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# Anti-ulcerogenic effect of *Momordica charantia* L. fruits on various ulcer models in rats

İlhan Gürbüz, Çiğdem Akyüz, Erdem Yeşilada *, Bilge Şener

*Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, Hipodrom 06330, Ankara, Turkey*

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**Abstract**

The mature fruits of *Momordica charantia* L. (Cucurbitaceae) are used externally for the rapid healing of wounds and internally for the treatment of peptic ulcers in Turkish folk medicine. For the evaluation of the latter activity, ethanol-induced ulcerogenesis model in rats was employed. The olive oil extract of the material as well as dried-powdered fruits in filtered honey showed significant and dose-dependent anti-ulcerogenic activity against this model. A potent and dose-dependent inhibitory activity was also observed by the administration of ethanol extract of the fruits. For the bioassay-guided fractionation, the material was first extracted with hexane and then by ethanol and both extracts were found active against the same ulcer model. Furthermore, ethanol extract of the fruits showed significant activity against HCl–EtOH induced ulcerogenesis in indomethacin-pretreated rats and diethyldithiocarbamate-induced ulcer models. 

*Keywords:* *Momordica charantia* L. fruit; Cucurbitaceae; Antiulcerogenic activity; Cytoprotective effect

## 1. Introduction

*Momordica charantia* L. (Cucurbitaceae) is cultivated throughout the tropics, particularly in India, China, East Africa and South America and used in many countries as a folk remedy for various ailments. Especially the vivid orange-colored and oblong-shaped fruits of the plant are in use as drug and vegetable. Unripe fruits of the plant are mainly used for diabetes and extensive investigations have shown that an extract of the fruits has marked hypoglycemic properties both in animals and human. Several constituents including charantin (mixture of sterol glucosides), vicine (pyrimidine nucleoside) and p-insulin (polypeptide) are reported as the active ingredients for this purpose (Oliver-Bever, 1986; Raman and Lau, 1996). In addition to this effect, it is used for stomach aches, colds and fevers, rheumatism, gout, and the induction of abortion. In Ayurvedic medicine, it has been prescribed as stimulant, blood purifier, laxative and antihelmintic. Powdered fruit is also claimed to be useful in healing wounds, leprous and malignant ulcers (Raman and Lau, 1996).

Although *M. charantia* is not native in Turkey, the fruits, popularly known as 'kudret narı', are

* Corresponding author. Fax: +90-312-2235018.
*E-mail address:* yesilada@tr-net.net.tr (E. Yeşilada)

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frequently used in folk medicine, especially in the western parts of Anatolia (Yeşilada et al., 1999a). However, most of the above-mentioned medicinal features of the fruits are not recognized in Turkish folk medicine. The matured fruits are used externally for the rapid healing of wounds and internally for the treatment of peptic ulcers (Baytop, 1984). The material is supplied either from the ornamental plants, which are cultivated at gardens in the warm locations or from herb dealers. Recently, the plant is also cultivated in the fields in the Marmara region (western Anatolia).

The aim of the present study is to evaluate the anti-ulcerogenic effect of the plant using various experimental ulcer models.

## 2. Materials and methods

### 2.1. Plant material

*M. charantia* L. mature fruits were purchased from a cultivator in Bursa (Akköy, Çelebi Çiftliği) in September, 1997. A voucher specimen is stored in the Herbarium of Gazi University, Faculty of Pharmacy (97D001). Fresh fruits were cut into small pieces and a portion was immediately put into deep-freezer to process later. Another portion of cut fruits was immersed into olive oil and put into refrigerator, while the other portion was dried at room temperature and powdered.

### 2.2. Chemical processing

The dried and powdered material (400 g) was extracted at room temperature with EtOH 96% (16 × 1.5 l) and the combined extracts was evaporated to dryness under reduced pressure (31.1 g; 7.8% yield). The residue was then fractionated with successive solvent extraction with hexane, diethyl ether and BuOH, but the yield of the latter two extracts was found to be too low for in vivo testing. Thus the fractionation process was carried out by first extracting the dried and powdered material (50 g) with hexane (8 × 200 ml) and the organic solvent evaporated under reduced pressure to give 0.52 g extract. Then the remaining material was extracted with EtOH 96% (2 × 700 ml) by continuous stirring at room temperature and the extract evaporated to dryness under reduced pressure (R-EtOH; 0.6 g). For the TLC analysis of the extracts a toluene:aceton (85:15) solvent system was used and spots were visualized by spraying with anisaldehyde-sulphuric acid reagent.

