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Compare Activities on Regulating Lipid-Metabolism and Reducing Oxidative Stress of Diabetic Rats of *Tremella Aurantialba* Broth's Extract (TBE) with Its Mycelia Polysaccharides (TMP)

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ABSTRACT: Abnormal lipid-metabolism and elevated oxidative stress are the familiar complications of diabetic mellitus. Regulated lipid-metabolism and decreased oxidative stress have become the key indices to cure diabetic complications. The activities of broth extract (TBE) and mycelia polysaccharides (TMP) of *Tremella aurantialba*, which is one of the best-known multipurpose medicinal fungi in China, were studied using alloxan-induced diabetic rats. TBE contains saponins, while TMP contains polysaccharides. Both TBE and TMP could reduce the blood glucose levels of diabetic rats; TBE had stronger abilities to reduce the levels of total cholesterol and total triglyceride in serum, those of malondialdehyde, and enhance the activities of superoxide dismutase and glutathione reductase in different tissues of diabetic rats ($P < 0.01$). TBE had slightly stronger abilities to enhance the total antioxidant capability, catalase, and glutathione peroxidase in different tissues of diabetic rats, but no significant difference was found between TBE and TMP groups. All these results indicated that TBE was more capable of regulating lipid-metabolism and decreasing oxidative stress.

Keywords: blood glucose, lipid-metabolism, oxidative press, *Tremella aurantialba*

Introduction

Diabetic mellitus, a leading metabolic disorder occurring worldwide, is characterized by hyperglycemia associated with impairments in insulin secretion and/or insulin action as well as alteration in intermediary metabolism of carbohydrates, protein, and lipids. Several reports indicated that the annual incidence rate of diabetic mellitus would increase worldwide in the future time (Stone and others 2007). It has been proposed that approximately 60 million Chinese people will be affected by diabetic mellitus by the year of 2015 in China (Huang 2007).

Dieting and medication are 2 important measures to control the mortality of diabetes. Edible and medical fungi are important sources of diet and medicine used for curing diabetes mellitus. In recent years some researchers have evaluated and identified the antidiabetic potential of traditionally used Chinese medicinal fungi in their animal experiments (Kiho and others 1993, 1995; He and others 2006; Lo and others 2006). Previous studies have also confirmed the efficacy of some medicinal fungi in the modulation of oxidative stress associated with diabetic mellitus (He and others 2006). However, in studies on bioactive components of these fungi, only limited studies were conducted concerning other components except for polysaccharides, such as saponins, terpenoids, flavonoids, sterols, and alkanoids with hypoglycermic and antihyperglycermic effects from medical and food fungi (Yamaguchi and others 2000).

The genus *Tremella* Pers. (*Tremellales*, higher basidiomycetes) belongs to the so-called "jelly mushrooms" group that forms gelatinous fruiting bodies. About 80 species have been recognized and none of them is known to be poisonous (Chen 1998; Kirk and others 2001). Jelly mushrooms from the genus *Tremella* have been used as food and folk medicines. In the past decades, they were investigated in detail to understand their medicinal properties *in vivo* and *in vitro* model system. Their clinical applications were studied with regard to different groups of patients (Lin and others 1982; Kiho and others 1995, 2000; Gao and others 1996a, 1996b). *T. aurantialba*, one of the genus *Tremella* Pers., has been used as diet and medical drugs for millenary in many Asian countries, including anticancer, treatment of thrombophebitis, atherosclerosis, and senile degradation of microvessels. In the reported article on the bioactive component, *T. aurantialba* was also not an exception. More attention was focused on the bioactivities of polysaccharides, such as regulating blood glucose and blood lipid (Kiho and others 1995), few paper was paid for other active components, such as saponins.

In our previous article, it had been reported that the extract from *T. aurantialba* broth (TBE) could regulate plasma glucose of type 2 diabetic rats (Zhang and Zhang 2006). In the current study, the effects of TBE, *T. aurantialba* mycelia polysaccharides (TMP), and metformin on the fast blood glucose levels, body weight, the metabolic profile of lipid, the activities of superoxide dismutase, glutathione reductase catalase, glutathione peroxidase, total antioxidant activity, and malondialdehyde level in some tissues, were compared in alloxan-induced diabetic mellitus rats. The study can not only facilitate to develop other active components whose

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activities may be more than those of polysaccharides from broth of food and medical fungi, but also provide a substitute alternative for the cure of diabetic mellitus.

