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# Anti-Oxidant Effects of Highly Bioavailable Curcumin Powder in High-Fat Diet Fed- and Streptozotocin-Induced Type 2 Diabetic Rats

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## Abstract

Curcumin is a hydrophobic polyphenol extracted from turmeric that exhibits a variety of biological functions has albeit with limited efficacy as a functional food material owing to its low absorption when administered orally. The newly developed curcumin powder formulation exhibits improved absorption rate *in vivo*. This study evaluates the anti-oxidant effects of Theracurmin® (TC), which is highly bio-available in curcumin powder. The antioxidant activity of TC was investigated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity, ferrous reducing antioxidant power (FRAP) assays, NO radical, superoxide radical, $H_2O_2$ scavenging activity, and total antioxidant capacity (TAC). Additionally, we evaluated the antioxidant activity of TC in high-fat diet (HFD)-fed streptozotocin (STZ)-induced Type 2 diabetic rats. As a result of oral administration of TC for 13 weeks in type 2 diabetic rats, the group administration of 2,000 mg/kg significantly increased FRAP, superoxide dismutase (SOD), and reduced the level of glutathione (GSH) in liver tissue 1.9, 1.2, and 1.2-times, respectively. Furthermore, serum TAC levels increased by 1.3-fold after the rats were administered with a dose of 500 mg/kg. These results were consistent with the *in vitro* assay results. In conclusion, TC exhibited its potential as a functional food material through its antioxidant properties.

Key words: curcumin, theracurmin, antioxidant activity, high-fat diet, streptozotocin

## Introduction

Diabetes mellitus (DM) is a major health problem worldwide. Type 2 diabetes mellitus (T2DM), which accounts for 90% of all diabetes, is characterized by increased blood glucose levels owing to a progressive decline in insulin action (insulin resistance) and pancreatic β-cell dysfunction (Srinivasan et al. 2005). Oxidative stress causes insulin resistance, β-cell dysfunction, and ultimately T2DM (Wright et al. 2006). Oxidative stress causes defective angiogenesis in response to ischemia, activates a number of proinflammatory pathways, and causes long-lasting epigenetic changes that drive persistent expression of proinflammatory genes even after glycemia is normalized ("hyperglycemic memory") (Giacco & Brownlee 2010). Based on this report, therapies to reduce oxidative stress may be helpful in patients with T2DM and those at risk of developing diabetes.

Curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione), is one of the representative constituents of turmeric, a traditional Indian spice, and is a hydrophobic polyphenol that imparts the characteristic yellow color to turmeric. The biological activities, such as radical scavenging, anti-inflammation, anti-tumor, anti-allergic and neuroprotective effects, of curcumin have been investigated extensively (Kuttan et al. 1987; Antony et al. 1999; Ram et al. 2003; Matsuda et al. 2004; Dairam et al. 2007). Due to the diverse physiological activities of curcumin, curcumin has been used as a functional food material.

Despite the biological activities of curcumin, the effect of curcumin as a functional food has not been fully understood, mostly

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because of curcumin's low bioavailability. In fact, 75% of ingested curcumin is excreted in the feces and the concentration of curcumin in the blood is very low, suggesting that curcumin has a low intestinal absorption rate (Wahlstrom & Blennow 1978; Park et al. 2015). In order to overcome this problem, a powdered formulation of curcumin, TC has been developed, which is increased in bio-absorption up to 28 times more than unmodified curcumin, as shown in tests involving humans (Sasaki et al. 2011; Park et al. 2015).

In this study, we evaluated the antioxidant activity of TC, which is curcumin material in powdered form, in high-fat diet (HFD)-fed STZ-induced Type 2 diabetic rat. Because, oxidative stress is increased in Type 2 diabetic animal model.

## Materials and Methods

### 1. Materials and reagents

High-fat diets (60% fat) were purchased from Research Diets Inc. (New Brunswick, NJ, USA). The ingredient composition of the HFD was as follows (%): Casein, 19.7; L-Cystine, 0.3; Maltodextrin 10, 12.3; Sucrose, 6.8; Soybean Oil, 5.5; Lard, 54.4; and Vitamin Mix V10001, 1. The energy supply of the HFD was as follows (%): fat, 60; carbohydrates, 19; protein, 20 (Table 1). Streptozotocin (STZ) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Commercial assay kits for estimating FRAP, malondialdehyde (MDA) content, SOD content, TAC, and hydrogen peroxide ($H_2O_2$) content were purchased from Cell biolabs. Inc. (San Diego, CA, USA). Catalase and glutathione peroxidase (GPx) were obtained from Cayman Chemical (Ann Arbor, MI, USA). Reduced GSH was purchased from Enzo Life Sciences, Inc. (Farmingdale, NY, USA). All other chemicals and reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless stated otherwise.

