Available online at www.tox.or.kr

Biological Safety and Anti-hepatofibrogenic Effects of *Brassica rapa* (Turnip) Nanoparticle

Dae-Hun Park[1,*], Lan Li[2,*], Hyung-Kwan Jang[3], Young-Jin Kim[4], Ja-June Jang[5], Yeon Shik Choi[6], Seung-Kee Park[7] & Min-Jae Lee[2]

[1]College of Pharmacy, Kangwon National University, 192-1 Hyoja-dong, Chuncheon-si, Gangwon-do 200-701, Korea
[2]School of Veterinary Medicine, Kangwon National University
[3]Department of Veterinary Infectious Diseases and Avian Disease, College of Veterinary Medicine, Chonbuk National University, Korea
[4]Traditional Food Research Group, Korea Food Research Institute, 516 Baekhyun-dong, Bundang-gu, Seongnam-si, Gyeonggi-do 463-746, Korea
[5]Department of Pathology, College of Medicine, Seoul National University, 28 Yeongeon-dong, Jongno-gu, Seoul 110-799, Korea
[6]Department of Laboratory Animals, Korea Bio Polytechnic, Nonsan-si, Chungcheongnam-do, Korea
[7]Department of Microbiology, College of Medicine, Hallym University, 39 Hallymdaehak-gil, Chuncheon-si, Gangwon-do 200-702, Korea
*These authors contributed equally to this work
Correspondence and requests for materials should be addressed to M. J. Lee (mjlee@kangwon.ac.kr)

Accepted 30 October 2009

Abstract

Hepatic fibrosis is one of chronic liver diseases which spread in worldwide and it has high risk to turn advanced cirrhosis and hepatocellualr carcinoma. *Brassica* family has been produced for commercial purpose and in Korea *Brassica rapa* (Turnip) is cultivated in Ganghwa County, Gyeonggi-do Korea and used for making Kimchi. Recently pharmacological effects of turnip have been known; diabete mellitus modulation, alcohol oxidization, and fibrosis inhibition. In previous study we found antifibrogenic effect of turnip water extract and in this study we made turnip nanoparticle to promote turnip delivery into liver. At the same time we assessed the biological safety of turnip nanoparticle. Thioacetamide (TAA) induced hepatic nodular formation and fibrosis (mean of fibrosis score: 4). However, 1% turnip nanoparticle inhibited TAA-induced hepatic nodular formation and fibrosis (mean of fibrosis score: 2-3). Activities of serum enzymes (aspartic acid transaminase (AST), alanine transaminase (ALT), and total bilirubin (T-Bil)), complete blood count (CBC), and the appearance of organs were not different from control and 1% turnip nanoparticle treatment. Conclusively 1% turnip nanoparticle significantly reduced TAA-induced hepatic fibrosis and was safe in 7-weeks feeding.

Keywords: *Brassica rapa*, Turnip nanoparticle, Biological safety, Hepatic fibrosis

Liver disease has 3 steps; hepatic fibrosis, hepatic cirrhosis, and hepatocellular carcinoma. Chronic liver disease (CLD) spreads extensively in worldwide and 20-40% among patients with CLD turn advanced hepatic fibrosis and hepatic cirrhosis[1]. The cause factors of hepatic fibrosis are very manifold; viral hepatitis (especially hepatitis B and C), alcohol abuse, drugs, metabolic diseases, congenital abnormalities[2]. The normal liver consists of an epithelial component (hepatocytes), an endothelial lining, tissue magrophages (Kupffer cells), and perivascular mesenchymal cells called as stellate cells (previously called Ito cell, lipocyte, perisinusoidal cell, or fat-storing cell). Among theses, stellate cells are the key component of hepatic fibrogenesis. Stellate cells activation result in hepatic fibrosis via various pathways; connective tissue growth factor (CTGF/CCN2) upregulation[3], Smad proteins activation by transforming growth factor beta (TGFβ)[4], cannabinoids modulation[5] etc. As the liver turns fibrotic, the constitution of hepatic extracellular matrix (ECM) is changed. The contents of collagens and noncollagenous elements increase 3-5-fold[6].