### 2.3. Biological methods

Wistar rats of either sex weighing 120–150 g were used in biological tests (Gülhane Military Academy of Medicine, Ankara). The animals were left for 48 h to acclimatize to the animal room conditions and were maintained on standard pellet diet and water ad libitum. The food was withdrawn 24 h before the experiment, but the animals were allowed free access of water. Coprophagy was prevented by fasting the rats in wire-bottomed cages. The control group animals received the same experimental handling as those of the test groups except that the drug treatment was replaced by administration of appropriate volumes of the dosing vehicle. Test samples were administered to animals in a 5 ml/kg bodyweight dosage as a suspension in either 0.5% carboxymethyl cellulose/distilled water or 0.5% of Tween 80 or olive oil by means of a gavage. On the other hand, as a result of the sticky character, the preparation of dried-powdered fruits in filtered honey was given to animals by means of a small spoon.

#### 2.3.1. Effects on the ulceration induced by ethanol

Test sample was administered orally 15 min before the oral administration of ethanol 96% (1 ml) to a group of six rats. Later (1 h), the animals were sacrificed with an over-dose of ether. The stomachs were then removed and inflated with 10 ml of 1% formalin solution and immersed in the same solution to fix the outer layer of stomach. Each stomach was opened along the great curvature, rinsed with tap water to remove gastric contents and blood clots and examined under a dissecting microscope (20 × 6.3 × ) with a square-grid eyepiece to assess the formation of ulcers. The sum of length (mm) of all lesions for each stomach was used as the ulcer index (UI), and the

inhibition percentage was calculated by the following formula;

$$[(UI_{Control} - UI_{Treated})/UI_{Control}] \times 100.$$

*2.3.2. Effects on the ulceration induced by HCl + ethanol in indomethacin-pretreated rats*

After 90 min the s.c. injection of 5 mg/kg of indomethacin to rats, test samples were administered orally and 30 min later each rat was orally treated with 150 mM HCl in 60% of ethanol (1 ml). After 1 h the induction of ulcerogenesis, rats were sacrificed and treated as described above (Yamomoto et al., 1992).

*2.3.3. Effects on the ulcerogenesis induced by diethyldithiocarbamate*

Test samples were administered to rats orally 30 min before the surgical operation. The rats were then anesthetized with ether and a midline laparotomy was performed to ligate the pylorus of each. Immediately after the operation, each rat was treated *s.c* with 1.5 ml of diethyldithiocarbamate (Sigma) in saline at a dose of 800 mg/kg and 1 ml oral dose of 0.1 N HCl. The rats were killed 5 h after the pylorus ligation with over-dose of ether. The stomach was removed and inflated by filling 10 ml of 10% formalin from the oesophagus. The ulcer index was measured as described before.

*2.4. Statistical analysis of data*

Results were expressed as mean ± S.E.M. The statistical difference between the mean ulcer index of the treated group and that of the control was calculated by using Student's *t*-test.

## 3. Results and discussion

The common preparation type, which is practiced in Turkish folk medicine is to use the oily extract; the whole matured fruits of *M. charantia* are cut into pieces and put inside a jar of pure olive or almond oil and left under sunshine until the seeds melted, for about 2 or 3 weeks, then they are homogenized by pressing with a hard material, i.e. spoon. This ointment is kept at home to use when necessary for the rapid healing of wounds, abscess and eczematous skin by applying two times a day, externally, as well as for the treatment of gastric and duodenal ulcers, internally (Baytop, 1984). For the latter purpose, a tablespoon of extract should be ingested 30 min before breakfast on an empty stomach every morning. The paste prepared by pounding the fresh fruits or dried-powdered fruits mixed with filtered pure honey is also prescribed to treat peptic ulcers and these preparations are also advised to be administered on an empty stomach every morning (Baytop, 1984).

In a previous study, anti-ulcerogenic activity of the oily extract of the fruits as well as solvent extracts (hexane-, chloroform-, ethanol- and water-fractions) was studied using immobilization plus cold-induced stress and indomethacin-induced ulcer models in rats; the material and fractions were found to be totally ineffective (Yıldırım, 1994). The authors even claimed that the olive oil or honey itself which are used as vehicle to prepare the drug are more effective than the oily extract of the fruits. Thus they attributed the healing effect of this folk remedy to these vehicles, in addition to a placebo effect.