### 2. Preparation of curcumin

The test material TC (kindly provided by Theravalues, Tokyo, Japan) had a curcumin content of 300 mg/g and was prepared by mixing gum ghatti, maltose, citric acid, and dextrin. Turmeric raw material (*Curcuma longa* L.) was cut and flaked, extracted with hexane and acetone, filtered and concentrated, and the turmeric oleoresin curcumin obtained after drying. Gum ghatti, maltose, citric acid, and dextrin were dissolved in water, and the primary obtained turmeric oleoresin curcumin was added mixed, and ground. The mixture was filtered, dried through a spray dryer, and final powdered.

### 3. Measurement of antioxidant effect by *in vitro* assays

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of TC was determined by the method of Kim et al. (2016) with some modifications. FRAP of TC was measured using com-

**Table 1. Composition of feed**

| Ingredients \ Group | Rodent diet with 60 % kcal fat | | Rodent diet with 10 % kcal fat | |
|---|---|---|---|---|
| | Gm | Kcal | Gm | Kcal |
| Casein, 80 mesh | 200 | 800 | 200 | 800 |
| L-Cystine | 3 | 12 | 3 | 12 |
| Corn starch | 0 | 0 | 315 | 1,260 |
| Maltodextrin 10 | 125 | 500 | 35 | 140 |
| Sucrose | 68.8 | 275.2 | 350 | 1,400 |
| Cellulose, BW200 | 50 | 0 | 50 | 0 |
| Soybean oil | 25 | 225 | 25 | 225 |
| Lard | 245 | 2,205 | 20 | 180 |
| Mineral mix S10026 | 10 | 0 | 10 | 0 |
| DiCalcium phosphate | 13 | 0 | 3 | 0 |
| Calcium carbonate | 5.5 | 0 | 5.5 | 0 |
| Potassium citrate, 1 $H_2O$ | 16.5 | 0 | 16.5 | 0 |
| Vitamin mix V10001 | 10 | 40 | 10 | 40 |
| Choline bitartrate | 2 | 0 | 2 | 0 |
| Total | 773.8 | 4,057 | 1,055 | 4,057 |

mercial kit according to the recommended protocol. The NO and superoxide radical scavenging activity of TC were measured by Marcocci et al. (1994) and Fontana et al. (2001), respectively. $H_2O_2$ scavenging activity and TAC of TC were measured using commercial kit according to the recommended protocol, respectively. Ascorbic acid and Trolox were used as reference drugs.

### 4. Animals

Male SD rats (150~180 g, 6 weeks old) were supplied from Orient Bio (Gyeonggi-do, Korea). All mice were housed in polycarbonate cages at 23±3℃ and 55±15% humidity, and fed standard laboratory chow and water *ad libitum*. Ten rats per group were used throughout the experiments after an initial acclimation period of at least 1 week. All animal experiments were approved by The Committee for the Care and Use of Laboratory Animals in GyeongGi Bio Center and performed in accordance with The GyeongGi Bio Center Guidelines for Laboratory Animals Care and Usage (IRB Number 2017-03-0001).

### 5. Preparation of experimental type 2 diabetic rats with increased oxidative stress

T2DM was induced by HFD and low-dose of STZ treatment as described previously (Srinivasan et al. 2005). Briefly, the rats were fed with HFD *ad libitum* for a period of 2 weeks and then injected with low dose of STZ (single dose of 35 mg/kg, i.p.). Seven days after STZ injection, the fasting blood glucose levels were estimated; those rats having blood glucose levels > 250 mg/dL were considered diabetic and were selected for further experiments. These rats were then continued on HFD until the end of the study. To measure the anti-oxidant effect of TC, type 2 diabetic rats were randomly divided into five groups: untreated control, T2DM control, and 500, 1,000, or 2,000 mg/kg TC treated groups. TC was administered orally once a day for a period of 13 weeks. After 13 weeks of treatment, the diets were removed from the cages 12 h before the animals were anaesthetized for the collection of serum or plasma samples. Rats were euthanized and liver tissues ($n$ = 10) were dissected out, cleaned, and washed in ice-cold phosphate buffer saline for analysis.

