Chinese Traditional Medicine Journal

ISSN : 2693 6356
2018 | Vol 1 | Issue 2

In Vitro Anti-Oxidant Study of Pure Mattifying Face Cream Using HEPG2 CellLine

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Abstract

Background: Herbal cosmetics are gaining a wide popularity in world market mainly due to the concern of consumers with regards to its safety and minimum or nil untoward effects. They are used for variety of reason in almost all the age group**. Aim:** The present study deals in vitro anti-oxidant assay of the poly-herbal formulation, Pure Mattifying Face Cream. **Materials & Methods:** Human liver cancer cell line (HepG2) was used as an in vitro model to evaluate the antioxidant capacity of the test substance. HepG2 cells were exposed to carbon tetra chloride (CCL_4) in order to induce hepatotoxicity. Cells were pre-treated with different concentrations of test substance to assess the antioxidative effects as opposed to control untreated cells. Investigation of cytotoxicity, and markers of oxidative stress (reduced glutathione content) was carried out. **Results:** The test substance showed low cytotoxicity, on the basis of CTC50 (the cytoxic concentration / dose that kills 50% of cells) value the concentration of the test substance considered for Ant-oxidant activity against HepG2 cell line. The test product showed dose dependent anti-oxidant activity. The test substance showed higher content of glutathione with 16.138 and 13.179 µM of glutathione content per mg of protein against toxicant CCL_4. **Conclusion:** The result suggested that the Pure Mattifying Face Cream did possess a positive anti-oxidant activity in HepG2 cell line. Which implies that the cream can be effectively used in oxidative stress due to free radicals damage to skin.

Keywords: *Anti-Oxidant assay, HepG2 cell line, In vitro study, Pure Mattifying Face Cream*

Introduction

cosmetics are products which are used for variety of reasons ranging from enhancing of beauty to minimizing the skin defect, with
later being the major reason. The Federal Food, Drug, and Cosmetic Act (FD&C Act) de◎nes cosmetics by their intended use, as "articles intended to be rubbed, poured, sprinkled, or sprayed on, introduced into, or otherwise applied to the human body...for cleansing, beautifying, promoting attractiveness, or altering the appearance" [FD&C Act, sec. 201(I)] [1].

The history of cosmetics dates back to time immemorial which undoubtedly was natural. The invent of synthetic or chemical substance has taken over the cosmetic market by storm. However the
recent ban on animal testing of cosmetics by European union has lead to a serious concern on the safety and adverse effects of these cosmetics [2].
Advances in technology has helped in reinventing the potential of natural substances as cosmetics in an applicable scienti◎c way. The fact is coupled byvarious clinical and laboratory studies which have
identi◎ed activities in many natural ingredients thathave potential bene◎cial activities for personal skin care [3-4].

Among various causes of skin aging, oxidative stress due to free radical damage which cannot be neutralized by internal defenses is said to be the most common and important one [5]. Pure Mattifying Face Cream is one such natural cosmetic enriched with natural revitalize, essential oils that nourish, renew and provide a long standing skin hydration. enrichment with all the natural goodness of Liquorice, Aloe vera, Lotus, Hibiscus and Coconut in a de◎nite balanced formulations that synergistically helps to revive dry lusterless skin and reveals youthfulness. Its continued application can help in fastening the renewal of skin cells and visibly reduces ◎ne lines, wrinkles and age spots making one look younger.

The present study is purported to assess the anti- oxidant activity of test substance in HepG2 cell line model.

Materials and Methods:

Test System: HepG2 (Human, Hepatocellular carcinoma) has been routinely used as a test system, which, when treated with CCL4 induces severe hepatotoxicity resulting in oxidative stress with reduced amounts of reduced glutathione (GSH).

Test culture preparation: Cell lines were cultured in MEM media supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100µg/ml) and amphotericin B (5 µg/ml) in a humidi◎ed atmosphere of 5% CO2 at 37C until confluent. The cells will be dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures will be grown in 25 cm2 culture flasks and all experiments will be carried out in 96 micro titre plates.

Test Product: Pure Mattifying Face Cream, each gram of Cream contains, Licorice (30 mg), Aloe vera (30 mg), Lotus ◎ower (20 mg) and Chinese rose (20 mg) and Coconut in a de◎nite balanced formulation.

