ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 11, Issue 10, 2018

Online - 2455-3891
Print - 0974-2441

Research Article

THE EFFECT OF GAMBIER EXTRACT ON THE LEVELS OF MALONDIALDEHYDE, SUPEROXIDE DISMUTASE, AND BLOOD GLUCOSE IN TYPE 2 DIABETES MELLITUS PATIENTS

YUNITA SARI PANE[1]*, RATNA AKBARI GANIE[2], DHARMA LINDARTO[3], AZNAN LELO[1]

[1]Department of Pharmacology & Therapeutics, Faculty of Medicine, Universitas, Sumatera Utara, Medan, North Sumatera, Indonesia, [2]Department of Clinical Pathology, Faculty of Medicine, Universitas Sumatera Utara, Medan, North Sumatera, Indonesia, [3]Department of Internal Medicine, Faculty of Medicine, Universitas Sumatera Utara, Medan, North Sumatera, Indonesia.
Email: yunitasaripane@yahoo.com

Received: 09 April 2018, Revised and Accepted: 06 June 2018

ABSTRACT

Objective: Diabetes mellitus is a disease associated with dysfunction of pancreatic β-cells and oxidative stress. A treatment which can reduce the impact of oxidative stress may be beneficial in the treatment of diabetes. Therefore, this study aimed to investigate the effect of gambier extract on the levels of malondialdehyde (MDA), superoxide dismutase (SOD), and blood glucose level (BGL) in type 2 diabetes mellitus (T2DM).

Methods: This research was a randomized clinical study consisted of two groups, namely placebo group (n=10) and gambier group (n=6). The blood samples were collected from the vein after fasting overnight and before consuming 100 g white bread to measure the levels of MDA, SOD, and BGL. The same procedure was conducted after fasting and 2-h postprandial on day 1 and day 14. The data obtained were analyzed with Student's t-test with a statistical significance level of $p<0.05$.

Results: The results showed that there was no change in MDA levels in the placebo group during the observation, but there was a significant decrease in MDA levels in the gambier group on day 14. In contrast, SOD levels increased in all measurements although there was no change on day 1 in the placebo group. The present study also found a significant increase of BGLs after consuming 100 g bread in both groups, but less BGL elevation in the gambier group.

Conclusion: It is concluded that gambier extract has special mechanisms in the treatment of T2DM as an antioxidant and BGLs reduction.

Keywords: Gambier extract, Catechin, Malondialdehyde, Superoxide dismutase, Blood glucose level, Type 2 diabetes mellitus.

 DOI: http://dx.doi.org/10.22159/ajpcr.2018.v11i10.26620

INTRODUCTION

Diabetes mellitus is a metabolic disease characterized by chronic hyperglycemia and related to the dysfunction of pancreatic β-cells and oxidative stress. Oxidative stress is reported to contribute to the dysfunction and effects of antioxidants on the pathogenesis of diabetes [1]. This occurs due to the release of superoxide which alters the electron transport in the mitochondria and increases the activity of nicotinamide adenine dinucleotide phosphate oxidase. Oxidative stress, through the production of reactive oxygen species (ROS), has been suggested as the root cause underlying the development of type 2 diabetes mellitus (T2DM) and the progression of long-term diabetes complications [2]. Thus, it is important to find complementary treatments such as herbal medicines which can reduce the impact of oxidative stress, so it will be beneficial in the treatment of diabetes. Gambier or *Uncaria tomentosa* (*Ut*, Cat's claw) has antioxidant characteristics [3].

The polyphenol catechins have strong radical-scavenging and antioxidative effects. These effects may contribute to the prevention of diseases such as diabetes [1].

Catechins may treat diabetes and its complications with the modification of oxidative stress [4]. It was reported previously that the consumption of catechin-rich green tea slightly inhibited postprandial elevation of blood glucose level (BGL) and oxidative products in postmenopausal women [5]. This finding suggests that the intake of green tea containing catechins could reduce the risk of type 2 diabetes. Among many medicinal plants which have been used for treatment, this research used a plant which was well known to the public, namely gambier in the form of extract. Gambier is an herbal medicine which is rich in antioxidants such as catechins.

