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Toxicological Evaluation of Synthetic Detergent-induced Effects in Rat Skin

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Abstract. Toxicological evaluation of synthetic detergent was carried out by exposing the rat skin to the test solution of the commercial detergent in three concentrations (1%, 2.5% and 5%). The skin was exposed daily for one hour for five consecutive days. The skin reaction to the detergent was scored daily, whereas the enzymatic activities in the treated and control skin segments were determined on the fifth day after scoring the skin reaction. The exposed skin showed oedematous changes with erythema and cracking and/or scaling of the skin. The enzymes acid phosphatase (AcPase), glucose-6-phosphate dehydrogenase (G6PDH), glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) were stimulated significantly in the skin of the treated rats. A dose dependent effect was observed in skin reactions and enzymatic activities. Precautionary measures for skin protection from detergents are suggested.

Introduction

Washing and maintenance of cleanness are very important for hygiene and for these purposes, various synthetic detergents are most widely used household and industrial substances. Poor quality detergents can cause severe skin reactions and dermatitis [1]. The irritation of the skin and dermal reactions due to detergents are well known [2-5]. Increase in the level of enzyme activities in the skin due to dermal application of detergents has been reported by some investigators [6, 7]. Enzyme release is accepted as a marker of the cellular injury to the skin [8-10].

The present study was undertaken to ascertain the skin reaction and the enzyme activities in the detergent exposed skin. Further, it was aimed to investigate as to if these effects were detergent concentration dependent.

Material and Method

Detergent

A synthetic detergent was obtained from the local market. The brand represented from an organized industry. The ingredients present in the detergent are linear alkyl benzene sulphonate (LAS), alkaline builders (soda ash, sodium carbonate), sodium silicate, sodium tripolyphosphate and fluorescent whitening agent and perfumes. A freshly prepared 1%, 2.5% or 5% treatment solution of this detergent in distilled water was used daily for topical application on the rat's skin.

Animals

Twenty-four male albino rats, weighing approximately 180±10g, were divided into four groups (Group I – Group IV) of 6 animals in each. Group I served as the control, whereas Groups II, III and IV were the groups treated with 1, 2.5 or 5% treatment solutions respectively. The animals were maintained in the animal house of the department at 22±1ºC in groups of 6 per cage, at 12 hours automated light and dark cycles. Pilsbury's diet and tap water were available *ad libitum* throughout the experiment except during the observation and intervention period.

Experimental washing treatment

The treatment solutions were prewarmed at 37±1ºC in water bath before each treatment. All animals were anesthetized with ether and were shaved on the entire dorsal and ventral surfaces of the body with an electric shaver. The shaved rats were gently immersed up to neck in treatment solutions (1, 2.5 and 5% for Group II, III and IV respectively) in glass jars of appropriate size. The pH of 1%, 2.5% and 5% solution of detergent was 10.55, 10.45 and 10.35 respectively, and the control animals were immersed in alkalized distilled water (pH 10.45) similarly. The glass jars were covered with fiber board and each board had a central opening of the size of the rat's neck. The fiber covering was designed in such a manner that it could be opened and closed into two halves on a hinged end. The inner edges of the central opening were padded with foam to make the rats neck comfortable after locking the two halves. Such a fiber covering helped in supporting the neck of the rats with maximum comfort, restrained the animals from getting submerged in solutions and helped in to keep the animals' faces dry. Each glass jar filled with respective solution and containing the restrained animal of respective groups was kept in water bath maintained at a constant temperature of 37±1ºC for 1 hour daily for five consecutive days. At the end of each immersion period, the animals were taken out of the containers, released gently from their neck restrainer, washed gently with lukewarm water, dried gently with paper towel and returned to their cages.

Measurement of skin reactions

Since the skin of different parts of the body vary in susceptibility to irritants, for the scoring purposes of the skin reaction to detergent solution, a uniformity was maintained in scoring the same sites in each animal for all five days in treated as well as in control animals. The changes in the central most part of the dorsal side as well as the ventral side

were recorded visually by scoring the reaction based on a 12-point scale [5]. The readings were observed 60 minutes following the completion of solution treatment and keeping the animals in their respective cages after washing and drying the skin with paper towels. The average scores on dorsal and ventral surfaces was considered as a single score for one animal per day. The mean of five scores for five consecutive days was considered as the final score of skin reaction for each animal. The 12 point scoring of the skin response is shown in Table 1.