Preparation of Test Doses:

For studies, each weighed test drugs were separately dissolved in 1% between 80 and volume was made up with MEM supplemented with 2% inactivated FBS to obtain a stock solution of 1 mg/ml concentration and sterilized by ◎ltration. Serial two fold dilutions wereprepared from this for carrying out cytotoxic studies.

Procedure: Determination of Cytotoxicity in HepG2 cell line The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 105 cells/ml using MEM containing 10% FBS. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was discarded and the cells were washed with medium and 100 µl of different test concentrations of test drugs were added on to the partial monolayer in microtitre plates. The plates were then incubated at 37o C in 5% CO2 atmosphere. After 24 h, the drug solutions in the wells were discarded and 50 µl of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37o C in 5% CO2 atmosphere. The supernatant was removed and 100 µl of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated and concentration of test drug needed to inhibit cell growth by 50% (CTC50) values is generated from thedose-response curves for each cell line [6].

In vitro anti-oxidant activity of extractsStep I: Cell Lysates (Treatment)

Cell lysates were prepared for the assessment of antioxidant activity. HepG2 cells were grown to confluency in 60mm petri dishes. Test substance (500, 250 µg/ml) along with CCL4 (1%) was added to the culture and incubated for 24 h. Cell samples were homogenized in lysis buffer containing Tris buffer. Cell homogenates were then centrifuged at 2000 RPM for 10 min at 4 °C. The clear supernatant was collected for determination of protein concentrations using theBradford Protein assay, using bovine serum albuminas a protein standard [7].

Step II: Reduced glutathione (GSH)

Reduced glutathione (GSH) activity was assayed according to the method of Ellman, 1959. To 0.1 ml of different cell supernatant, 2.4 ml of 0.02 M EDTA solution was added and kept on ice bath for 10 min. Then 2 ml of distilled water and 0.5 ml of 50 %w/v TCA were added. This mixture was kept on ice for 10- 15 min, and then centrifuged at 3000 g for 15 min. To 1 ml of supernatant, 2.0 ml of Tris buffer (0.4M) was added. Then 0.05 ml of DTNB solution (Ellman'sreagent; 0.01M DTNB in methanol) was added and

vortexed thoroughly. OD was read (within 2-3 min after the addition of DTNB) at 412 nm in spectrophotometer against a reagent blank.

Results

The test substance showed low cytotoxicity, on the basis of CTC50 value, the concentration of the test substance which is considered for anti-oxidant activity against HepG2 cell line (Table 1).

Table 1: Cytotoxic properties of test drugs against HepG2 cell line

Sl. No	Name of sample	Test Conc.(µg/ml)	% Cytotoxicity	CTC50 (µg/ml)
1	**R** 2272 (IHC/2014/AC)	1000	20.81±4.6	>1000
		500	16.02±3.0	
		250	13.11±3.0	
		125	8.05±3.5	
		62.5	7.46±2.8	

ingredients, many of which are carcinogenic and toxic in nature, can potentially result in various degenerative diseases. Of-late, interest in research towards exploring natural antioxidants for skin care has greatly risen. Principal sources in this discovery process are herbal and medicinal plants which are touted to be safe and effective. Thus, relying on herbal based cosmetic preparations over their synthetic counterparts that come with a potential tag of side effects, is always welcome. The test substance used in this study is a multi-herbal formulation intended to minimize pre-mature ageing and improve skin complexion keeping the skin soft and supple. The test substance exhibited potent antioxidant actions and found to be non-toxic. The combinatorial effect of the different constituent herbs and the respective active ingredients might have been mainly responsible for the observed effects. The constituent herbs and their active principles in the enriched formulation – (i) Licorice (Glabridin and other phytonutrients); (ii) Aloe vera (amino acids, various minerals like Ca, Mg, Na, bioactive enzyme alosein and polysaccharides); (iii) Lotus ◎ower (the ◎ower acid, AHA (alpha hydroxyl acid)) and (iv) Chinese rose (alpha-hydroxy- acids (AHA)) - might offer a synergistic effect towards antioxidant features complimented by in◎ammatory and antibacterial properties, which probably could bethe mode of action of the substance studied[9-12].

