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NEW ANTI-AGING AND ANTI-WRINKLE COSMETIC INGREDIENT : INNER NUTSHELL OF *CASTANEA MOLLISIMA* BL (CHESTNUT)

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ABSTRACT

Inner nutshell of *Castanea mollisima* BL (chestnut) has been used as an anti-aging and anti-wrinkle agent from the ancient time in east Asia. In order to develop new anti-aging and anti-wrinkle, ethanolic extract of inner nutshell of *Castanea mollisima* BL (Cor-285) was prepared and various biological activities were evaluated. Cor-285 showed potent antioxidant activity. Especially, Cor-285 possessed potent free radical scavenging activity *in vitro* (IC50:7.6 g/ml) compared to gallic acid (IC50:12.5 g/ml). Cor-285 showed the preventive effect against UV-induced cytotoxicity of fibroblast at concentration of 25~250 g/ml. When Cor-285 was evaluated for its anti-allergic activity, it effectively inhibited histamine release from mast cells induced by compound 48/80 (86% inhibition at 10mg/ml). The inhibitory activity was stronger than that of glycyrrhiznate. Cor-285 also showed *in vivo* inhibition against delayed hypersensitivity as well as croton-oil induced ear edema in mice when topically applied. These results strongly suggest that Cor-285 may reduce immunoregulatory / inflammatory skin trouble. From the attempts to isolate the constituents, citropten (simple coumarin) and ellagic acid, a well known radical scavenger, were isolated. In a clinical trial of twenty healthy volunteers with aged skin, 6 weeks application of Cor-285 (3% cream) decreased wrinkle about 26% and increased moisturizing 20% on the skin. All of these results indicate that Cor-285 may be an effective anti-aging and anti-wrinkle agent.

INTRODUCTION

Many anti-aging agents used in the cosmetic industry are claimed to possess anti-oxidative and/or free radical scavenging activities. These anti-oxidative properties may prevent peroxidation of various cellular components, which eventually leads to aging process. Therefore, it is valuable to investigate anti-oxidative and free radical scavenging activities of various natural products for finding new cosmetic ingredients in the purpose of anti-aging. In the course of our screening program from plant extracts, ethanolic extract (Cor-285) of inner nutshell of *Castanea mollishima* was found to possess anti-oxidative and free radical scavenging activities. Because of this preliminary result and literatural background of using inner nutshell of *Castanea mollishima* (Chestnut) as a popular remedy for anti-aging as well as anti-wrinkle agent from the ancient time in Korea, we selected Cor-285 as a candidate for new potential anti-aging and anti-wrinkle agent. In this study, various biological activities such as anti-inflammation, inhibition of delayed hypersensitivity and anti-wrinkle effects of Cor-285 were investigated. And it was found that Cor-285 possessed anti-inflammatory activity and

inhibitory activity of delayed hypersensitivity *in vivo*. In addition, Cor-285 showed anti-wrinkle effects in 6-week clinical trial. Since retinol and AHA showed adverse effects such as skin irritation despite wide use as an anti-wrinkle agent in skin care products, Cor-285 may be a new and safe substitute based on the plant sources for anti-aging as well as an anti-wrinkle agent without serious side-effects.

MATERIALS AND METHODS

SPF male Sprague-Dawley rat (90-110g) and mice (18-22g) were obtained from Korea experimental animal center (Seoul, Korea). The animals were maintained in our animal facility (KNU) under the conditions of 22 1 C and 55 5% relative humidity with 12hr light/dark cycle for at least 7 days prior to use. Croton oil, arachidonic acid (99%), butylated hydroxytoluene (BHT), prednisolone, indomethacin, thiobarbituric acid and compound 48/80 were purchased from Sigma Chem. Co. (St. Louis, MO). Picryl chloride was a product of nacalai tesque (Japan). The other reagents used in this study were reagent grade chemicals available.

