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Full Length Research Paper

Antioxidant and chemoprotective properties of *Momordica charantia* L. (bitter melon) fruit extract

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Momordica charantia, commonly known as bitter melon, is used as a vegetable in number of countries. Extracts of *M. charantia* plant, fruit pulp, and seed have been reported to have a wide medicinal use in the traditional medical systems, most often as hypoglycemic and anti-diabetic agents. We have studied the effect of *M. charantia,* collected from Kazdaglari (Mount Ida) in Balikesir, fruit extract on glutathione S-transferases (GSTs), cytochrome P450s (CYPs), and antioxidant enzymes in rats. Male Wistar rats, aged 12 weeks and weighing 200-250 g, were given 200 mg *M. charantia* fruit extract per kg body weight, i.p., for four consecutive days. At the end of the experimental period, the animals were sacrificed, and liver, kidney, and lung were isolated. Our results have indicated significant increase in especially hepatic antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities. The strongest increase (about 9-fold) was observed in GPx activities while about 2 to 5-fold increases were observed in SOD and CAT. *M. charantia* fruit extract also exhibited hepatoprotective effects in CCl_4-intoxicated rats. In addition, about 50% increase was also noted with hepatic cytosolic GSTs. On the other hand, treatments of rats with *M. charantia* significantly reduced both ethoxyresorufin *O*-deethylase (EROD) and methoxyresorufin *O*-deethylase (MROD) activities in rat liver microsomes, which are known to be catalyzed by CYP1A isoforms These results suggest that the *M. charantia* fruit extract possesses the anti-oxidant effects besides having protective activities in rats.

Key words: *Momordica charantia*, antioxidant enzymes, glutathione S-transferases, cytochrome P450, chemoprotection.

INTRODUCTION

It is generally accepted that reactive oxygen species (ROS) and/or free radicals play an important role in the development of tissue damage and pathological events in living organisms (Kehrer, 1993; Halliwel and Gutteridge, 1999). There are increasing evidences that increased consumption of fruits and vegetables and intake of certain nonnutrients that are present in foods reduce the risk of various pathological events such as cancer (Goodwin and Brodwick, 1995; Steinmetz and Potter, 1996) and cardio- and cerebro-vascular diseases (Rimm et al., 1996). This is often attributed to the antioxidants in the fruits and vegetables such as vitamin C, E, carotenoids, lycopenes and flavonoids that prevent free radical damages (Stahelin et al., 1991; Steinberg, 1991; Willett, 1994). Thus, much attention has been focused on the investigation of antioxidants that can scavenge ROS, especially natural antioxidants, phenolic and flavonoids from plants which are mostly used as protective agents against free radical-mediated diseases (Shahidi and Wanasundra, 1992; Rice-Evans et al., 1996). In addition, the traditional use of medicinal plants or their crude extracts in the prevention and/or treatment of several chronic diseases has been increasingly practiced in various different ethnic societies worldwide.

This study was designed to specifically investigate the antioxidant and chemoprotective efficacy of *Momordica charantia* fruit extract by investigating antioxidant enzymes, cytochrome P450s (CYPs) and glutathione S-transferases (GSTs) in Wistar rat liver, lung and kidney

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tissues when it is administered *in vivo*.

The antioxidant enzymes (AOE) include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and indirectly glutathione reductase (GR). Their role as protective enzymes are well known and have been extensively investigated both *in vivo* and *in vitro* in model systems. Cytochrome P450 (CYP, EC 1.14.14.1) enzymes are active in monooxygenation and hydroxylation of various xenobiotics, including drugs, carcinogens, environmental pollutants as well as many endogenous substrates, fatty acids, arachidonic acids, steroids, prostaglandins, leukotrienes (Nebert and Gonzales, 1987; Zimniak and Waxman, 1993). Glutathione S-transferases (GST, EC 2.5.1.18) are a complex multigene family of enzymes that are widely distributed in the animal kingdom (Snyder and Maddison, 1997). The most important function of GST is detoxification, conjugating reduced glutathione with a large number of electrophilic metabolites derived from a variety of xenobiotics, including carcinogens, toxins and drugs. GSTs are also involved in the metabolism of endogenous substances such as leukotriene and prostaglandins.

