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Single and mixture administration of white tea (*Camellia sinensis*) and moringa (*Moringa oleifera*) as antihyperglycemia in diabetic male rats (*Sprague-dawley*) induced by streptozotocin

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Abstract.Hyperglycemia can increase oxidative stress by increasing the formation of reactive oxygen species (ROS) and alter redox reactions. Excessive ROS production leads to oxidative stress that can increase the product of lipid peroxidation. ROS production can be measured indirectly by measuring lipid peroxidation metabolites such as malondialdehyde (MDA) and F2-Isoprostan. The aim of this study was to determine the effect of single and mixture of white tea and moringa on oxidative stress markers (MDA and F2-Isoprostan) on streptozotocin-induced diabetic rats and compared with tea controls (green tea). Male *Sprague-dawley* rats, consist of 30 rats, were divided into six groups. Normal group was healthy rats without treatment, negative control group (NC) was streptozotocin-induced diabetic group without treatment, white tea treatment group (WT), moringa treatment group (M), mixture white tea and moringa treatment group (WT+M). The treatment were daily administrated by orally with 100 mg/kg BW epigallocatechingallate (EGCG) for 21 days. MDA was measured by UV-VIS spectrophotometer at 535 nm and F2-Isoprostane level was measured by ELISA. The Change of MDA levels (Δ MDA) between treatment groups showed that WT, M and WT+M were significantly different with NC group ($p < 0.05$). Compared to the control tea group (GT) showed that MDA levels of WT or M groups were different with Δ MDA GT group ($p < 0.05$). WT+M group that decreased MDA levels did not differ significantly with GT ($p > 0.05$). Change of F2-Isoprostan level between treatment groups (Δ F2-Isoprostan) showed that there were no significantly different to NC group ($p > 0.05$). It can be concluded that administration of white tea, moringa dan mixture of white tea+moringa may prevent or reduce MDA level in streptozotocin diabetic rats but not in F2-Isoprostane level. The combination of white tea+moringa (WT+M) has a better ability to decrease MDA level compared with single treatment and not significantly different with the control tea group (green tea).

1. Introduction

Hyperglycemia can increase oxidative stress by increasing the formation of reactive oxygen species (ROS) and alter redox reactions. Several studies have shown ROS as a result of hyperglycemia leading many secondary complications and oxidative damage of peripheral tissue [1]. Excessive ROS production leads to oxidative stress that can increase the product of lipid peroxidation [2][3]. Lipid peroxidation is thought to play an important role to cause endothelial dysfunction. Lipid peroxidation will leads to breakdown of fatty acid chains into various toxic compounds and cause damage of cell



membranes[4]. It is difficult to measure the present of free radicals in the laboratory because of its very short half-live time. ROS production can be measured indirectly by measuring lipid peroxidation metabolites such as malondialdehyde (MDA) and F2-Isoprostane [5].MDA can be formed when radicalhydroxyl react with the fatty acid component of the cell membrane.F2-Isoprostan is also a metabolite of the peroxidation of polyunsaturated fatty acids, especially arachidonic acid (AA) directly catalyzed by ROS [6][7][8].

Mc Kay and Blumberg report that the ability of polyphenols to capture free radicals is 100 times more effective than vitamin C and 25 times more effective than vitamin E [9]. White tea and moringa are sources of antioxidants that contain various polyphenolic compounds. The main compositions of tea are protein, polysaccharide, polyphenols, minerals, trace elements, organic amino acids , lignans, theaflavins, thearubigin, caffeine, theophylline and theobromine [10][11]. Catechins(flavanols) are the most phenol componentin the fresh tea leaves. The types ofcatechinarecatechin (C), galliccatechin (GC), epicatechin (EC), epigallocatechin (EGC), epicatechingallate (ECG), danepigallocatechingallate (EGCG) [12]. Moringaalso contains 46 powerful antioxidants that protect the body from free radicals, containing 18 amino acids (eight essentials) that the body needs to build new cells, 36 anti-inflammatory compounds, and 90 other nutrients such as vitamins and minerals [13]. This study aims to determine the effect of single and mixtureadministration of white tea and moringa on oxidative stress markers (MDA and F2-Isoprostan) on streptozotocin-induced diabetic rats and compared with green tea as control.

2. Tools and materials

Tools used in the experiment werebaker glass, filter paper, water heater, termometer, gastric tube, cages, digital scales, drinking bottles, 1 ml and 3 ml syringes,ependorf, vacutainer tube, glucometer (Nesco), gluco strip, spectrophotometer and ELISA reader. This research used materials that consisted of green tea, white tea that were obtainedfrom Tea and Quinine Research Center, Gambung and moringa (Kelorina) were obtained from PT Moringa Organik Indonesia, Blora, 30 male rats strain *Sprague-Dawley* aged 12 weeks with average weights of 200-300 g, streptozotocin (STZ) (Bioworld 41910012-3), citrate buffer, bedding, purified water,trichloroacetic acid (TCA), thiobarbituric acid (TBA) and ELISA kit (BT Labs E1179Ra).