Materials and Methods

Chemicals

Alloxan was purchased from Sigma-Aldrich (Shanghai, China). The diagnostic kits for glucose, fructosamine, total cholesterol, phospholipids, triglyceride, superoxide dismutase, glutathione reductase, catalase and glutathione peroxidise, total antioxidant capability, and malondialdehyde were obtained from Rongsheng Biotechnology Co. Ltd. (Shanghai, China). AB-8 macro-porous resin was purchased from the Chemical Plant of Nankai Univ. (Tianjin, China).

Animals

Wistar strain rats, varying in weight between 225.7 ± 7.89 g, were purchased from the Central Animal House, Chinese Academy of Sciences (Nanjing, China). The animals were kept in the steel cages at a temperature of 25 to 30 °C and a relative humidity of 45% to 55% in a light/dark (12/12 h) alternating room. The animals were fed on a standard pellet diet that contained: 4.5% corn oil, 23% casein, 31.9% corn starch, 30.4% sucrose, 5.3% cellulose, 1.2% vitamin complex, and 3.7% mineral mixture, and water was freely available. Procedures involving animals and their care were conducted in conformity with the institutional guidelines of School of Medicine, Jiangsu Univ., China.

Preparation and qualitative analysis of crude extract from *T. aurantialba* broth (TBE)

Submerged incubation and fermentation. A strain of *T. aurantialba* JSU-05, kindly provided by Weijing Qu, Professor of East China Normal Univ., China, was used in this study. The stock culture was maintained on potato dextrose agar (PDA) slants and subcultured once every 6 mo. The slants were incubated at 27 °C for 7 d and then stored at 4 °C. *T. aurantialba* UJS-05 initially grew at 25 °C for 7 d on the solid seed medium containing 15 g bran and 20 mL water, and then transferred into the seed culture medium. During the culturing course, the flask was shaken once a day, starting from the 3rd to the 5th day.

Five grams of such solid culture mentioned previously was inoculated in 100 mL seed medium containing 20 g/L sucrose, 10 g/L corn powder, and 5 g/L peptone. The seed culture experiments were done at 27 °C with a rotary shaker (Shanghai Pharmaceutical Industrial Academy, China) at 150 rpm for 2 d. The fermentation medium was inoculated with 10% (v/v) of the seed culture and then cultivated at 27 °C in 250 mL flasks containing 100 mL of fermentation medium containing 40 g/L sucrose, 4 g/L peptone, 10 g/L corn powder, 10 g/L bran, 1.5 g/L KH_2PO_4, and 0.75 g/L $MgSO_4$.

Preparation of crude extract. When fermentation was over, the fermented broth was adjusted to pH = 2.5 with HCl, and then centrifuged at 5000 × *g*. The supernatant (3000 mL) was applied to the AB-8 macroporous resin column (26 × 300 mm) balanced with 70% ethanol dipping, 2% NaOH solution, and 2% HCl solution. After the sorption process, stepwise elution was conducted with a discontinuous gradient of water, 20%, 40%, 60%, 80%, and 100% ethanol solutions at 3 mL/min. TBE was obtained by collecting and lyophilizing the 40% ethanol fraction. TBE obtained were stored at 4 °C for further use.

Preparation of polysaccharides from TMP. Polyaccharides from TMP were prepared in accordance with the hot water extracting method (Kiho and others 2001; Wang and others 2002). Mycelia, obtained from the culture broths by centrifugation for the isolation of water-soluble endopolysaccharides, were washed 3 times with distilled water and dried at 80 °C till constant weight. Dry mycelia (200 meshes) were degreased with ethanol at 80 °C for 12 h in a water bath (mycelium/ethanol ratio: 1:10). The residues, collected via centrifugation (4500 × *g* for 15 min) and dried at 80 °C, were extracted 3 times with distilled water for 4 h in a boiling water-bath (residue/distilled water ratio: 1:10), and centrifuged at 4500 × *g* for 15 min. The supernatants were collected and concentrated to 50 mL under reduced pressure by means of a rotary evaporator. The concentrated solution was deproteinized by being mixed with the same volume of the Sevage reagent, chloroform:n-butanol (4:1, v/v), then shaken for 5 min and centrifuged at 3000 × *g* for 10 min, the above-mentioned procedure was repeated 8 times. The precipitate, referred to as TMP, was obtained by being precipitated with 4 volumes of 95% alcohol solution (v/v), washed with alcohol, acetone, and ether, and finally dried till constant weight.