In our opinion, the model employed by these researchers may not be convenient for the correct activity assessment of the material. It is reported that stress-induced gastric lesions are mainly as a result of the increased acid output and are markedly inhibited by antisecretory drugs (Brodie et al., 1962; Okabe et al., 1977). Considering that the fruit is also effectively used for the rapid healing of wounds in folk medicine, which may be an indication of some cytoprotective activity, it seemed more logical to employ ulcer models induced by corrosive agents. Indomethacin-induced ulcerogenesis employed in the aforementioned study is also known as a representative model of this type, but any healing effect against this model could not be observed as well. Thus, we decided to test the activity of the fruits using another model of this type and employed the ethanol-induced ulcerogenesis model in rats (Robert et al., 1979).

As shown in Table 1, the olive oil extract of *M. charantia* fruits showed a dose dependent and

significant anti-ulcerogenic activity (94.5–98% inhibition). We also tested the effect of the dried fruits when mixed with filtered honey and observed that the stomachs were completely prevented from the formation of ulcers (Table 2). In both experiments the vehicles themselves, i.e. olive oil and honey, also exhibited some inhibitory activity against this model of ulcer, 58.1 and 94.3%, respectively, when compared to ulcer index obtained through the administration of 0.5% carboxymethylcellulose suspension (Table 3). This conclusion was also pointed out by Yıldırım (1994), but the author reported the inhibitory activity of the olive oil to be more prominent than that of the oily extract of fruits.

The doses administered to rats in the present study were estimated from human dose (a desertspoon of dried and powdered fruit weighted about 4.76 g). In the study of Yıldırım (1994), however, the olive oil extract and each of the solvent extracts, i.e. hexane, chloroform, ethanol and water, were administered in equal doses (500 mg/kg), which were very close to those administered in the present study (we employed 330 and 660 mg/kg of olive oil extract and 310 and 620 mg/kg of EtOH extract for activity assessment). Thus, the different results observed in these two studies are not caused by the administration of different doses.

Table 1
Anti-ulcerogenic activity of olive oil extract of *M. charantia* fruits against ethanol-induced ulcerogenesis

| Test sample | Dose per os (mg/kg) | Ulcer index (mean ± S.E.M.) | Prevention ratio[a] | Inhibition (%) |
|---|---|---|---|---|
| Control (olive oil) | | 415.8 ± 197 | 0/6 | |
| Olive oil extract | 330 | 22.5 ± 16.5** | 2/6 | 94.5 |
| Olive oil extract | 660 | 8.75 ± 8.75*** | 3/6 | 97.9 |

[a] Number of stomachs completely prevented from any bleeding or lesion.
** $P<0.01$ significant from control.
*** $P<0.001$ significant from control.

Table 2
Anti-ulcerogenic activity of dried and powdered fruits of *M. charantia* in filtered honey against ethanol-induced ulcerogenesis

| Test sample | Dose per os (mg/kg) | Ulcer index (mean ± S.E.M.) | Prevention ratio[a] | Inhibition (%) |
|---|---|---|---|---|
| Control (filtered honey) | | 55.7 ± 24.3 | 0/3 | |
| Dried-powdered fruit/honey | 4760 | 0.0 ± 0.0* | 3/3 | 100.0 |

[a] Number of stomachs completely prevented from any bleeding or lesion.
* $P<0.05$ significant from control.

Table 3
Anti-ulcerogenic activity of ethanol extract of *M. charantia* fruits and fractions against ethanol-induced ulcerogenesis

| Test sample | Dose per os (mg/kg) | Ulcer index (mean ± S.E.M.) | Prevention ratio[a] | Inhibition (%) |
|---|---|---|---|---|
| Control (0.5% CMC) | | 993.0 ± 247.0 | 0/6 | |
| EtOH extract | 310 | 443.1 ± 170.2* | 2/6 | 55.7 |
| EtOH extract | 620 | 18.8 ± 18.8*** | 5/6 | 98.1 |
| Hexane extract | 290 | 228.0 ± 127.1* | 1/5 | 77.0 |
| R-EtOH extract[b] | 330 | 198.3 ± 120.8* | 2/6 | 80.0 |

[a] Number of stomachs completely prevented from any bleeding or lesion.
[b] After hexane extraction the residue was extracted with ethanol 96%.
* $P<0.05$ significant from control.
*** $P<0.001$ significant from control.