3. Methods

3.1. Preparing steeping white tea, moringa, mixture white tea+moringa and green tea

White tea, moringa, mixture white tea+moringa and green tea were taken 20 g and brewed with 200 ml of water with a temperature of approximately 90°C for the next five minutes and then were filtered using filter paper. The filtratesof white tea, moringa, white tea+moringa and green tea were separated from the solvent using a rotary evaporator at a temperature of 58-60°C for 20 minutes [14].

3.2. Animal's trial

Each rat was adapted in a cage for about 14 days. After passing the adaptation period, the rats were divided into normal group (5 rats) which are healthy rats group without treatment and STZ-induced diabetes group (25 rats). The STZ solution was prepared in citrate buffer and given by intraperitoneal injection at dosage 40 mg/kg of body weight after overnight fasting. The first blood glucose measurements were made on the 3rd day after STZ induction to announce hyperglycemia (blood glucose \geq 126 mg/dl).After hyperglycemia, the rats were divided into five groups consisted of negative control (NC) which was not treated, control of tea that was given by green tea (GT),white tea (WT) group,moringa (M) group and mixed white tea and moringa (WT+M) group. At the beginningone rat was killed to measure the MDA and F2-isoprostane level as based line.Four other rats were given treatment that administered by oral with reference content of ECGG 100 mg / kgBW for 21 days. Administration conducted in the morning at 09.00-10.00 o'clock. All groups were fed standard laboratory and drank purity water ad libitum. The procedures undertaken in this study have received approval from the IPB ethics committee (66-2017 IPB).

3.3. Blood collection

After 21 days of treatment, 4-5 ml blood samples were collected from cardiac about 4-5 ml. Blood were collected in vacutainer tube and allowed to clot 30 -120 min at room temperature and then the tube were centrifuged at 3000 rpm for 10 minute. Supernatant was separated and transfered to eppendorf and frozen at -60°C until analysis.

3.4. Malondialdehyde and F2-Isoprostane analysis

The MDA measurement method refers to the Capeyron *et al* method [15]. Blood serum (supernatant) (100 μ L) was added with 2.45 mL trichloroacetic acid (TCA) and 2.45 mL thiobarbituric acid (TBA) and then heated with boiling water at 100 ° C for 20 minutes, then centrifuged for 10 minutes at 8000 rpm. The level of MDA was measured spectrophotometrically on UV-VIS spectrophotometer at 535 nm. The pink supernatant reads its absorbance with a 2.45 mL TCA and 2.45 mL TBA as a blank.

Quantification of F2-Isoprostane in blood serum was used the immunological approach by enzyme-linked immunosorbent assay (ELISA) [16]. F2-Isoprostane quantification procedures were performed in accordance recommendation of 8-iso-Prostaglandin F2 Enzyme Immunoassay Kit (E1179Ra), Bioassay Technology LAB, China. MDA and F2-Isoprostane levels are expressed as ng/ml concentrations using calibrating curves and expressed of decrease in MDA and F2-isoprostane levels compared to negative control.

3.5. Data analysis procedure

The quantitative data were analysed using SPSS software. Paired T-test was used to determine whether the mean difference between before and after intervention. ANOVA variety analysis method and then Duncan's test was used to find the existence of significant difference ($p < 0.05$) among the treatment groups.

4. Result and discussion

The results of the malondialdehyde level in serum showed in table 1. Mean MDA levels in STZ-induced diabetic rats before obtaining treatment were 78.62 ng/ml and 78.42 ng/ml for the normal group of mice.

Table 1. Changes malondialdehyde levels in serum (ng/ml).

Group	Before treatment (D-0)	After treatment (D-22)	Δ MDA	<i>p</i> value
NC	74.25	99.25 \pm 2.04 ^a	25.00 \pm 2.04 ^c	0.000*
GT	84.25	78.00 \pm 9.68 ^b	-6.25 \pm 9.68 ^a	0.287
WT	74.25	81.75 \pm 5.40 ^b	7.50 \pm 5.40 ^b	0.069
M	81.75	91.12 \pm 11.30 ^a	9.37 \pm 11.30 ^b	0.194
WT+M	78.62	79.87 \pm 5.54 ^b	1.25 \pm 5.54 ^{ab}	0.681
Normal	78.42	78.00 \pm 3.06	-0.42 \pm 3.06	0.802
<i>p</i> value		0.006**	0.001**	

*) Paired t-test, significant at $p < 0.05$

**) Anova continued post hoc Duncan, significant at $p < 0.05$, the alphabetic difference showed significantly different

Comparison of MDA level measurements before and after intervention showed that only the NC group increase significantly in MDA levels ($p < 0.05$) while the GT, WT, M and WT+M groups did not significantly increase MDA level. Based on ANOVA analysis of changes in MDA level (Δ MDA) between treatment groups showed that GT, WT, M and WT+M group had MDA levels significantly different with NC group ($p < 0.05$). MDA levels of WT and M groups were significantly different compared to Δ MDA GT group ($p < 0.05$) but MDA levels of WT+M group did not differ significantly with GT group ($p > 0.05$).