Preparation of Cor-285

Inner nutshell of was obtained from the oriental market place in seoul, Korea. The dried products were soaked in ethanolic solution for 3 days and evaporated to dryness in vacuum after filtration (Cor-285). From these extracts, citropten and ellagic acid were successfully isolated using standard column chromatography (silica gel and Sephadex LH-20)

in vitro biological activities

For measuring anti-oxidative activity, a lipid peroxidation system was induced by Fenton reagent. Briefly, each sample (100µl) and ethyl linolate (10µl) was added to incubation medium (4.89 ml) containing 2% SDS, 1uM ferrous chloride and 0.5mM hydrogen peroxide. After incubation at 55 C for 16 hrs, oxidation was measured using thiobarbituric acid (TBA) assay according to the method of Ohkawa *et al*[1]. For measuring scavenging activity, sample (2 ml) was added to 2 ml of 60uM 1,1-diphenyl-2-picryl hydrazyl(DPPH) ethanolic solution and kept at room temperature for 30 min according to the procedure of Fugita *et al*[2]. To examine the inhibitory activity against type I hypersensitivity *in vitro*, effects of Cor-285 on histamine release from mast cells isolated following the experimental procedure of Uvans[3] were studied. After preincubation of 1.8 ml of rat intraperitoneal mast cells for 10 min, Cor-285 was added and incubated for 5 min. Compound 48/80 (10µg/ml) was added and incubated further for 10 min, followed by termination by rapid cooling. The histamine content in the supernatant from centrifugation at 1,200 rpm for 5 min was measured according to the previously published method of Hidehiko *et al.*[4] using fluorescence spectroscopy.

Anti-inflammatory activity and inhibition of delayed hypersensitivity *in vivo*

For measuring topical anti-inflammatory activity, mouse ear edema assay was employed. According to the slightly modified method of Kim *et al.* [5] of original procedure of Tonneli *et al*[6], Cor-285 was topically applied to right ears of mice. In 30 min, 2.5% croton oil or 2% arachidonic acid dissolved in acetone was applied as an inflammagen and ear thickness was measured at 5 hr after treatment of croton oil or 1hr after treatment of arachidonic acid. Ear thickness was measured using

dial thickness gauge (Lux Scientific Instrument USA). Inhibitory activity of delayed hypersensitivity was examined using the procedure of Tarayre *et al.*[7]. Briefly, 3% picryl chloride solution was applied to the shaved abdomen of mice. One week later, picryl chloride solution was applied to ears of mice and ear thickness was measured at 24 hr after the treatment picryl chloride. Cor-285 was applied to ears of mice once every day for 7 days starting from day 0. The differences between ear thickness of the extract treated group and the control group only with picryl chloride was regarded as an inhibitory activity.

Application of Cor-285 on healthy volunteers

Twenty healthy women volunteers were selected. Their ages ranged from 19 to 36 (mean=26). In left forearm, Cor-285 dissolved in 50% 1,3-butylene glycol was topically applied on the intact skin twice a day for the indicated time period, while right forearm received only vehicle for the same period of time. The effects of Cor-285 from the clinical trial were examined as follows.

Effects on skin moisturizing, skin elasticity and wrinkle formation

In order to evaluate the skin moisturizing effect, the degree of stratum corneum hydration was measured by capacitance method using a Corneometer CM 820 (C+K electronic GmbH, Germany) according to the previously published procedure[8-11]. For measuring skin elasticity, suction method was used with Cutometer SEM 575 (C+K electronic GmbH, Germany)[12]. The degree of wrinkle improvement was evaluated by measuring skin roughness using Skin-Visiometer SV 400 (C+K electronic GmbH, Germany[13-14].

RESULTS AND DISCUSSION

In order to establish the biological action profile of the ethanolic extract of inner nutshell of chestnut (Cor-285), various *in vitro* and *in vivo* activities such as histamine release, anti-inflammation and wrinkle improvement were carried out.

