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In Vivo Xylitol Primary Dermal Irritation and Phototoxicity Evaluation

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SUMMARY. **Xylitol is a widely studied sugar with therapeutic properties and is effective against microorganisms. Despite a variety of toxicological data being available about this compound, dermal toxicological tests cannot be found. Here, the aim was to carry out *in vivo* assays to verify xylitol skin application safety. Primary dermal irritation studies were done with rabbits using 5 and 10% (w/w) xylitol, in either cream or gel form. Phototoxicity assays were also performed with guinea pigs, using only 10% (w/w) xylitol, in both forms. Primary dermal irritation studies revealed that xylitol topically used (5 and 10%) did not induce erythema or edema formation, but did show phototoxicity properties. Xylitol is an adequate alternative compound to be applied for skin disease control, since this application will be done together with sunscreen.**

INTRODUCTION

Xylitol is a sugar-alcohol largely used as a sweetener, due to its non- and anti-cariogenic properties [1,2] and its metabolism is insulin independent, which allows xylitol be used by diabetic patients [3]. A variety of works can be found reporting xylitol applied to treat otitis media [4-6] and increase bone density [7-10]. Besides the more widespread uses, xylitol is also indicated for patients deficient in glucose-6P-dehydrogenase enzyme [11], in parenteral nutrition [12], cystic fibrosis [13] and in immobilization of trauma [14].

Concerning xylitol toxicity and tolerance, it has been observed that individuals who used this compound as their only sweetener for two years did not present serious side effects [15]. Xylitol was granted Generally Regarded as Safe (GRAS) status for use as a food additive by the Food and Drug Administration (FDA). The median lethal dose (LD_{50}) obtained from studies in rats is 25.7 g /kg body weight. Acute toxicity assays performed on animals, in which xylitol was administered orally, have indicated that this compound showed very low toxicity. Furthermore, conventional teratogenicity and embryotoxicity tests, as well as the *in vitro* and *in vivo* assays for mutagenicity and clastogenicity demonstrated that xylitol presented very low toxicity [16].

Data about xylitol dermal toxicity is not yet available and there is a great potential for human skin exposure to this compound. This is an important administration route to be studied, as a potential microbicidal growth control property was previously investigated by our group [17] and others [18,19]. The current study was conducted in order to evaluate xylitol primary dermal irritation and phototoxicity. To achieve this, primary irritation and phototoxicity assays were carried out with two different bases (cream and gel) and different xylitol concentrations (5 and 10%, w/w).

MATERIAL AND METHODS

These procedures were conducted in accordance with Ethical Principles in Animal Research adopted by the Brazilian College of Animal Experimentation (COBEA) and were approved by the Ethical Committee for Animal Research of the Federal University of Juiz de Fora (Brazil).

Primary dermal irritation studies

Test material and animals

Xylitol (Fluka BioChemika, Switzerland) was administered at 5 and 10% (w/ w) incorporated in cream or gel through a 60% (w/w) mixture in ultra-pure water. Three adult male and three adult female New Zealand albino rabbits, weighting between 1.5 and 2.5 kilograms, were obtained from a local supplier (Fazenda Experimental Professor Hélio Barbosa, UFMG) and

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were used for each experimental condition: 5% xylitol in cream; 10% xylitol in cream; 5% xylitol in gel and 10% xylitol in gel. The animals were housed individually in cages in a temperature-controlled (20–25 °C) and humidity-monitored (45–65%) environment. The rabbits were provided continuous access to tap water and fed commercial chow.

Procedure

Two approximately 6 cm^2 test sites (one intact and one abraded) were delineated on each animal. Xylitol incorporated in cream or gel and 0.5 g of the test substance was placed on a 2 cm^2 gauze pad. One pad was then applied to each abraded and intact skin dosing site and held in place for 4 h with non-allergenic, occlusive tape. The patch was then removed, and the degree of erythema and edema was evaluated according to the Draize method [20] at approximately 1, 24, 48, and 72 h and so on each day, until the 11th day after patch removal. Animals weight was monitored at the beginning (1st day), the middle (6th day) and the final (11th day) of the experiments.

