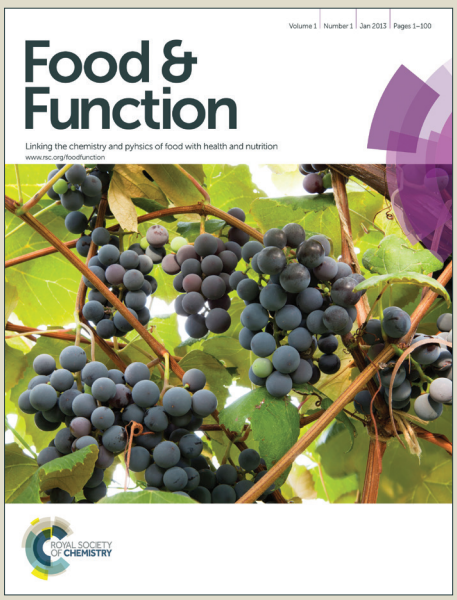
Food & Function

Accepted Manuscript



This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/foodfunction

Potential Protective Effect of Highly Bioavailable Curcumin on Oxidative stress Model induced by Microinjection of Sodium Nitroprusside in Mice Brain

Qand Agha Nazari [a], Yuki Takada-Takatori [b], Tadashi Hashimoto [c], Atsushi Imaizumi [c], Yasuhiko Izumi [a], Akinori Akaike [a, d] and Toshiaki Kume [a, *]

[a]Department of Pharmacology, Graduate School of Pharmaceutical Sciences, Kyoto University, 46-29 Shimoadachi-cho, Sakyo-Ku, Kyoto 606-8501, Japan
[b]Department of Pharmacology, Faculty of Pharmaceutical Sciences, Doshisha Women's College, Kyoto 610-0395, Japan
[c]Theravalues Corporation, 1st floor, Kioicho Building 3-12 Kioicho, Chiyoda-Ku, Tokyo 102-0094, Japan.
[d]Graduate School of Pharmaceutical Sciences, Nagoya University, Chikusa-Ku, Nagoya 464-8601, Japan

Address correspondence to Toshiaki Kume, PhD,
Tel: 81-75-753-4576;
Fax: 81-75-753-4579;
E-mail: tkume@pharm.kyoto-u.ac.jp.

Abstrast

Curcumin, a polyphenolic compound has several pharmacological activities such as anti cancer, anti-inflammatory and antioxidant. However, curcumin shows poor oral bioavailability. The purpose of this study was to investigate protective effects of highly bioavailable curcumin, THERACURMIN and curcumin, against sodium nitroprusside (SNP) -induced oxidative damage in mice brain. Intrastriatal microinjection of THERACURMIN or curcumin with SNP significantly protected SNP-induced brain damage and motor dysfunction. Oral administration of THERACURMIN (1 and 3 g/kg, containing 100 and 300 mg/kg curcumin, respectively) significantly protected SNP-induced brain damage and motor dysfunction. However, oral administration of 300 mg/kg curcumin did not protect motor dysfunction induced by SNP. These results suggest that curcumin and THERACURMIN have protective effects against SNP-induced oxidative damage. Moreover, oral administration of THERACURMIN, potently protected brain damage, suggesting higher bioavailability of THERACURMIN following oral administration.

Keywords curcumin, THERACURMIN, sodium nitroprusside, brain damage, motor dysfunction.

Introduction

Oxidative stress has been implicated in the progression of neurodegenerative disorders, including Parkinson's disease, Alzheimer's disease and Huntington's disease, as well as stroke and trauma [1-6]. Oxidative stress is caused in situation where there the balance between production of reactive oxygen species (ROS) and the level of antioxidants is largely disturbed and results in the damage to the cells by excessive ROS production. The brain and nervous system are more vulnerable to oxidative stress than other tissues, due to high consumption of oxygen and the consequent generation of large amounts of ROS and limited antioxidant capacity [7, 8]. However, cells normally employ a number of defense mechanisms against ROS such as antioxidant enzymes, vitamin E, vitamin C and glutathione. When the antioxidant defense is not enough, the lipids, proteins and DNA undergo damage and subsequently cell death occurs [9]. Since many degenerative disorders are closely related to free radical overloading and intracellular oxidative stress, antioxidant supplements can reduce ROS in cells and are consequently useful for treatment of neurodegenerative diseases [10]. Thus, natural antioxidants may serve as useful protective agents against oxidative stress associated with neurologic disorders.

Curcumin, 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, is a yellow colored polyphenolic compound derived from the *Curcuma longa*. Curcumin is used in wide regions of the South and Southeast Asia, as well as other parts of the world [11]. Recently, several reports have suggested that curcumin can be used as a potential drug in the treatment of cancer, cardiovascular disease, inflammatory conditions and neurodegenerative disorders [12-15]. Moreover, several other reports have demonstrated the ability of curcumin against oxidative damage in cell culture and animal model [16-18]. However, one of the major weak points of curcumin as a potential therapeutic agent in the treatment of systemic diseases is its poor aqueous solubility [19], which is the main reason for its lower bioavailability following oral administration [20]. Therefore, poor bioavailability of curcumin through oral administration is one of the main reasons of its failure to exert protective effects in clinical trials.