Brassica family has been produced for consumption in worldwide and in Korea *Brassica rapa* (Turnip) among them is cultivated in Ganghwa County, Gyeonggi-do Korea and used for making Kimchi. Turnip has pharmacological functions; it suppressed type 2 abetes mellitus by glucose and lipid metabolism modulation[7], it oxidized alcohol[8], and its extract inhibited hepatic fibrogenesis[9]. Various flavonoids and hydroxycinnamic derivatives were characterized from Turnip[10,11]; One-methoxyindole-3-acetonitrile and indole-3-acetonitrile had anticancer effect[12], turnip extract fractions inhibited hepatic fibrogenesis[13], ρ-coumaroylglucose and

Table 1. Turnip particle size and percentage of nanoscale according to pulverization time

Pulverization time (hr)	Mean of particle size (diameter, μm)	Percentage of nanoscale (%)
0	87.9	0.0
96	1.1	49.3
264	0.96	60.6

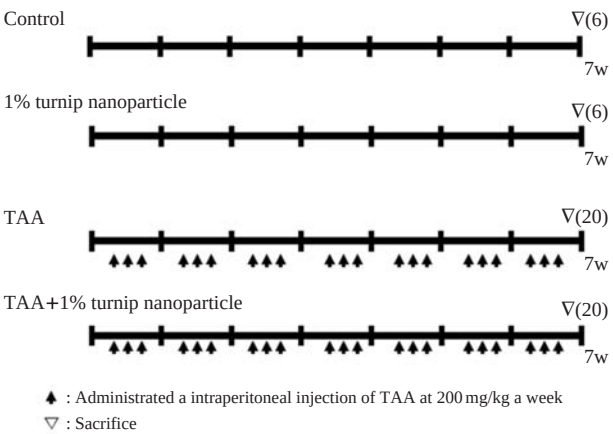

Figure 1. The protocol of TAA-induced hepatic fibrogenetic animal model. Arrows mean administration into intraperitoneal injection of TAA at a 50 mg/kg triple a week. Number in round bracket is animal number in each group.

feruloyl-glucose had anticancerous, antioxidant and anti-inflammatory effects[14,15], and d-galactosamine protected liver disease via DPPH scavenging, catalases and superoxide dismutase (SOD) decreasing, and serum enzymes regulating[16].

Nanotechnology is one of fast growing fields from economy to science. More than 200 nanomaterials have been used for medical and industrial purposes[17,18]. Nanomaterials are easy to delivery and to maintain in biological target sites because of the advantage of size. However as these materials are respirable size it is possible to spread pollution environment and/or bio-organisms including human being[19,20].

In this study, we examined anti-fibrogenic effects and biosafety of turnip nanoscale powder.

Brassica rapa (Turnip) Nanoparticle Is Safe in 7-weeks Synchronous Feeding in Rats

At 96 hr after pulverization mean of particle size reached about 1.1 μm and percentage of nanoscale was 49.3% (Table 1). At 264 hr mean of particle size was 0.96 μm and percentage of nanoscale was 60.6%. Morphology of freeze-dried turnip powder was large fragments, rough surface, and sharp edges but shape of turnip nanoparticle was small spherical (data not shown).

Body weights of thioacetamide (TAA) treated groups (TAA treatment group and TAA and 1% turnip nanoparticle cotreatment group) were slighted compared with them of TAA untreated group (control and 1% turnip nanoparticle treatment group) and 1% turnip nanoparticle recovered TAA-induced body weight's decrease ($P < 0.05$) (Figure 2A). Activities of liver function-related serological enzymes-aspartic acid transaminase (AST), alanine transaminase (ALT), and total bilirubin (T-Bil)-in TAA treated groups increased than those of TAA untreated groups (Figure 2B). Activity of AST in TAA and 1% turnip nanoparticle treatment group slightly decreased. Complete blood count (CBC) and the appearance of organs were not different from control and 1% turnip nanoparticle treatment (data not shown).