Phototoxicity assays

Test material and animals

Only 10% (w/w) xylitol, either in cream or gel, was used. Male Durkin-Hartley albino guinea pigs, weighting between 300 to 360 g, were obtained from a local supplier (FIOCRUZ, Brazil). The animals were housed individually in stainless steel cages in temperature-controlled (19–23 °C) and humidity-monitored (50-60%) quarters. Test animals were provided continuous access to tap water and commercial chow. Four animals were used as test and as positive control and two animals as a negative control, for each formulation tested. The positive control employed was 2 mg/g of 8-methoxypsoralen (Sigma-Aldrich, United States of America), incorporated in cream or gel.

Procedure

The experiments were conducted according to Brito [21], Pinto *et al.* [22] and Okumura *et al.* [23] with modifications. Each animal had four application sites measuring 1.5 cm^2 to which aliquots (0.5 g/ site) of test/control substances were applied in duplicate (one aliquot on each side). Chemical substances used were 10 % xylitol, 8 - methoxypsoralen (8-MOP), which was used as a positive control, and base only. After topical application, the animals were placed into restrainers to be exposed to UVA light. Sunscreen was placed over the left back of each guinea pig for protection against irradiation while the right back was left uncovered to allow exposure to UVA at a target dose of 200 J/cm^2 during 15 min. The spectrum contained an emission peak at 355 nm, using a 15 Watts UVA potency lamp (Phillips, Denmark). The distance between the guinea pig skin surface and the light source was about 30 cm. After completion of the exposure period, the sunscreen was removed. Test sites were graded at approximately 1, 24, 48 and 72 h after the initiation of the UVA exposure using a Draize scoring system [20]. The weight of animals was observed at the beginning and end of the experiments.

Statistical analysis

Statistical analysis were performed only to check if animal weight varied significantly during the assays. Analysis of variance and Student-t test for dependent samples were done for primary dermal irritation studies and phototoxicity assays, respectively. For the toxicology assays, the design of the experiments performed is such that statistical analysis was not necessary using the Draize evaluation.

RESULTS AND DISCUSSION

Body weight

No statistically significant difference was observed between the weights measured at the three times ($p = 0.769$) in primary dermal irritation studies. A slightly lower mean body weight relative to test and control animals ($p = 0.021$) was observed from the first day of the experiment until the end of the study.

Primary dermal irritation studies

Two sums were obtained for each formulation tested: one concerning erythema (total A) and the other edema (total B). Both values were added, divided into four (erythema and edema on abrased skin and not) and divided into ten (number of days of application). If the result is lower than 1, the formulation is considered not irritative; if the score is between 1 and 2, it is considered slightly irritative; if the score is between 2 and 3, it is considered mild to moderately irritative and if the score is upper than 4, the formulation is considered severely irritative. In Table 1 the final values related to each situation can be observed.

With respect to the classification proposed by Draize [20], all the tested formulations were classified as not irritative. Thus, this result allows xylitol to be used to treat skin diseases with security.

In a retrospective study conducted by Derelanko *et al.* [24] with 224 cases of dermal toxicity studies using six rabbits per experimental group, it was noted when reducing this number to five or four that the agreement declined to a little below 90 % and when three animals were used, the agreement was close to 70 %. Thus, the sample used in these trials (n = 6) is satisfactory and allows a reliable result to be found. For the same substance tested at different concentrations, Craig *et al.* [25] studied the dermal acute toxicity of oils extracted from *Juniperus occidentalis* and *Chamaecyparis lawsoniana* plants at concentrations of 0.5, 5 and 50 % in albino New Zealand rabbits and found absence of toxicity, except for 50% *J. occidentalis,* in which case a positive toxicity reaction was found. This result illustrates the usefulness of testing a higher concentration than the therapeutic one, to ensure the safety of the therapeutic dose. This situation on those assays was also observed in the present study.

Phototoxicity assays

To calculate the phototoxic index for each animal, the sum obtained in the irradiated site was subtracted from the sum obtained in the non-irradiated site, for xylitol, 8-MOP and controls (cream or gel).

The results presented in this study were analyzed according to the observations of Mercier [26] and Brito [21]. According to Mercier [26], when the difference in values obtained subtracting scores for redness and swelling of an irradiated area from that for the area not irradiated is not greater than or equal to 2, the reaction is positive, or there was phototoxicity; where the value is 0 or 1, the reaction is negative and, in other cases, reactions are doubtful. According to Brito [21] from the result of each individual animal, the set is then classified, using the percentage of animals in which the reaction is positive: 0-10% → formulation is not phototoxic; 10-30% → formulation has slight phototoxicity; 30-70% → formulation has moderate phototoxicity; 70-100% → formulation has high phototoxicity. Table 2 shows the number of animals which presented positive and negative reactions when each formulation was applied.