Table 1. Reaction of rat skin following exposure to treatment solutions of a synthetic detergent for five consecutive days

Skin Reactions	Scores after exposure to various concentrations of treatment solution of synthetic detergent*			
	0%	1%	2.5%	5%
Erythema	0	2	3	6
Oedema	0	1	2	4
Cracking/Scaling	0	1	2	4

* Each score value is the mean of the dorsal and ventral score of one animal x 5 days x 6 animals per treatment group.

Score grades for skin reaction:

No reaction	= 0	Becoming well developed	= 6
Marginal/very slight	= 1	Well developed	= 8
Slight	= 2	Becoming severe	= 10
Fairly distinct	= 3	Severe	= 12
Quite distinct	= 4		

Enzyme assays in the skin

On the fifth day, after the completion of the solution treatment, animals were sacrificed by decapitation. A 2x2 cm area of the skin was removed from the central most part of the dorsal and ventral side of the shaven area and kept in ice-cold petridishes. The adhered subcutaneous fatty tissues were removed with a sterilized scalpel. After proper, gentle and quick scrapping, the clean tissue of the skin was blotted, weighed, finely minced and homogenized in chilled 0.25M sucrose solution. A 10% homogenate was centrifuged at 1000 g for 10 minutes at 4ºC. The clear supernatant was used for the enzyme and protein assays. Activities of acid phosphatase (AcPase), glucose-6-phosphatase (G-6-Pase), glutamate oxaloacetate (GOT) and glutamate pyruvate transaminase (GPT) were determined spectrophotometrically using methods of Bergmeyer *et al*. [11], Lohr and Walker [12] and Frankel and Reitman [13] respectively. The protein content of the homogenate was determined by the method of Lowry *et al.* [14] using bovine serum albumin as the standard.

Results

The results of the score of rat skin reactions after five consecutive exposures in treatment solution are shown in Table 1. Treatment solution in 1% concentration produced slight erythema and marginal oedema and cracking/scaling, whereas in 2.5% concentration, the treatment solution caused a fairly distinct erythema but slight oedema and cracking/scaling. Further in the 5% concentration, the treatment solution had the highest degree of effect producing redness in erythematic reactions that was becoming well developed whereas oedema and skin cracking/scaling were quite distinct. No skin reactions were noticed in the control animals. The severity of skin reactions after treatment solution exposure was found to be dose dependent.

The levels of enzyme activities in rat skin after exposure to treatment solutions in three concentrations are presented in Table 2. The AcPase activity was found to be elevated significantly at all three concentrations of the treatment solution. The activity of G-6-Pase was significantly increased at the medium (2.5%) and high (5%) concentrations only. GOT and GPT activities were significantly stimulated at all three concentrations of the treatment solution. Likewise the superficial skin reactions and enzyme activities in the treated skin, due to treatment solution exposure, were also found to be dose dependent in their effects.

Table 2. Effect of various concentrations of treatment solutions of a synthetic detergent, on the enzymes of rat skin exposed for five consecutive days

Enzymes	Concentrations of the treatment solution of synthetic detergent						
	0% (Control)	1%	Percent change	2.5%	Percent change	5%	Percent change
AcPase (nmoles phenol lib/min/mg protein)	36.75 ±2.38	70.56** ±2.84	92%	78.35** ±4.51	113.2%	83.64*** ±3.71	127.5%
G-6-Pase (mu/mg protein)	17.35 ±2.81	26.62* ±4.93	53.4%	35.92** ±4.74	107%	39.82*** ±2.36	129.5%
GOT (nmoles hydrozones formed/min/mg protein)	19.86 ±3.16	41.72** ±5.65	110.1%	49.19** ±3.96	147.6%	51.88*** ±4.32	161.2%
GPT (nmoles hydrozones formed/min/mg protein)	12.73 ±1.51	16.83* ±1.62	32.2%	19.37** ±2.93	52.1%	23.53*** ±2.18	84.8%

* $p<0.05$; ** $p<0.01$ and *** $p<0.001$; when compared to control as evaluated by Student's t-test.
Each value is the mean ±SEM of six animals in each group.

