Encapsulated Curcumin Enhances Intestinal Absorption and Improves Hepatic Damage in Alcoholic Liver Disease-Induced Rats.

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ABSTRACT: Encapsulated curcumin (ENCC) was prepared from a commercial curcuminoids complex and was evaluated for its intestinal permeability and hepatoprotective effects. Intestinal permeability was evaluated using a Caco-2 intestinal cell monolayer system and the non-everted gut sac method. The hepatoprotective effect was evaluated in experimental rats administered alcohol for 4 weeks. The intestinal permeability results suggested that encapsulation is a useful method for enhancing adsorption of curcumin via the intestinal epithelium. ENCC administration resulted in the significant reduction of various serum indicators. Notably, most of the indicators elevated by ethanol decreased below normal levels when rats were administered a high dose of ENCC. Oral administration of ENCC also augmented the activity of glutathione peroxidase in the liver, and both normal curcumin and ENCC significantly alleviated high levels of malondialdehyde. Our results demonstrate a significant hepatoprotective effect of ENCC *in vivo* owing to its ability to improve bioavailability of curcumin.

Keywords: curcumin, encapsulation, intestinal permeability, bioavailability, hepatoprotective effect

INTRODUCTION

Curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione] is one of the principal curcuminoids that was initially isolated from the rhizomes of turmeric (*Curcuma longa* L.), and exists as a bright yellow phenolic compound (Nabavi et al., 2014). Curcumin has been extensively used for centuries as a spice, food preservative, and yellow colorant in the food, drugs, and cosmetic industries (Shishu and Maheshwari, 2010). Beside its culinary uses, curcumin has been considered a medicinal remedy in several countries for centuries, owing to its various physiological activities, such as antioxidant (Menon and Sudheer, 2007; Feng and Liu, 2009; Nabavi et al., 2011; Nabavi et al., 2012; Shakeri and Boskabady, 2017), anti-depressant (Kulkarni et al., 2008; Bhutani et al., 2009; Kulkarni et al., 2009), anti-inflammatory (Menon and Sudheer, 2007; Shakeri and Boskabady, 2017), antimicrobial (Mathew and Hsu, 2018; da Silva et al., 2018), anticancer (Bar-Sela et al., 2010; Wilken et al., 2011; Vallianou et al., 2015), and immunomodulatory properties (Gautam et al., 2007; Shakeri and Boskabady, 2017).

Despite its large potential for medicinal use, the clinical and industrial application of the curcumin is highly limited owing to several disadvantages, including its low water solubility, poor bioavailability, fast metabolization, and susceptibility to degradation in alkaline conditions and when exposed to light (Anand et al., 2007; Douglass and Clouatre, 2015). Such unstable characteristics and low bioavailability are due to natural curcumin being a tautomeric compound that exists in an enol form in organic solvents and in a keto form in water (Manolova et al., 2014; Nelson et al., 2017). To overcome these limitations, recent studies have focused on developing new methods to improve the stability and bioavailability of curcumin. A multitude of methods have been devised to enhance the pharmacokinetic and delivery profiles of three major curcuminoids (curcumin, demethoxycurcumin, and bisdemethoxycurcumin) (Douglass and Clouatre, 2015). These efforts can be separated into the following four strategies: glucuronidation/metabolism interference via adjuvants; liposomes, micelles, and phospholipid complexes; nanoparticles; and emulsifying or dispersing

agents (Anand et al., 2007; Douglass and Clouatre, 2015). Specifically, recent attempts have aimed encapsulate curcumin in nanoparticles, micelles, and hydrogels (Altunbas et al., 2011; Duan et al., 2015). Shaikh et al. (2009) recently reported that encapsulated curcumin improves oral bioavailability at least 9-fold compared with curcumin administered with piperine as an absorption enhancer, and Bisht et al. (2007) reported that polymeric nanoparticle-encapsulated curcumins, namely nanocurcumin, show potential for expanding the clinical repertoire of the efficacious agent by enabling ready aqueous dispersion. Araiza-Calahorra et al. (2018) reviewed and summarized recent advances in the application of encapsulation technology used for curcumin with regard to their dispersion and encapsulation efficiency. Despite the high potential of encapsulated curcumin (ENCC) to enhance curcumin bioavailability, few studies have evaluated the effect of ENCC for improving disease. Moreover, few studies have investigated the effect of curcumin on hepatic diseases; investigations in this field should be encouraged because liver disorders are one of the main causes of mortality worldwide (Rivera-Espinoza and Muriel, 2009).