Characterization of TBE. The TLC analysis was performed with the method of Wagner and others (Ou and others 2007). The developing solvent was n-butanol:acetic acid:water (40:10:50; upper phase). Fractions were dissolved in the developing solvent and applied to aluminum-backed plates of silica gel (layer thickness 0.2 mm, 10 × 20 cm; Branch of Qingdao Ocean Plant, Qingdao, Shandong, China). After development, the plates were air-dried, sprayed with methanol: acetic acid: sulfuric acid: anisaldehyde (85:10:5:0.1), sulfuric acid: $CHCl_3$ (1:9), and CCl_3COOH, respectively, and then heated at 100 °C for 5 min. Upon heating, the exhibited color was observed.

Analysis of the contents of protein, saponin, reducing sugar, and polysaccharides in TBE and TMP. The content of polysaccharides was equal to the gross sugar content minus reducing sugar content. Gross sugar and reducing sugar content were determined with the anthrone sulfate method (Texier and others 1984) and 3.5-dinitro-salicylic acid method (Miller 1959), respectively. Gross protein contents were determined by biuret reaction (Joyce and Sanford 1970). Saponin content was determined by colorimetric method (Xiang and others 2001)

Induction of diabetes in rats

Diabetes in rats was induced by intraperitoneal injection of alloxan at a dose of 180 mg/Kg body weight. The alloxan solution was freshly prepared in 0.1 mol/L citrate buffer (pH = 4.5) in a volume of 1 mL/Kg, kept on ice, and injected immediately. Five days later, fasted blood was drawn from the tail vein and used for the measurement of blood glucose levels. The rats, which were considered to be diabetic mellitus-diseased only if their blood glucose levels exceeded 13 mmol/dL, were selected for study. All rats were not treated with insulin in this study.

Experimental design

In the experiment, a total of 50 rats (10 normal; 40 alloxan diabetically survived rats) were used. The rats were divided into 5 groups.

Group 1 (10 rats): Normal untreated rats.
Group 2 (10 rats): Alloxan-treated diabetic rats intragastrically administered TMP at a dose of 100 mg/Kg body weight.
Group 3 (10 rats): Alloxan-treated diabetic rats intragastrically administered TBE at a dose of 80 mg/Kg body weight.
Group 4 (10 rats): Alloxan-treated diabetic rats intragastrically administered metformin at a dose of 100 mg/Kg body weight.
Group 5 (10 rats): Alloxan-treated diabetic rats intragastrically administered an equivalent volume of distilled water as control.

The blood glucose levels were checked periodically for 28 d to confirm whether hyperglycemia was brought under control. After 28 d of treatment, all rats were fasted overnight (18 to 24 h), and blood was drawn from the orbital sinus after anesthetization with ether and collected into potassium oxalate and sodium fluoride tubes for analysis of the blood glucose, total cholesterol, phospholipids, triglycerides, superoxide dismutase, glutathione reductase, total antioxidant capability, glutathione peroxidise activities, and malondialdehyde. Liver, kidney, and pancreas tissues were excised immediately and were stored at −70 °C until required. Before using, tissue samples were weighed and homogenized on ice using a homogenizer in the appropriate buffer [1/10 parts (w/v)]. The homogenates were centrifuged at 10000 × *g* for 15 min at 4 °C to discard any cell debris. The supper liquid that was provided for analysis comprised superoxide dismutase, glutathione reductase, total antioxidant capability and glutathione peroxidise activities, and malondialdehyde.

Analytical methods

The levels of blood glucose, total cholesterol, phospholipid, and triglycerides in the serum of diabetic rats were determined using their corresponding colorimetric assay kit according to their manufacturer's protocol, respectively.

The superoxide dismutase and glutathione reductase, total antioxidant capability, catalase and glutathione peroxidise activities, and malondialdehyde levels of supernatants were immediately determined using their corresponding colorimetric assay kit and protein in supernatants was also analyzed. All assays were carried out in triplicates.

Statistical analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT). The results were expressed as mean ± SD for 10 rats in each group. *P*-values < 0.05 were considered to be significant.

