

Effects of trypsin inhibitor on plasma antioxidant activity and lipid levels in mice from sweet potato roots

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Abstract

BACKGROUND: Several inflammatory diseases are thought to be related to oxidative injury and reactive oxygen species have been proposed as important causative agents of heart disease and ageing. This study was designed to investigate the effects of sweet potato trypsin inhibitor (SPTI) on antioxidant enzymes, lipid peroxidation and lipid profiles in mice.

RESULTS: Twenty mice were randomly divided into four groups and fed with TI (10, 50 and 100 mg kg⁻¹ BW) as treatment and with saline as a control in addition to regular diets. After 35 days, Trolox equivalent antioxidant capacity (TEAC), triglyceride (TG) and cholesterol levels in plasma and superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx), thiobarbituric acid reactive substances (TBARS) in liver were measured. Serum from the group that had received the the highest oral dose of SPTI (100 mg kg⁻¹ BW) had the highest total antioxidant activity (expressed as 3.59 ± 0.237 mmol L⁻¹ TEAC). The SOD, catalase and GPx activity of SPTI groups were significantly increased compared with the control group. Malondialdehyde (MDA) was significantly lower in all experimental groups compared with the control one. No significant differences in the concentration of low-density lipoprotein (LDL)-cholesterol was found, but high density lipoprotein (HDL)-cholesterol, triglyceride (TG) and total cholesterol tended to decrease.

CONCLUSION: This study showed that the oral intake of SPTI in mice may trigger inflammatory responses which result in an increase in antioxidant enzyme activities, and a decrease in MDA, TG and total cholesterol, which are known risk factors of inflammatory and heart disease.

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Keywords: sweet potato; trypsin inhibitor; antioxidant; lipid

INTRODUCTION

It is commonly accepted that in a situation of oxidative stress, reactive oxygen molecules such as superoxide (O₂^{•-}, HOO^{•-}), hydroxyl (OH[•]) and peroxy (ROO[•]) radicals are generated. The reactive oxygen molecules play an important role in the degenerative or pathological processes of various serious diseases, such as cancer, coronary heart disease, Alzheimer's disease,^{1,2} neurodegenerative disorders, atherosclerosis, cataracts, inflammation³ and ageing.⁴ The decrease of the tissue antioxidant enzyme system activity may cause an increase in lipid peroxidation. Atherosclerosis is caused not only by the deposition of cholesterol but also by inflammation. Polyunsaturated fatty acids of the membrane are peroxidised by free radical-mediated reactions and lipid peroxidation is

an autocatalytic process, which is a common cause of cell death. The biological effects of reactive oxygen molecules are controlled *in vivo* by enzymatic (superoxide dismutase, catalase and glutathione peroxidase) and non-enzymatic (vitamins A, C, E and reduced glutathione) defence mechanisms. Anti-inflammatory drugs used to treat arthritis may also reduce the risk of heart disease, which means that inflammation plays an important role in heart disease. Cyclooxygenase-2 (COX-2) inhibition reduces low-grade chronic inflammation, improves endothelium-dependent vasodilation and oxidative stress in coronary artery disease, suggesting that COX-2 inhibition has the pharmaceutical potential in cardiovascular disease.⁵ Because antioxidant nutrients such as vitamin E, vitamin C, carotenoids and numerous polyphenolic compounds

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directly scavenge reactive oxidants, it is hypothesised that they constitute a vital endogenous defence against oxidative cellular and tissue injury caused by toxic and carcinogenic chemicals. Pharmacological modulations, including lipid lowering and antioxidant agents have been shown to improve endothelial function in humans.