Table 1 and Fig. 1 clearly demonstrated the anti-oxidative and free radical scavenging activity of Cor-285. Cor-285 showed the anti-oxidative activity (IC50 = 48μg/ml), while BHT was the most potent inhibitor (IC50=0.2μg/ml). Especially, Cor-285 possessed potent free radical scavenging activity *in vitro* (IC50=7.6μg/ml) compared to gallic acid (IC50= 12.5μg/ml). There have been numerous reports[15-21] that certain plant extracts possess anti-oxidative and free radical scavenging activities. Most of these active plant extracts contain phenolic or polyphenolic compound such as flavonoids and tannins that would contribute to their anti-oxidative and radical scavenging activities. Citropten (coumarin) and ellagic acid were successfully isolated from Cor-285. Ellagic acid is a well-known free radical scavenger, while citropten did not show the anti-oxidative and radical scavenging activity. Cor-285 showed potent inhibition of histamine release from rat mast cells activated by compound 48/80 (data not shown). In addition, Cor-285 possessed anti inflammatory activity and inhibitory activity in delayed hypersensitivity *in vivo* (Table 2 and 3). In a 6-week clinical trial, Cor-285 also increased skin moisturizing about 20% compared to control (Fig. 2). Cor-285 also increased skin elasticity about 10% compared to control (Fig. 3) and roughness of skin wrinkle about 26% (Fig. 4).

All of these results strongly suggested that Cor-285 is a new anti-aging and anti-wrinkle agent having potential anti-inflammatory activity useful for various skin troubles.

REFERENCES

1. Ohakawa, T., Ohishi, N., and Yagi, K., *Anal. Biochem.*, 1997, *95*, 351
2. Fugita, Y., Uera, I., Morimoto, Y., Nakajima, M., Hatano, C., and Okuda, T.,: *Yakugaku Zasshi*, 1998, *108*, 129
3. Uvans, I.L, Thon, *Exp. Cell Res.*, 1959, *18*, 512
4. Hidehiko, H., Ryouichi, E., and Yukimasa, O., *Japaness J. Allergy*, 1970, *19(3)*, 193-198
5. Kim, H.K., Namgoong, S.Y., and Kim, H.P., *Arch Pharm. Res.*, 1993, *16*, 18
6. Tonneli, G., Thiabalt, L., and Ringgler, I., *Endocrinal.*, 1965, *77*, 625
7. Tarayre, J.P., Barbara, M., Aliaga, M., and Tisneversailles, *J. Arzneim. Forsch./Drug Res.*, 1990, *40(11)*, 10, 1125
8. Courage, W. : Hardware and measuring principle: CORNEOMETER. In: *Bioengineering of the skin*: water and the stratum corneum: Elsner P., Berardesca, E., and Maibach H.I., Eds., CRC, Boca Raton, 1994, 171-176
9. Barel, A., Clarys, P.: Measurement of epidermal capacitance. In: *Handbook of non-invasive methods and the skin* ; Serup, J. and Jemec, GBE, Eds., CRC, Boca Raton, 1995, 165-197
10. Distante, F., Berardesca, E.: Hydration. In: *Bioengineering of the skin*: Methods and instrumentation: Berardesca, E., Elsner, P., Wilhelm, K.P., and Maibach, H.I., Eds., CRC, Boca Raton,1995
11. Stab, F., Sauermann, G., Hoppe, U.: Evaluation of moisturizers. In: *Bioengineering of the skin*: Skin surface imaging and analysis; Wilhelm, K.P., Elsmer, P., Berardesca, E, and Maibach, H.I, Eds., CRC, Boca Raton, 1997, 315-330
12. Elsner, P.: Skin elasticity. In: Berardesca, E., Elsner, P., Wilhelem, K.P., and Maribach, H.I., Eds., : *Bioengineering of the skin* : Methods and instrumentation : CRC, Boca Raton, FL, 1995, Chapter 6, 53-64
13. Nissen, H.P., Comparative studies of skin roughness measurements by profilometry and a new image analysis system: *Cosmetics and toiletries manufacture world wide*, 1995, 247-248
14. Gassmueller, J., Kecskes A., Jahn, P., : Stylus method for skin surface contour measurement., *Hand-book of non-invasive methods and the skin*, 1995, 83-87
15. Davies, K.T.A. : Oxidative damage and repair, Pergamon press, New York, 1991
16. Simic, M.G., and Bergtold, D.S. : *Mutation Res.*, 1991, *250*, 17
17. Hartman, P.E., and Shankel, D.M. : *Environ. Mol. Mutagen.*, 1990, *15*, 145
18. Wattenberg, L.W. : *Cancer Res.*, 1992, *52*, 2015s
19. Masaki, H. : Active oxygen scavenging activity in plant extracts, *Fragrance J.*, 1995, *8*, 64
20. Fukuda, T., and Kitada, Y. : Reactive oxygen species scavenging effect of crude drug, *Fragrance J.*, 1995, *18*, 75
21. Yoshikawa, M., and Yamahara, J. : *Chem. Pharm. Bull.*, 1992, *40(8)*, 2248