To increase the systemic bioavailability of curcumin, recently a micro-particle and surface controlled drug delivery system of curcumin, named THERACURMIN has been developed. THERACURMIN exhibits good dispersibility in water, and its oral administration exhibited significantly higher bioavailability than that of conventional curcumin in rat model and human [21, 22]. However, there is no report to evaluate protective effects of THERACURMIN against central nervous system disorders. Recently we have established an *in vivo* brain oxidative stress model, in order to evaluate effects of antioxidant substances [23]. Therefore, the aim of our study was to investigate the protective effects of conventional curcumin or highly bioavailable curcumin, THERACURMIN against oxidative stress using our in viv*o* oxidative stress model.

Materials and Methods

Materials

ICR mice were obtained from Nihon SLC (Shizuoka, Japan). Sodium nitroprusside, 2, 3, 5-triphenyltetrazolium chloride (TTC), dimethyl sulfoxide were purchased from nacalai tesque (Kyoto, Japan). Curcumin and gum ghatti were obtained from Sigma (St. Louis, MO, USA). Nembutal (Sodium Pentobarbital) was obtained from Dainippon Sumitomo Pharma (Osaka, Japan). THERACURMIN was obtained from Theravalues Corporation (Tokyo, Japan).

Animals and Oral Administration

Six-week-old male ICR mice (25 - 30 g) were used in the present study. The animals were housed in the control temperature room; lightening maintained on a 12-h light-dark cycle with ambient temperatures maintained at 20°C - 22 °C. Food was also freely available *ad libitum*. The experiments were conducted in accordance with the Ethical Guidance of the Kyoto University Animal Experimentation Committee, and the Guidance of the Japanese Pharmacological Society. THERACURMIN solution consisted of 12% w/w of curcumin, 46% glycerin, 4% gum ghatti, and 38% of water. Mice were orally administered curcumin (100 - 300 mg/kg; dispersed in 1% gum ghatti solution) or THERACURMIN (0.3 - 3 g/kg containing 30 - 300 mg/kg curcumin) by gavage. One day after oral administration of drugs, SNP (10 nmol, in 0.9% saline) at a volume of 1-μl was injected into the mice striatum and 24 h after SNP injection, the behavior tests and TTC staining were performed.

Rotarod test

The rotarod test is used to assess motor coordination and balance in mouse [24]. In the present study, the rotational speed tested in steady state mode was 20 rpm for 180 s. The time the mouse was able to walk on the rod before falling was collected (180 s).

Locomotor activity test

The locomotor activity test is used to assess spontaneous activity in mouse [25]. The locomotor activity was tested individually in an open field using boxes, equipped with infrared beams. The mice were placed into the box and their movements were measured for 5 min. The interruptions of photo beams for 5 min per mice were registered as the number of transitions (horizontal activity). In the same time, the number of rearings (vertical activity) was counted.

Surgery and intrastriatal microinjection

Mice were anesthetized with Nembutal (sodium pentobarbital, 60 mg/kg, i.p.) and placed in the stereotaxic frame. An incision was made along the midline of the skull to expose

the skull. Coordinates for injections were: AP +0.2 mm, ML +2 mm, DV -3.5 mm. Then into the skull the holes were drilled and a 30-gauge blunt tip needle was inserted into striatum. Curcumin (10 - 100 μg) plus SNP (10 nmol) and THERACURMIN (30 - 300 μg, containing 3 - 30 μg curcumin) plus SNP (10 nmol) l was injected into the right striatum. After injection, the needle was held in place for additional 5 min to prevent back flow and allow diffusion of the drugs.

Histological examination

Mice were decapitated 24 h after microinjection of drugs under deep anesthesia with sodium pentobarbital (60 mg/kg i.p.). Brains were rapidly removed after intracardial infusion of phosphate-buffered saline (PBS). The brains then were coronally cut into eight (1 mm thickness) slices, using a brain slicer. The brain slices were immediately incubated in 2% TTC solution for 30 min at 37 °C. The unstained areas in slices were quantified with Image J 1.42 program, and the damage volume was calculated by summing up the damaged area in all slices.

Statistical analyses

The results were expressed as mean ± S.E.M. One way analyses of variance (ANOVA) were used followed by Tukey's post test to determine statistical significant among three or more groups. Between two groups of mice, Student's *t*-test was performed to determine statistical significance. Results were considered statistically significant at $p < 0.05$. All statistical analyses were conducted using GraphPad InStat (GraphPad Software Inc., San Diego, CA, USA).

Results and Discussion

Effect of intrastriatal microinjection of THERACURMIN and curcumin on SNP-induced motor dysfunction and brain damage

To evaluate the effect of highly bioavailable curcumin, THERACURMIN, and curcumin against SNP-toxicity, we first co-injected THERACURMIN or curcumin with SNP (10 nmol) into the mice striatum. In rotarod test, co-injection of THERACURMIN (300 μg, containing 30 μg of curcumin) or curcumin (100 μg) with SNP significantly prevented SNP-induced impairment in mice performance (Fig. 1A and B). In locomotor test, co-injection of THERACURMIN or curcumin did not alter horizontal activities (transitions), but significantly prevented SNP-induced impairment in vertical activities (rearings) (Fig. 1C - F). Quantitative analysis of TTC staining showed that injection of both THERACURMIN (300 μg) and curcumin (100 μg) significantly protected brain damage induced by SNP-induced toxicity (Fig. 2).