Protease inhibitors in plants may be important in regulating and controlling endogenous proteases and in acting as protective agents against insect and/or microbial proteases⁶. Sohnie and Bhandarker⁷ reported for the first time the presence of trypsin inhibitor (TI) in sweet potato (SP). Later, it was indicated that TI activities in SP are positively correlated with concentrations of water-soluble protein,⁸ and that TI activities increase in response to drought.⁹ Polyamines, including cadaverine, spermidine and spermine, were bound covalently to SPTI, which might participate in regulating the growth and developmental processes of SP.¹⁰ TI in SP storage roots account for about 60% of total water-soluble proteins and could be recognised as storage proteins.⁹ Matsuoka *et al.*¹¹ identified sporamin as the major storage protein in SP root, accounting for 80% of the total proteins there. A dramatic decrease of the amount of sporamin to 2% of the original value was found during sprouting. Lin¹² considered sporamin as one form of TI in SP, a finding confirmed later by Yeh *et al.*¹³ In our report, sweet potato TI exhibited dehydroascorbate reductase, monodehydroascorbate reductase and antioxidant activities.¹⁴ In the present study, the antioxidant and lipid-lowering effects of TI were examined *in vivo*. After 35 days of administration of TI to mice, antioxidant enzyme activities (SOD, catalase, malondialdehyde (MDA)) and lipid profiles (MDA, TG, HDL-cholesterol and total cholesterol) were measured.

MATERIALS AND METHODS

Chemicals

Tris and electrophoretic reagents were purchased from Merck (Darmstadt, Germany); Seebule prestained markers for SDS-PAGE including myosin (250 kDa), phosphorylase (148 kDa), BSA (98 kDa), glutamate dehydrogenase (64 kDa), alcohol dehydrogenase (50 kDa), carbonic anhydrase (36 kDa), myoglobin red (22 kDa), lysozyme (16 kDa), aprotinin (6 kDa) and insulin b chain (4 kDa) were from Invitrogen (Groningen, the Netherlands); total cholesterol, triglycerides (TG), HDL-cholesterol and LDL-cholesterol were measured by enzymatic methods. Cholesterol liquicolour, triglyceride liquicolour, LDL-cholesterol liquicolour and HDL-cholesterol liquicolour test kits were purchased from Human (precipitant and standard; Human, Wiesbaden, Germany). Coomassie brilliant blue G-250, other chemicals and reagents were purchased from Sigma (St Louis, MO, USA).

In our laboratory the water used in experiments was purified via two major steps using Millipore devices (Billerica, MA, USA). The first step was through reverse osmosis (RO). Tap water was pumped sequentially through 5 µm and 1 µm filters; then through one pair of a reverse osmosis pack (1 µm carbon) to obtain water purified by reverse osmosis. In the second step, this purified water was treated through one pair of Q-gard filters (Q-gard is a registered pack name of the Millipore company); then deionised through a Ultrapure Oxganex Cartridge (MA, USA); finally through 0.22 µm filter cup to obtain Milli-Q (MQ) water, which was ready for use.

Plant materials

Fresh storage roots of sweet potato (*Ipomoea batatas* Lam. 'Tainong 57') were purchased from a local market. Samples were washed and peeled, and then cut into strips that were extracted immediately.

Purification of sweet potato trypsin inhibitor

Extraction and purification of TI from sweet potato storage roots was carried out at 4 °C according to the method of Huang *et al.*¹⁵ The storage roots were cut into strips that were extracted immediately with four volumes (wt/vol) of 100 mmol L⁻¹ Tris-HCl buffer (pH 7.9) containing 100 mmol L⁻¹ NaCl, 1% (w/v) ascorbate and 1% (w/v) polyvinylpyrrolidone (PVPP) in a homogeniser for 30 s (four times). The homogenates were filtered through four layers of cheesecloth and centrifuged twice at 12 000 × *g* for 30 min. The crude extracts were loaded directly onto a trypsin-Sepharose 4B affinity column (1.0 × 10 cm), and the adsorbed TI were eluted by changing pH value with 0.2 mol L⁻¹ KCl buffer (pH 2.0).¹⁶ The extracts were desalted and concentrated with Centricon 10 and then lyophilised for further use.

