J. Food Sci. Nutr.
Vol. 5, No. 1, p. 37~41 (2000)

Effects of Ethylacetate Fraction of Persimmon Leaves on Experimentally-induced Gastric Mucosal Damage and Gastric Ulcers in Rats

Myung-Hee Choo, Hyun-Suk Choi, Kil-Man Shin, Soon-Teck Jung*, Kyong-Su Kim and Myung-Yul Lee†

Department of Food and Nutrition, Chosun University, Kwangju 501-759, Korea
**Department of Food Science and Technology, Mokpo National University, Chonnam 534-729, Korea*

Abstract

The protective effects of the ethylacetate fraction of persimmon leaves (PEF) against experimentally induced gastric mucosal damage and gastric ulcers were evaluated in rats. In the prophylatic study, 100 mg/kg ethylacetate fraction of persimmon leaves (PEFH) exhibited a total protection of 73.8% and 65.7% against HCl-ethanol and 0.2 N NaOH-induced gastric mucosal membrane lesions, respectively, which was superior to cimetidine 50 mg/kg, a commonly used anti-ulcer drug. PEFH showed excellent anti-ulcer effects against pylorus ligation induced gastric ulcers, compared to the control group, however, 50 mg/kg ethylacetate fraction of persimmon leaves (PEFL) and PEFH did not affect ulcers induced by water immersion stress, and that is inferior to cimetidine 50 mg/kg. In conclusion, the results suggest that the ethylacetate fraction of persimmon leaves can be used both in prevention and treatment of experimentally induced gastric mucosal damage and ulcers.

Key words: persimmon leaves, ethylacetate fraction, prevention and treatment, HCl-ethanol, water immersion stress, gastric ulcer, mucosal damage

INTRODUCTION

Persimmon leaves (leaves of *Diospyros kaki* Tunberg.) have been used as a herb medicine for the treatment of coagulation, apoplexy, hematemesis, chilblain, asthma, atherosclerosis and diabetes mellitus (1,2); its usage is expanded for the preparation of herb tea (3,4) and traditionally employed for the treatment of hypertension (5-7). It has been found that condensed tannins and flavonoids from persimmon leaves are mainly responsible for the antihypertensive, antimutagenic, anticarcinogenic and antioxidative actions (8-10). Four flavonoids such as astragalin, kaempferol-3-O-(2″-O-gallyl)-(2″-O-gallyl)-glucoside, isoquercetin and quercetin-3-O-(2″-O-gallyl) glucoside, have been isolated from persimmon leaves (11). Especially flavonoid aglycones in persimmon leaves, such as catechin, kaempferol, and quercetin reportedly possess strong antioxidative activities acting as oxygen radicals scavenger, metal chelator and lipid peroxidation inhibitors (12-14). And kaempferol inhibits the synthesis of nucleic acid in tumor cells and transcription by RNA polymerase II (15). However, more systematic studies on the characterization of persimmon flavonoid glycosides and their antioxidative activity are not available yet.

Recently, flavonoids contained in herb medicines were found to be effective in cases of experimentally-induced gastric mucosal damage and gastric ulcers in rats (16). However, very few studies have examined their effectiveness in pharmacological experiments.

The objective of this study was to investigate the effects of the ethylacetate fraction of persimmon leaves on gastric mucosal membrane protection and the recovery from gastric damage and ulcers.

MATERIALS AND METHODS

Materials

Fresh leaves of persimmon, *Diospyros kaki* Tunberg. (Ebenaceae), were extracted twice with methanol-water (80 : 20, v/v), and the extracts were then fractionated with n-hexane, chloroform, ethylacetate, n-butanol and water. The ethylacetate fraction showed the highest antioxidative activity, evaluated by the DPPH test, among these extracts and was divided two groups of doses ; 50 mg/kg, 100 mg/kg, respectively.