Results and Discussion

Effect of TBE, TMP, and metformin on blood glucose

High blood glucose level was a significant and dramatic character of diabetic mellitus and the control of blood glucose levels was a key step to cure diabetic mellitus and its complications. In the present study, an experimental model of diabetic rats was induced by intraperitoneal administration of alloxan. TBE was administered at the dose of 100 mg/Kg body weight. This dose of TBE was chosen since the results obtained by us in the earlier related studies suggested that it would be effective (Zhang and Zhang 2006). At the start of experiment, all groups of rats had nearly the same blood glucose levels ($P > 0.05$). In contrast with the blood glucose level of normal rats, administration of alloxan led to 5-fold elevation of blood glucose levels, which was maintained over a period of 4 wk. It was attested that the model rats were stable and usable. In comparison to the untreated group, the blood glucose levels of administrating TBE, TMP, and metformin were significantly decreased ($P < 0.05$). However, there was no significant difference between the blood glucose levels of TBE, TMP, and metformin groups ($P > 0.05$) (Table 1).

Effect of TBE, TMP, and metformin on body weight

The basal body weight was 200.8 ± 15.64 (Table 2) and there is no variation from one group to another. The body weight of the untreated and TMP groups was slightly changed at the end of the 4th week ($P > 0.05$) in comparison with initial body weight. But intragastrically administrating of TBE and metformin for 4 wk resulted in a significant increase in body weight ($P < 0.05$) as compared with the untreated group. No significant difference was observed between TBE, TMP, and metformin groups. While enhancing body weight, TBE also rectified the abnormal food intake efficiency of diabetic rats (Table 2). A significant difference in both body weight gain and food intake was observed between the control groups and normal animals. The food intake amount of the diabetic untreated rats significantly increased in contrast with the

Table 1—Effect of TBE on blood glucose in diabetic rats.

	Blood glucose levels (mmol/L)				
Group	0 d	7 d	14 d	21 d	28 d
Normal	5.89 ± 0.89	5.97 ± 1.03	5.93 ± 1.06	5.97 ± 1.08	6.01 ± 1.14
Untreated	22.34 ± 6.24	23.71 ± 6.11	24.63 ± 7.02	25.79 ± 5.12	27.05 ± 3.84
TBE	22.58 ± 5.63	13.71 ± 6.22[a]	13.24 ± 6.75[a]	14.83 ± 6.76[a]	15.48 ± 6.52[a]
TMP	22.76 ± 5.60	15.80 ± 5.21[a]	16.89 ± 5.34[a]	18.21 ± 3.82[a]	20.29 ± 3.09[a]
Metformin	22.68 ± 5.29	15.49 ± 7.03[a]	15.87 ± 6.87[a]	16.38 ± 7.01[a]	17.88 ± 6.89[a]

Each value is mean ± S.E. for 10 rats in each group. Values with superscript letter a within a column are significantly different at $P < 0.05$ (DMRT) in contrast with the untreated group.

Table 2—Effect of TBE, TMP, and metformin on the body weight gain and food intake in diabetic rats for 28 d.

	Body weight (g)							
Group	0 d	7 d	14 d	21 d	28 d	Body weight gain (g/d)	Food intake (g/d)	Food efficiency ratio[d]
Normal	215.6 ± 16.5	223.5 ± 17.6	239.8 ± 19.2	256.4 ± 21.6	273.2 ± 24.9	2.06 ± 0.3	7.63 ± 0.65	0.27 ± 0.04
Untreated	193.2 ± 13.04	194.5 ± 18.9	201.5 ± 20.8	206.4 ± 26.5	208.1 ± 27.8	1.06 ± 0.51[a]	11.24 ± 0.92[a]	0.09 ± 0.05[a]
TBE	200.2 ± 11.82	216.3 ± 15.1[b]	232.5 ± 27.6[b]	246.5 ± 27.5[b]	254.0 ± 31.1[b]	1.92 ± 0.37[bc]	8.94 ± 0.74[abc]	0.21 ± 0.04[abc]
TMP	199.3 ± 11.26	207.5 ± 18.47	214.4 ± 20.5	224.7 ± 24.8	234.2 ± 26.5[b]	1.25 ± 0.32[ab]	9.76 ± 0.83[ab]	0.13 ± 0.03[ab]
Metformin	210.8 ± 19.89	220.0 ± 30.4	234.8 ± 27.7[b]	248.4 ± 25.64[b]	259.1 ± 29.6[b]	1.73 ± 0.28[abc]	8.85 ± 0.86[a]	0.20 ± 0.03[ac]