Protein and activity stainings of TI on 15% denaturing polyacrylamide gels

TI was detected with both protein and activity stainings on 15% SDS-PAGE gels. Samples were mixed with sample buffer, namely 60 mmol L⁻¹ Tris-HCl buffer (pH 6.8) containing 2% SDS, 25% glycerol, and 0.1% bromophenol blue with or without 2-mercaptoethanol. Coomassie brilliant blue G-250 was used for protein staining.¹⁷ For TI activity staining, the gel was stained according to the method of Hou *et al.*¹⁸ When SDS-PAGE was finished, gel was immersed and shaken twice in 25% vol/vol isopropanol in 10 mmol L⁻¹ Tris buffer (pH 7.9) for 10 min each. The gel was then dipped into 10 mmol L⁻¹ hydrogen peroxide in the same buffer at least 30 min with gentle shaking, and finally washed in 10 mmol L⁻¹ Tris buffer (pH 7.9) for 10 min. Then the gel was incubated in trypsin solution (50 µg bovine trypsin/mL, 10 mmol L⁻¹ Tris buffer pH 7.9) at 37 °C for 20 min. After rinsing with the same buffer to remove excess trypsin, the gel was incubated in the dark at 37 °C for at least 30 min with 160 mL of

substrate dye solution immediately prepared before use. The substrate dye solution consisted of 40 mg *N*-acetyl-D,L-phenylalanine β -naphthyl ester (APNE) in 16 mL of *N,N*-dimethylformamide that was brought to 160 mL with 144 mL of 10 mmol L⁻¹ Tris buffer (pH 7.9), in which 80 mg tetrazotised *o*-dianisidine were dissolved. The gel was destained with 10% acetic acid for 30 min.

Experimental mice

Male BALB/cByJNarl mice (6 weeks old) were obtained from the National Laboratory Animal Center, National Applied Research Laboratories, National Science Council in Taipei, Taiwan, and maintained in the Institute of Plant and Microbial Biology at Academia Sinica in Taipei, Taiwan. The mice were kept in cages and fed on a chow diet (laboratory standard diet; BioLASCO Taiwan Co., Ltd.). The animal room was kept under a 12-h light and 12-h dark cycle with constant temperature (25 ± 2 °C) and 50–60% relative humidity. The body weights were measured once a week during the 5-week period.

The doses of TI were based on the earlier cell culture studies (100 µg mL⁻¹ multiplied by 1000 is 100 mg mL⁻¹). TI was dissolved in normal saline and the doses used were described by Huang *et al.*¹⁶

Experimental design

A total of 20 mice were divided into four groups with five mice in each group. All the mice were fed a regular diet and orally treated once every 2 days by intragastric tube. Control group was orally treated with 0.2 mL control solution containing 2.7 mmol L⁻¹ potassium chloride, 1.47 mmol L⁻¹ potassium dihydrogen phosphate, 7.7 mmol L⁻¹ disodium hydrogen phosphate, and 137 mmol L⁻¹ NaCl; while the high dose, the medium dose, and the low dose groups were orally treated with 100, 50 and 10 mg TI kg⁻¹ body weight (BW), respectively, in 0.2 mL control solution. After 35 days of experiment, blood was collected into heparinised tubes. Samples were immediately centrifuged at 1500 × *g* for 5 min and the plasma was separated. Total antioxidant activity, triglyceride (TG), high density lipoprotein (HDL)-cholesterol, low density lipoprotein (LDL)-cholesterol and cholesterol levels were determined in plasma samples.

Preparation of tissue homogenates

Mice were anaesthetised with ether. After opening the abdomen and thorax, liver was removed and stored at -70 °C. For enzyme activity assay, liver tissues were homogenised in 50 mmol L⁻¹ potassium phosphate, pH 7.0, containing 0.1% Triton-X 100 with a Polytron homogeniser, followed by sonication on ice for 30 s with a microprobe at maximum power. The tissue homogenate was clarified by centrifugation at 20 000 × *g* for 10 min. Protein content in each

sample was determined by a bicinchoninic acid (BCA) protein assay kit (Pierce, Rockford, IL, USA).