1% Turnip Nanoparticle Inhibits TAA-induced Rat Hepatic Fibrogenesis

The relative liver weight to body weight in TAA treated groups (TAA treatment group & TAA and 1% turnip nanoparticle cotreatment group) was smaller than that in TAA untreated groups (control & 1% turnip nanoparticle treatment group) (Figure 3A). 1% turnip nanoparticle did not affect the relative liver weight to body weight.

1% turnip nanoparticle did not induce the hepatic morphological changes, but TAA formed nodulations in the majority of liver and 1% turnip nanoparticle inhibited TAA-induced hepatic nodular formations (Figure 3B-H & E). In order to confirm the level of fibrosis we conducted Masson's trichome staining and assessed fibrosis scoring according to the fibrotic area. The photo of TAA treatment group was shown like typical hepatic fibrosis and mean of fibrosis scoring was score 4 (Figure 3C). 1% turnip nanoparticle suppressed hepatic fibrogenesis (Figure 3B-MT) and the fibrosis score of TAA and 1% turnip nanoparticle treatment group resulted in 2-3.

Discussion

Turnip is used as a material for making Kimchi and that has various pharmacological functions; type 2 diabetes mellitus suppression[7], alcohol oxidization[8], antiproliferative effect against cancer cells[12], antioxidant and anti-inflammatory effects[14,15], and DPPH scavenging effect[16]. Hepatic fibrosis is one of chronic liver diseases which spread in worldwide. Although there are lots of studies to find agents for preventing hepatic fibrosis, it is not easy to find candidates which have antifibrogenic effect and biological safety. Turnip Kim-