We developed a new ENCC that should improve the stability, bioaccessibility, and bioavailability of commercial curcuminoid complexes. The purpose of this study was to investigate whether intestinal permeability is greater for ENCC than normal curcumin (NCC), and ENCC could improve liver function of rats induced with alcoholic liver disease.

MATERIALS AND METHODS

Reagents
The commercial curcuminoids complex "Curcumin C3 Complex", which comprises over 95% curcuminoids (curcumin 79.13%, demethoxycurcumin 18.33%, and 2.54% bisdemethoxycurcumin), was obtained from Neocrema Co., Ltd. (Seungnam, Korea) and used as the NCC sample. The ENCC suspension containing approximately 12% (w/v) total curcuminoids was prepared and supplied from Neocrema Co., Ltd.. To make NCC micelles, 120 mg of NCC was suspended in 1 mL water to make a dose of 120 mg/mL (12%, w/v). Because NCC had poor solubility in water (<0.1 mg/mL), 0.1% Tween 20 was added as a dispersion medium.

Quantification of curcumin
A spectrophotometric method was used to determine the concentration of curcumin in biological samples. In brief, various biological samples containing curcumin were directly measured by an UV spectrophotometer (422 nm) using a microplate reader (Tecan, Männedorf, Switzerland). To calculate curcumin concentrations, the coefficient equation ($y=1.113e^{-1}\chi$) reported by Shishu and Maheshwari (2010) was applied.

Cell line and culture
The human intestinal Caco-2 cell line (ATCC HTB-37[TM]) was obtained from American Type Culture Collection (Manassas, VA, USA). Cells were cultured in Dulbecco's modified Eagle's medium (Thermo Fisher Scientific, Waltham, MA, USA) containing 1% non-essential amino acids (Life Technology Co., Carlsbad, CA, USA), 100 U/mL penicillin/streptomycin (Thermo Fisher Scientific), and 10% heat-inactivated fetal bovine serum (Thermo Fisher Scientific). The cells were cultured in a 95% air, 5% CO_2 humidified atmosphere at 37°C.

Animals and experimental design
Six-week-old male Sprague-Dawley rats (SD rats, Orient Bio, Sungnam, Korea) were housed in standard cages under controlled conditions of temperature (22±2°C) and humidity (60±5%), and with a 12-h light/dark cycle. The rats were provided free access to water and commercial diets (Daehan Bio, Wonju, Korea). All rats were fasted the day before euthanasia. All animal studies were approved by the Korea University guidelines (KUIACUC-2017-135) for the ethical treatment of laboratory animals.

Assessment of *in vitro* absorption using Caco-2 cells
Transparent polyethylene terephthalate inserts (0.4 μm) and 24-well cell culture plates were obtained from BD Biosciences Co. (San Diego, CA, USA). For assessment of *in vitro* ENCC permeability, Caco-2 cell suspensions (300 μL; 1×10^6 cells/mL) was plated onto transwell inserts (in the apical compartment) to form a cell monolayer and 900 μL of the culture medium was subsequently added to the lower side (basolateral layer). The culture medium was changed every 2 to 3 days until the cells were fully differentiated, and the transepithelial electrical resistance (TEER) value was measured every day to confirm the formation of the *in vitro* intestinal monolayer. When the TEER reached 500 Ωcm^2, curcumin samples were added to the apical compartments, and the culture medium from both compartments were collected to determine curcumin concentrations at different time points (30, 60, and 90 min).