For 10% xylitol either in cream or gel, three out of four animals showed positive reaction. For 8-methoxypsoralen, in the form of cream, all the animals presented positive reaction, and, in the form of gel, three out of four presented positive reaction. All the controls, both cream and gel (four, in total), presented negative reaction These data indicate that xylitol, when incorporated in cream and gel, although cumulative skin toxicity is not present, has moderate phototoxicity. In the controls, phototoxicity reactions were not observed.

Gia *et al.* [27] tested phototoxicity properties of furanocumarins, a chemical class to which 8-methoxypsoralen belongs, and assures the use of this kind of compound as the positive control in these assays. Santos *et al.* [28] tested the action against phototoxicity of a sunscreen containing a mixture of three organic filters and 8-methoxypsoralen in guinea pigs after exposure to UVA radiation for two hours. It was observed that the mixture of sunscreen prevented any phototoxicity reaction in guinea pigs, but 8-methoxypsoralen showed a phototoxicity effect compared with those found in the present study. Here, the irradiation is 100 times higher than the dose that a person could be exposed to on a summer day at noon [28] and the controls reacted as predicted (positive control showed positive reactions and negative controls – only bases – showed negative reactions).

There is also a need to verify whether a compound with a pharmacological property is safe for dermal application. In a similar experiment, Okumura *et al.* [23] tested solutions containing ketoprofen and 8-methoxypsoralen dissolved in acetone in a treated area which was exposed to UVA and UVB radiation. Only in the sites where 8-methoxypsoralen had been administered, erythema and edema were observed. For ketoprofen skin application, there

Pharmaceutical formulation	Xylitol concentration (w/w)	Values referenced to erythema	edema	Sum	Final value
Cream	5.0%	0.17	0	0.17	0.004
	10.0%	0	0	0	0
Cream control		1.02	0.16	1.18	0.03
Gel	5.0%	3.8	0.49	4.29	0.107
	10.0%	9.45	0.64	10.09	0.253
Gel control		10.16	0.66	10.82	0.270

Table 1. Final values related to data obtained from Primary dermal irritation studies.

Pharmaceutical form	Drug	Number of animals which presented positive or negative reaction		Number of animals
		Positive reaction	Negative reaction	
Cream	10% xylitol	3	1	4
	8-methoxypsoralen	3	1	4
	control	0	2	2
Gel	10 % xylitol	3	1	4
	8-methoxypsoralen	4	0	4
	control	0	2	2

Table 2. Final values related to data obtained from phototoxicity assays.

was no need for special care. The phototoxicity properties of tobacco smoke were evidenced by Placzek *et al.* [29]. The authors related this property to aging, and also to the consumption of cigarettes. This fact indicated that tobacco smoke, besides causing a negative effect on the lungs, also caused adverse reactions in the skin of people exposed to it, as demonstrated in *in vitro* tests using red blood cells.

CONCLUSIONS

This is the first study targeting xylitol skin toxicity. These results demonstrated that xylitol, either in cream or gel, has phototoxic properties elicited by radiation rich in UVA. Despite a thorough search of the literature for xylitol –related effects, we could not find references to primary dermal irritation or phototoxicity. So, these novel values will be useful when xylitol skin applications are tested.