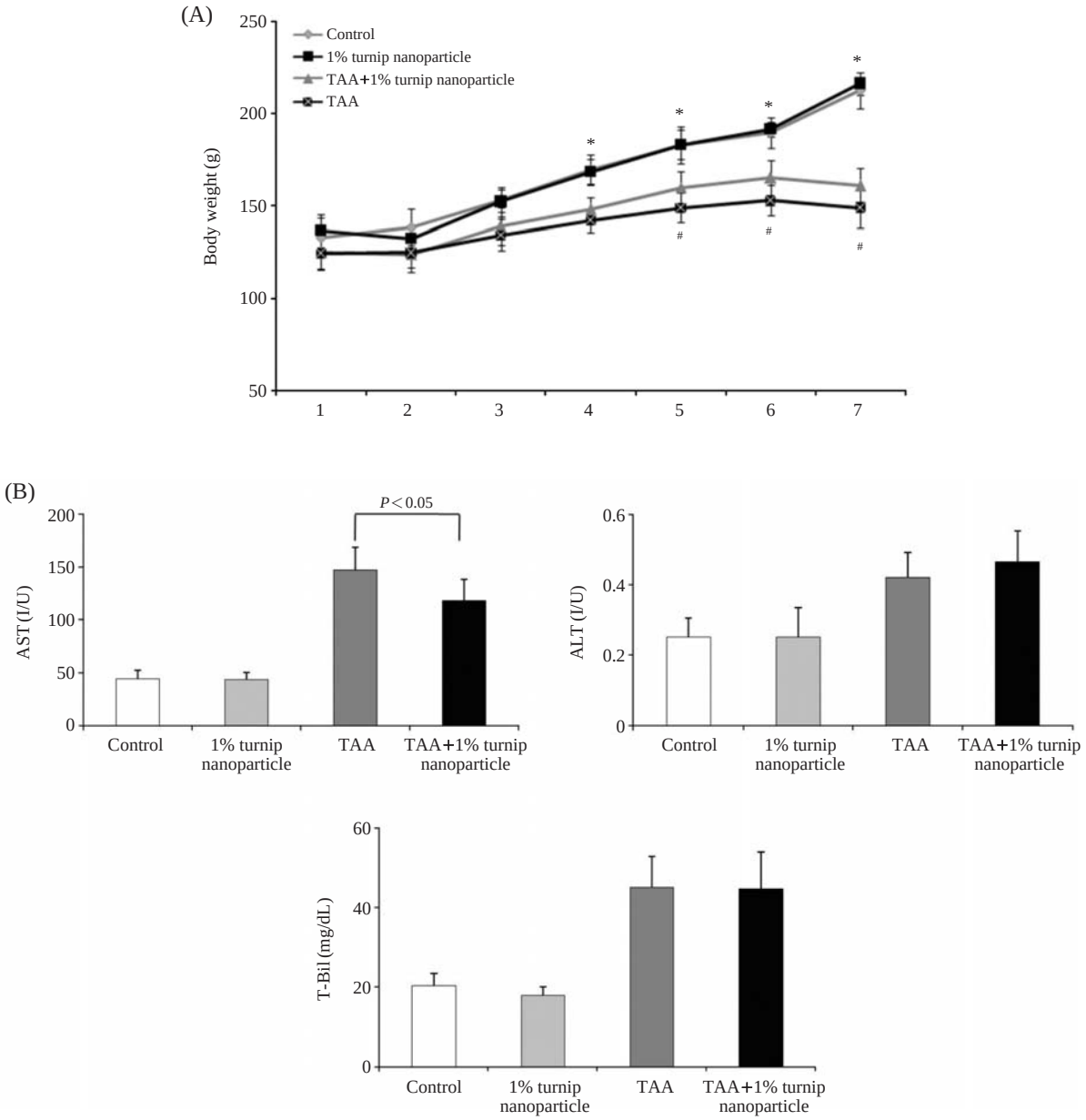

Figure 2. Turnip nanoparticle is safe in rats. (A) 1% turnip nanoparticle does not affect the body weight's change. The body weights in TAA untreated group (control & 1% turnip nanoparticle treatment group) are larger than those in TAA treated groups (TAA treatment group & TAA+1% turnip nanoparticle treatment group) ($P < 0.05$). TAA decreases the body weight but 1% turnip nanoparticle rescues the body weight loss ($P < 0.05$). All value are expressed as mean ± SD. *$P < 0.05$, TAA untreated groups *vs.* TAA treated group, $^{\#}P < 0.05$, TAA treatment group *vs.* TAA+1% Turnip Nanoparticle treatment group (B) It is not different from activities of serum enzymes of control and those of 1% nanoparticle treatment group. TAA results in hepatic disorder and then activities of serum enzymes like as AST, ALT, and T-Bil increase. 1% turnip nanoparticle slightly decreases TAA-induced abnormal rise of activity of AST. All values were expressed as mean ± SD. $P < 0.05$, TAA untreated groups *vs.* TAA treated group.

chi is a famous and almost daily eaten food. As turnip is very biological safe, turnip Kimchi is made with turnip.

1% turnip nanoparticle inhibited TAA-induced hepatic nodular formation and fibrosis. TAA induced nodular formation in the majority of liver and mean of fibrosis score was 4 but in TAA and 1% turnip nanoparticle cotreatment group hepatic nodules almost disappeared