Animals

Male Sprague-Dawley rats (200~250 g), aged 7 weeks, purchased from the experimental animal center of Chosun University, were used in this study. The animals were kept under standard laboratory conditions and allowed access to rodent chow (Che-il, Korea) and drinking water. Animals were divided into 4 groups : control group (CON), ethylacetate fraction of persimmon leaves 50 mg/kg administered group (PEFL), ethylacetate fraction of persimmon leaves 100 mg/kg administered group (PEFH) and cimetidine 50 mg/kg administered group (CMT).

HCl-ethanol induced gastric mucosal membrane lesions

Male rats weighing approximately 200 g were divided into 4 groups of 5 animals. After 24 hr of fasting, they were orally

†Corresponding author. E-mail: mylee@mail.chosun.ac.kr
Phone: 82-62-230-7722, Fax: 82-62-224-8880

administered test samples. After 30 min of administration, each rat was treated with 1 ml of 150 mM HCl-60% ethanol. Each rat was then killed by ether 6 hr after the treatment of the necrotizing agent, and the stomach was excised and filled in 2%-formaline solution (17). Lesion index was calculated by adding the length (mm) of the lesions in the fundus region. Student's *t*-test was used for the statistical analysis.

0.2 N NaOH induced gastric mucosal membrane lesions

The examination was the same method as in the HCl-ethanol induced gastric mucosal membrane lesions (17) except that 0.2 N NaOH was used as the necrotizing agent instead of HCl-ethanol.

Indomethacin induced gastric mucosal membrane lesions

The examination was the same method as in the HCl-ethanol induced gastric mucosal membrane lesions except that test samples were given orally 10 min before treatment of indomethacin (20 mg/kg, s.c.) suspended in 1% CMC (carboxy methyl cellulose) as the necrotizing agent instead of HCl-ethanol (18).

Pylours ligation induced gastric ulcers

Male rats were divided into 4 groups of 4~6 animals. After 48 hr of fasting, rats were anesthetized with ether and the abdomen was opened. The pylours region of the stomach was ligated, followed by administration of test samples into the duodenum. After 6 hr of administration, the stomach was excised and fixed in 2%-formaline solution. The severity of the ulcer was determined based on the ulcer formation in the forestomach as follows : 0: normal; 1: local bleeding; 2: two or more sites of small ulceration (<3 mm); 3: five or more sites of small ulceration or one or more sites of large ulceration; 4: two or more sites of large ulceration; and 5: gastric perforation (19). The perforated stomach was scored 50% in all.

$$\text{Inhibition ratio (\%)} = \frac{\text{ulcer index (control) - ulcer index (test samples)}}{\text{ulcer index (contorl)}} \times 100$$

Water immersion stress induced gastric ulcers

Male rats were divided into 4 groups of 4~6 animals. After 24 hr of fasting, test samples were administered orally, and each animal was put into a vinyl chloride pipe, which is covered at the top and bottom with metal mesh, and immersed in water (22°C) to the neck. After 5 hr the animals were sacrificed and the stomachs were excised. Each stomach was then fixed in 2%-formaline solution (20). The ulcer index and statistical analysis were determined by the same method as for pylorus ligation induced gastric ulcers.

Adaptive cytoprotection on absolute ethanol-induced gastric mucosal damage

After 24 hr of fasting, male rats were divided into 6 groups of 5 animals as follows:

I. absolute ethanol treated group;
II. 10% ethanol administered group before absolute ethanol treatment;
III. ethylacetate fracton of persimmon leaves (50 mg/kg) administered group before 4 hr of absolute ethanol treatment;
IV. ethylacetate fracton of persimmon leaves (50 mg/kg) administered group before 3.5 hr of 10% ethanol and 4 hr of absolute ethanol treatment;
V. cimetidine (50 mg/kg) administered group before 4 hr of absolute ethanol treatment;
VI. cimetidine (50 mg/kg) administered group before 3.5 hr of 10% ethanol and 4 hr of absolute ethanol treatment.

After 1 hr the animals were sacrificed and the stomachs were excised and fixed in 2%-formaline solution (21). Lesion index and the statistical analysis were calculated by the same method as for HCl-ethanol induced gastric mucosal membrane lesions.