Each value is mean ± S.E. for 10 rats in each group. Values with different superscript letters within a column are significantly different at $P < 0.05$ (DMRT). [a]In contrast with the normal group; [b]in contrast with the untreated group; [c]in contrast with the TMP group; [d]body weight gain/food intake.

normal rats ($P < 0.05$). After administrating TBE, TMP, and metformin, the amount of food intake was markedly lower as compared with diabetic untreated group. Especially in TBE group, the weight gain was not lower than in TMP and metformin, but the food intake was lower than in TMP and metformin. This result demonstrated that TBE could significantly increase food efficiency ration and be in favor of animal growth.

Effect of TBE, TMP, and metformin on lipid levels

Abnormal lipid metabolism is a common syndrome of diabetic mellitus. There is evidence that not only excessive consumption of fats may eventually lead to the development of insulin resistance (Uauy and Diaz 2005) but also endogenous lipid molecules induce cytosolic phosphoenolpyruvate carboxykinase gene transcription, which plays key roles in gluconeogenesis, glyceroneogenesis, and cataplerosis, and attenuate the insulin action (Chen 2007). Clinically, it has been observed that there is present altered fat metabolism in type 1 and type 2 diabetes leading to the variation of serum cholesterol and triglyceride levels (Eisenbarth and others 1994). Hypercholesterelomia and hypertriglyceredemia have been reported to occur in streptozotocin-induced diabetic rats (Sharma and others 1997). In insulin-deficient subjects, insulin deficiency fails to activate lipoprotein lipase and causes hypertriglyceredemia. Hence, it is necessary to estimate serum cholesterol and triglyceride in the rats suffering chronic type 1 diabetic mellitus. Comparisons with untreated diabetic rats and TMP rats, the levels of total cholesterol, phospholipid, and triglyceride in the TBE group were significantly lower ($P < 0.01$), almost reaching normal levels ($P > 0.05$) (Table 3). These results demonstrated that TBE could decrease the levels of total cholesterol and triglyceride in the blood of diabetic rats.

Attenuation of activities of TBE, TMP, and metformin and its effect on oxidative stress

Recently, much attention has been paid to the role of oxidative stress, which resulted from an imbalance in oxygen free radical production, and it has been suggested that oxidative stress may be the key and common factor constraining the pathogenesis of different diabetic complications (Ding and others 2007; Cvetkovic and others 2008). Under hyperglycemic conditions such as diabetes, free radical production may arise from glucose self-oxidation (Hunt and others 1988), oxidative degradation of Amadori products (Elgawish and others 1996), advanced glycation end-proteins–receptor of glycation end-proteins interaction (Tan and others 2007), and redox potential increase (Linnane and Eastwood 2006). If not completely scavenged, redundant free radicals can bring about great damages to DNA, protein, or lipids. Eucaryotic cells contain antioxidant defenses that can protect these cells from oxidative damage. Antioxidants may be either nonenzymatic (vitamins C and E and reduced glutathione) or enzymatic (glutathione reductase and superoxide dismutase). In the course of diabetes, both types of antioxidants have been reported to be reduced (Makar and others 1995; Mooradian 1995) or enhanced (Sechi and others 1997), depending on the tissues studied or diabetes duration. So, in our investigations, superoxide dismutase, glutathione reductase, catalase and glutathione peroxidise, total antioxidant capability, and malondialdehyde were indexed to compare the impact of TBE, TMP, and metformin on oxidative press in diabetic rats.

The total antioxidant capability of diabetic untreated group was significantly debased compared with normal group in plasma, liver, kidney, and pancreas ($P < 0.05$) (Table 4). When giving tested drugs, total antioxidant capabilities in different tissue showed uptrend, but the uptrend displayed markedly significant difference in liver

Table 3—Effect of TBE, TMP, and metformin on levels of cholesterol, phospholipids, and triglyceride in the plasma of diabetic rats.