Assessment of *ex vivo* permeability using the non-everted sac method
The *ex vivo* permeability of curcumin samples was compared using a non-everted sac method (Shishu and Maheshwari, 2010). Prior to the test, NCC and ENCC were dispersed with micelles at a ratio of 1:6, and were prepared using the method reported by Suresh and Srinivasan (2007). SD rats were fasted for 12 h and euthanized, and the small intestinal tissue (from the duo-

denum to jejunum) was collected and washed inside and outside with phosphate buffered saline (PBS). Subsequently, the intestines were cut into sacs of 10 cm length, and stored in Krebs-Hanseleit buffer (pH 7.4) until the experiment. To assess *ex vivo* permeability, the sacs were filled with NCC or ENCC (1 mL of 120 mg/mL) and tied tightly with thread. Each sac was placed in a conical tube filled with buffer (20 mL), and were shaken in a 37°C water bath. The buffer solution inside and outside the sac was collected, and the curcumin concentration was analyzed using the spectrophotometric method described above.

Hepatoprotective effect of ENCC in alcoholic liver damage-induced rats

For *in vivo* experiments, SD rats (average weight 190 g) were randomly divided into six groups with seven rats per group (Table 1). To induce liver damage in rats, 800 μL of 30% ethanol was mixed with the curcumin sample solutions (NCC or ENCC). After acclimation for 1 week (when the rats were 7 weeks old), samples were mixed with ethanol and were orally administered to the rats for 4 weeks, at doses of 100 and 300 mg per kg body weight, respectively. PBS (800 μL) and 30% ethanol (800 μL) without sample solution were administered to the negative control group and liver damage-induced group, respectively. The rats were euthanized on day 29, and the major organs, such as the liver, kidney, heart, and spleen, were weighed. A blood sample was collected by venipuncture and the serum was extracted by centrifugation (3,000 g, 15 min, and 4°C). Serum markers for liver damage, such as the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), total cholesterol (TCHO), and triglyceride (TG) were then assessed using a biochemistry analyzer (Dri-Chem 3500i, Fujifilm, Tokyo, Japan). In addition, to confirm the antioxidant activity induced by the oral administration of the curcumin samples liver tissue was collected and washed with PBS. A 150 mg sample was then homogenized with 1.5 mL Tris-HCl buffer (pH 7.4), and the homogenates were centrifuged at 10,000 g for 15 min at 4°C to separate the clear supernatant containing antioxidant enzymes. The supernatant was then

aliquoted and stored at 80°C until the experiment. Glutathione peroxidase (GSH-px) and malondialdehyde (MDA) levels were measured according to the method reported by Paglia and Valentine (1967), and using a thiobarbituric acid reactive substances assay (Armstrong and Browne, 1994) with slight modifications, respectively.

Statistical analyses

Results of permeability tests are presented as the mean ±standard deviation (SD) of three independent experiments carried out in triplicate. Results of biological tests are presented as mean±standard error (SE) of three independent experiments carried out in triplicate. Statistical assessment was performed using the Statistical Package for Social Sciences version 12.0 (SPSS Inc., Chicago, IL, USA), and significant differences were evaluated by one-way analysis of variance and Duncan's multiple range tests. All differences were considered significant at $P<$ 0.05.

RESULTS

In vitro absorption test using intestinal Caco-2 cells

To compare the permeation and absorption of NCC and ENCC across the intestinal mucosal barrier, an *in vitro* intestinal epithelial model was established using the Caco-2 cell line. The Caco-2 monolayer system, which is recommended by the US Food and Drug Administration, is widely accepted as a suitable *in vitro* tool for predicting intestinal epithelial cell absorption, transport, and metabolism of various medical substances (Westerhout et al., 2014; Liu et al., 2016).

As shown in Fig. 1A, the curcumin concentration inside the transwell (apical side) decreased in a time-dependent manner for both the NCC and ENCC groups. When the Caco-2 monolayer was treated with NCC, the curcumin concentration decreased in a time-dependent manner from 30 min to 90 min. A similar trend was observed with ENCC treatment. There was no significant difference in the decrease in curcumin concentration between the NCC and ENCC groups.