RESULTS AND DISCUSSION

Effects of ethylacetate fraction of persimmon leaves on HCl-ethanol induced gastric mucosal membrane lesions

As shown in Table 1, the ethylacetate fraction of persimmon leaves at 100 mg/kg significantly inhibited the gastric mucosal membrane lesions induced by the treatment of HCl-ethanol, which is used for the examination of cellular protective actions, as compared to the control group. That was superior to cimetidine 50 mg/kg, a commonly used anti-ulcer drug. It has been shown that drugs which are effective against HCl-ethanol induced gastric mucosal membrane lesions can possess gastric mucosal membrane protective actions (22,23). The anti-ulcer effects of the ethylacetate fraction of persimmon leaves were shown in a dose-dependant manner. These results suggest that the ethylacetate fraction of persimmon leaves may activate the gastric mucosal membrane protective

Table 1. Effects of persimmon leaves ethylacetate fraction on HCl-ethanol induced gastric lesions in rats

Treatment[1)]	Dose (mg/kg, p.o.)	No. of rats	Gastric lesion (mm)	Inhibition (%)
CON	-	6	211.4±10.7[2)]	-
PEFL	50	6	154.6±14.1*[3)]	26.9
PEFH	100	6	55.6± 7.9**[4)]	73.8
CMT	50	6	105.9±25.4*	49.9

Gastric lesions were induced by treatment of 1ml of HCl-ethanol(60% ethanol+150 mM HCl). Each sample was given orally 1 hr before HCl-ethanol treatment. Animals were sacrificed 1 hr after HCl-ethanol treatment.

[1)]PEFL : Persimmon ethylacetate fraction (50 mg/kg, b.w./day, p.o.)
PEFH : Persimmon ethylacetate fraction (100 mg/kg, b.w./day, p.o.)
CMT : Cimetidine (50 mg/kg, b.w./day, p.o.)

[2)]Mean±S.E.

[3)]Significantly different from control at $p<0.05$.

[4)]Significantly different from control at $p<0.01$.

mechanism and thereby exhibit anti-ulcer effects.

Effects of ethylacetate fraction of persimmon leaves on 0.2 N NaOH induced gastric mucosal membrane lesions

As shown in Table 2, the oral administration of ethylacetate fraction of persimmon leaves at 100 mg/kg significantly abolished the gastric lesions induced by 0.2 N NaOH treatment by 65.7% as compared to the control group.

Effects of ethylacetate fraction of persimmon leaves on indomethacine induced gastric mucosal membrane lesions

Indomethacine produced moderate mucosal damage in the gastrointestinal tract including the stomach and small and large intestine (24). The gastric lesion scores of 50 mg/kg and 100 mg/kg of ethylacetate fraction of persimmon leaves, and cimetidine 50 mg/kg were 10.5±2.2, 4.5±0.3, and 7.4±0.7, respectively, shown in Table 3. All the test samples were proved to be effective against indomethacine induced gastric mucosal membrane lesions, but the ethylacetate fraction of persimmon leaves at 100 mg/kg markedly attenuated the disease activity index as compared to the control group.

Effects of ethylacetate fraction of persimmon leaves on pylorus ligation induced gastric ulcers.

As shown in Table 4, ethylacetate fraction of persimmon leaves (50 mg/kg) did not show any significant inhibitory action against pylorus ligation induced gastric ulcers, but the ethylacetate fraction of persimmon leaves (100 mg/kg) inhibited significantly the ulceration by 63.0% as compared to the control group, and that was superior to the cimetidine 50 mg/kg administered group.