Momordica charantia L. (Cucurbitaceae) known as bitter melon, balsam pear, Karela, etc. is a vegetable indigenous to tropical areas including India, Asia, South America and Africa and cultivated throughout South America as food and medicine. Various preparations of *M. charantia* from injectable extracts to fruit juice to dried fruit bits have been traditionally used worldwide, particularly for blood-sugar lowering effects (Welihinda et al., 1986; Raman and Lau, 1996). In addition, it has been reported to exhibit diverse biological activities such as antioxidant, antimicrobial, antiviral, antihepatotoxic and antiulcerogenic activities which are attributed to an array of biologically active plant chemicals including triterpenes, pisteins and steroids (Grover and Yadav, 2004).

Although *M. charantia* is not a native plant in Turkey, the fruits, known as “kudret nari”, are frequently used in folk medicine, especially in west and southwest part of Anatolia for the treatment of peptic ulcers and tumors. Recently, the plant is being cultivated in Western Anatolia. In the view of ethnomedical reports *M. charantia* is being used in folkloric medicine on various ulcers, diabetes and infections (Gurbuz et al., 2000; Scartezzini and Speroni, 2000; Beloin et al., 2005). Thus, present study was undertaken to further characterize the biological activities of *M. charantia*.

MATERIALS AND METHODS

Plant material

The fruits of *M. charantia* were collected in the fall of 2003 and 2004 from the foot of Kazdaglari (Mount Ida), Balikesir, Turkey.

Mount Ida, which is also very popular in mythology due to its role in legendary Trojan War, is 1774 m. Height Mountain and is located near the Edremit Bay between Ayvalik and Edremit.

Extraction

The collected fruits, air-dried and fine powdered, were extracted with hexane using soxhlet extraction apparatus according to the Soxhlet’s method where materials are extracted by repeated percolation which lasts about 6 - 8 h with hexane under reflux in a special glassware. At the end of extraction process, the solvent is then evaporated and the remaining mass is measured. The percentage yields are calculated as mg per g dried fruits. Finally, residues were dissolved in dimethysulfoxide (DMSO) : water (8:2) mixture.

Animal

Male Wistar rats (12-14 weeks old) weighing 200-250 g were used. They were housed University Animal House in standard conditions and fed with standard diet with water ad libitum. All experimental procedures in animals such as administration of substances by i.p., collection of blood and tissue etc. are performed to the national standards under appropriate regimes with Veterinary services and licensed projects. Rats were randomly assorted into the following four groups (each group consisting of 8-20 rats). Group I (control) some rats were treated with DMSO-water mixture, i.p. daily for 4 days; Group II (Mom) rats were treated with *M. charantia* fruit extract 200 mg/kg, i.p. daily for 4 consecutive days; Group III (CCl_4) rats were administered with CCl_4 to induce hepatotoxicity at dose of 10 mg CCl_4/kg, i.p. daily for 2 consecutive days; and Group IV (Mom + CCl_4) rats were pretreated with 200 mg/kg; i.p. daily for 4 consecutive days prior to administration of CCl_4 10 mg CCl_4/kg, i.p. daily for 2 consecutive days.

At the end of experimental period, the rats were anesthetized with ether following a 16-hr fasting. Blood samples were taken from aorta to determine the serum enzymes. The livers, lungs and kidneys were removed and rinsed with cold physiological saline and stored at -80°C until analyzed.

Chemicals

1-chloro-2,4-dinitrobenzene (CDNB), alanine, aspartic acid, bovine serum albumin (BSA), glutathione reduced (GSH), H_2O_2, glutathione reductase, methoxy- and ethoxyresorufin, cholic acid, β-nicotinamide adenine dinucleotide phosphate (β-NADPH) and pyrogallol were purchased from Sigma (USA). All other chemicals and solvents were of highest purity and analytical grade.

Preparation of tissues subcellular fractions

The tissues were homogenized in 5 parts homogenization solution (1.15% KCl containing 250 mM EDTA, 100 mM phenylmethylsulphonylfluoride, 100 mM butylated hydroxytoluene, 0.025% cholate) using a tissue homogenizer with a teflon pestle at 4°C. The subcellular fractions of rat tissues were prepared by a standard differential centrifugation procedure as described previously (Sen and Kirikbakan, 2004). The amounts of proteins in various fractions were measured using the method of Lowry et al. (1951) with BSA as the standard.

Determination of total antioxidant response

Antioxidant capacity of *M. charantia* was determined by measuring total antioxidant response (TAR) against potent free radical reaction as described by Erel (2004).