Table 4
Effects of ethanol extract of *M. charantia* fruits against diethyldithiocarbamate-induced ulcerogenesis in rats

| Test sample | Dose per os (mg/kg) | Ulcer index (mean ± S.E.M.) | Prevention ratio[a] | Inhibition (%) |
|---|---|---|---|---|
| Control (0.5% CMC) | | 23.1 ± 5.4 | 0/6 | |
| EtOH extract | 310 | 5.0 ± 1.1* | 0/4 | 78.4 |
| EtOH extract | 620 | 12.9 ± 4.2 | 2/6 | 44.2 |

[a] Number of stomachs completely prevented from any bleeding or lesion.
* $P < 0.05$ significant from control.

To verify the effect of the fruits, the ethanol extract prepared from the dried fruits was also administered to animals. As shown in Table 3, a dose-dependent and significant activity was observed. For the bioassay-guided fractionation of the active principle(s), the dried fruit was fractionated with successive solvents, hexane, diethyl ether and butanol, but the yields of the latter two extracts were too low for further in vivo testing. Thus, fractionation was performed by extracting the material first with hexane and then with ethanol (R-EtOH). Both fractions showed equally significant activity against the same ulcer model. TLC examination revealed that the hexane-soluble fraction consisted of carotenoids. But carotenoids were not completely extracted by hexane, and R-EtOH extract also contained carotenoids in addition to several more polar compounds.

It has been suggested that active oxygen species may be involved in the pathogenesis of gastric mucosal injuries (Szelenyi and Brune, 1988). Consequently, some radical scavengers were shown to have a protective effect against the mucosal injuries induced by active oxygen species (Oka et al., 1991). Oka et al. (1990) reported an in vivo ulcer model for the assessment of effects of antioxidant compounds on the prevention from gastric injuries. They used a potent metal chelator, diethyldithiocarbamate (DDC), as the inhibitor of cytosolic copper, zinc-SOD to produce gastric mucosal injury.

Carotenoids are known to possess potent antioxidant activity and have been previously reported to prevent the gastric mucosa from the development of injury produced by different noxious agents (Garamszegi et al., 1989; Vincze et al. 1989; Kiraly et al., 1992). Since, high carotenoid content was detected in the active fractions of the *M. charantia* fruits, we also investigated the effect of EtOH extract on DDC-induced ulcerogenesis in rats to explain the involvement of antioxidant effect in the anti-ulcerogenic activity of the material.

As shown in Table 4, the EtOH extract of the fruits significantly inhibited DDC-induced ulcerogenesis at 310 mg/kg dose (78.4% inhibition), but the activity was not consistent and decreased to 44.2%, in a higher dose (620 mg/kg). The results of this experiment may also indicate that the antioxidant components of the fruits, i.e. carotenoids, may have a role in the anti-ulcerogenic effect of this remedy.

As discussed above, various extracts and preparations obtained from the fruits of *M. charantia* showed prominent effect against ethanol- and DDC-induced ulcerogenesis in rats, which may be indication of a cytoprotective activity. We also tested the effect of the active fractions, ethanol and olive oil extracts, on the ulceration induced by HCl + ethanol in indomethacin-treated rats, using more severe conditions of ulcerogenesis. As shown in Table 5, EtOH extract showed a significant anti-ulcerogenic effect and inhibited the formation of ulcer in 45.8%.

On the other hand, involvement of an anti-*Helicobacter pylori* activity was studied in a previous study (Yeşilada et al., 1999b). Chloroform fraction of the fruits showed some inhibitory activity against standard and clinical strains of *H. pylori*.

In conclusion, in spite of a negative previous report about the anti-ulcerogenic effect of the *M. charanthia* fruits, folkloric utilization of the material has been confirmed in the present study by

Table 5
Effects of active fractions of *M. charantia* fruits against HCl+ethanol-induced ulcerogenesis in indomethacin pretreated rats

| Test sample | Dose per os (mg/kg) | Ulcer index (mean ± S.E.M.) | Prevention ratio[a] | Inhibition (%) |
|---|---|---|---|---|
| Control (0.5% Tween 80) | | 106.5 ± 17.2 | 0/6 | |
| EtOH extract | 620 | 57.7 ± 11.6* | 0/6 | 45.8 |
| Olive oil extract | 660 | 102.0 ± 16.6 | 0/6 | 0.9 |
| Famotidine | 20 | 123.3 ± 16.1 | 0/6 | – |

[a] Number of stomachs completely prevented from any bleeding or lesion.
* $P < 0.05$; significant from control.

using different experimental models in rats as well as various extracts and preparations of the material. Thus, the present results also demonstrated that a correct model employment may be crucial for the activity assessment.