Antioxidant activity by ABTS assay

Total antioxidant status of the plasma was measured using 2,2'-azinobis[3-ethylbenzthiazoline]-6-sulfonic acid (ABTS) assay.¹⁹ An aqueous solution of 7 mmol L⁻¹ ABTS was oxidised using potassium peroxodisulfate (2.45 mmol L⁻¹) for 16 h in the dark. The ABTS⁺ solution was diluted with ethanol to an absorbance of 0.7 ± 0.05 at 734 nm (UV-visible spectrophotometer, Model DU640B; Beckman, Glenrothes, UK). A standard calibration curve was obtained for Trolox at concentrations of 0, 0.1, 0.2, 0.5, 1.0 and 2.0 mmol L⁻¹. An aliquot (10 µL) of each sample (10 mg mL⁻¹) was mixed with 1.0 mL of ABTS⁺ radical cation solution in cuvette and the absorbance was read at 734 nm after 1 min. Antioxidant properties of serum were expressed as trolox equivalent antioxidant capacity (TEAC), calculated from at least three different concentrations of extract tested in the assay giving a linear response.

Measurement of superoxide dismutase activity

The activity of superoxide dismutase (SOD) was determined by monitoring the inhibition of the autoxidation of pyrogallol.²⁰ The rate of pyrogallol oxidation was measured by recording absorbance of 320 nm at 25 °C using a Beckman UV-visible spectrophotometer, Model DU640B. One unit of SOD activity was defined as the amount of enzyme that inhibited the oxidation of pyrogallol by 50%. Specific SOD activity was expressed as U mg⁻¹ protein.

Measurement of catalase activity

Catalase activity was determined according to Aebi.²¹ Briefly, 50 mmol L⁻¹ potassium phosphate buffer (pH 7.0) and 30 mmol L⁻¹ H₂O₂ were added to the extract of liver and the change of absorbance at 240 nm in 1 min at 25 °C was measured. One unit of catalase activity was defined as the amount that decomposes 1 µmol of H₂O₂ in 1 min at 37 °C. Catalase activity was expressed as U mg⁻¹ protein.

Measurement of glutathione peroxidase activity

Glutathione peroxidase (GPx) activity was determined by the method of Paglia and Valentine.²² Reaction mixtures contained 250 mmol L⁻¹ potassium phosphate buffer, pH 7.4, 40 mmol L⁻¹ GSH, 20 mmol L⁻¹ NADPH, 5 U mL⁻¹ glutathione reductase, 15 mmol L⁻¹ cumene hydroperoxide and the extract of liver. Reaction rate was recorded by using spectrophotometry to follow the NADPH-related decrease of absorbance values at 340 nm in 1 min. One unit of GSH-PX was defined as the amount that reduced the level of GSH by 1 µmol L⁻¹ in 1 min per mg protein. Specific GPx activity was expressed as U mg⁻¹ protein.

Lipid peroxidation intermediates

Thiobarbituric acid reactive substances (TBARS) are products of the oxidative degradation of polyunsaturated fatty acids; in particular, MDA. Lipid peroxidation was assayed by the measurement of MDA levels via absorbance at 535 nm on the basis of MDA reacting with thiobarbituric acid, according to Tatum and Chow.²³ Briefly, 0.4 mL of the liver extract was mixed with 0.4 mL thiobarbituric acid reagent (consisting of 0.4% thiobarbituric acid (TBA) and 0.2% butylated hydroxytoluene (BHT)). The reaction mixture was placed in water at 90 °C for 45 min then cooled. An equal volume of *n*-butanol was added, the mixture centrifuged and then the absorbance of the supernatant was recorded at 535 nm. A standard curve was obtained with a known amount of 1,1,3,3-tetraethoxypropane (TEP), using the same assay procedure.

Triglyceride and cholesterol levels

The concentrations of TG, LDL-cholesterol, HDL-cholesterol and total cholesterol in plasma were determined enzymatically using commercially available test kits (precipitant and standard, Human, Wiesbaden, Germany).

Statistical analysis

Means of triplicates were calculated. Student's *t*-test was used for comparison between two treatments. All data (expressed as per cent of control value) were means \pm SE. A difference was considered to be statistically significant when $P < 0.05$, $P < 0.01$ or $P < 0.001$.

RESULTS

Extraction and purification of TI from sweet potato storage root TI was purified from sweet potato tubers according to the Huang *et al.*¹⁶ The inset of Figure 1 shows protein staining (lane 1) and activity staining (lane 2) of purified TI without 2-mercaptoethanol on a 15% SDS-PAGE gel.