Determination of serum AST and ALT and LDH activities

In order to evaluate hepatocellular damage and antihepatotoxic potential of the *M. charantia* fruit extract enzymatic leakage of transaminases [ALanine aminoTransferase (ALT) and ASpartate aminoTransferase (AST)] and Lactate DeHydrogenase (LDH) in serum were measured by the method of Raitman and Frankel (1957) and Wroblewski and La Due (1955), respectively. Blood was centrifuged at 4000 rpm at 4°C for 10 min to get serum.

Determination of antioxidant enzyme activities

SOD activity was measured by the method of Marklund and Marklund (1974). CAT activity was measured using Abei's method (1974). The GPx activity was assayed by using Paglia and Valentine's method (1967).

Determination of phase I and phase II enzyme activities

Cytochrome P450 dependent 7-ethoxyresorufin *O*-deethylase (EROD) and 7-methoxyresorufin *O*-deethylase (MROD) activities were determined by the fluorometric measurements of resorufin formed using the method of Burke and Mayer (1974) as optimized by Sen and Arinc (2000). GST activities using CDNB as substrate were determined as described by Habig et al. (1974).

Statistical analysis

Results are expressed as mean ± SD of at least three determinations for each data point. One way ANOVA, Tukey and Dunnett tests were applied for analyzing the significance of difference between and among different groups.

RESULTS AND DISCUSSION

M. charantia fruit extract was studied for its antioxidant and chemoprotective effects by investigating antioxidant enzymes, cytochrome P450s and glutathione S-transferases as well as its protective effects on hepatocellular damage in CCl_4-intoxicated rats were evaluated.

Free radical scavenging effects of *M. charantia* fruit extract was tested by measuring total antioxidant status of *M. charantia* using a colorimetric method for the total antioxidant response (TAR) as described by Erel (2004). The relative total antioxidant response of *M. charantia* was determined 1.43 ± 0.02 mmol Trolox equiv/L, which was higher than 1 mM GSH, indicating potential antioxidant properties of *M. charantia* fruit extract. The effects of *M. charantia* extract on the serum transaminases and LDH in control and CCl_4-intoxicated rats are given in Table 1. Treatment of rats with *M. charantia* fruit extract alone did not alter the enzyme activities while in the CCl_4-intoxicated groups transaminases (ALT and AST) and LDH activities significantly increased ($p<0.001$). However, the rats pre-treated with *M. charantia* decreased significantly these elevated transaminases and LDH activities (Table 1). CCl_4 is a known hepatotoxic compound working through the generation of reactive free radicals. Treatment with *M. charantia* fruit extract was found to up regulate different antioxidant and detoxifying enzymes in liver of rats challenged with CCl_4 as compared to control. Therefore, these results demonstrated that the fruit of *M. charantia* has protective function against CCl_4 toxicity in rat liver when administered *in vivo* at a dose of 200 mg / kg/day i.p, for 4 consecutive days prior to CCl_4 intoxication. Similar results have been reported for some other ethnobotanical fruits and herbs (Hsiao et al., 2003; Kim et al., 2003; Jung et al., 2004).

Antioxidant actions of food may be through inhibitory actions on generation of ROS or by direct scavenging of free radicals. In addition, the levels of endogenous antioxidants may also be up regulated by increasing expression of the genes encoding the antioxidant enzymes SOD, CAT and GPx. AOE and antioxidant molecules can inhibit free radical production by chelating the transition metal catalysts, breaking chain reactions, reducing concentrations of ROS and/or scavenging initiating radicals (Aruoma, 1994; Halliwel and Gutteridge, 1999). In order to investigate whether the antioxidant activities of *M. charantia* fruit and its related constituents are mediated by an increase in antioxidant enzymes, we measured SOD, CAT and GPx and GST activities in different tissues of rats treated with *M. charantia* fruit extract. In the present study, treatment of rats with *M. charantia* fruit extract significantly increased rat liver, lung and kidney SOD, CAT and GPx activities (Table 2). There were significant ($p<0.001$) increase in all hepatic CAT, SOD and GPx activities. More than four-fold increases were observed with *M. charantia* fruit extract treatment in hepatic antioxidant enzymes suggesting that liver plays predominant role in protection against free radicals. Although similar significant levels of elevations were observed in GPx activities in different tissues, CAT and SOD activities were found to be increased to lesser degrees in kidney and lung tissues (Table 2). SOD, CAT and GPx are major free radical scavenging enzymes that have shown to be reduced in a number of pathophysiological processes and diseases such as diabetes (Cohen and Heikkila, 1994). Thus, activation of these AOE by the administration of *M. charantia* fruit extract clearly shows that *M. charantia* fruit contains free radical scavenging activity, which could exert a beneficial action against pathophysiological alterations caused by the presence of superoxide and hydroxide radicals.