Therefore, the present study aimed to investigate the effect of gambier (*Uncaria Gambir* Roxb.) extract on malondialdehyde (MDA), superoxide dismutase (SOD), and BGL in T2DM patients.

METHODS

This study has received ethical approval from the Health Research Ethical Committee in University of Sumatera Utara (No:572/TGL/KEPKFK USU-RSUP HAM/2017). The study group consisted of uncontrolled T2DM subjects with metformin monotherapy. The inclusion criteria were T2DM, aged between 35 and 70 years old, BGL at fasting >126 mg/dl, and the 2-h postprandial >200 mg/dl, whereas the exclusion criteria were the subjects consumed other antidiabetics, glycated hemoglobin (HbA1C) <7, and had a history of kidney or liver disease. The subjects recruited in the study had a 14-day follow-up with daily monitoring of drug consumption by phone. In addition, the time of administration, dosages, and presence or absence of the side effects of metformin or gambier extract were also monitored. 16 T2DM patients were randomly assigned to two treatment groups, namely the placebo group (500 mg metformin + placebo) (n=10) and the gambier group (500 mg metformin + 375 mg gambier extract) (n=6). All drugs were consumed twice a day.

After fasting overnight and before consuming 100-g white bread, vein blood samples were collected to measure MDA, SOD, and BGL levels of the subjects. The same procedure was conducted at the 2-h postprandial on day 1 and day 14.

BGL levels were determined with the glucose oxidase method while MDA and SOD concentrations were determined with spectrophotometric assay kit. All procedures were performed according to the

manufacturer's instructions. BGL levels were measured by the GLUC 2 Roche procedures. MDA levels were measured using thiobarbituric acid assay (R and D System procedures), whereas SOD levels were measured using the BioAssay system procedures.

The data obtained were presented as a mean ± standard deviation. Student's t-tests were performed for mean comparisons. $p<0.05$ was considered as statistically significant.

RESULTS

The general characteristics of subjects participated in the present study are presented in Table 1. There were no significant differences in all variables measured before the intervention for the placebo and gambier groups.

Table 2 summarizes the changes in the levels of MDA, SOD, and BGL on the day of observation. There was a declining trend in MDA levels in both groups. However, there was only a significant decrease in MDA levels in the gambier group on day 14 ($p=0.001$). In addition, MDA levels in the gambier group were lower than the placebo group. Overall, there was no significant difference, except the MDA level in the gambier group was significantly lower than the placebo group at the 2-h postprandial on day 14.

On the other hand, the levels of SOD significantly ($p<0.05$) increased in all measurements, but there was no change only on the 1st day in the placebo group. Furthermore, SOD levels were higher in the gambier group than in the placebo group, but it was not statistically significant ($p>0.05$) (Table 2).

2 h after consuming 100 mg white bread, all BGLs were significantly increased (Table 2)

Table 3 summarizes data about the levels of MDA, SOD, and BGL based at the fasting hour and 2-h postprandial on the observation day. As shown, there were significantly higher MDA levels at fasting hour on day 14 than on day 1 in both groups. However, there was no difference ($p=0.35$) in MDA levels at the 2-h postprandial between day 1 and day 14 in the gambier group.

In contrast, Table 3 also summarizes that there was no significant difference in SOD levels between day 1 and day 14, but SOD levels at the fasting hour on day 14 were significantly higher than on day 1 in the gambier group.

As shown in Table 3, there was a significant lower in the BGLs at fasting hour in both groups. The 2-h postprandial BGL was only lower in the gambier group.

Table 4 summarizes the percentage of decreasing MDA levels; however, no significant difference was found in the percentage of decrease in MDA levels for each treatment between day 1 and day 14, although the percentage of decrease in MDA levels was two times higher in gambier group. But there was a significant difference in the percentage of decreasing MDA levels between the gambier group compared to the placebo group on day 14.