Effects of TI on the plasma antioxidant activity

Figure 2 shows that the serum of mice group with oral treatment of the highest dose of TI (100 mg kg⁻¹ BW) had the highest total antioxidant activity (expressed as 3.59 ± 0.237 mmol L⁻¹ TEAC). Control displayed the lowest one (3.18 ± 0.092 mmol L⁻¹ TEAC). There was a significant increase in the antioxidant activity of groups treated with TI (50 or 100 mg kg⁻¹ BW, $P < 0.01$ or $P < 0.05$) when compared with the control group.

Effects of TI on the antioxidant enzyme activities

SOD converts superoxide to hydrogen peroxide and is a major defence for aerobic cells in combating the toxic effects of superoxide radicals.²⁴ As presented in Figure 3, administration of TI at 100 mg kg⁻¹ BW for

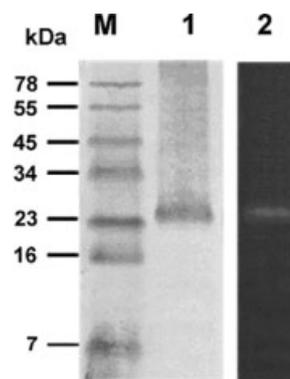


Figure 1. The protein (lane 1) and activity (lane 2) stainings of the trypsin inhibitor from sweet potato storage root on SDS-PAGE gels with 2-mercaptoethanol. The gel system contained 2.5 cm, 4% stacking gel and 4.5 cm, 15% separating gel. M indicates the Seeblue™ prestained markers of SDS-PAGE. Ten micrograms of TI were loaded in each well.

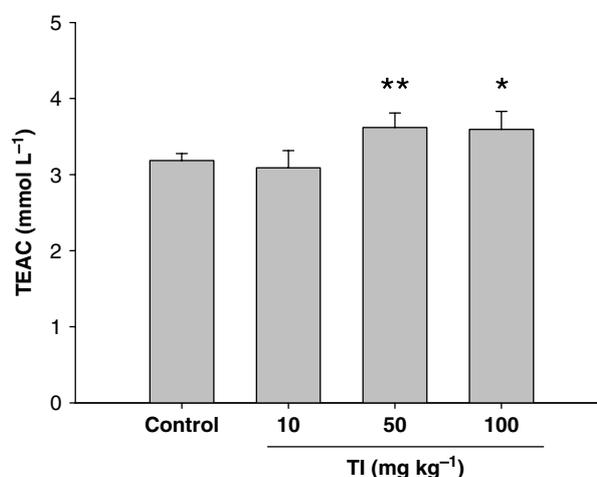


Figure 2. Effects of TI on the plasma antioxidant activity. Total antioxidant activity in the plasma of mice 5 weeks after oral administration of the high dose of TI (100 mg kg⁻¹ BW), the medium dose of TI (50 mg kg⁻¹ BW) and the low dose of TI (10 mg kg⁻¹ BW). Results represent the means \pm SE from at least three separate experiments. * $P < 0.05$ and ** $P < 0.01$ (unpaired *t*-test) compared with samples of control group.

35 days significantly increased the activity of SOD ($P < 0.05$), while the SOD activities of the other experimental groups did not show any significant change when compared with the control group.

Catalase decomposes hydrogen peroxide and protects the tissues from highly reactive hydroxyl radicals.²⁵ Figure 4 illustrates the activity of catalase in the plasma of control and experimental groups. There was either a significant increase in the catalase activity of group treated with 10 mg TI kg⁻¹ BW ($P < 0.05$), or highly significant increase of groups treated with 50 or 100 mg kg⁻¹ BW of TI when compared with the control group ($P < 0.001$).