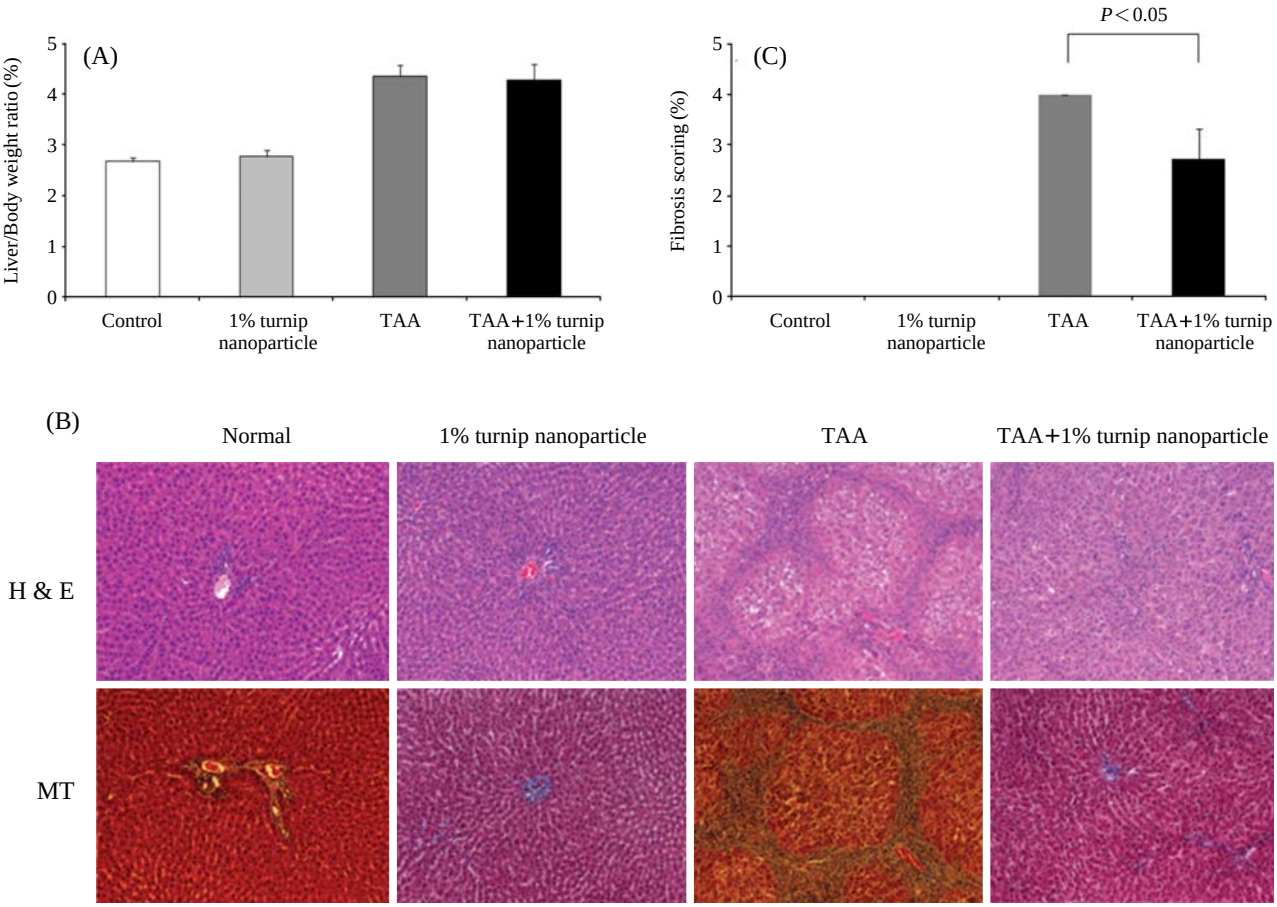

Figure 3. 1% turnip nanoparticle inhibits TAA-induced hepatic nodular formation and fibrosis. (A) The ratio of liver/body weight in TAA treated groups (TAA treatment group & TAA+1% turnip nanoparticle treatment group) is larger than that in TAA untreated group (control & 1% turnip nanoparticle treatment group). All values were expressed as mean ± SD. $P < 0.05$, TAA untreated groups *vs.* TAA treated group (B) Histopathological analysis. Photos of livers in control and 1% turnip nanoparticle treatment group are normal and that in TAA treatment is shown typical hepatic fibrosis (nodular formation). 1% turnip nanoparticle almost inhibits TAA-induced nodular formation and fibrosis. (C) Fibrosis scores in each group. Semi-quantitative scoring system is adopted to evaluate the degree of liver fibrosis (0: no, score 1: portal fibrosis, score 2: periportal fibrosis, score 3: septal fibrosis, score 4: cirrhosis). Mean of fibrosis scores in TAA treatment group is 4 but 1% turnip nanoparticle decreases mean of fibrosis scores by 2-3. All values were expressed as mean ± SD. $P < 0.05$, TAA untreated groups vs. TAA treated group.