Group	Cholesterol (mg/dL)	Phospholipid (mg/dL)	Triglyceride (mg/dL)
Normal	90.1 ± 12.6	87.3 ± 8.5	85.3 ± 6.1
Untreated	143.9 ± 10.8[a]	135.6 ± 13[a]	175.2 ± 27.4[a]
TBE	94.3 ± 9.4[bc]	91.2 ± 8.5[bc]	82.5 ± 13.6[bc]
TMP	108.6 ± 10.7[ab]	121.6 ± 9.8[ab]	143.1 ± 18.9[ab]
Metformin	99.8 ± 8.5[b]	104.2 ± 11[abc]	131.4 ± 17.6[ab]

Each value is mean ± S.E. for 10 rats in each group. Values with different superscript letters within a column are significantly different at $P < 0.05$ (DMRT). [a]In contrast with the normal group; [b]in contrast with the untreated group; [c]in contrast with the TMP group.

Table 4—Effect of TBE, TMP, and metformin on total antioxidant activities in tissues of diabetic rats (U/mL).

Group	Plasma	Liver	Kidney	Pancreas
Normal	21.89 ± 5.09	1.786 ± 0.15	1.029 ± 0.064	0.819 ± 0.176
Untreated	9.37 ± 6.74[a]	0.514 ± 0.33[a]	0.566 ± 0.143[a]	0.577 ± 0.165[a]
TBE	15.76 ± 5.97[a]	1.017 ± 0.43[ab]	0.976 ± 0.231[b]	0.704 ± 0.143
TMP	13.54 ± 6.27[a]	0.894 ± 0.32	0.872 ± 0.187[b]	0.639 ± 0.107
Metformin	12.86 ± 6.16[a]	0.943 ± 0.52[b]	0.738 ± 0.149[a]	0.677 ± 0.143

Each value is mean ± S.E. for 10 rats in each group. Values with different superscript letters within a column are significantly different at $P < 0.05$ (DMRT). [a]In contrast with the normal group; [b]in contrast with the untreated group; [c]in contrast with the TMP group.

Table 5—Effect of TBE, TMP, and metformin on SOD activities in tissues of diabetic rats (U/mL).

Group	Plasma	Liver	Kidney	Pancreas
Normal	166.63 ± 24.11	17.75 ± 1.78	28.36 ± 3.48	11.23 ± 2.14
Untreated	91.78 ± 12.87[a]	9.86 ± 2.04[a]	12.56 ± 3.53[a]	6.87 ± 2.04[a]
TBE	156.87 ± 11.83[bc]	16.34 ± 2.13[bc]	25.43 ± 4.72[bc]	9.65 ± 2.63[b]
TMP	121.56 ± 17.82[ab]	10.48 ± 2.41[b]	18.64 ± 3.72[ab]	8.97 ± 2.32[b]
Metformin	132.84 ± 13.76[ab]	14.18 ± 1.86[ab]	22.38 ± 4.27[abc]	8.45 ± 1.75[b]

Each value is mean ± S.E. for 10 rats in each group. Values with different superscript letters within a column are significantly different at $P < 0.05$ (DMRT). [a]In contrast with the normal group; [b]in contrast with the untreated group; [c]in contrast with the TMP group.

and kidney, and the significant difference between TBE and TMP was not observed.

Plasma, liver, kidney, and pancreas are endowed with innate antioxidant defense mechanisms, such as the presence of the enzymes catalase, superoxide dismutase, and glutathione peroxidase. A reduction in the activities of these enzymes is associated with the accumulation of highly reactive free radicals, leading to deleterious effects such as loss of integrity and function of cell membranes (Reedy and Lokesh 1992; Krishnakantha and Lokesh 1993; Sheela and Angusti 1995). Administration of alloxan leads to generation of reactive oxygen species such as H_2O_2, $O_2^{\bullet}-$, and $HO^{\bullet}$, which are associated with inactivation of superoxide dismutase, catalase, and glutathione peroxidise. This probably explains the significantly reduced activities of superoxide dismutase, catalase, and glutathione peroxidise observed by us in rats challenged with alloxan (untreated group in Table 5 to 7). In rats receiving TBE, TMP, and metformin, Significant increase of superoxide dismutase was noticed in the TBE and metformin groups in different tissue ($P < 0.05$), simultaneous obvious differences also existed between the TBE and TMP groups ($P < 0.05$) in all examined tissue (Table 5); significant increase of catalase in the TBE, TMP, and metformin was only found in the liver and kidney ($P < 0.05$), and no markedly difference was found between TBE and TMP groups ($P > 0.05$) (Table 6); while significant increase of glutathione peroxidise activities was only found in all tissues of TBE group, as well as liver and plasma in the TMP group(Table 7).