In the placebo group, the percentage of increase in the SOD levels was markedly on day 14, but it was not statistically significant compared to that on day 1. On the contrary, in the gambier group, the percentage of increase in SOD levels on day 14 was lower than that on day 1. Although the percentage of increase in SOD levels in the gambier group was higher than the placebo group, a significant difference was only found on day 1 (Table 4).

The percentage of increase in BGL at the 2-h postprandial occurred in both groups; however, there was a double increase of BGL in the placebo group ($p=0.02$). There was no significant of the percentage increase in BGL between two groups.

Table 1: Baseline data of participants before an intervention in both groups (placebo group and gambier group)

Variable	Group		p
	Placebo	Gambier	
Number of subjects (male/female)	10 (6/4)	6 (3/3)	-
Age (year old)	53.50±8.54	54.83±10.78	0.788
Education			
Academic	3	3	
High school	7	3	
BMI	23.75±4.65	25.31±3.74	0.499
High (cm)	157.50±10.99	161.83±12.40	0.478
Weight (kg)	59.60±11.34	63.33±9.44	0.510
HbA1C	10.97±2.37	9.8±1.98	0.329
	0.71±0.10	0.68±0.06	0.575
SOD (U/ml)	5.33±1.58	5.37±2.13	0.448
Fasting blood glucose level (mg/dl)	238.50±87.64	234.00±72.71	0.917
AST (SGOT) (U/L)	15.50±4.84	25.67±15.96	0.191
ALT (SGPT) (U/L)	26.50	16.40±8.50	0.072
Ureum (mg/dl)	32.15±13.51	24.87±13.14	0.310
Creatinine (U/L)	1.05±0.61	0.95±0.52	0.411
Time diagnosed (year)	5.90±1.39	7.50±2.08	0.510
Abdominal circle (cm)	89.40±10.93	92.33±12.48	0.629

BMI: Body mass index, HbA1C: Glycated hemoglobin, SOD: Superoxide dismutase, AST: Aspartate aminotransferase, SGOT: Serum glutamic oxaloacetic transaminase, ALT: Alanine transaminase, SGPT: Serum glutamic-pyruvic transaminase

Table 2: The levels of malondialdehyde, superoxide dismutase, and blood glucose on the day of observation

Variable	Day 1		p	Day 14		p
	Fasting hour	2-h postprandial		Fasting hour	2-h postprandial	
Malondialdehyde (nmol/dl)						
Placebo	0.71±0.10	0.67±0.18	0.36	1.05±0.62	0.82±0.09	0.44
Gambier	0.68±0.06	0.60±0.20	0.12	0.91±0.10	0.67±0.04	0.001*
p	0.58	0.23		0.19	0.001*	
Superoxide dismutase (U/ml)						
Placebo	5.33±1.58	5.76±2.14	0.39	4.29±2.27	5.73±2.81	0.001*
Gambier	5.36±2.13	8.19±2.18	0.03*	5.82±1.95	8.84±4.11	0.02*
p	0.45	0.13		0.07	0.09	
Blood glucose (mg/dl)						
Placebo	238.50±87.4	329.30±125.71	0.001*	196.00±88.64	333.90±73.89	0.001*
Gambier	234.00±72.71	326.33±67.49	0.001*	194.33±53.76	302.83±55.71	0.001*
p	0.92	0.96		0.59	0.39	

*Significance ($p<0.05$)

Table 3: The levels of malondialdehyde, superoxide dismutase, and blood glucose based on the fasting hour and 2-h postprandial