and mean of fibrosis score was 2-3. Activities of serum enzymes (aspartic acid transaminase (AST), alanine transaminase (ALT), and total bilirubin (T-Bil), complete blood count (CBC), and the appearance of organs were not different from control and 1% turnip nanoparticle treatment.

Generally the purpose of using nanoparticle is to improve the agent's retention time and delivery efficiency in target site. In our previous study we found the result that turnip water extract prevents hepatofibrogenesis, through this study we hoped that turnip have antihepatofibrogenic efficacy in spite of configuration change.

In conclusive, we got the results that 1% turnip nanoparticle significantly reduced TAA-induced hepatic fibrosis and in addition was safe in 7-weeks feeding. We suggest turnip nanoparticle is possible to be safely used as an antifibrogenic agent.

Materials & Methods

Turnip Nanoparticle Production

Turnips were collected, washed with clean tap water, dried in dark- and cold-room, chopped small size, and freeze-dried. The freeze-dried powder (150 g) was pulverized with 1,000 mL EtOH with 1 kg ϕ20 mm porcelains in porcelain mill pot (DS-BM5L, DongSeo Science Co., Korea) at 30 rpm for 11 days. The distribution of turnip nanoparticles was analyzed by particle

size analyzer (CILAS 1064, CILAS, France), and the structure of nanoparticles was photographed using a scanning electron microscope (Hitachi 238N, Hitach, Japan).

Animals

Ten male F344 rats were purchased from Orient Bio (Sungnam, Korea) and acclimated for 7 days. All animals were housed in a temperature and relative humidity-controlled environment ($22 \pm 3^{\circ}C$, 12-hr light/dark cycle) during acclimation fed *ad libitum* with AIN76 diet and water.

The experimental protocol for animal treatment by thioacetamide (TAA, Sigma, St. Louis, MO, USA) is outlined in Figure 1 and was approved by Institutional Animal Care and Use Committee (IACUC) Kangwon National University. The rats divided into control (6 heads), 1% turnip nanoparticle group (6 heads), TAA group (20 heads), and 1% turnip nanoparticle+TAA group (20 heads). Rats were injected with saline or TAA (200 mg/kg) three times a week intraperitoneally for 7 weeks and fed *ad libitum* with water and AIN76 diet or AIN76 with 1% turnip nanoparticle.

Histopathological Analysis

The body weights of them were checked every 3 days. After 1 days of final injection, rats were weighed body weight, judged the appearance, anesthetised with isofluran, collected whole blood through intracardic route, and sacrificed using isofluran. After sacrificing rats, all organs were checked with the unaided eyes for analysis pathological changes, and fixed hearts, lungs, livers, kidneys, spleens, urinary bladders, testises and ovaries with 10% neutral formalin. Fixed samples were embedded with paraffin using Tissue-Tek VIP (Sakura, Japan) and sliced 4 μm thickness. Histopathological study was performed on all slices stained with H & E and by light microscopy and was done on liver stained with MT stain. Semi-quantitative scoring system was adopted for evaluation of the degree of liver fibrosis (0: no, score 1: portal fibrosis, score 2: periportal fibrosis, score 3: septal fibrosis, score 4: cirrhosis).

Biological Toxicity Analysis

Using the collected whole blood, CBC was measured with Hemavet950 (Drew Scientific Group, USA) and the serum levels of AST, ALT, and T-Bil activities were determined using Fuji Dri-Chem 3500i analyzer (Fuji-film, Japan).

Statistical Analysis

All data are presented as mean ± SD. Statistical comparison among groups was made by Student's T-test ($P < 0.005$ was considered significant).