Curcumin has been reported to be a useful agent against oxidative stress insults in cell culture and animal models [16-18]. Curcumin has also been reported to prevent amyloid β-induced toxicity in rat cortical culture [26]. We have already reported that SNP induces brain oxidative damage by iron related radical reactions [23]. Our results showed that co-injection of THERACURMIN or curcumin significantly protected mice brain against SNP-induced oxidative damage. The behavioral and histological examinations confirmed the protective effects of both THERACURMIN and curcumin. These results indicate that THERACURMIN and curcumin are quite effective in the prevention of the damage induced by SNP in brain.

Effect of oral administration of THERACURMIN and curcumin on striatal injection of SNP-induced motor dysfunction.

Since curcumin is a common dietary constituent and the oral route is the preferred route of administration for food and drugs, we then investigated the effect of THERACURMIN and curcumin after oral administration. To evaluate the effect of oral administration of THERACURMIN and curcumin against SNP-induced motor dysfunction and brain damage, these drugs were orally administered and SNP (10 nmol) was injected into the mice striatum 24 h after the administration. As shown in Figure 3A, oral administration of THERACURMIN (1 - 3 g/kg, containing 100 - 300 mg/kg of curcumin) dose-dependently prevented SNP-induced impairment in mice performance. In locomotor test, administration of THERACURMIN (1 – 3 g/kg) did not alter horizontal activities (transitions), but significantly increased vertical activities (rearings) (Fig. 3C and E). On the other hand, curcumin (100 – 300 mg/kg) did not prevent SNP-induced motor dysfunction (Fig. 3B, D and F). As shown in Figure 4, quantitative analysis of TTC staining showed that oral administration of THERACURMIN (1 - 3 g/kg) significantly protected brain damage induced by SNP. However, curcumin did not affect brain damage caused by SNP (data not shown).

Curcumin has been reported to exhibit several biological and pharmacological activities including potent antioxidant, anticancer and anti-inflammatory effects [27]. It has been reported that oral administration of curcumin (400 mg/kg) for 10 days significantly protected intrastriatal injection of quinolic acid-induced neurotoxicity [28]. In another study, oral administration of curcumin (80 mg/kg) for 3 weeks was protective against striatal injection of 6-hydroxydopamine-induced brain damage [29]. These reports suggest that curcumin has potential to protect brain damage, but needs chronic treatment with higher doses to exert its protective effects against neurological disorders. In our results, oral administration of curcumin for 24 h was not protective against SNP-induced toxicity, indicating lower bioavailability of curcumin following oral administration. The main reasons for lower bioavailability of curcumin are its poor aqueous solubility [19], and rapidly reduction by hepatic and intestinal enzymes [30]. Moreover, poor bioavailability of curcumin through oral administration is one of the causes of its failure to exert protective effect in clinical trials. For example, in a clinical trial, following oral administration of 0.5 - 8 g of curcumin in each healthy subject, curcumin was not detected in the serum. However, only lower levels of curcumin were detected in two subjects administered 10 or 12 g of curcumin [31].

Highly bioavailable curcumin has improved its water solubility and plasma bioavailability following oral administration in animal models [32, 33]. Moreover, in previous reports, the pharmacokinetic studies showed that the plasma bioavailability of curcumin was considerably higher after oral administration of THERACURMIN than that of curcumin in human and rat model. These pharmacokinetic studies showed a plasma concentration of about 30-fold higher than that of curcumin at the same administered doses [21]. Our results showed that oral administration of THERACURMIN to mice elicited an improvement of motor function, demonstrating protective effects of THERACURMIN against the deficiency of behavioral performance induced by striatal injection of SNP. This behavior improving actions of THERACURMIN was confirmed by histological detections by TTC staining on brain slices, indicating that THERACURMIN has a protective effect against oxidative stress following oral administration. Brain curcumin concentration was below the limit of detection after oral administration of 3 g/kg THERACURMIN (data not shown). Previous study reported that brain concentration of curcumin reached 4-5 μg/g brain 20-40 min after intraperitoneal injection (100 mg/kg), while that was below the limit of detection after oral administration (50 mg/kg) [34]. We administrated THERACURMIN at higher doses than that in above mentioned report. Thus, our results suggest that THERACURMIN can be absorbed after oral administration in sufficient concentration and exerts protective effect against SNP-induced oxidative stress. Furthermore, previous reports indicated that curcumin or highly bioavailable curcumin can cross the blood brain barrier and reach to the CNS. For example, after intravenous injection in rat, the curcumin and highly bioavailable curcumin crossed the blood brain barrier and the retention time of highly bioavailable curcumin in the brain was longer than that of curcumin [35]. Moreover, after oral administration of curcumin with piperine

in the rat, the intestinal absorption of curcumin was increased and it was detected in the brain for 96 h [36]. In addition, curcumin has been reported to cross blood brain barrier and induce its neuroprotective effects in the treatment of Parkinson's diseases [37]. These reports further support our study that THERACURMIN might reach the CNS in sufficient concentration to exert its protective effect against SNP-induced toxicity.

As Chin et al. discussed in the previous review [38], adverse effects such as carcinogenic and prooxidant activity cannot be completely ruled out, especially during long-term supplementation at very high doses of curcumin. In the present study, single administration prevented brain damage induced by oxidative stress. Thus, the proper application of curcumin would be required although many have reported that curcumin have a relatively low toxicity profile [39].