The change in curcumin concentration outside of the

Table 1. Experimental design for the oral administration of normal and encapsulated curcumins in a rat model of chronic hepatic disease induced by ethanol

Group	Induction of alcoholic cirrhosis	Sample treatment
NOR	Saline with 1% Tween 20	—
CON	0.8 g of 30% EtOH with 1% Tween 20	—
NCC-L	0.8 g of 30% EtOH with 1% Tween 20	100 mg/kg of NCC
NCC-H	0.8 g of 30% EtOH with 1% Tween 20	300 mg/kg of NCC
ENCC-L	0.8 g of 30% EtOH with 1% Tween 20	100 mg/kg of ENCC
ENCC-H	0.8 g of 30% EtOH with 1% Tween 20	300 mg/kg of ENCC

NOR, normal control; CON, alcoholic cirrhosis control; NCC, normal curcumin; ENCC, encapsulated curcumin.
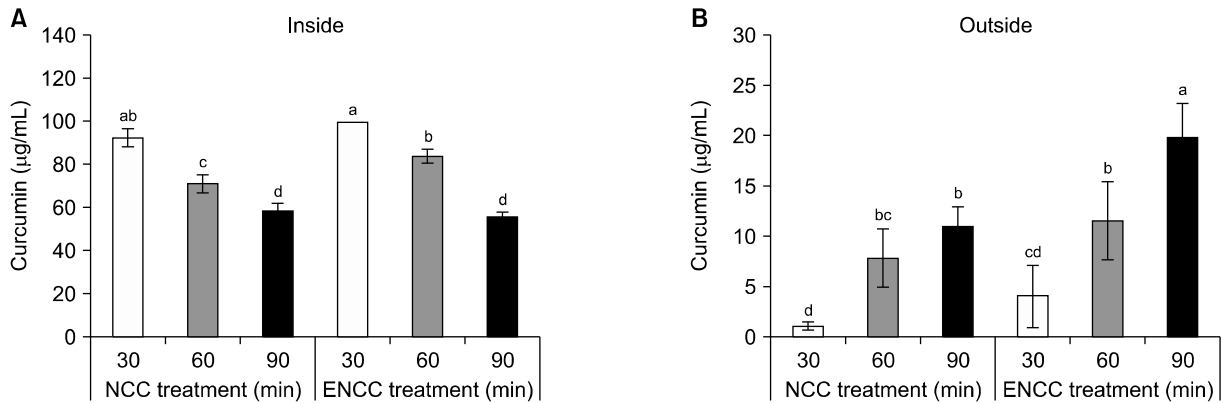
Fig. 1. *In vitro* permeability of normal and encapsulated curcumins in Caco-2 intestinal epithelial cells over time. Caco-2 cells were grown in transwell insert plates to form an intestinal monolayer. At 30, 60, and 90 min following treatment of the cell monolayer, the level of curcumin was determined both (A) inside cells (curcumin not absorbed through the monolayer) and (B) outside of the cells (curcumin through the monolayer).

transwell (basolateral side) is represented in Fig 1B. After NCC treatment, curcumin concentrations increased from 30 min to 60 min, whereas treatment with ENCC showed higher permeability. In particular, after 90 min of treatment, the curcumin concentration on the basolateral side was significantly higher for cells treated with ENCC higher compared with NCC. These results suggest that ENCC is more permeable than NCC through Caco-2 monolayers.