### 6. Measurement of antioxidant effects

FRAP, SOD, catalase and Reduced GSH were measured in frozen liver tissues using commercial kits for each according to the recommended protocol. TAC was measured in serum using a commercially available kit and the recommended protocols.

### 7. Statistical analysis

Data are indicated as mean±S.E. Statistical analyses of the data were performed using SPSS ver. 22 for medical science. Student's $t$-test was used for the parametric comparisons. $p<0.05$ was considered to be statistically significant.

## Results and Discussion

Oxidative stress causes the activation of 5 major pathways: polyol pathway flux, formation of advanced glycation end products (AGEs), expression of the receptor for AGEs and its activating ligands, activation of protein kinase C isoforms, and hexosamine pathway (Giacco & Brownlee 2010). These mechanisms caused by oxidative stress are closely related to pathogenesis and complications of T2DM (Brownlee M 2001).

TC exhibited significant free radical scavenging activity of DPPH, NO and $H_2O_2$, *in vitro* (Fig. 1A, C and E). IC50 values

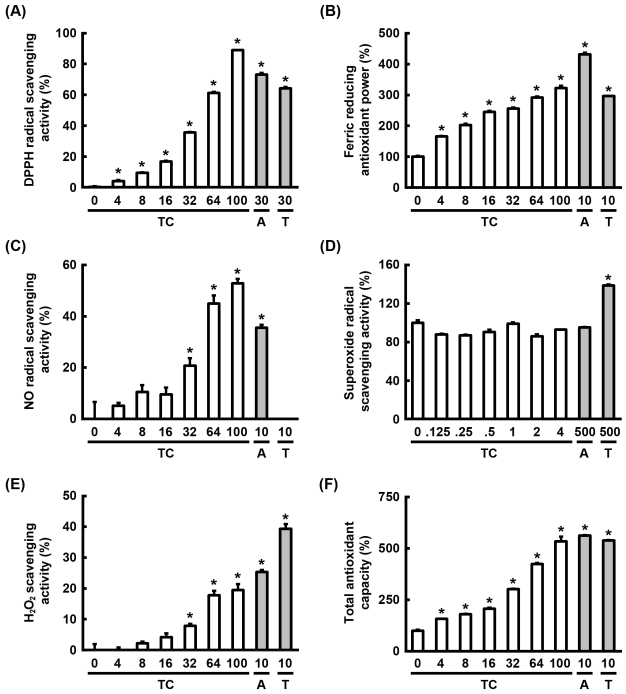


**Fig. 1. *In vitro* antioxidant effects of Theracurmin® (TC).** DPPH radical scavenging activity (A), FRAP (B), NO radical scavenging activity (C), Superoxide radical scavenging activity (D), $H_2O_2$ radical scavenging activity (E), and TAC (F). A and T mean ascorbic acid and Trolox, respectively. The concentration of TC is μg/mL, and the concentrations of A and T are μM. * $p<0.05$ and ** $p<0.01$ versus not treated with TC. Data were expressed as mean±S.E. The results were analyzed by Student's $t$-test.

for DPPH, NO and $H_2O_2$ assay were calculated (Probit) to be 39.9, 96.9 and 432.3 μg/mL, respectively. TC, however, did not show superoxide radical scavenging activity. Ascorbic acid used as a control drug was also not effective at 500 μM. In addition, TC increased FRAP and TAC concentration-dependently (Fig. 1B and F). This *in vitro* assay showed that TC exhibited an antioxidant effect by not only free radical scavenging activity but also increasing FRAP and TAC.

Based on the *in vitro* assay results, the antioxidant effect of TC was confirmed by *in vivo* assay using experimental type 2 diabetic rats. The ratios of the rats relative to the liver weights are presented in the Table 2. Blood glucose levels were checked once a week during TC administration period. The T2DM induced group showed a significant increase in blood glucose levels compared with that in the control group from day 1 to week 13 ($p$<0.01). TC 1,000 mg/kg treated group showed significantly decreased blood glucose levels at 8 weeks, but no significant difference at 13 weeks (Table 2). Most of the antioxidant experiments with HFD Fed- and STZ-induced type 2 diabetic rats are performed within 8 weeks. In this study, high fat diet was administered for 13 weeks. Administration of long-term high-fat diets continued to increase oxidative stress. This is the reason why the blood glucose lowering effect of TC was reduced at 13 weeks compared to 8 weeks. Since most antioxidant studies are conducted within 8 weeks, TC may be transient, but exhibits a hypoglycemic effect.