Conclusions

Our results indicate that THERACURMIN and curcumin have protective effect against SNP-induced oxidative stress in mice brain. Furthermore, oral administration of THERACURMIN potently protected brain damage induced by SNP, suggesting higher bioavailability of THERACURMIN following oral administration. The potent protective effect of THERACURMIN following oral administration suggests that highly bioavailable curcumin is one of the best ways of delivering curcumin to the plasma and brain.

Acknowledgements

This study was supported in part by JSPS KAKENHI Grant number 24390139 and by a grant from the Smoking Research Foundation, Japan.

References

(1) A. C. Cristóvão, S. Guhathakurta, E. Bok, G. Je, S. D. Yoo, D. H. Choi, Y. S. Kim, NADPH Oxidase 1 Mediates α-Synucleinopathy in Parkinson's Disease. *J. Neurosci.* 2012, **32**, 14465-14477.

(2) P. Sompol, W. Ittarat, J. Tangpong, Y. Chen, I. Doubinskaia, I. Batinic-Haberle, H. M. Abdul, D. A. Butterfield, D. K. A. St, Clair, A neuronal model of Alzheimer's disease: an insight into the mechanisms of oxidative stress- mediated mitochondrial injury. *Neuroscience*. 2008, **22**, 120-130.

(3) T. H. Sanderson, C. A. Reynolds, R. Kumar, K. Przyklenk, M. Hüttemann, Molecular Mechanisms of Ischemia–Reperfusion Injury in Brain: Pivotal Role of the Mitochondrial Membrane Potential in Reactive Oxygen Species Generation. *Mol. Neurobiol*. 2013, **47**, 9-23

(4) X. D. Chao, Y. H. Ma, P. Luo, L. Cao, W. B. La, B. C. Zhao, F. Han, W. Liu, W. D. Ning, N. Su, L. Zhang, J. Zhu, Z. Fei, Y. Qu, Up-regulation of Heme oxygenase-1 attenuates brain damage after cerebral ischemia via simultaneous inhibition of superoxide production and preservation of NO bioavailability. *Exp Neurol.* 2013, **239**, 163-169

(5) T. M. Andriessen, B. Jacobs, P. E. Vos, Clinical characteristics and pathophysiological mechanisms of focal and diffuse traumatic brain injury. *J. Cell. Mol. Med.* 2010, **14**, 2381-2392.

(6) A. Durukan, T. Tatlisumak, Acute ischemic stroke: Overview of major experimental rodent models, pathophysiology, and therapy of focal cerebral ischemia. *Pharmacol. Biochem. Behav.* 2007, **87**, 179-197.

(7) K. Cui, X. Luo, K. Xu, M. R. Ven, Murthy, Role of oxidative stress in neurodegeneration: recent developments in assay methods for oxidative stress and nutraceutical antioxidants. *Prog. Neuropsychopharmacol. Biol Psychiatry.* 2004, **28**, 771-799.

(8) B. Uttara, A. V. Singh, P. Zamboni, R. T. Mahajan, Oxidative Stress and Neurodegenerative Diseases: A Review of Upstream and Downstream Antioxidant Therapeutic Options. *Curr. Neuropharmacol*. 2009, **7**, 65-74.

(9) M. Valko, D. Leibfritz, J. Moncol, M. T. Cronin, M. Mazur, J. Telser, Free radicals and antioxidants in normal physiological functions and human disease. *Int. J. Biochem. Cell. Biol.* 2007, **39**, 44-84.

(10) A. T. Diplock, J. L. Charleux, G. Crozier-Willi, F. J. Kok, C. Rice-Evans, M. Roberfroid, W. Stahl, J. Viña-Ribes, Functional food science and defence against reactive oxidative species. *Br. J. Nutr.* 1998, **80**, 77-112.

(11) A. Goel, A. B. Kunnumakkara, B. B. Aggarwal, Curcumin as ''Curecumin'': From kitchen to clinic. *Biochem. Pharmacol.* 2008, **75**, 787-809.

(12) A. Shehzad, F. Wahid, Y. S. Lee, Curcumin in Cancer Chemoprevention: Molecular Targets, Pharmacokinetics, Bioavailability, and Clinical Trials. *Arch. Pharm. (Weinheim)*. 2010, **343**, 489-499.

(13) N. P. Wang, Z. F. Wang, S. Tootle, T. Philip, Z. Q. Zhao, Curcumin promotes cardiac repair and ameliorates cardiac dysfunction following myocardial infarction. *Br J Pharmacol*. 2012,**167**, 1550-1562.

(14) J. S. Jurenka, Anti-inflammatory Properties of Curcumin, a Major Constituent of *Curcuma longa:* A Review of Preclinical and Clinical Research. *Altern. Med. Rev*. 2009, **14**, 141-153.

(15) G. M. Cole, B. Teter, S. A. Frautschy, Neuroprotective Effects of Curcumin. *Adv. Exp. Med. Biol.* 2007, **595**, 197-212.