Ex vitro absorption using the non-everted intestinal sac method

The non-everted sac method used in the present study involves placing the solution in the rat gut sac without everting (Liu et al., 2016). After fixed time intervals (30, 60, and 90 min after treatment), intestinal absorption was determined by measuring the decrease of curcumin content within the gut and the increase of curcumin content outside the gut (Fig. 2A and 2B, respectively). The curcumin contents both inside and outside the intestinal sac did not differ between the NCC and ENCC groups within 30 min of treatment. However, at 60 min, the curcumin content outside the sac treated with ENCC were slightly increased compared with those treated with NCC, but the difference was not statistically significant (Fig. 2A). However, a significant difference was observed at 90 min after experiment. The curcumin content outside of ENCC-treated sacs were significantly increased approximately 4-fold compared with those of the NCC group (Fig. 2B), and the curcumin content within sacs treated with ENCC were significantly decreased compared with those in the NCC group (Fig. 2C). Of particular note, ENCC treatment enhanced absorption into intestinal tissue in a time-dependent manner, whereas the effects of NCC treatment did not show significantly differ over time.
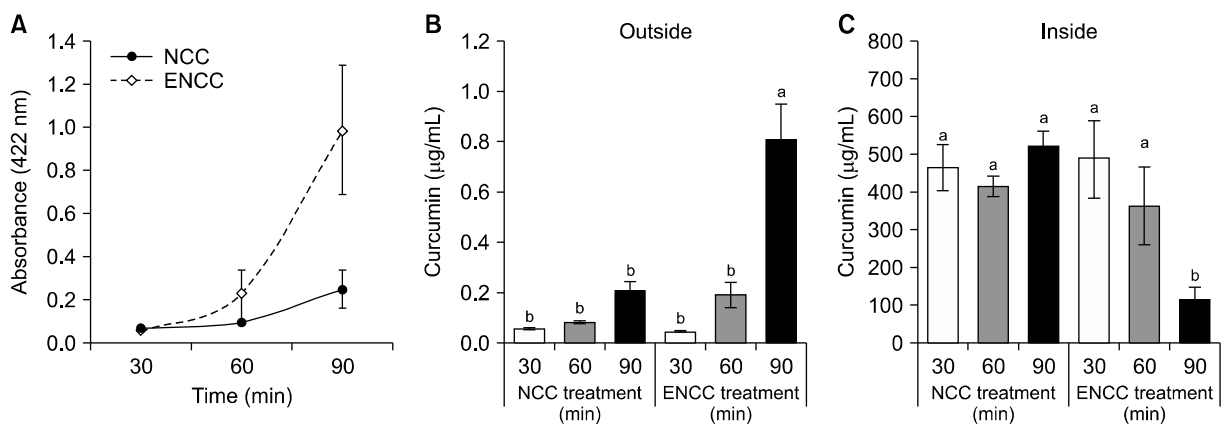

Fig. 2. Comparison of absorption rate between normal and encapsulated curcumins using the non-everted intestinal sac method. A 10-cm intestinal sac was randomly prepared from SD rats, and normal and encapsulated curcumins were injected inside of the sac. At 30, 60, and 90 min following sample injection inside the sac, the level of curcumin was determined both inside (curcumin not-absorbed through the intestinal sac) and outside of the sac (curcumin absorbed through the intestinal sac). (A) Change of absorbance measured at 422 nm outside of the sac over time. (B) Curcumin concentration calculated from absorbance. (C) Change of curcumin concentration inside of the sac over time.

Changes of organ weight induced by constant alcohol consumption

Low and high doses of both curcumin samples were orally administered to the rats for 4 weeks together with constant alcohol intake (Table 1). After euthanization, the major organs, such as the heart, liver, spleen, and kidney, were immediately weighed to confirm the influence of excessive alcohol adaptation. As shown in Table 2, there was no significant difference between the normal (NOR) and alcohol-administered control (CON) groups with respect to liver and kidney weight. However, the heart and spleen weights of the CON group showed a significant difference compared with those of the NOR group. There was no significant difference in the weights of any organs from rats in the NCC- or ENCC-treated groups administered alcohol and those in the NOR group. This result indicates that both NCC and ENCC do not induce organ toxicity in rats and are likely to inhibit alcohol-induced heart and kidney damage.