Acknowledgements

This work was supported by a grant (Code C1006670-01-01) from Korea Institute of Toxicology, Republic of Korea.

References

1. Zhou, K. & Lu, G. Assessment of fibrosis in chronic liver diseases. *J Digestive Diseases* **10**:7-14 (2009).
2. Friedman SL. in *Diseases of the Liver* (Schiff, E., Sorrell, M. & Maddrey, W. eds.) 8th Ed., Lippincott-Raven, Philadelphia, 371-386 (1998).
3. Paradis, V. *et al*. High glucose and hyperinsulinemia stimulate connective tissue growth factor expression: a potential mechanism involved in progression to fibrosis in nonalcoholic steatohepatitis. *Hepatology* **34**: 738-744 (2001).
4. Breitkopf, K. *et al*. TGF-beta/Smad signaling in the injured liver. *Z Gastroenterol* **44**:57-66 (2006).
5. Mallat, A. *et al*. Cannaboid receptors as new targets of antifibrosing strategies during chronic liver diseases. *Expert Opin Ther Targets* **11**:403-409 (2007).
6. Friedman, S. L. Molecular regulation of hepatic fibrosis, an integrated cellular response to tissue injury. *J Biol Chem* **275**:2247-2250 (2000).
7. Jung, U. J. *et al*. Effects of the ethanol extract of the roots of *Brassica rapa* on glucose and lipid metabolism in C57BL/KsJ-*db*/*db* mice. *Clinical Nutrition* **27**:158-167 (2008).
8. Kim, D. H. *et al*. Effects of alcohol oxidation of *Brassica rapa* L. extraction process in Kang-Hwa. *Kor J Medicinal Crop Sci* **14**:45-48 (2006).
9. Shin, H. W. *et al*. Inhibitory effect of turnip extract on thioacetamide-induced rat hepatic fibrogenesis. *Cancer Prevention Research* **11**:265-272 (2006).
10. Romani, A. *et al*. HPLC-DAD/MS characterization of flavonoids and hydroxycinnamic derivatives in turnip tops (*Brassica rapa* L. subsp. *sylvestris* L.). *J Agric Food Chem* **54**:1342-1346 (2006).
11. Kim, J. S. *et al*. Chemical constituents from the root of *Brassica campestris* ssp *rapa*. *Kor J Pharmacogn* **35**:259-263 (2004).
12. Sones, K. *et al*. An estimate of the mean daily intake of glucosinolates from cruciferous vegetables in the UK. *J Sci Food Agric* **35**:712-720 (1984).
13. Shin, H. W. *et al*. Inhibitory effect of turnip extract on hepatic fibrogenesis. *Lab Anim Res* **22**:279-288 (2006).
14. Stobart, A. K. & Thomas, D. R. Chlorophyllase in tissue cultures of *kalanchoë crenata*. *Phytochemistry* **7**: 1963-1972 (1968).
15. Xu, Y. N. *et al*. Components from the roots of *Chaenomeles japonica. Kor J Pharmacogn* **33**:267-271 (2002).
16. Choi, H. J. *et al*. Hepatoprotective effects of *Brassica*

rapa (Turnip) on d-galactosamine induced liver injured rats. *Kor J Pharmacogn* **37**:258-265 (2006).
17. Griffitt, R. J. *et al*. Exposure to copper nanopaticles causes gill injury and acute lethality in zebrafish (Danio rerio). *Environ Sci Technol* **41**:8178-8186 (2007).
18. Brumfiel, G. Consumer products leap aboard thenano band-wagon. *Nature* **440**:262 (2006).
19. Stone, V. *et al*. Air pollution, ultrafine and nanoparticle toxicology cellular and molecular interactions. *IEEE Trans Nanobioscience* **6**:331-340 (2007).
20. Müblfeld, C. *et al*. Translocation and cellular entering mechanisms of nanoparticles in the respiratory tract. *Swiss Med Wkly* **138**:387-391 (2008).