(16) R. Barreto, S. Kawakita, J. Tsuchiya, E. Minelli, K. Pavasuthipaisit, A. Helmy, F. Marotta, Metal-induced oxidative damage in cultured hepatocytes and hepatic lysosomal fraction: beneficial effect of a curcumin/absinthium compound. *Chin. J. Dig. Dis*. 2005, **6**, 3-6.

(17) H. C. Huang, C. J. Lin, W. J. Liu, R. R. Jiang, Z. F. Jiang, Dual effects of curcumin on neuronal oxidative stress in the presence of Cu(II). *Food. Chem. Toxicol.* 2011, **49**, 1578-1583.

(18) S. Rong, Y. Zhao, W. Bao, X. Xiao, D. Wang, A. K. Nussler, H. Yan, P. Yao, L. Liu, Curcumin prevents chronic alcohol-induced liver disease involving decreasing ROS generation and enhancing antioxidative capacity. *Phytomedicine.* 2012, **19**, 545-550.

(19) H. H. Tønnesen, M. Másson, T. Loftsson, Studies of curcumin and curcuminoids. XXVII. Cyclodextrin complexation: solubility, chemical and photochemical stability. *Int J Pharm.* 2002, **244**, 127-135.

(20) R. A. Sharma, A. J. Gescher, W. P. Steward, Curcumin: The story so far. *Eur, J. Cancer.* 2005, **41**, 1955-1968.

(21) H. Sasaki, Y. Sunagawa, K. Takahashi, A. Imaizumi, H. Fukuda, T. Hashimoto, H. Wada, Y. Katanasaka, H. Kakeya, M. Fujita, K. Hasegawa, T. Morimoto, Innovative Preparation of Curcumin for Improved Oral Bioavailability. *Biol Pharm. Bull*. 2011, **34**, 660-665.

(22) M. Kanai, A. Imaizumi, Y. Otsuka, H. Sasaki, M. Hashiguchi, K. Tsujiko, S. Matsumoto, H. Ishiguro, T. Chiba, Dose-escalation and pharmacokinetic study of nanoparticle curcumin, a potential anticancer agent with improved bioavailability, in healthy human volunteers. *Cancer. Chemother.Pharmacol.* 2012, **69**, 65-70.

(23) Q. A. Nazari, K. Mizuno, T. Kume, Y. Takada-Takatori, Y. Izumi, A. Akaike, In Vivo Brain Oxidative Stress Model Induced by Microinjection of Sodium Nitroprusside in Mice. *J Pharmacol. Sci*. 2012, ***120***, 105-111.

(24) G. Rozas, E. López-Martín, M. J. Guerra, J. L. Labandeira-García, The overall rod performance test in the MPTP-treated-mouse model of Parkinsonism. *J. Neurosci Methods*. 1998, **83**, 165-175.

(25) F. Richter, M. Hamann, A. Richter, Chronic Rotenone Treatment Induces Behavioral Effects but No Pathological Signs of Parkinsonism in Mice. *J. Neurosci. Res*. 2007, **85**, 681-691.

(26) X. Y. Qin, Y. Cheng, L. C. Yu, Potential protection of curcumin against intracellular amyloid β-induced toxicity in cultured rat prefrontal cortical neurons. *Neurosci. Lett*. 2010, **480**, 21-24.

(27) H. Hatcher, R. Planalp, J. Cho, F. M. Torti, S. V. Torti, Curcumin: From ancient medicine to current clinical trials. *Cell. Mol. Life. Sci*. 2008, **65**, 1631-1652.

(28) I. Carmona-Ramírez, A. Santamaría, J. C. Tobón-Velasco, M. Orozco-Ibarra, I. G. González-Herrera, J. Pedraza-Chaverrí, P. D. Maldonado, Curcumin restores Nrf2 levels and prevents quinolinic acid-induced neurotoxicity. *J. Nutr. Biochem.* 2013, **24**, 14-24.

(29) G. Khuwaja, M. M. Khan, T. Ishrat, A. Ahmad, S. S. Raza, M. Ashafaq, H. Javed, M. B. Khan, A. Khan, K. Vaibhav, M. M. Safhi, F. Islam, Neuroprotective effects of curcumin on 6-hydroxydopamine-induced Parkinsonism in rats: Behavioral, neurochemical and immunohistochemical studies. *Brain Res*. 2011, **1368**, 254-263.

(30) M. H. Pan, T. M. Huang, J. K. Lin, Biotransformation of Curcumin Through Reduction And Glucuronidation In Mice. *Drug. Metab. Dispos.* 1999, **27**, 486-494.

(31) C. D. Lao, D. Normolle, D. D. Heath, S. I. Murray, J. M. Bailey, M. E. Boggs, J. Crowell, C. L. Rock, D. E. Brenner, Dose escalation of a curcuminoid formulation. *Complement. Altern Med.* 2006, **6**, 10.

(32) X. Xie, Q. Tao, Y. Zou, F. Zhang, M. Guo, Y. Wang, H. Wang. Q. Zhou, S. Yu, PLGA Nanoparticles Improve the Oral Bioavailability of Curcumin in Rats: Characterizations and Mechanisms. *J. Agric. Food. Chem*. 2011, **59**, 9280- 9289.

(33) L. Zhongfa, M. Chiu, J. Wang, W. Chen, W. Yen, P. Fan-Havard, L. D. Yee, L. D.; K. K. Chan, Enhancement of curcumin oral absorption and pharmacokinetics of curcuminoids and curcumin metabolites in mice. *Cancer. Chemother. Pharmacol*. 2012, **69**, 679-689.