Effect of curcumin samples on liver transaminase, LDH, TCHO, and TG contents in alcoholic liver damage-induced rats

The effects of curcumin samples on hepatic function was investigated using a liver disease-induced rat model by continuously administering ethanol. The results of bio-

chemical indicators of liver function, such as AST, ALT, LDH, TCHO, and TG, are summarized in Table 3. Constant ethanol administration resulted in severe hepatotoxicity in the rats, as evidenced by significant elevations of serum indicators (Table 3). Co-administration of ethanol with either NCC or ENCC significantly suppressed these indicators, suggesting that both NCC and ENCC can improve the liver damage induced by ethanol consumption. Although a dose-dependent change was rarely observed in serum ALT and AST levels of rats in the group administered NCC, the rats administered ENCC and ethanol showed significant and dose-dependent reductions in serum indicators. Specifically, levels of ALT, LDH, and TCHO in mice administered a high dose of ENCC were significantly decreased compared with those in other groups, including the normal control. This result suggests that oral administration of ENCC may be more effective than NCC for alleviating liver damage in experimental rats, which is likely due to an enhanced rate of absorption via the encapsulation process.

Effect of curcumin samples on antioxidant activity in alcoholic liver damage-induced rats

The effects of curcumin on GSH-px, an antioxidant enzyme, and MDA, a marker for oxidative stress, in the liver tissue were evaluated. As shown in Fig. 3A, the concentration of hepatic GSH-px in livers of the CON group was significantly lower than that of livers from the NOR group, indicating that the activity of GSH-px was significantly reduced by alcohol administration. GSH-px activity was slightly increased in both NCC-treated groups (NCC-L and NCC-H); however, it was not significantly different compared with rats from the CON group. Oral administration of ENCC with alcohol treatment augmented the activity of GSH-px in a dose-dependent manner. Specifically, administeration of high dose ENCC (ENCC-H) significantly increased the GSH-px activity degraded by continuous alcohol administration. As shown in Fig. 3B, the level of MDA in liver tissue was significantly increased in the CON group compared with those from rats in the NOR group, indicating that liver damage

Table 2. Effects of the oral administration of normal and encapsulated curcumins in a rat model of chronic hepatic disease induced by ethanol

Group[1]	Heart	Liver	Spleen	Kidney
NOR	0.37 ± 0.01^b	4.30 ± 0.05^{ns}	0.21 ± 0.02^a	0.80 ± 0.04^{ns}
CON	0.44 ± 0.01^a	4.13 ± 0.10	0.19 ± 0.01^{ab}	0.76 ± 0.01
NCC-L	0.39 ± 0.01^b	4.25 ± 0.06	0.18 ± 0.00^b	0.79 ± 0.01
NCC-H	0.39 ± 0.00^b	4.19 ± 0.09	0.18 ± 0.01^{ab}	0.78 ± 0.03
ENCC-L	0.39 ± 0.01^b	4.15 ± 0.10	0.18 ± 0.01^{ab}	0.81 ± 0.00
ENCC-H	0.39 ± 0.01^b	4.38 ± 0.08	0.19 ± 0.00^{ab}	0.79 ± 0.02

[1]Group names refer to those described in Table 1.
Different letters (a,b) in a column indicate a statistically significant difference among groups ($P<0.05$).
[ns]Not significant.

Table 3. Biochemistry analysis in serum isolated from liver disease-induced experimental rats

Group[1]	ALT (U/L)	AST (U/L)	LDH (U/L)	TCHO (mg/dL)	TG (mg/dL)
NOR	30.25 ± 0.48^{cd}	44.50 ± 0.65^d	212.25 ± 6.92^b	86.75 ± 6.80^c	118.00 ± 6.22^d
CON	43.25 ± 0.63^a	62.00 ± 0.41^a	315.00 ± 12.48^a	245.75 ± 15.20^a	219.25 ± 3.57^a
NCC-L	35.75 ± 1.38^b	55.75 ± 0.25^b	242.75 ± 15.77^b	173.50 ± 16.54^b	206.75 ± 7.10^b
NCC-H	34.75 ± 0.63^{bc}	53.75 ± 0.25^b	162.25 ± 11.48^c	100.00 ± 2.16^c	148.25 ± 14.56^c
ENCC-L	32.00 ± 2.86^{bc}	54.75 ± 1.03^b	225.25 ± 15.52^b	90.00 ± 4.92^c	187.25 ± 8.92^b
ENCC-H	25.50 ± 2.25^c	48.50 ± 1.26^c	115.50 ± 6.08^d	83.75 ± 1.11^c	126.50 ± 8.65^d