Glutathione reductase, the enzyme responsible for recycling glutathione disulfide to glutathione, significantly decreased in plasma, liver, kidney, and pancreas of diabetic rats as compared with the normal rats ($P < 0.01$) (Table 8). However, after given drugs, TBE significantly changed almost to the normal levels as compared with the normal rats ($P > 0.05$), and the activities were stronger than the effects of TMP ($P < 0.05$).

These results described previously possibly confer that these protective activities of TBE, by dampening the generation of free radicals induced by alloxan, were stronger than those of TMP and metformin.

Peroxidation of free radical mediated cell membrane lipids has been implicated under pathological conditions such as increased cell membrane rigidity, decreased cellular deformability, and lipid fluidity. Overproduction of reactive oxygen species due to glucose oxidation in the presence of the transition metals can cause membrane damage through the peroxidation of membrane lipid and protein glycation (Kolanjiappan and others 2002). Elevated lipid peroxidation in liver and kidney has been well demonstrated in alloxan-induced diabetic rats (Ananthan and others 2004). The extent of tissue damage has been assessed in terms of the measurements of lipid peroxidation products such as malondialdehyde and antioxidants (Gutteridge 1995). Thus, the observed increase of malondialdehyde in different tissues could be used as an index of lipid peoxidation.

Alloxan administration led to a significant increase in the malondialdehyde level of plasma, liver, kidney, and pancreas as compared with the normal rats ($P < 0.01$) (Table 9). The administration of TBE ameliorated the alloxan-induced elevation of lipid peroxidation in plasma, liver, and pancreas as compared to the untreated rats. However, no significant decrease was observed in MDA in TMP and metformin rats. The previously mentioned results presented in Table 9 show that TBE could decrease the oxidative stress induced by alloxan and protect tissues from damages mediated by free radicals.

Conclusions

Abnormal lipid-metabolism and enhanced oxidative stress were 2 major factors contributing to familiar complications of diabetic mellitus. In this article, the regulation activities on

Table 6—Effect of TBE, TMP, and metformin on catalase activities in tissues of diabetic rats (U/mL).

Group	Plasma	Liver	Kidney	Pancreas
Normal	20.894 ± 2.768	4.163 ± 0.276	1.784 ± 0.253	0.419 ± 0.176
Untreated	15.438 ± 2.515[a]	2.264 ± 0.282[a]	1.068 ± 0.199[a]	0.269 ± 0.147[a]
TBE	20.837 ± 2.587[b]	3.216 ± 0.276[ab]	1.497 ± 0.277[ab]	0.327 ± 0.174
TMP	18.364 ± 2.139	3.061 ± 0.307[ab]	1.412 ± 0.218[ab]	0.316 ± 0.156
Metformin	16.549 ± 2.132[a]	2.976 ± 0.284[ab]	1.423 ± 0.255[ab]	0.301 ± 0.163

Each value is mean ± S.E. for 10 rats in each group. Values with different superscript letters within a column are significantly different at $P < 0.05$ (DMRT). [a]In contrast with the normal group; [b]in contrast with the untreated group; [c]in contrast with the TMP group.

Table 7—Effect of TBE, TMP, and metformin on glutathione peroxidase activities in tissues of diabetic rats (U/mL).

Group	Plasma	Liver	Kidney	Pancreas
Normal	159.771 ± 13.168	67.131 ± 9.171	40.518 ± 5.946	10.342 ± 2.673
Untreated	100.494 ± 12.611[a]	41.953 ± 10.622[a]	30.673 ± 6.783[a]	6.414 ± 2.917[a]
TBE	139.715 ± 12.839[ab]	61.739 ± 9.735[b]	38.361 ± 6.539[ab]	9.313 ± 2.37[b]
TMP	117.623 ± 11.723[ab]	58.552 ± 8.371[b]	35.412 ± 5.811	7.02 ± 2.356[a]
Metformin	197.28 ± 12.35.8[a]	58.269 ± 8.661[b]	34.347 ± 6.114	7.177 ± 2.537[a]

Each value is mean ± S.E. for 10 rats in each group. Values with different superscript letters within a column are significantly different at $P < 0.05$ (DMRT). [a]In contrast with the normal group; [b]in contrast with the untreated group; [c]in contrast with the TMP group.