(34) C. Schiborr, G. P. Eckert, G. Rimbach, J. Frank, A validated method for the quantification of curcumin in plasma and brain tissue by fast narrow-bore high-performance liquid chromatography with fluorescence detection. *Anal. Bioanal. Chem.* 2010 **397**, 1917-1925.

(35) Y. M. Tsai, C. F. Chien, L. C. Lin, T. H. Tsai, Curcumin and its nano-formulation: The kinetics of tissue distribution and blood–brain barrier penetration. *Int J Pharm*. 2011**,** *416*: 331-338.

(36) D. Suresh, K. Srinivasan, Tissue distribution & elimination of capsaicin, piperine & curcumin following oral intake in rats. *Indian. J. Med. Res.* 2010, **131**, 682-691.

(37) R. B. Mythri, M. M. Bharath, Curcumin: A Potential Neuroprotective Agent in Parkinson's Disease. *Curr. Pharm. Des*. 2012, *18*, 91-99.

(38) D. Chin, P. Huebbe, K. Pallauf, G. Rimbach, Neuroprotective properties of curcumin in Alzheimer's disease--merits and limitations. *Curr Med Chem.* 2013, **20**, 3955-3985.

(39) T. Esatbeyoglu, P. Huebbe, I. M. Ernst, D Chin, A. E. Wagner, G. Rimbach, Curcumin--from molecule to biological function. *Angew Chem Int Ed Engl.* 2012, **51**, 5308-5332.

Figure legends

Fig 1 Effect of intrastriatal microinjection of THERACURMIN and curcumin on SNP-induced motor dysfunction. Behavioral changes were examined 24 h after microinjection of drugs. In rotarod test, intrastriatal injection of THERACURMIN (300 μg) or curcumin (100 μg) prevented SNP-induced impairment in mice performance (A and B). In locomotor test, both THERACURMIN and curcumin did not alter horizontal activities (transitions) (C and D), but increased vertical activities (rearings) (E and F). $^{**}P < 0.01$, $^{***}P < 0.001$ compared with vehicle, $^{\#}P < 0.05$, compared with SNP treatment. Values represent mean ± S.E.M. (n = 5 - 7).

Fig 2 Effect of intrastriatal microinjection of THERACURMIN and curcumin on brain damage induced by SNP. SNP (10 nmol) (A) or THERACURMIN (300 μg) with SNP (B) was injected into the striatum and 24 h after injection of drugs TTC staining was performed. Quantitative analysis showed a significant protective effect of THERACURMIN(C) or curcumin (D) compared with SNP treatment. $^{**}P < 0.01$, compared with Vehicle. $^{\#}P < 0.05$, compared with SNP treatment. Values represent mean ± S.E.M. (n = 5 – 7). Scale bar = 1 cm.

Fig 3 Effect of oral administration of THERACURMIN and curcumin on SNP-induced motor dysfunction. Behavioral changes were examined 24 h after microinjection of drugs. In rotarod test, THERACURMIN (1 - 3 g/kg, p.o.) prevented SNP-induced impairment in mice performance (A). In locomotor test, THERACURMIN did not alter horizontal activities (transitions) (C), but increased vertical activities (rearings) (E). In both rotarod (B) and locomotor (D and F) tests, curcumin did not prevent SNP-induced motor dysfunction . $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, compared with vehicle, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, compared with SNP treatment. Values represent mean ± S.E.M. (n = 7 - 9).

Fig 4 Effect of oral administration of THERACURMIN on brain damage induced by SNP. Vehicle (A) or THERACURMIN (3 g/kg) (B) was orally administrated and SNP (10 nmol) was injected 24 h after administration. Twenty four hour after SNP injection, TTC staining was performed. Quantitative analysis showed a significant protective effect of THERACURMIN(C) compared with SNP treatment. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, compared with SNP treatment. Values represent mean ± S.E.M. (n = 7 – 9). Scale bar = 1 cm.