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REFERENCES

1. Wen, Z.T., C. Browngardt, & R.A. Burne (2001) *FEMS Microbiol. Lett.* **205**: 337-42.
2. Iwata, C., H. Nakagaki, J. Morita, T. Sekiya, M. Goshima, T. Abe, A. Isogai, M. Hanaki, M. Kuwahara, M. Tatematsu & C. Robinson (2003) *Arch. Oral Biol.* **48**: 389-95.
3. Ylikahri, R. (1979) *Adv. Food Res.* **25**: 159-80.
4. Uhari, M., T. M. Kontiokari, M. Koskela & M. Niemela (1996) *Brit. Med. J.* **313**: 1180-1183.
5. Leiras, O. M. Uhari & T. Kontiokari. *Use of xylitol and pharmaceutical compositions therefore*. Patent N° US6066677.
6. Sawaguchi M. & Y. Sawaguchi. Sawaguchi, M., Y. Sawaguchi, K. Nakao, H. Kuromaru & H. Hirofumi. Xylitol nose spray. Patent N° JP2001172150.
7. Mattila, P.T., M.L.E. Knuutila & M. J. Svanberg (1998a) *Metabolis.* **47**: 578-83.
8. Mattila, P.T., M.J. Svanberg, P. Põkkã & M. L. E. Knuttila (1998b) *J. Nutr.* **128**: 1811-4.
9. Tischler, A.S., J.F. Powers, J.C. Downing, J.C. Riseberg, M. Shahsavari, J. Ziar & R.M. McClain (1996) *Toxicol. Appl. Pharm.* **140**: 115-23.
10. Mattila, P.T., H. Kangasmaa & M.L.E. Knuutila (2005) *Metabolis.* **54**: 548-551.
11. Wang, Y.M. & J. van Eys (1981) *Annu. Rev. Nutr.* **1**: 435-75.
12. Georgieff, M., E. Pscheidl, L.L. Moldawer, B.R. Bistrian & G.L. Blackburn (1991) *Eur. J. Clin. Invest.* **21**: 249-58.
13. Zabner, J., M.P. Seiler, J.L. Launspach, P. H. Karp, W.R. Kearney, D.C. Look, J.J. Smith & M.J. Welsh (2000) *Proc. Natl. Acad. Sci. USA* **97**: 11614-9.
14. Sanromán, M.A., H. Dominguez, M. J. Núnez & J.M.Lema (1991) *Afinidad* **434**: 215-22.
15. Voirol, F. (1977) *Food Eng. Intl.* **2**: 40-1.
16. WHO/ FAO (1977) *"Summary of toxicological data of certain food additives, Twenty- first Report of the Joint FAO/ WHO Expert Committee on Food Additives."* Geneva: WHO Technical Report Series (Word Health Organization) **617**: 124-47.
17. Ferreira, A.S., M.A. Souza, N.R. Barbosa & S.S. Silva (2008) *Exp. Parasit.* **119**: 74-9.
18. Masako, K., I. Hideyuki, O. Shigeyuki & I. Zenro (2005a) *J. Dermatol. Sci.* **38**: 197-205.
19. Masako, K., K. Yusuke, I. Hideyuki, M. Atsuko, M. Yoshiki, M. Kayoko & K. Makoto (2005b) *J. Dermatol. Sci.* **38**: 207-13.
20. Draize, J.H. (1959) *"Dermal toxicity",* in: Appraisal of the safety of chemicals in foods. Washington (DC): Association of Food and Drug Officials of the United States. p. 46-59.
21. Brito, A.S. (1994) *"Manual de ensaios toxicológicos in vivo"*. Editora da UNICAMP, Brazil.
22. Pinto, T.J.A., T.M. Kaneko & M.T. Ohara (2003) *"Controle biológico de qualidade de produtos farmacêuticos, correlatos e cosméticos"*. 2th edition. Atheneu, Brazil.
23. Okumura, Y., S. Yamauchi, S. Takayama, H. Kato & M. Kokubu (2005) *J. Toxicol. Sci.* **30**: 19-28.
24. Derelanko, M.J., C.E. Finegan & B.J. Dunn (1993) *Fund. Appl. Toxicol.* **21**: 159-63.
25. Craig, A.M., J.J. Karchesy, L.L. Blythe, M.P. González-Hernández & L.R. Swan (2004) *Toxicol. Lett.* **154**: 217-24.
26. Mercier, O. (1991) *"Test to evaluate the phototoxic and photoallergic potentials by topical applications in the guinea-pig"* Les Oncins, France.
27. Gia, O., M.S. Magno, H. Gonzalez-Diaz, E. Quezada, L. Santana, E. Uriarte & L.D. Via (2005) *Bioorgan. Med. Chem.* **13**: 809-17.
28. Santos, E.P., S. Garcia, Z.M.F. Freitas & A.L. Barth (2001) *Rev. Visão Acadêmica* **2**: 71-6.
29. Placzek, M., U. Kerkmann, S. Bell, P. Koepke & B. Przybilla (2004) *Brit. J. Dermatol.* **150**: 991-3.