Table 8—Effect of TBE, TMP, and metformin on GR activities in tissues of diabetic rats (U/mL).

Group	Plasma	Liver	Kidney	Pancreas
Normal	221.99 ± 11.15	83.65 ± 5.35	37.5 ± 6.4	8.72 ± 1.13
Untreated	177.59 ± 12.56[a]	49.54 ± 6.23[a]	18.54 ± 5.98[a]	5.12 ± 1.29[a]
TBE	218.93 ± 12.13[bc]	78.65 ± 6.87[bc]	32.23 ± 6.12[bc]	7.67 ± 1.08[bc]
TMP	188.34 ± 10.13[a]	57.13 ± 6.94[ab]	25.41 ± 5.83[ab]	6.02 ± 1.14[ab]
Metformin	197.28 ± 11.86[ab]	70.54 ± 6.07[abc]	30.65 ± 6.82[ab]	7.1 ± 0.97[abc]

Each value is mean ± S.E. for 10 rats in each group. Values with different superscript letters within a column are significantly different at $P < 0.05$ (DMRT). [a]In contrast with the normal group; [b]in contrast with the untreated group; [c]in contrast with the TMP group.

Table 9—Effect of TBE, TMP, and metformin on malondialdehyde levels in tissues of diabetic rats (nmol/mL).

Group	Plasma	Liver	Kidney	Pancreas
Normal	3.85 ± 1.67	0.64 ± 0.42	0.98 ± 0.31	0.23 ± 0.17
Untreated	6.26 ± 1.56[a]	1.25 ± 0.47[a]	1.56 ± 0.35[a]	0.48 ± 0.18[a]
TBE	4.18 ± 1.32[bc]	0.77 ± 0.48[bc]	1.32 ± 0.34	0.25 ± 0.14[b]
TMP	5.59 ± 1.09[a]	1.19 ± 0.33[a]	1.61 ± 0.38[a]	0.28 ± 0.19
Metformin	5.13 ± 1.14	1.06 ± 0.26	1.47 ± 0.34[a]	0.31 ± 0.17

Each value is mean ± S.E. for 10 rats in each group. Values with different superscript letters within a column are significantly different at $P < 0.05$ (DMRT). [a]In contrast with the normal group; [b]in contrast with the untreated group; [c]in contrast with the TMP group.

lipid-metabolism and attenuation activities on oxidative stress of diabetic rats of TBE and TMP, as well as metformin were compared. TBE possesses stranger function in decreasing blood glucose level and the concentration of cholesterol, phospholipids, and triglyceridon of plasma of diabetic rats. TBE could increase the superoxide dismutase, catalase, glutathione peroxidise, and glutathione reductase and decrease the malondialdehyde concentration in determined tissue. It was shown that TBE could perform more significant function in rectifying abnormal lipid-metabolism, reducing oxidant press, and controlling diabetic complications.

Unlike the hypoglycermic component of TMP that contained 47.6% polysaccharides, there was little polar substance in TBE, such as protein, reducing sugar, and polysaccharide. TBE could be more easily dissolved in boiled water, 40% to 60% ethanol and alkaline solution. TBE could give rise to durable foam. TLC indicated that the band with the same R_f value turned red after reaction with methanol:acetic acid:sulfuric acid:anisaldehyde (85:10:5:0.1), green after reaction with sulfuric acid:$CHCl_3$ (1:9), and yellow after reaction with CCl_3COOH. Based on these characteristics, it was speculated that some components of TBE were saponins (Xiao 1997). Saponins content in TBE was estimated at 23.1% by colorimetric method (Xiang and others 2001). About the structure of the saponins in TBE would be reported in other articles.

In many reported studies, saponins and polysaccharides were thought of possessing potent activities to regulate blood glucose levels, blood lipid levels, and antioxidative stress (Lang and others 2005; Zhang and others 2007). These studied results also supported our conclusion obtained in the experiment. Since TBE and TMP were 2 kinds of different metabolites simultaneously produced in submerged culture of *T. aurantialba* and the difference of their function was so significant that the same mechanism may not be appropriate to explain the difference. The mechanism for TBE to regulate blood glucose levels, blood lipid levels, and alleviate oxidative stress remained unclear and further studies are required to elucidate their operating mechanisms.

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