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## References

Baytop, T., 1984. Türkiye' de Bitkilerle Tedavi (Phytotherapy in Turkey, Past and Present). İstanbul Üniversitesi Yayınları (Publication), Istanbul.

Brodie, D.A., Marshall, R.W., Moreno, O.M., 1962. Effects of restraint on gastric acidity in the rat. Am. J. Physiol. 202, 812.

Garamszegi, M., Javor, T., Suto, G., Vincze, A., Toth, Gy., Mozsik, Gy., 1989. Effect of atropine, $PGF_{2\alpha}$ and cimetidine on the β-carotene induced cytoprotection in ethanol-treated rats. Acta Physiol. Hung. 73, 221–224 CA. 112:69747x (1990).

Kiraly, A., Suto, G., Vincze, A., Toth, Gy., Matus, Z., Mozsik, Gy., 1992. Correlation between the cytoprotective effect of β-carotene and its gastric mucosal level in indomethacin-treated rats with or without acute surgical vagotomy. Acta Physiol. Hung. 80, 213–218 CA. 121:552y (1994).

Oka, S., Ogino, K., Hobara, T., Yoshimura, H., Okazaki, Y., Takemoto, T., Ishiyaöa, H., Imaizumi, T., Yamasaki, K., Kanbe, T., 1990. Role of active oxygen species in diethyldithiocarbamate-induced gastric ulcer in the rat. Experientia 46, 281–283.

Oka, S., Ogino, K., Hobara, T., Yoshimura, S., Okazaki, Y., Takemoto, T., Iida, Y., 1991. Effects of various mucosal protective drugs on diethyldithiocarbamate-induced antral ulcer in rats. Eur. J. Pharmacol. 197, 99–102.

Okabe, S., Takeuchi, K., Urushidani, T., Takagi, K., 1977. Effects of cimetidine, a histamine $H_2$-receptor antagonist, on various experimental gastric and duodenal ulcers. Am. J. Dig. Dis. 22, 677–684.

Oliver-Bever, B., 1986. 'Medicinal Plants in Tropical West Africa'. Cambridge University Press, Cambridge.

Raman, A., Lau, C., 1996. Anti-diabetic properties and phytochemistry of *Momordica charantia* L. Phytomedicine 2, 349–362.

Robert, A., Nezamis, J.E., Lancester, C., Hanchar, A.J., 1979. Cytoprotection by prostaglandins in rats. Gastroenterology 77, 433.

Szelenyi, I., Brune, K., 1988. Possible role of oxygen free radicals in ethanol-induced gastric mucosal damage in rats. Dig. Dis. Sci. 33, 865.

Vincze, A., Garamszegi, M., Javor, T., Suto, G., Tigyi, A., Toth, Gy., Zsoldos, T., Mozsik, Gy., 1989. The free-radical mechanism in β-carotene induced gastric cytoprotection in hydrochloric acid model. Acta Physiol. Hung. 73, 351–355 CA. 112:69749z (1990).

Yamomoto, K., Kakegawa, H., Ueda, H., Matsumoto, H., Miki, T., Satoh, T., 1992. Gastric cytoprotective anti-ulcerogenic actions of hydroxychalcons in rats. Planta Med. 58, 389–393.

Yeşilada, E., Sezik, E., Honda, G., Takaishi, Y., Takeda, Y., Tanaka, T., 1999a. Traditional medicine in Turkey IX: folk medicine in north-west Anatolia. J. Ethnopharmacol. 64, 199–206.

Yeşilada, E., Gürbüz, İ, Shibata, H., 1999b. Screening of Turkish anti-ulcerogenic folk remedies for anti-*Helicobacter pylori* activity. J. Ethnopharmacol. 66, 289–293.

Yıldırım, Ö.F., 1994. *Momordica charantia* L. (Kudret Narı) Bitkisinin Çeşitli Ekstrelerinin Siçanlarda Deneysel Olarak Oluşturulan Peptik Ülserde Etkisinin Incelenmesi (Studies on the Effect of *Momordica charantia* L. on the Experimentally-Induced Peptic Ulcer in Rats). Yüksek Lisans tezi (Master thesis), Gazi Üniv. Sağlık Bilimleri Enstitüsü (Gazi University, Institute of Medical Sciences).