[1]Group names refer to those described in Table 1.
Different letters (a–d) in a column indicate a statistically significant difference among groups ($P<0.05$).
ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; TCHO, total cholesterol; TG, triglyceride.
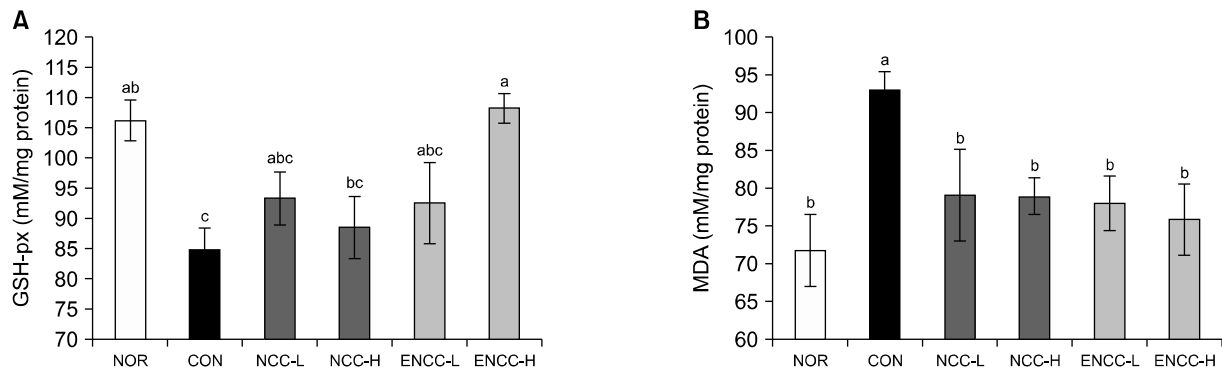
Fig. 3. Effect of oral administration of normal and encapsulated curcumin on levels of (A) malondialdehyde (MDA) and (B) glutathione peroxidase (GSH-px) in liver tissue. Liver tissue was homogenized with Tris-HCl buffer, and MDA and GSH-px in the homogenates were analyzed. Group names refer to Table 1.

was induced by alcohol administration. Interestingly, both NCC and ENCC significantly alleviated the enhanced levels of hepatic MDA produced by alcohol administration at both doses with no significant differences among the four groups. Nevertheless, the inhibitory effect of MDA peaked in the ENCC-H group (75.8 mM per mg protein; 18.4% inhibition compared with the CON group).

DISCUSSION

To overcome the limitations of the industrial application of curcumin, food ingredient formulators have started employing a variety of approaches to enhance the absorption, bioactivity, and bioavailability of curcumin (Douglass and Clouatre, 2015). Among these methods, microencapsulation is a process by which tiny particles or droplets are surrounded by a coating to obtain small capsules with many useful properties. However, few studies have investigated the encapsulation of curcumin. Therefore, we compared a novel ENCC formulation with NCC for intestinal permeability and potential for improving hepatic function. The *in vitro* intestinal permeability test using a Caco-2 monolayer system showed no difference in curcumin concentration on the apical side of the transwell between the NCC and ENCC treatment groups; however, the curcumin concentration toward the basolatoral side significantly increased in the ENCC group compared with the NCC group (Fig. 2). These results indicated that normal curcumin is likely to degrade when it is transported through the intestinal monolayer, whereas ENCC is chemically more stable and shows enhanced permeability compared with NCC.