Glutathione peroxidase (GPx) catalyses the reduction of hydroperoxides, including hydrogen peroxides, by reduced glutathione and functions to protect the cell from oxidative damage.²⁶ Figure 5 illustrates the activity of GPx in the liver of control and experimental mice groups. There was a significant increase

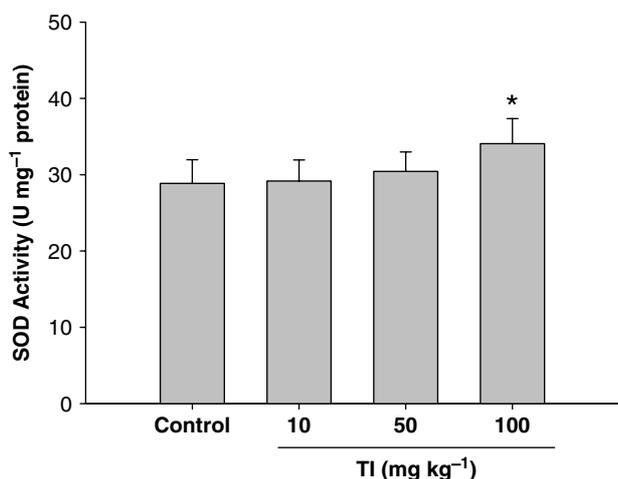


Figure 3. Superoxide dismutase (SOD) activity in the liver of mice 5 weeks after oral administration of the high dose of TI (100 mg kg⁻¹ BW), the medium dose of TI (50 mg kg⁻¹ BW) and the low dose of TI (10 mg kg⁻¹ BW) Results represent the means \pm SE from at least three separate experiments. * $P < 0.05$ (unpaired *t*-test) compared with samples of control group.

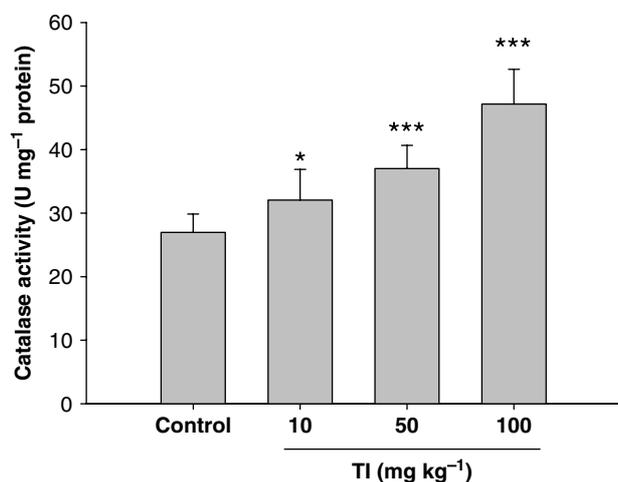


Figure 4. Catalase activity in the liver of mice 5 weeks after oral administration of the high dose of TI (100 mg kg⁻¹ BW), the medium dose of TI (50 mg kg⁻¹ BW) and the low dose of TI (10 mg kg⁻¹ BW) Results represent the means \pm SE from at least three separate experiments. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ (unpaired *t*-test) compared with samples of control group.

in activity of GPx of group treated with 10 mg TI kg⁻¹ BW ($P < 0.05$), or highly significant increase of groups treated with 50 or 100 mg kg⁻¹ BW of TI when compared with the control group ($P < 0.001$).

Lipid peroxidation has been established as a major mechanism of cellular injury in many biological systems of plant and animal origin. The mechanism involves a process whereby unsaturated lipids are oxidised to form additional radical species as well as toxic by-products that can be harmful to the host system. Polyunsaturated lipids are especially susceptible to this type of damage when in an oxidising environment and they can react to form lipid peroxides. Polyunsaturated fatty acid peroxides further react to form MDA, which is found in most biological samples including foodstuffs, serum, plasma, tissues

