminonitrogen in skin. However, these alterations were more marked when the skin was simultaneously exposed to SLS and Ni indicating additive toxic effects (Mathur et al 1988). In the present study there was increased lipid peroxidation with concomitant decrease in SOD activity in the skin of guinea pigs following 7 or 14 days of exposure to SLS+Ni. These findings corroborate well with the histological damage observed in the skin. Lipid peroxidation has been identified as a deteriorative process leading to pathological manifestations by way of the action of free radicals on membrane lipids. A decrease in SOD activity show failoure of skin to scavange the superoxide radicals resulting in inhibition of oxidative defence mechanism. SOD is thought to play an important role in the protection of cells against the toxic insult of superoxide anion radicals. The enzyme is distributed in respiring cells (Fridovich 1976; 1978). Superoxide anion induces lipid peroxidation of biological membranes and this reaction is inhibited by SOD (Zimmermann et al 1973; Svengen et al 1978;McCord 1979). Superoxide radicals are constantly generated in the body tissues and failure in their immediate removal can initiate a damaging effect on the polyunsaturated fatty acids and structural proteins of plasma membrane (Maestro 1980). In the present study exposure to Ni or SLS does not alter the SOD activity but combined exposure (Ni+SLS) however brings about significant decrease in SOD activity both at 7 and 14 days due to the production of excessive superoxide radicals. Although there was signficant increase in glutathione contents at 14 days in skin it could not provide protection against free radicals attack on the membrane was quite evident histopathologically.

In the present study treatment with SLS or SLS+Ni caused increase in histamine content in skin at 7 or 14 days. The way in which surfactant release histamine from skin mast cells is thought to be similar to that of some chemicals and lipophilic drugs (Rohlech et al 1971). The release of histamine from mast cells in skin would mediate cutaneous inflammation (Wilhelm 1973). The mechanism of its release from the mast cells has been elucidated for certain specific stimuli such as compound 48/80 (Lagunoff 1972; Rohlich et al 1971). allergens (Bach 1974), certain cytotoxic chemicals, drugs and venom (Bloom & Haegermark 1967; Frish-Holmberg 1971). The histological findings observed in skin further support the cutaneous injury mediated additionally by the release of histamine. The increase in Ni content shows that SLS help in the absorption of the metal.

The data derived from the present study in guinea pigs therefore suggested that excessive exposure of industrial workers or populations to Ni and SLS may bring about cutaneous damage and when these are present together more damage can be predicted. However, low level exposure to these chemicals may produce slight damage to skin.

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