Table 2. Effects of persimmon leaves ethylacetate fraction on 0.2 N NaOH induced gastric lesions in rats

Treatment[1)]	Dose (mg/kg, p.o.)	No. of rats	Gastric lesion (mm)	Inhibition (%)
CON	-	5	49.9±8.4[2)]	-
PEFL	50	5	41.4±7.1	17.1
PEFH	100	5	17.1±5.3**[4)]	65.7
CMT	50	5	26.1±7.8*[3)]	27.6

Gastric lesions were induced by treatment of 1 ml of 0.2 N NaOH. Each sample was given orally 1 hr before 0.2 N NaOH treatment. Animals were sacrificed 1 hr after 0.2 N NaOH treatment.
[1)]See the legend of Table 1.
[2)]Mean±S.E.
[3)]Significantly different from control at $p<0.05$.
[4)]Significantly different from control at $p<0.01$.

Table 3. Effects of persimmon leaves ethylacetate fraction on indomethacin induced gastric lesions in rats

Treatment[1)]	Dose (mg/kg, s.c.)	Gastric lesion (mm)	Inhibition (%)
CON	-	26.7±3.8[2)]	-
PEFL	50	10.5±2.2**[3)]	60.5
PEFH	100	4.5±0.3**	83.2
CMT	50	7.4±0.7**	72.3

Gastric lesion were induced by giving indomethacin (20 mg/kg, s.c.). Each sample was given orally 10 min before indomethacin treatment. Animals were sacrificed 7 hr after indomethacin treatment.
[1)]See the legend of Table 1.
[2)]Mean±S.E.
[3)]Significantly different from control at $p<0.01$.

Table 4. Effects of persimmon leaves ethylacetate fraction on gastric ulcer in pylorus ligated rats

Treatment[1)]	Dose (mg/kg, i.d.)	Ulcer index	Inhibition (%)
CON	-	4.8±0.7[2)]	-
PEFL	50	3.6±0.5	25.0
PEFH	100	1.8±0.3**[4)]	63.0
CMT	50	2.5±0.4*[3)]	48.9

Each sample was given intraduodenally immediately after pylorus ligation. Animals were sacrificed 6 hr after pylorus ligation.
[1)]See the legend of Table 1.
[2)]Mean±S.E.
[3)]Significantly different from control at $p<0.05$.
[4)]Significantly different from control at $p<0.01$.

Effects of ethylacetate fraction of persimmon leaves on water immersion stress induced gastric ulcers

Table 5 shows that the oral administration of the ethylacetate fraction of persimmon leaves at 50 mg/kg and 100 mg/kg did not have any inhibitory effect on the stress induced ulcers, whereas a dose of 50 mg/kg cimetidine significantly prevented stress induced ulcers. The reduction of the ulcer index by the ethylacetate fraction of persimmon leaves at 10 mg/kg was half that of cimetidine (50 mg/kg).

Effects of ethylacetate fraction of persimmon leaves on adaptive cytoprotection by absolute ethanol-induced gastric mucosal damage

As shown in Table 6, the cytoprotective effects of the ethylacetate fraction of persimmon leaves at 50 mg/kg against absolute ethanol- induced gastric mucosal damage was significantly diminished by 66.7% as compared to the control group, which is an absolute ethanol-single treated group. And it was found that the ethylacetate fraction of persimmon leaves at 50 mg/kg significantly enhanced the adaptive cyto-

Table 5. Effects of persimmon leaves ethylacetate fraction on stress induced gastric ulcer in rats

Treatment[1)]	Dose (mg/kg, p.o.)	Ulcer index	Inhibition (%)
CON	-	2.3±0.4[2)]	-
PEFL	50	1.9±0.2	17.4
PEFH	100	1.6±0.2*[3)]	30.4
CMT	50	0.7±0.3**[4)]	69.6

Gastric ulcers were induced by immersing animals into the water bath (22°C) up to the xiphoid process. Each sample was given orally 10 min before stress. Animals were sacrificed 5 hr after immersion into the water bath.
[1)]See the legend of Table 1.
[2)]Mean±S.E.
[3)]Significantly different from control at $p<0.05$.
[4)]Significantly different from control at $p<0.01$.