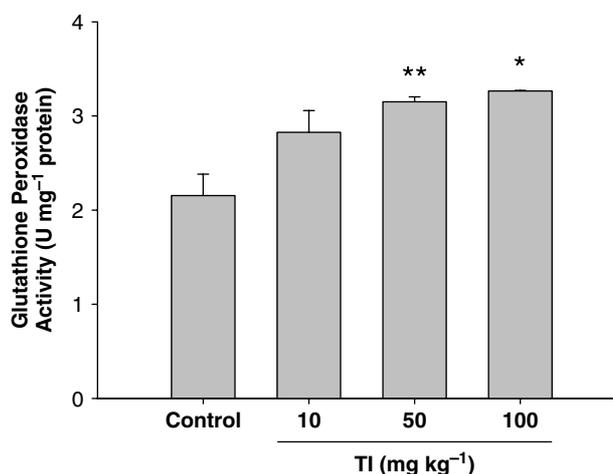


Figure 5. Glutathione peroxidase (GPx) activity in the liver of mice 5 weeks after oral administration of the high dose of TI (100 mg kg⁻¹ BW), the medium dose of TI (50 mg kg⁻¹ BW) and the low dose of TI (10 mg kg⁻¹ BW) Results represent the means \pm SE from at least three separate experiments. * $P < 0.05$ and ** $P < 0.01$ (unpaired *t*-test) compared with samples of control group.

and urine, as a result of lipid hydroperoxides, and has become one of the most widely reported analytes for the purpose of estimating oxidative stress effects on lipids. Figure 6 shows MDA levels in the liver of control and experimental mice groups. TI very significantly decreased the MDA level in liver at dose of 10 or 50 mg kg⁻¹ body ($P < 0.01$), while it significantly decreased the MDA level at dose of 100 mg kg⁻¹ body ($P < 0.05$) when compared with the control group.

Effects of TI on the plasma lipid levels

Lipoproteins are macromolecules of lipid and protein that transport lipids (including cholesterol and triglycerides) through the vascular and extravascular body fluids. They are involved in a diversity of processes such as immune reactions, coagulation and

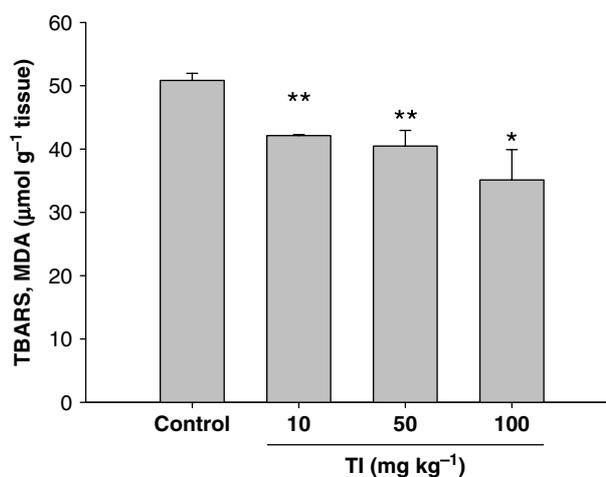


Figure 6. Malondialdehyde (MDA) levels in the liver of mice 5 weeks after oral administration of the high dose of TI (100 mg kg⁻¹ BW), the medium dose of TI (50 mg kg⁻¹ BW) and the low dose of TI (10 mg kg⁻¹ BW) Results represent the means \pm SE from at least three separate experiments. * $P < 0.05$ and ** $P < 0.01$ (unpaired *t*-test) compared with samples of control group.

Table 1. Concentrations of lipids in plasma

Treatment	Plasma concentration (mmol L ⁻¹)			
	Triglyceride	Total cholesterol	HDL-cholesterol	LDL-cholesterol
Control	9.4 ± 1.37	21.7 ± 0.85	6.6 ± 0.32	2.7 ± 0.20
TI (10 mg kg ⁻¹)	9.4 ± 1.99	22.6 ± 2.65	6.5 ± 0.30	3.9 ± 0.56
TI (50 mg kg ⁻¹)	8.6 ± 1.18	14.3 ± 0.7**	4.3 ± 0.69*	2.8 ± 0.15
TI (100 mg kg ⁻¹)	6.0 ± 0.52*	11.6 ± 2.69*	4.1 ± 0.77*	2.6 ± 0.16

HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Results represent the means ± SE from at least three separate experiments.