The test product showed dose dependent anti-oxidant activity. HepG2 cells pre-treated with test substance had higher glutathione content of 16.138 and 13.179 micromoles/mg protein, at 250 and 500µg/ml test substance respectively, against toxicant CCL4, as mentioned in Table 2.

Table 2: Anti-oxidant activity of test substance in HepG2 cells against CCl4 induced toxicity.

{¡ No	Samples	Concentrationtested	Glutathione (micromoles/mgprotein)

Conclusion

Pure Mattifying Face Cream has shown positive anti-oxidant activity in HepG2 cell line and found to be non-toxic. Hence it can be advocated in condition of

1	**IHC/2014/AC**	500 µg/ml 250 µg/ml	
2	CCl_4	1%	7.92±0.103
3	Control	-	10.364±0.993

Discussion16.13±1.169
13.17±0.902oxidative stress due to free radical damage to skin.

References

1. Is it a Cosmetic, a Drug, or Both? (Or Is It Soap?). [Internet]. US Food and Drug Administration. [Cited on 05-01-2016]. Available from http://www.fda.gov

Skin is the most apparent organ that reveals our physical appearance. In modern day's stressful lifestyle that has adverse effects on our skin, it is of great concern to make efforts towards preventing skin deterioration. There is nurturing scientific evidence to support the theory of free radicals (oxidative stress) weakening the skin's endogenous defense system and leading to various forms of skin damage including rapid skin ageing[8]. Antioxidants are believed to block the process of free radical generation and subsequently protect the overall health of the skin. Chemical based cosmetics contain hazardous/Cosmetics/GuidanceRegulation/LawsReg ulations/ucm074201.htm

2. Ban on Animal Testing. [Internet]. European Commission. [Cited on 05-01-2016]. Available from http://ec.europa.eu/growth /sectors/cosmetics/animal-testing /index_ en.htm
3. Fowler JF, Woolery-Lloyd H, Waldorf H, Saini R. Innovations in natural ingredients and their use in skin care. J Drugs Dermatol. 2010; 9 (6 Suppl): S72–81.
4. Baumann L, Woolery-Lloyd H, Friedman A. Natural" ingredients in cosmetic dermatology. J Drugs Dermatol. 2009; 8 (6 Suppl): s5–9.
5. Charlene De Haven, Oxidative Stress and Free Radical Damage to Skin, [Internet]. 2014. [Cited on 05-01-2016]. Available from http://www.isclinical.com/whitepapers/WhitePaper_OxidativeStressFreeRadicalDamage_June2014.pdf
6. Francis D and Rita L. Rapid colorometric assay for cell growth and survival modi◎cations to the tetrazolium dye procedure giving improved sensitivity and reliability. Journal of Immunological Methods; 1986; 89: 271-277.
7. George L. Ellman. Tissue sulfhydryl groups. Archives of Biochemistry and Biophysics; 959;82(1): 170–77.
8. Biesalski H K. Free radical theory of ageing. Current Opinion in Clinical and Metabolic Nutrition. Nutrition and Metabolic Care, 2002; 5 (1): 5-10.
9. Yin Wei Mak, Li Oon Chuah, Rosma Ahmad and Rajeev Bhat. Antioxidant and antibacterial activities of hibiscus (Hibiscus rosa- s inensis L.) and Cassia (Senna bicapsularis L.) ◎ower extracts. Journal of King Saud University - Science; 2013; 25(4):

275–282.
10. Fu Y, Chen J, Li YJ and Zheng YF, Li P. Antioxidant and anti-inflammatory activities of six flavonoids separated from licorice. Food Chem. 2013 Nov 15;141(2):1063-71.
11. Benson KF, Newman RA and Jensen GS. Antioxidant, anti-inflammatory, anti-apoptotic, and skin regenerative properties of an Aloe vera-based extract of Nerium oleander leaves. 2015; 8: 239-48.
12. Nishkruti R Mehta, Ekta P Patel Pragnesh V Patani and Biren Shah. Nelumbo Nucifera (Lotus): A Review on Ethanobotany, Phytochemistry and Pharmacology, Indian Journal of Pharmaceutical and Biological Research. 2013; 1 (4): 152-67.