TABLES AND FIGURES

Table 1. Anti-oxidative effects of the EtOH extracts of COR-285

Group	*Treatment*	*Absorbance, OD535nm*	*% inhibition*
Control (vehicle only)		0.351 0.002	-
Cor-285	5 g/ml	0.291 0.010	16.9
	50 g/ml	0.178 0.010**	50.9
	100 g/ml	0.130 0.005**	63.0
d/-α-tocopherol	5 g/ml	0.275 0.025	22.7
	30 g/ml	0.184 0.035	47.6
	50 g/ml	0.121 0.028	65.6
l-ascorbic acid	100 g/ml	0.287 0.007**	18.3
	200 g/ml	0.188 0.006*	46.5
	300 g/ml	0.139 0.028	60.4
BHT	1 g/ml	0.114 0.010	67.5
	10 g/ml	0.054 0.022	85.6
	100 g/ml	0.009 0.001**	97.5

**: P < 0.05, **: P < 0.01, significantly different from control (n=3)*

Table 2. Mouse ear edema inhibition of the EtOH extracts of COR-285

	Dose/ear	Thickness	Increase	% inhibition
Control	-	0.23	0.02mm	-
Prednisolone	0.1mg	0.18	0.03mm*	22
	1.0mg	0.10	0.02mm*	57
YP-70% EtOH	0.2mg	0.18	0.02mm*	22
	1.0mg	0.15	0.01mm*	35
Citropten	0.2mg	0.21	0.01mm	-
	1.0mg	0.21	0.02mm	-

croton-oil induced ear edema *: P < 0.05, n = 10

Table 3. Inhibition of delayed hypersensitivity by the EtOH extracts of COR-285

	Dose/ear	Thickness	Increase	% inhibition
Control	-	0.13	0.01mm	-
Prednisolone	0.01mg	0.10	0.005mm**	23
	0.10mg	0.07	0.005mm**	46
YP-70%-EtOH	0.2mg	0.10	0.007mm*	23
	1.0mg	0.08	0.005mm*	38
Citropten	0.01mg	0.11	0.008mm	8
	0.05mg	0.11	0.007mm	8

** : $P < 0.01$, * : $P < 0.05$, n = 10

Figure 1. Free radical scavenging activity of chestnut extracts determined by DPPH free radical generating system.