In addition, the *ex vivo* permeability test using a non-everted sac method was further conducted to predict *in vivo* absorption of the curcumin in humans. There are two types of intestinal sac methods; an everted gut sac method (Wilson and Wiseman, 1954) and non-everted gut sac

method (Tariq et al., 2015). After testing both methods in a preliminary study (data not shown), we decided to adopt the non-everted sac method for assessing the permeability potential of the curcumin samples, since the everted intestinal sac model has several disadvantages, including morphological damage to the intestinal tissue while everting. Dixit et al. (2012) reported the several advantages of a non-everted sac method to recommend it as the preferred method for permeability studies: greater simplicity, lower sample volume requirements, and amenable toward successive collection of serosal samples with less intestinal morphological damage as a result of the absence of eversion. Through the *ex vivo* permeability test using a non-everted sac method, we also confirmed that ENCC inside the gut could be more effectively transported outside of the gut compared with NCC. Taken together, both the *in vitro* and *ex vivo* absorption tests demonstrated that encapsulation is a useful method for curcumin to be more effectively absorbed via the intestinal epithelium, demonstrating its potential to enhance curcumin bioavailability for to benefit human health.

Although curcumin is known to suppress glutathione degradation, as observed frequently in both alcoholic liver disease and in carbon tetrachloride (CCl_4)-induced injury, there are few reports on the protective effect of processed curcumin for enhancing bioavailability in the context of liver disease. Based on our *in vitro* and *ex vivo* absorption tests, we further evaluated the possibility of oral administration of ENCC compared with NCC for enhancing hepatoprotective activity in liver-damaged experimental rats. Liver function tests are well-known groups of serum tests that provide information about liver damage. Among the various serum tests available, transaminases (ALT and AST) are particularly useful for diagnosing liver injury with some degree of intact liver function (Johnston, 1999). LDH is present in many kinds of organs and tissues throughout the body, including the liver, heart, pancreas, kidneys, skeletal muscles, lymph tissue, and blood cells, but is released into the bloodstream

upon injury to specific organs and tissues, causing a rise in blood LDH levels (You et al., 2010). In addition, serum levels of TCHO and TG were analyzed to confirm the development of a fatty liver caused by ethanol consumption. Given that the levels of serum transaminases and LDH were significantly more suppressed in the group administered ENCC compared with NCC, we could conclude that the increase of curcumin absorption by encapsulation might help to reslove liver damage triggered by alcohol consumption. In addition, the changes of TCHO and TG levels suggested that ENCC is more effective for resolving alcoholic fatty liver compared with NCC.

Numerous *in vitro* studies have indicated that curcumin exerts potent antioxidant and anti-inflammatory properties, which may account for its protective effect in chronic liver diseases (Vera-Ramirez et al., 2013). Therefore, to investigate the possibility of the curcumin-mediated enhancement of antioxidant activity in the hepatic tissue, liver tissue was collected, homogenized, and centrifuged after rats were treated with ethanol for 4 weeks. Only rats treated with high dose ENCC could recover hepatic GSH-px activity, which protects the liver against oxidative damage. Consistent with our results, Bisht et al. (2011) reported that a novel formulation of curcumin (NanoCurc^{TM}) decreased mRNA levels of inflammatory cytokines and enhanced the antioxidant capacity in the mouse liver tissue damaged by intraperitoneal injection of CCl$_4$. Importantly, the encapsulation technique is considered an invaluable tool in the cosmetic and/or pharmaceutical industries, and provides great flexibility in the choice of delivery mechanisms and excipients that can be used. One of the great advantages of the encapsulation process is that it allows for convenient and low-cost production of a range of solid lipid nanoparticles that offer flexibility in formulation, resulting in enhanced bioavailability.

Despite numerous studies on the physiological activity of curcumin, this is the first to investigate the *in vivo* hepatoprotective effect of ENCC conferred by improving bioavailability. However, further studies on the dose-activity relationship, pharmacokinetic properties, and molecular mechanism of our ENCC formulation are needed to explore potential clinical application.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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