Antioxidant enzymes such as SOD inhibit lipid peroxidation, inhibit the inactivation of sulfhydryl-containing enzymes, and inactivate or remove ROS causing cross-linking of essential proteins. These actions of antioxidants work as a defense mechanism against ROS and play an important role in preventing and alleviating diabetic complications (Cha et al. 2008; Jang et al. 2014). Therefore, the antioxidant effects including SOD activity of TC were measured *in vivo*. FRAP, SOD activity, catalase activity and reduced GSH were significantly decreased in the liver of HFD Fed- and STZ-induced type 2 diabetic rats. TC significantly increased these, but catalase activity did not show any significant difference. In addition, oral administration of TC 500 mg/kg significantly increased TAC in serum of type 2 diabetic rats (Table 2). This *in vivo* assay showed that oral administration of TC exhibited antioxidant effects by increasing reduced FRAP, SOD, Reduced GSH and TAC owing to the induction of T2DM in liver and serum. The *in vivo* antioxidant effect of TC is also expected to be better if measured within 8 weeks of the usual antioxidant assay periods. The results confirmed on week 13 demonstrate the powerful antioxidant effect of TC.

To summarize, oxidative stress causes the pathogenesis and complications of T2DM, and TC can be developed as a functional food material for prevention of T2DM and diseases related to oxidative stress because it can prevent oxidative stress.

## Conclusion

TC showed an antioxidant effect *in vitro* by not only free radical scavenging activity but also increasing FRAP and TAC. In addition, TC increases antioxidant activity and antioxidant enzyme activity by increasing FRAP, SOD, Reduced GSH, and TAC in HFD fed- and STZ-induced type 2 diabetic rat, a power-

**Table 2. Effect of Theracurmin® (TC) on blood glucose levels and antioxidant enzyme activities in liver tissue and serum in normal and T2DM rats**

| | | NOR | CON | T5 | T10 | T20 |
|---|---|---|---|---|---|---|
| Blood glucose (mg/dL) | 8 week | 97.8±3.2 | 493.3±40.4$^{\#}$ | 412.3±33.0 | 374.2±28.1$^{*}$ | 446.6±38.2 |
| | 13 week | 96.5±2.5 | 455.7±47.1$^{\#}$ | 407.7±36.1 | 403.6±20.9 | 451.1±42.5 |
| Relative liver weight (w/w) | 13 week | 0.028 | 0.031$^{\#\#}$ | 0.027$^{*}$ | 0.029$^{*}$ | 0.030 |
| Liver FRAP (μM) | 13 week | 49.5±9.0 | 27.5±2.0$^{\#}$ | 36.8±2.6$^{*}$ | 39.2±3.3$^{**}$ | 53.0±2.5$^{**}$ |
| Liver SOD activity (%) | 13 week | 35.6±2.0 | 23.9±0.9$^{\#\#}$ | 28.2±1.0$^{**}$ | 27.7±0.6$^{**}$ | 27.9±0.8$^{**}$ |
| Liver catalase activity (nM/min/mL) | 13 week | 23.5±1.8 | 19.1±0.7$^{\#}$ | 21.8±1.2 | 20.9±2.0 | 18.4±1.5 |
| Liver reduced GSH (pM) | 13 week | 497.1±4.4 | 277.0±11.5$^{\#\#}$ | 377.8±8.2$^{**}$ | 272.3±13.9 | 321.2±12.2$^{*}$ |
| Serum TAC (μM) | 13 week | 201.2±20.9 | 153.2±6.0$^{\#}$ | 195.3±12.4$^{**}$ | 158.2±6.2 | 165.2±10.7 |

$^{\#}$ $p$<0.05 and $^{\#\#}$ $p$<0.01 versus control group.
$^{*}$ $p$<0.05 and $^{**}$ $p$<0.01 versus T2DM induced group.
Data were expressed as mean±S.E. The results were analyzed by Student's $t$-test.

ful oxidative stress model. Because of these antioxidant effects, it is possible that TC can be a functional food material, which prevent some of the complications associated with T2DM and diseases related to oxidative stress.

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