Figure 1

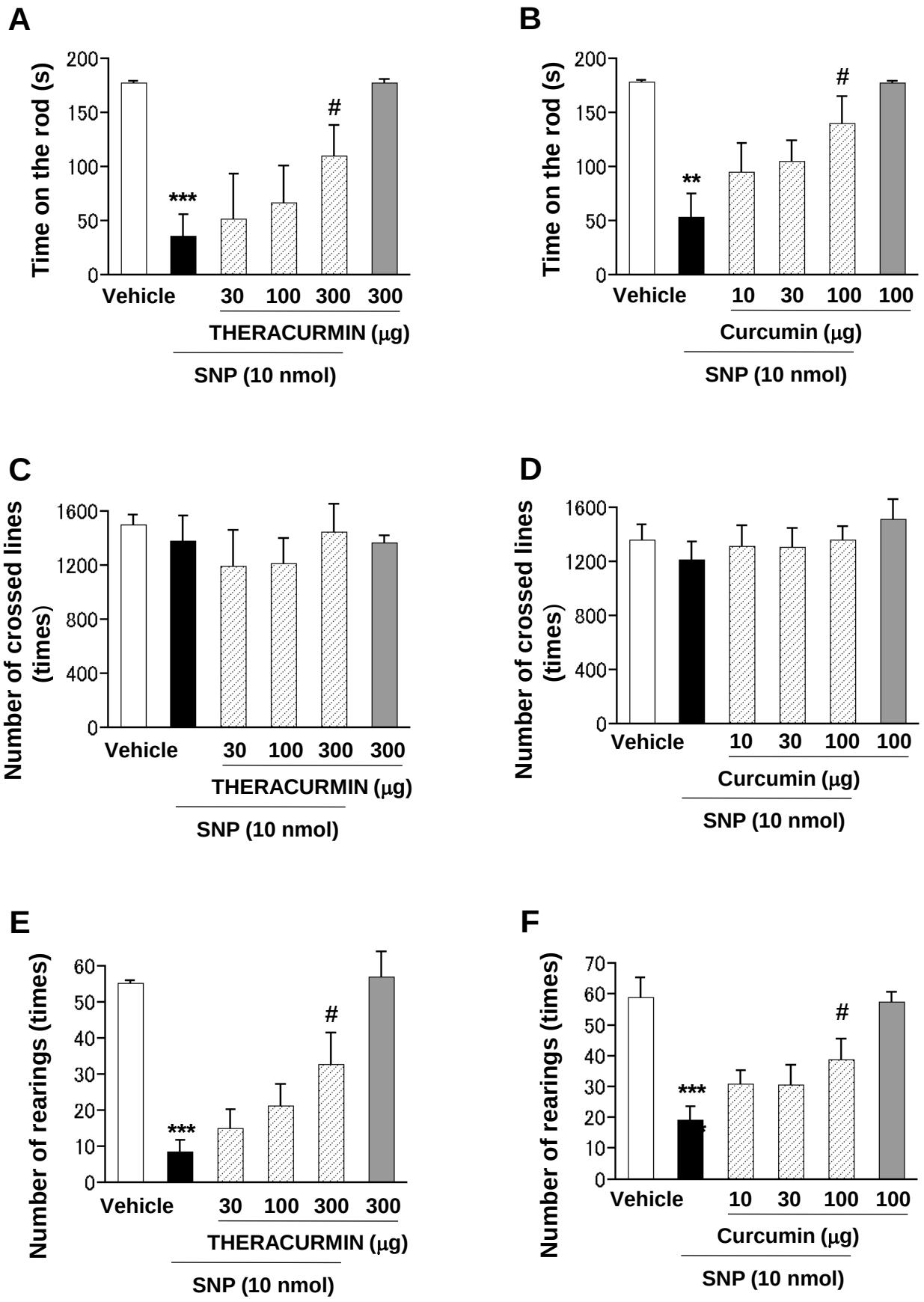


Figure 2

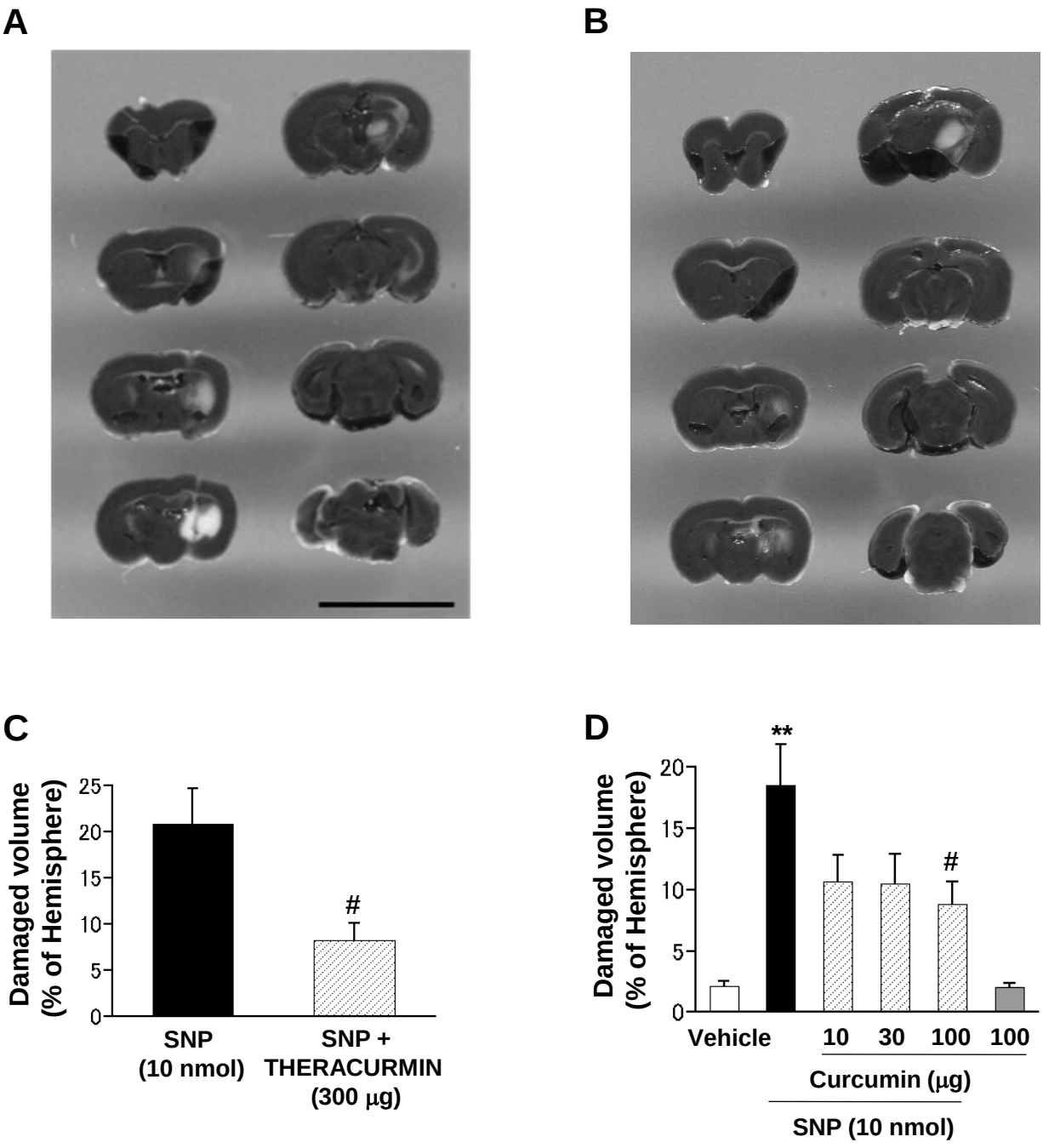


