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Evaluation of *in vivo* efficacy of topical formulations containing soybean extract

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Abstract

In the present study it was evaluated the: (i) functional stability of the soybean extract as a raw material and dispersed in two different topical formulations, (ii) skin retention using modified Franz diffusion cells, and (iii) *in vivo* activity of these formulations to inhibit 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) increases in the skin of hairless mice. The physico-chemical stability was evaluated by pH, globule size and centrifugation test. Furthermore, functional stability was also evaluated by antilipoperoxidative activity. The two topical formulations were stored at 4 °C, 30 °C/60% RH and 40 °C/70% RH for 6 months. The evaluation of the antiperoxidative stability of soybean extract itself and incorporated in formulations did not demonstrate loss of activity by storage at 4 °C/6 months. During 6 months of the study in different storage conditions the formulations 1 and 2 added or not with soybean extract were stable to physico-chemical tests. The effect of antioxidant compounds detected by the inhibition of MDA formation was time-dependent for formulation 2 as detected in the skin retention study. Pretreatment with formulation 1 or 2 significantly diminished TPA-induced H_2O_2 and MDA generation. In conclusion, the present results suggest for the first time that formulations containing soybean extract may be a topical source of antioxidant compounds that decrease oxidative damages of the skin.

Keywords: Antioxidant; Isoflavonoid; *In vitro* retention; Stability test; Skin; Topical formulation

1. Introduction

Skin is a highly metabolic tissue that presents the largest surface area in the body and serves as the protective layer for internal organs (Kohen and Gati, 2000). It is designed to give both physical and biochemical protection and is equipped with a large number of defense mechanisms (Halliwell and Gutteridge, 1990).

On the other hand, the skin is very susceptible to oxidative stress, because it presents susceptible biological targets for such reactions. It is exposed to a variety of damaging oxidative species, from outer environment; skin itself, and various endogenous sources (Kohen and Gati, 2000). Elevation in cellular concentration of reactive oxygen species (ROS) such as superoxide anion, hydrogen peroxide and hydroxyl radicals characterizes the oxidative stress (Salem et al., 1999). These ROS seem deleterious since they were related to many skin disorders such as cancer, cutaneous autoimmune diseases and skin aging (Lopez-Torres et al., 1998; Fuchs and Packer, 1991).

Researches have focused on the potential use of some enzymes and secondary compounds of higher plants as free radical scavengers to prevent oxidative skin damage (Casagrande et al., 2006a), and thus, their topical application has been of considerable interest (Saija et al., 1998). In this sense, antioxidants from natural products provide novel possibilities

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for the treatment and prevention of oxidative stress-mediated skin diseases (Aquino et al., 2002).

Among the natural products, flavonoids, a group of polyphenolic compounds possessing broad biological properties, exert beneficial effects due to their capability to interact with protein phosphorylation, and the antioxidant, iron-chelant and free radical scavenging activities (Saija et al., 1998). Furthermore, flavonoids are claimed to be free of toxicity and side effects, and particularly, harmless to the skin (Bonina et al., 1996; Fuchs and Packer, 1991). In this context, the subclass of the more ubiquitous flavonoids, isoflavonoids (Messina, 2000) include genistein and daidzein. There are evidences suggesting that these compounds may prevent cancer (Wang et al., 2002) and ROS-induced human skin damages. In fact, genistein and daidzein as well as polyphenols present antioxidant activity and are found in the extract of soybean (Messina, 2000). The use of the extract of soybean instead of the purified genistein and daidzein can increase the antioxidative potential of the purified molecules alone. Furthermore, plant extracts acquisition is less expensive than the purification of specific substances.

The evaluation of topical formulations added with soybean extract by antioxidant activity is a crucial issue in the study of new pharmaceutical products for skin oxidative damage treatment. Furthermore, there is no evidence on *in vivo* use of topical formulation containing soybean extract to prevent oxidative damages. Thus, the present study was designed to evaluate both physico-chemical and antioxidant activity stability of different formulations containing soybean extract as well as *in vitro* percutaneous absorption. Finally, the *in vivo* protection against TPA-induced oxidative stress was assessed. The TPA model was used since it is recognized as an inflammatory agent (Sultana and Saleem, 2004) and tumor promoter that has been shown to generate superoxide anion, hydrogen peroxide and lipid hydroperoxides leading to oxidative damage (Sharma and Sultana, 2004).

2. Materials and methods

2.1. Chemicals

Commercial soybean extract (Isoflavin Beta®) and raw materials for formulations were obtained from Galena (Campinas, SP, Brazil) and are presented in the formulation section. Genistein, daidzein, thiobarbituric acid (TBA), 12-*O*-tetradecanoyl phorbol-13-acetate (TPA), phenol-red and horse radish peroxidase were obtained from Sigma Chemical CO. (St. Louis, MO, USA). 2-deoxy-D-ribose was obtained from Acros (New Jersey, USA) and hydrogen peroxide 30% was purchased from Calbiochem (California, USA). All other reagents used were of pharmaceutical grade or HPLC grade.

2.2. Test formulations

The formulations were developed varying its lipidic content. The non-ionic emulsion with high lipid content (formulation 1) was prepared with commercially available self-emulsifying wax Polawax® (cetostearyl alcohol and polyoxyethylene derivative of a fatty acid ester of sorbitan 20E). The emulsion with low lipid content was prepared with low concentration of Polawax® (formulation 2) and an anionic hydrophilic colloid (carboxypolymethylene, Carbopol® 940) was also added as a stabilizing agent and triethanolamine as neutralized. Both formulation contained macadamia nut oil as emollient and propylene glycol as a moisturizer. The preservative used was a mixture of parabens and imidazolidinyl urea. Deionized water qsp was used for the preparation of all formulations (Table 1). Extract of soybean (2.00%) was firstly solubilized in propylene glycol and next incorporated to the formulations at the room temperature. The control formulations did not contain the extract. All formulations were allowed to equilibrate for 24 h prior to use in the study.

Table 1
Percent composition (w/w) of the emulsions

Components	Formulation 1 (%)	Formulation 2 (%)
Self-emulsifying wax	10.00	2.00
Carbopol® 940	–	0.18
Macadamia nut oil	2.00	2.00
Propylene glycol	6.00	6.00
Soybean lecithin	4.00	4.00
Urea	4.00	4.00
Methylparaben	0.15	0.15
Propylparaben	0.02	0.02
Imidazolidinyl urea	0.30	0.30
Deionized water	73.53	81.35

2.3. Physico-chemical stability

The physico-chemical stability was performed according to World Health Organization. Formulations containing or not the extract were stored at 4 °C, 30 °C/60% relative humidity (RH), and 40 °C/70% RH for 6 months (Casagrande et al., 2007; Singh, 1999).

2.3.1. pH measurements

The pH of formulations diluted 1:10 in deionized water was measured using a Digmed DMPH-2 pHmeter. All measurements were made at room temperature in triplicate for each analyzed sample (Anchisi et al., 2001; Di Mambro and Fonseca, 2007).

2.3.2. Centrifugation assay

The samples were centrifuged at 1660 × *g* for 30 min at room temperature and the phase separation was analyzed visually (Anchisi et al., 2001).

2.3.3. Globule size measurement

The sizes of the emulsions globules were examined microscopically (Olympus microscope fitted with a 40X objective lens). The formulations were diluted 100 times using propylene glycol/water (1:1), and after, the lipofilic stain Sudan II 1% was added. One droplet of the diluted emulsion was put into Neubauer chamber and the average numbers of globules in each square ($\tilde{n}$) were determined. The results were put into Eq. (1) to determine the numbers of globules in each gram of the emulsion