Discussion

Since washing and cleaning are inseparable habits of human beings for maintaining a hygienic condition, the use of various synthetic detergents is an important factor for hygienic lifestyle. Thus, the selection of suitable washing detergent is very important especially in atopic dermatitis patients, because incorrect selection of washing products cause irritation [1, 15].

In the present study, animals showed erythema, oedema, cracking and scaling of the skin after exposure for five consecutive days to test the solution of detergent. The animals developed quite a distinct cracked abdominal skin with hard leathery flanks and dorsal skin. Similar skin reactions showing a badly affected flank and dorsal region have been reported in guinea pigs using four synthetic detergents [6]. The scoring of skin reaction and measurement of enzyme activities in the present study were done from the similar superficial dermal sites to nullify the possibility of different anatomical sites influencing the irritation response as reported in animals [16] and human beings [17]. Mathias and Maibach [18] have described earlier that the amount of material applied to skin surface, vehicle, period and method of exposure are important factors that influence an irritation response. The skin reactions may be attributed to the removal of surface lipids or water soluble substances and denaturation of scleroproteins of the horny layer [15, 19] leading to progressive skin irritation and damage.

The presence of epidermal oedema along with enhanced enzyme release and ultra structural changes in rat skin have been reported as a result of surfactant treatment [20]. Other studies have also reported the release of enzymes accompanied by the development of erythema and oedema in dermis [1, 2, 5, 8] when surfactants were applied to the mouse skin for 28 consecutive days. An increase in the enzymic activities of G-6-Pase, succinic dehydrogenase (SDH), deoxyribonucleic acid (DNA), phosphogluconate dehydrogenase (PGDH) and monoamine oxidase (MAO) have also been reported under similar conditions in human as well as animal studies [6]. In the present study, the activities of lysosomal enzyme AcPase, GOT and GPT, which are found in both the cytoplasmic and mitochondrial compartments of the cells, were found to be stimulated dose-dependently stimulated as a result of the treatment of skin with the detergent solution, and such finding is in agreement with the result of previous studies [1, 3, 4]. These enhanced enzymatic activities may be due to the effect of skin damage and alterations in carbohydrate and protein metabolism. However, such an increase in enzymatic activities in skin has been reportedly related to epidermal repair due to the increased rate of mitosis in epidermal layers [21] or epidermal thickening [22-24].

The correlation of some skin diseases, such as atopic dermatitis, with epidermal barrier function [25-29] has demonstrated the importance of detergents. Thus, the present study on rat skin may help in evaluating the skin irritancy to the claiming of the mildness of a detergent. Also, these results provide a basis for developing further testing of other parameters like patch test [3, 4, 30-33] and epidermal barrier dysfunction test

[32, 33]. However, as a precaution, treating the skin with moisturizers after washing is the most usual procedure to prevent skin irritation and disruption.

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تقييم سمية التأثيرات المستحثة بمنظف اصطناعي في جلد الجرذان

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قسم علم الحيوان، كلية العلوم، جامعة الملك سعود، الرياض، المملكة العربية السعودية

(قدم للنشر في ١٤٢٦/٦/٢١هـ؛ وقبل للنشر في ١٤٢٦/٨/١٥هـ)

ملخص البحث. تم تقييم السمية الناتجة عن التعرض لمنظف اصطناعي وذلك بتعريض جلد الجرذان لثلاثة تراكيز مختلفة من المحلول المنظف لمدة ساعة يومياً ولخمسة أيام متتابعة.. تم تسجيل التفاعلات الجلدية الناتجة عن المنظف بشكل يومي، بينما تم تحديد النشاطات الإنزيمية في عينات الجلد من المجموعات المعالجة والمجموعات الضابطة في اليوم الخامس. أظهر الجلد المعرض للمنظف تغيرات إرتشاحية والتهابات جلدية و/أو تشققات مصحوبة بتقشر الجلد، كما أظهرت إنزيمات الفوسفاتاز الحمضي (AcPase) وإنزيم نازع الهيدروجين جلوكوز ٦ فوسفات (G6PDH) وجلوتاميت اكسالواستيت (GOT) وجلوتاميت بيروفيت ترانسامينيز (GPT) زيادة معنوية في نشاطها في عينات الجلد المعالج. ولقد لوحظ أن التغيرات الجلدية والنشاطات الإنزيمية كانت معتمدة على الجرعة المعطاة. تم اقتراح إجراءات احترازية لحماية الجلد من المنظفات.