Figure 3

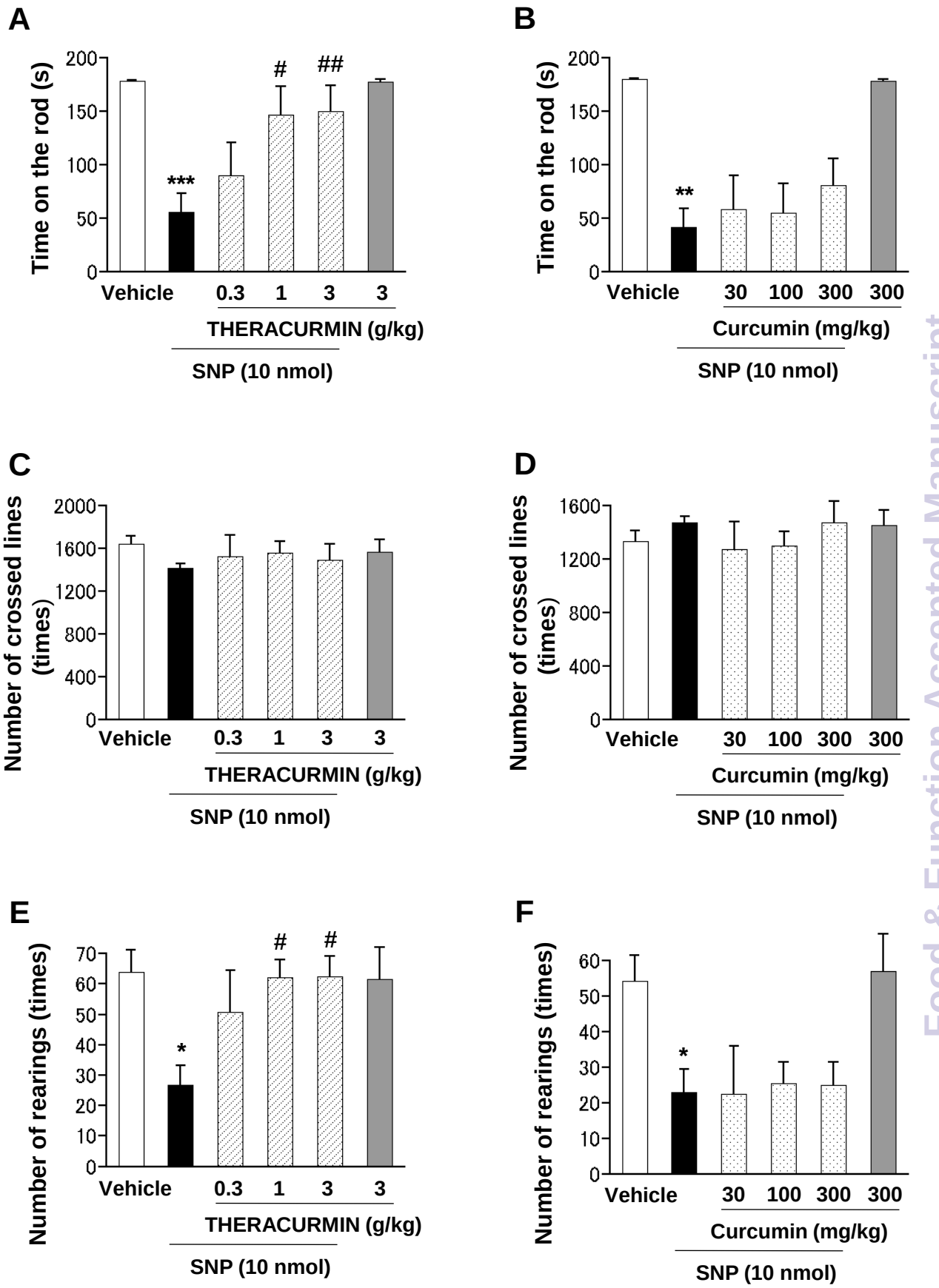


Figure 4