Table 6. Adaptive cytoprotective effects of persimmon leaves ethylacetate fraction on absolute ethanol-induced gastric mucosal damage in rats

Treatment	Dose (mg/kg, p.o.)	NO. of rats	Gastric lesion (mm)	Inhibition (%)
EtOH	-	5	223.8±35.6[1)]	-
10% EtOH+EtOH	-	5	113.2±15.3**[2)]	49.4
PEFL+EtOH	50	5	191.2±12.8	14.6
PEFL+10% EtOH+EtOH	50	5	74.6±958**	66.7
Cimetidine+EtOH	50	5	167.1±20.2	25.3
Cimetidine+10% EtOH+EtOH	50	5	82.7±7.3**	63.1

Gastric lesions were induced by treatment of 1ml of EtOH. Each sample was given orally 4 hr before EtOH treatment. Animals were sacrificed 1 hr after EtOH treatment.
[1)]Mean±S.E.
[2)]Significantly different from control at $p<0.01$.

protective action of 10% ethanol against the absolute ethanol-induced gastric mucosal damage, though its exact underlying mechanism remains to be clarified (25). The present findings demonstrate the ethylacetate fraction of persimmon leaves exerts gastric protective actions for the stomach against absolute ethanol treatment, but more experimental studies about the level of prostaglandins and NO (nitric oxide) in the gastric mucosal membrane in order to examine the protective effects should be undertaken.

ACKNOWLEDGEMENTS

This work was supported by the Food Industrial Technology Research Center, Mokpo National University, for which the authors are deeply appreciative.