In addition *M. charantia* fruit extract caused significant ($p<0.01$) elevations on hepatic and nephritic cytosolic GSTs which are responsible for the metabolism of numerous xenobiotics and play a major cellular antioxidant role. GSTs also have peroxidase and isomerase activity; they bind covalently with reactive metabolites formed from carcinogens and noncovalently with lipophilic compounds, thereby offering protection against oxidative stress (Hayes and Pulford, 1995).

In this study we have also attempted to examine whether *M. charantia* fruit extract were able to affect some CYP isoforms in male rat livers by using highly specific substrates to CYP isozymes. Treatments of rats

Table 1. The effects of *M. charantia* fruit extracts on serum LDH, AST and ALT.

Groups	Enzyme activities (Units)		
	LDH	AST	ALT
Control	670 ± 96	188.85 ±29	152.22 ± 19
Momordica	690 ± 90*	142.93 ± 15*	117.78 ± 14*
CCl_4	1,680 ± 166***	528.30 ± 74***	406.81 ± 63***
CCl_4 + Momordica	210 ± 15*	185.33 ± 21*	164.17 ± 16*

* $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Table 2. The effects of *M. charantia* fruit extracts on anti-oxidant enzyme activities.

Enzymes	Liver		Kidney		Lung	
	Control	Momordica	Control	Momordica	Control	Momordica
CAT (nmol/min/mg protein)	19.47 ± 2.79	92.01 ± 9.13***	11.79 ± 2.79	24.17 ± 3.17**	14.24 ± 1.84	3.12 ± 0.87*
GPx (nmol/min/mg protein)	0.96 ± 0.26	4.77 ± 0.44***	0.41 ± 0.10	3.42 ± 0.13***	1.18 ± 0.24	2.67 ± 0.19***
GST (nmol/min/mg protein)	257.8 ± 13.6	365.9 ± 27.6**	77.6 ± 4.4	150.3 ± 7.5**	156.7 ± 6.8	148.2 ± 10.9*
SOD (Unit/mg protein)	1.21 ± 0.06	5.09 ± 0.09***	0.23 ± 0.05	0.28 ± 0.05**	ND	ND

* $p<0.05$, ** $p<0.01$, *** $p<0.001$.

with *M. charantia* significantly reduced both EROD ($p<0.01$) and MROD ($p<0.05$) activities in rat liver microsomes, which are known to be catalyzed by CYP1A isoforms (Table 3). Although species differences make it difficult to extrapolate the inhibitions from rats to human, selective inhibition of CYP1A isoforms could be considered as one possible mechanism of the chemo-protective action of *M. charantia* because they are pri-marily involved in the metabolism and activation of carci-nogens and procarcinogens (Ioannides and Lewis, 2004). In addition, caution should be paid to the possible drug interactions in people who concurrently using *M. charantia.* However, further studies are required to be carried out on this CYP field to clearly elucidate which isoforms and how they are involved in the modulatory eff-ects.

These findings clearly demonstrated the antioxidant and chemoprotective activities of *M. charantia* fruit extract in experimental rat models. Although further studies are required to identify active components involved in the antioxidant and chemoprotective activity of this fruit, results strongly suggest that *M. charantia* fruit extract has chemoprotective action against CCl_4-induced toxicity. In addition, even if it is indirect, inhibition of CYP1A dependent activities could be considered as a promising cancer chemoproventive action by lowering metabolic activation of various carcinogens and/or procarcinogens. Based on our knowledge, the present study is the first reporting the effect of *M. charantia* fruit extract on CYP1A dependent enzyme activities, namely EROD and MROD.

In conclusion, the present study showed that *in vivo* treatment of rats with *M. charantia* fruit extract enhanced both AOE and GST activities while lowering CYP1A dependent enzyme activities.

Table 3. The effects of *M. charantia* fruit extracts on CYP1A dependent enzyme activities.

Groups	Enzyme activities (pmol resorufin/min/mg protein)	
	EROD	MROD
Control	106.5 ± 9.62	47.07 ± 3.53
Momordica	70.51 ± 9.57**	41.59 ± 8.16*

* $p<0.05$, ** $p<0.01$, *** $p<0.001$

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