Variable	Fasting hour		p	2-h postprandial		p
	Day 1	Day 14		Day 1	Day 14	
Malondialdehyde						
Placebo	0.71±0.10	1.05±0.62	0.001*	0.67±0.18	0.82±0.09	0.001*
Gambier	0.68±0.06	0.91±0.10	0.001*	0.60±0.20	0.67±0.04	0.35
Superoxide dismutase						
Placebo	5.33±1.58	4.29±2.27	0.07	5.76±2.14	5.73±2.81	0.95
Gambier	5.36±2.13	5.82±1.95	0.03*	8.19±2.18	8.84±4.11	0.58
Blood glucose						
Placebo	238.50±87.64	196.00±88.64	0.001*	329.30±125.71	333.90±73.89	0.80
Gambier	234.00±72.71	194.33±53.76	0.04*	326.33±67.49	302.83±55.71	0.02*

*Significance (p<0.05)

Table 4: The percentage of change in the levels of malondialdehyde, superoxide dismutase, and blood glucose in the placebo and gambier group

Variable	Day 1	Day 14	p
Malondialdehyde			
Placebo	−7.17±20.63	−7.14±26.52	0.29
Gambier	−13.13±27.15	−25.66±8.56	0.35
p	0.33	0.05	
Superoxide dismutase			
Placebo	9.75±23.42	34.86±26.86	0.07
Gambier	59.74±41.78	47.46±18.17	0.57
p	0.001*	0.33	
Blood glucose			
Placebo	37.52±6.00	84.77±36.75	0.02*
Gambier	44.17±22.33	59.48±23.78	0.22
p	0.38	0.19	

*Significance (p<0.05)

DISCUSSION

To the best of our knowledge, the present study was the first to examine the effect of gambier extract intake on oxidative stress (MDA and SOD) and BGL in uncontrolled T2DM patients. At the baseline data, all participants were completely uncontrolled T2DM with the fasting BGL hyperglycemia >200 mg/dl (238.50±87.64 mg/dl in the placebo group and 234.00±72.71 mg/dl in the gambier group). The HbA1C values for both groups were higher than 7 (10.97±2.37 in the placebo group and 9.8±1.98 in the gambier group). There were similar values found in the variables measured between the two groups (p>0.05) as shown in Table 1.

A complementary and alternative medicine has been used by many diabetic patients in the world. The herbal medicine gambier (Cat's claw) is used as CAM because it is rich in catechin. Gambier, which is called *Asen-'yaku* in Japan, previously, has a role in the normalization of BGL [6]. Another study demonstrated that daily consumption of green tea which is rich in catechin decreased serum MDA-low-density lipoprotein concentrations [7].

The present study showed that there was a decreasing trend in MDA levels in both groups. However, there was no statistically significant change in MDA levels for the placebo group during the observation, but there was a significant decrease in MDA levels in the gambier group only on day14 (p<0.05) (Table 2).

As shown in Table 3, the comparison of MDA levels at fasting hour and 2-h postprandial demonstrated that there were significant higher MDA levels at fasting hour on day 14 than day 1 in both groups (p<0.05). In contrast, in the gambier group, there was no difference found (p=0.35) in MDA levels at the 2-h postprandial between day 1 and day 14 (Table 3).

In addition, the levels of MDA in the gambier group appeared to be lower than the placebo group. Although there was no significant difference, the levels of MDA in the gambier group were significantly lower than the placebo group at the 2-h postprandial on day 14 (Table 2).

The percentage of decrease in MDA levels on day 14 was double compared to that on day 1; however, it was not statistically significant (Table 4). Furthermore, there was a significant difference in the percentage of decrease in MDA levels on day 14 between the placebo group (−7.14±26.52%) and the gambier group (−25.66±8.56%). This evidence indicates that gambier containing catechin suppressed the production of MDA after 2 weeks of ingestion.

The *in vitro* study of Manju *et al.* (2013) demonstrated that catechin were found in Acorus calamus rhizome. The total phenol content was expressed as mg of catechin/g of extract and the corresponding values for A. calamus methanolic and aqueous extract was found to be 12.17±1.47 mg/g and 19.86 ± 1.45 mg/g respectively. [8].