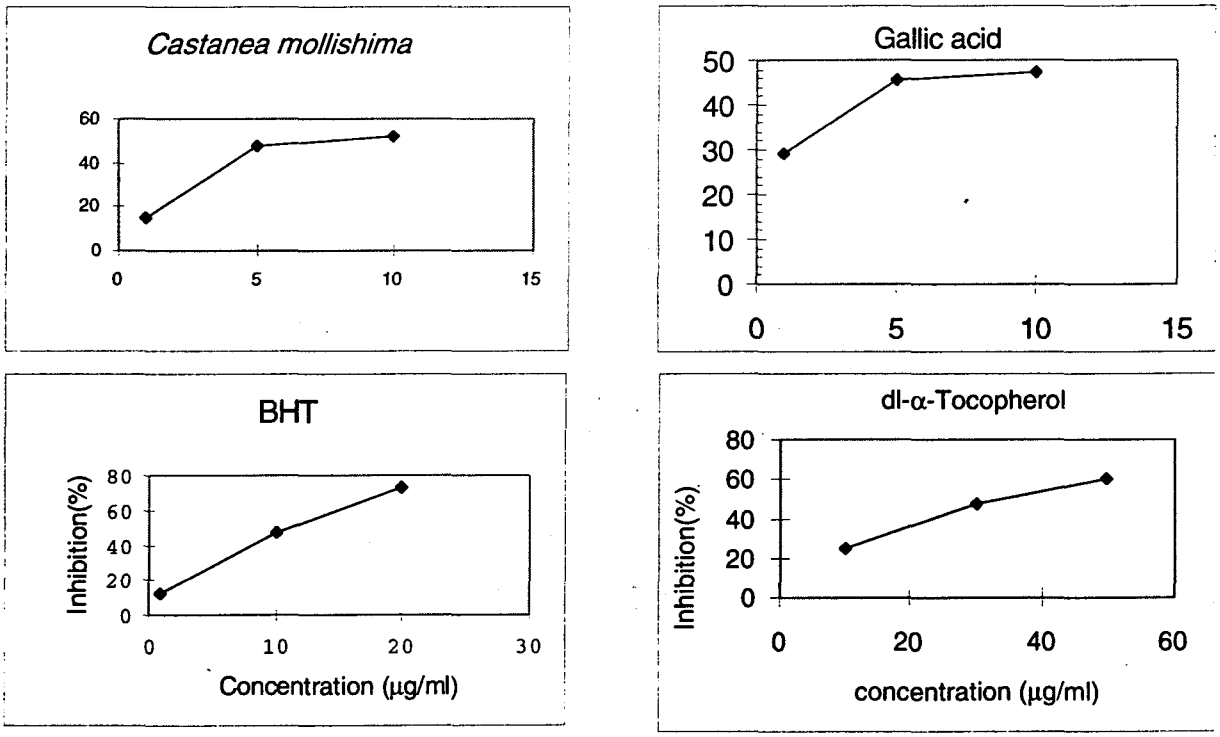

Figure 2. Skin moisturizing effect after twice-daily application of cream at 3% chestnut extracts (A) against placebo (B) for 6 weeks

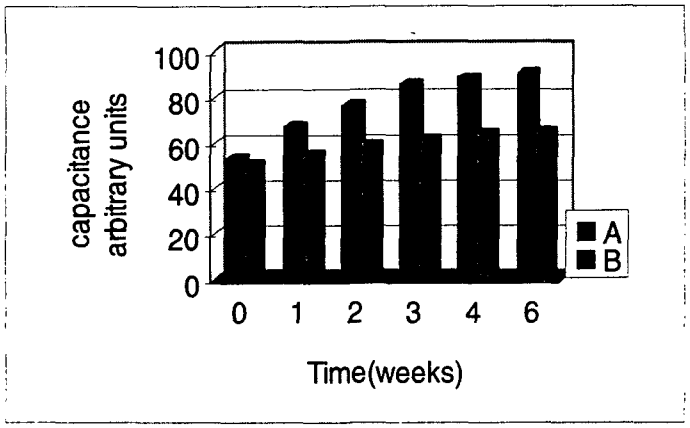

Figure 3. Skin elasticity effect after twice-daily application of cream at 3% chestnut extracts (A) against placebo (B) for 6 weeks

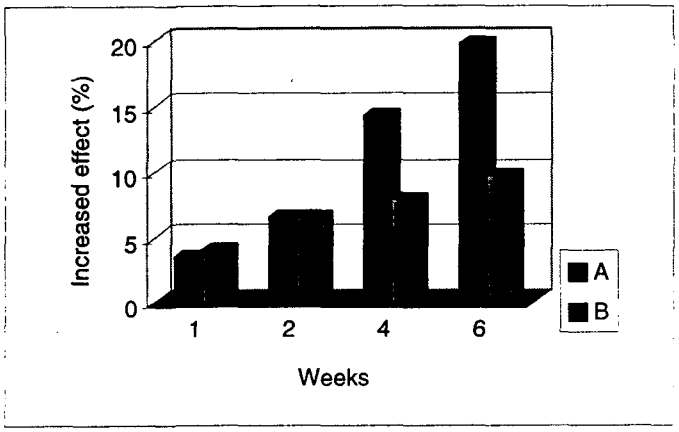

Figure 4. Skin anti-wrinkle effect after twice-daily application of cream at 3% chestnut extracts (A) against placebo (B) for 6 weeks