* $P < 0.05$ and ** $P < 0.01$ (unpaired *t*-test) compared with samples of control group.

tissue repair.²⁷ Table 1 shows the effects of TI on plasma lipid profiles following administration in mice. No significant differences in the concentration of LDL-cholesterol was found, but HDL-cholesterol, triglyceride (TG) and total cholesterol tended to decrease. Compared with the control group, TI very significantly decreased TG level at dose of 100 mg kg⁻¹ body ($P < 0.01$), or significantly decreased cholesterol level at dose of 50 or 100 mg kg⁻¹ BW ($P < 0.01$ or $P < 0.05$). There was a significantly decreased HDL-cholesterol level at TI dose of 50 or 100 mg kg⁻¹ BW ($P < 0.01$ or $P < 0.05$) in the plasma compared with the control group.

DISCUSSION

This study has shown that TI significantly reduces lipid peroxidation and improves plasma antioxidant capacity as shown by measurements of MDA as a lipid peroxidation indicator. It is known that impaired glucose metabolism leads to oxidative stress, proteins glycation and formation of free radicals.²⁸ Thus, an augmentation of plasma antioxidant capacity decreases plasma free radicals, as shown by this and other studies when consuming herbal extracts containing antioxidants.²⁹ This decrease in MDA levels may increase the activity of GPx in treated rats and hence prevent lipid peroxidation reactions.³⁰ It has been proposed that only some lipids produce MDA by peroxidation.³¹ The large decrease of MDA levels which reflects decrease of lipid peroxidation suggests that other factors such as reduction of lipids in the liver and plasma by TI may also have a role in decreasing lipid peroxidation and MDA levels.

The results of this study indicate that oral administration of mice with TI is capable of enhancing/maintaining the activity of hepatic enzymes which are involved in combating reactive oxygen species. Our results are in agreement with the results that oral administration with polyphenol-rich extracts may preserve antioxidant enzyme activities due to the free-radical scavenging activity, and/or induction of defence enzyme expression.³² Therefore, we propose that TI may also serve as an inducer beyond its known biological functions.

For all the antioxidant parameters studied TI showed significant antioxidant action, which could

exert a beneficial action against pathological alterations, especially in inflammatory diseases. The increase in the antioxidant enzyme activity and the reduction in the lipid peroxidation by TI may result in a reduction of a number of deleterious effects due to the accumulation of oxygen radicals. A potential mechanism of oxidative damage is the nitration of tyrosine residues of proteins, peroxidation of lipids, degradation of DNA and oligonucleosomal fragments.³³ Antioxidant enzymes, SOD, catalase and GPx offer protection against oxidative tissue damage. SOD converts O₂⁻ to H₂O₂. GPx and catalase consequently metabolise H₂O₂ to non-toxic products. Under oxidative stress, GPx and catalase are activated in the defence against oxidative injury.³⁴ Therefore, TI might contribute towards the total or partial alleviation of some clinical disorders.

Therapeutic strategies aimed at enhancing cholesterol efflux from the arterial wall may be of additional benefit for patients with atherosclerosis. Numerous epidemiological studies have associated increased HDL with an inverse risk for coronary artery disease. This protective effect of HDL may be due in part to an inhibition of the oxidative modification of LDL.^{35,36} It was generally accepted that low HDL-cholesterol might be a marker for the metabolic syndrome, an enhanced atherosclerotic disease state that is also associated with an impaired response to insulin, hypertriglyceridaemia and abnormal obesity.

In the present study, every 2 days intake of TI (10, 50, 100 mg kg⁻¹ BW) for 35 days resulted in a significant decrease in HDL-cholesterol, TG and total cholesterol effects which may be beneficial in the prevention of ischaemic heart disease. There is evidence that COX-2 may be a source of oxygen radicals itself, and therefore inhibition of the enzyme activity may reduce oxidative stress. Furthermore, antioxidative agents such as vitamin C reverse endothelial dysfunction in patients with coronary artery disease or coronary risk factors.^{37,38} It may be related to changes in tissue concentrations of antioxidant metabolites and in the specific activities of other enzymes involved in antioxidant metabolism, which could be caused by treatment with TI. In conclusion, our findings have shown that liver antioxidant enzyme activities, lipid peroxidation and HDL cholesterol levels are affected by administration

of TI in mice. Therefore, TI used in this study may reduce the risk of inflammation-related diseases, and the development of new treatments for both heart disease and endothelial dysfunction will be possible.

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