An animal study found that SOD activity significantly increased after an intraperitoneal administration, but catechins administration had no effect on MDA levels which remained stable during the study. SOD activity showed a moderate negative correlation with glutathione peroxidase activity [9].

This study also found that the levels of SOD increased in all measurements, and only no change was found on the 1st day of the placebo group. In addition, the levels of SOD were higher in the gambier group than in the placebo group, but it was not statistically significant (p>0.05) (Table 2). Table 3 summarizes that the levels of SOD at fasting hour on day 14 were higher significantly than on day 1 in the gambier group. In contrast, Table 3 also summarizes that there was no significant difference in SOD levels between day 1 and day 14, except the fact that SOD levels at fasting hour on day 14 were significantly higher than day 1 in the gambier group. The percentage of increase in SOD levels in the gambier group was higher than that in the placebo group; in particular, the percentage of increase in SOD levels in the gambier group on day 1 was 6 times higher than that in the placebo group (Table 4).

This is supported by a study conducted by Widodo *et al.* using herbal medicine *Nigella sativa* seed extract doses of 125 and 250 mg/kg showed antihyperglycemic effects, increased antioxidant activity, as well as pancreatic regeneration of organ damage in alloxan-induced diabetic rats [10]. While, Farias *et al.* found no differences in oxidative stress of the antioxidant enzyme SOD in colorectal cancer patients with and without the gambier therapy [11].

Catechin was identified as the major bioactive compound in gambier. One of the main importances is that the antioxidant in gambier is safe. The dose of catechin dependently decreased the serum levels of MDA and increased SOD in the catechin-treated diabetic groups versus the untreated diabetic group (p<0.05) [4]. The present study, however, was not able to demonstrate this phenomenon because of the relatively low dose of gambier extract used (375 mg).

Catechins are dietary polyphenolic compounds associated with a wide variety of beneficial health effects *in vitro*, *in vivo*, and clinical. These therapeutic characteristics have been attributed to catechins' antioxidant and free radical scavenging effects. Another herbal medicine, cocoa contains polyphenolic components, such as catechin, epicatechin, and procyanidin B2. These components have strong antioxidative activity and may improve type 2 diabetes [12].

Ramirez-Sanches *et al.* (2013) examined oxidative stress-related alterations in skeletal muscle of heart failure and type 2 diabetes patients when compared with healthy controls and evaluated the effect of 3-month treatment with (-)-epicatechin rich cocoa (ERC). There were severe alterations in oxidative stress regulatory systems in the skeletal muscle of heart failure and type 2 diabetes patients when compared with healthy controls. Treatment with (-)-ERC induced recovery in glutathione levels and increased in SOD [13].

It is suggested that the fluctuating blood glucose concentrations, as those observed during postprandial glycemic excursions in people with T2DM, may contribute significantly to oxidative stress. Oxidative stress has been implicated as the underlying cause of both the macrovascular and microvascular complications associated with T2DM and developing diabetes [2].

On the other hand, the present study showed that there was a significant increase in BGL after consuming 100 g bread in both groups, but there was no significant difference in BGL between the two groups (Table 2). Table 3 summarizes that BGLs were significantly lower on day 14 than on day 1. However, no change in BGLs was found at the 2-h postprandial in the placebo group. The percentage of increase in BGL at the 2-h postprandial in the placebo group was double and statistically significant (p=0.02). The percentage of increase in BGL between the two groups was not significant (Table 4). The present study also demonstrated that gambier extract suppressed the elevation of BGL postprandially. Other study showed that the intake of catechin-rich green tea slightly inhibited an increase in the postprandial BGLs and oxidative products in postmenopausal women [5]. These effects suggest that the intake of green tea containing catechins could reduce the risk of type 2 diabetes.

CONCLUSION

The present study showed that an acute ingestion of catechins containing herbal gambier reduced MDA levels and the 2-h postprandial plasma glucose concentrations, but it increased SOD levels in uncontrolled T2DM patients. The present findings also indicate that the intake of gambier extract containing catechins improves the 2-h postprandial hyperglycemia and redox homeostasis in uncontrolled T2DM patients.