REFERENCES

1. Shanghai Science and Technologic Publisher and Shougakukan : *The dictionary of Chinese drugs.* Shougakukan, Tokyo, p.15 (1978)
2. Matsuo, Y. and Ito, S. : The chemical structure of kaki tannin from immature fruit of the persimmon (*Diospyros kaki* L.). *Agric. Biol. Chem.*, **42**, 1637 (1978)
3. Chung, S.H., Moon, K.D., Kim, J.K., Seong, J.H. and Sohn, T.H. : Changes of chemical components in persimmon leaves during processing persimmon leaves tea. *Korean J. Food Sci. Technol.*, **26**, 141 (1994)
4. Park, Y.J., Kang, M.H., Kim, J.L., Park, O.J., Lee, M.S. and Jang, H.H. : Changes of vitamin C and superoxide-like activity of persimmon leaf tea by processing method and extraction condition. *Korean J. Food Sci. Technol.*, **27**, 281 (1995)
5. Kameda, K., Takaku, T., Okuda, H. and Kimura, Y. : Inhibitory effects of various flavonoids isolated from leaves of persimmon on angiotensin-converting enzyme activity. *J. Natural Products*, **50**, 680 (1987)
6. Uchida, S., Ohta, H., Niwa, M., Mori, A., Nonaka, G., Nishioka, I. and Ozaki, M. : Prolongation of life span of stroke-prone spontaneously hypertensive rats (SHRSP) ingesting persimmon tannin. *Chem. Pharm. Bull.*, **38**, 1049 (1990)
7. Funayama, S. and Hikino, H. : Hypotensive principles of *Diospyros kaki* leaves. *Chem. Pharm. Bull.*, **27**, 2865 (1979)
8. Moon, S.H. : The antimutagenic and anticarcinogenic effects of persimmon leaves. *Ph. D. thesis*, Pusan University, Pusan, Korea (1993)
9. Kim, B.G., Rhew, T.H., Choe, E.S., Chung, H.Y., Park, K.Y. and Rhee, S.H. : Effects of selected persimmon leaf components against Sarcoma 180 induced tumor in mice. *J. Korean Soc. Food Nutr.*, **22**, 334 (1993)
10. Choi, S.W., Chung, S.K. and Kang, W.W. : Antioxidative activity of catechin derivatives. the 53th Korean Soc. of Food Sci. and Technol. Congresss, Oral Presentation, November 5 (1994)
11. Choi, S.W., Kang, W.W., Chung, S.K. and Cheon, S.H. : Antioxidative activity of flavonoids in persimmon leaves. *Foods and Biotechnology*, **5**, 119 (1996)
12. Bors, W. and Saran, M. : Radical scavenging by flavonoid antioxidants. *Free Radic. Res. Commun.*, **2**, 289 (1987)
13. Morel, I., Lescoat, G., Cogrel, P., Sergent, O., Pasdeloup, N., Brissot, P., Cillard, P., Cogrel, P. and Cillard, J. : Antioxodants and iron-chelating activities of the flavonoids catechin, quercetin and diosmetin on iron-loaded rat hepatocyte cultures. *Biochem. Pharmacol.*, **45**, 13 (1993)
14. Das, N.P. and Ratty, A.K. : Effects of flavonoids on induced non-enzymatic lipid peroxidation. In "*Plant Flavonoids in Biology and Medicine*" Cody, V., Middleton, E. and Harbome, J.B. (eds.), Alan R. Liss, New York, p.243 (1986)
15. Nose, K. : Inhibition by flavonoids of RNA synthesis in permeable W1-38 cells and of transcription by RNA polymerase II. *Biochem. Pharm.*, **33**, 3823 (1984)
16. Oh, T.Y., Ahn, B.O., Ko, J.I., Ryu, B.K., Son, M.W., Kim, S.H., Kim, W.B. and Lee, E.B. : Studies on protective effect of DA-9601, an Artemisiae Herba extract, against ethanol-induced gastric mucosal damage and its mechanism. *J. Applied Pharmacol.*, **5**, 202 (1997)
17. Robert, A., Nezamis, J.E., Lancaster, C. and Hanchar, A.J. : Cytoprotection by prostaglandins in rats: Prevention of gastric necrosis produced by alcohol, HCl, NaOH, hypertonic NaCl and thermal injury. *Gastroenterology*, **77**, 433 (1979)
18. Matsumoto, T., Iida, M., Nakamura, S., Hizawa, K., Kuroki, F. and Fujishima, M. : An animal model of longitudal ulcers in the small intestine induced by intracoloically administered inodmethacine in rats. *Gastroenterologia Japonica*, **28**, 10 (1993)
19. Yamahara, J., Konoshima, T., Sawada, T. and Fujimura, H. : Biologically active principles of crude drugs. Pharmacological actions of *Swertia japobica* extracts, swertiamarin and gentianine. *Yakugaku Zasshi*, **98**, 1446 (1978)
20. Yamahara, J., Kobayashi, M., Miki, K., Kozuka, M., Sawada, Y. and Fujimura, H. : Cholagogic and antiulcer effect of *Saussureae Radix* and its active components. *Chem. Pharm. Bull.*, **33**, 1285 (1985)
21. Robert, A., Nezamis, J.E., Lancaster, C. and Davis, J.P. : Mild irritants prevent gastric necrosis through adaptive cytoprotection mediated by prostaglandins. *Am. J. Physiol.*, **245**, G113 (1983)
22. Oates, P.J. and Hakkinen, J.P. : Studies on the mechanism of ethanol-induced gastric damage in rats. *Gastroenterology*, **94**, 10 (1988)
23. Mizui, T. and Doteuchi, M. : Effects of polyamines on acidified

ethanol-induced gastric lesions in rats. *Japan J. Pharmacol.*, **55**, 939 (1983)
24. Yamasaki, K., Kanbe, T., Chijiwa, T., Ishiyama, H. and Morita, S. : Gastric mucosal protection by OPC-12759, a novel antiulcer compound, in the rat. *Eur. J. Pharmacol.*, **142**, 23 (1987)
25. Wallace, J.L., Morris, J.P., Krausse, E.J. and Greaves, S.E. : Reduction by cytoprotetive agents of ethanol-induced damage to the rat gastric mucosa: a correlated morphological and physiological study. *Can. J. Physiol. Pharmacol.*, **60**, 1686 (1982)

(Received January 12, 2000)