ACKNOWLEDGMENT

The authors would like to thank Laboratorium Terpadu, Faculty of Medicine, Universitas Sumatera Utara, for providing the place and facilities to conduct the research.

AUTHOR'S CONTRIBUTIONS

YSP, AZL, and DL designed this study. YSP conducts experiments. YSP, AZL, and RAG determine the model and analyze the data. YSP and AZL wrote the manuscript and have been discussed with DL and RAG. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

The authors declare that they have no competitive interest.

REFERENCES

1. Umeno A, Horie M, Murotomi K, Nakajima Y, Yoshida Y. Antioxidative and antidiabetic effects of natural polyphenols and isoflavones. Molecules 2016;21:pii: E708.
2. Wright E Jr., Scism-Bacon JL, Glass LC. Oxidative stress in Type 2 diabetes: The role of fasting and postprandial glycaemia. Int J Clin Pract 2006;60:308-14.
3. Pilarski R, Zieliński H, Ciesiołka D, Gulewicz K. Antioxidant activity of ethanolic and aqueous extracts of *Uncaria tomentosa* (Willd.) DC. J Ethnopharmacol 2006;104:18-23.
4. Samarghandian S, Azimi-Nezhad M, Farkhondeh T. Catechin treatment ameliorates diabetes and its complications in streptozotocin-induced diabetic rats. Dose Response 2017;15:1559325817691158.
5. Takahashi M, Miyashita M, Suzuki K, Bae SR, Kim HK, Wakisaka T, *et al.* Acute ingestion of catechin-rich green tea improves postprandial glucose status and increases serum thioredoxin concentrations in postmenopausal women. Br J Nutr 2014;112:1542-50.
6. Sugiyama S. Pharmacological action of gambir. Yakushigaku Zasshi 2005;40:29-33.
7. Hirano-Ohmori R, Takahashi R, Momiyama Y, Taniguchi H, Yonemura A, Tamai S, *et al.* Green tea consumption and serum malondialdehyde-modified LDL concentrations in healthy subjects. J Am Coll Nutr 2005;24:342-6.
8. Manju S, Chandran RP, Shaji PK, Nair GA. *In vitro* free radical scavenging potential of *Acorus calamus* L. Rizome from Kuttanad Wetlands, Kerala, India. Int J Pharm Pharm Sci 2013;5 Suppl 4:376-80.
9. Simos YV, Verginadis II, Toliopoulos IK, Velalopoulou AP, Karagounis IV, Karkabounas SC, *et al.* Effects of catechin and epicatechin on superoxide dismutase and glutathione peroxidase activity, *in vivo*. Redox Rep 2012;17:181-6.
10. Widodo GP, Herowati R, Perangin-angin JM, Kamlasi JE. Antihyperglycemic, antioxidant, and pancreas regeneration activities of black cumin (*Nigella sativa* L.) seeds ethanol extract in alloxan-induced diabetic rats. Int J Pharm Pharm Sci 2016;8:37-40.
11. Farias IL, Araújo MC, Farias JG, Rossato LV, Elsenbach LI, Dalmora SL, *et al.* Uncaria tomentosa for reducing side effects caused by chemotherapy in CRC patients: Clinical trial. Evid Based Complement Alternat Med 2012;2012:892182.
12. Satoh T, Igarashi M, Yamada S, Takahashi N, Watanabe K. Inhibitory effect of black tea and its combination with acarbose on small intestinal α-glucosidase activity. J Ethnopharmacol 2015;161:147-55.
13. Ramirez-Sanchez I, Taub PR, Ciaraldi TP, Nogueira L, Coe T, Perkins G, *et al.* (-)-epicatechin rich cocoa mediated modulation of oxidative stress regulators in skeletal muscle of heart failure and Type 2 diabetes patients. Int J Cardiol 2013;168:3982-90.