Comparison between F2-Isoprostane levels before and after treatment showed that only NC group increased significantly in F2-Isoprostane level ($p < 0.05$) while group GT, WT, M and WT+M did not change significantly ($p > 0.05$). Based on ANOVA analysis F2-Isoprostane level (Δ F2-Isoprostane) between treatment groups showed that there was no significantly different between the treatment groups and NC group ($p > 0.05$) (tabel 2).

Table 2. Changes F2-Isoprostane levels in serum (ng/ml).

Kelompok	Before intervention (D-0)	After intervention (D-22)	Δ F2-Isoprostane	<i>p value</i>
NC	15.77	17.99 \pm 0.66	2.22 \pm 0.66	0.007*
GT	14.70	14.82 \pm 2.21	0.12 \pm 2.21	0.917
WT	14.47	14.08 \pm 2.54	-0.40 \pm 2.54	0.777
M	15.08	16.16 \pm 1.39	1.08 \pm 1.39	0.218
WT+M	15.01	15.38 \pm 1.37	0.36 \pm 1.37	0.636
Normal	9.08	7.63 \pm 4.74	-1.45 \pm 4.74	0.308
<i>p value</i>		0.060	0.309	

*) Paired t-test, significant at $p < 0.05$

**) Anova continued post hoc Duncan, significant at $p < 0.05$, the alphabetic difference showed significantly different

Lipid peroxidation is a complex process due to the reaction of polyunsaturated fatty acid on cell membranes with reactive oxygen compounds. If it is not stopped quickly, there will be damage the cell membrane. Increasing lipid peroxidation in STZ-induced diabetic rats was characterized by elevated MDA and F2-isoprostane levels. Under normal circumstances MDA levels range from 0.83-1.01 $\mu\text{mol/L}$ or equivalent to 59.81-72.78 ng/ml [17]. Research on STZ-induced diabetic rats also showed increased levels of F2-Isoprostane than normal rats in both plasma [18] and urine [19].

The body naturally has a free-radical defense system, an intracellular endogenous antioxidant composed of enzymes synthesized by the body such as superoxide dismutase (SOD), catalase and glutathione peroxidase (GSH-Px) [20]. In the pathological state, enzymes that function as endogenous antioxidants may decrease. The STZ induction was able to decrease the antioxidant enzyme level of SOD, glutathione peroxidase (GSH-Px) and catalase in pancreas [21] so that in the STZ-induced diabetic rats group without intervention (NC) there was a significant increase in MDA and F2-Isoprostane levels due to the decreasing of defense system body in warding off free radicals.

Excessive ROS production requires exogenous antioxidants to neutralize it. Catechins and theaflavins are capable of interacting with cell membranes through the formation of hydrogen bonds with proteins and lipids of cell membranes [22][23]. Catechins and theaflavins react with ROS by donating hydrogen atoms so that free radical formation can be reduced and can increase antioxidant enzymes such as superoxide dismutase, ascorbic acid, α -tocopherol, and glutathione in order to inhibit free radical [24].

Epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG) and epicatechin (EC) have the same ability to increase the amount of glutathione and endogenous antioxidant activity such as SOD and GSH-Px [25]. Previous research by Osada *et al* [26] also suggests that EGCG and ECG have been able to reduce the oxidation of cholesterol, linoleic acid and arachidonate, thereby decreasing the formation of lipid peroxidation products. In addition to products of lipid peroxidation, MDA is also a byproduct of cyclooxygenase activity in platelets, which produce thromboxane [27]. Yamabe *et al.* [28] stated that EGCG is capable of lowering the regulation of cyclooxygenase (COX-2) thereby reducing MDA formation.

In contrast to MDA, F2-Isoprostane is formed nonenzymatically. F2-Isoprostane is formed in situ on cell membrane phospholipids where ROS attacks then broken down by phospholipase enzymes, released into extracellular, circulating in the blood and excreted through urine [29]. EGCG in tea has the ability to decrease F2-isoprostane levels. Increasing F2-isoprostane levels in rats due to sodium iodate injection are dramatically decreased after being given green tea extract intervention with

additional EGCG or single content of EGCG. The amount of EGCG required to decrease the F2-Isoprostan level is 387.8 mg/kgBW whereas if given 50.3 mg/kgBW didn't show a significantly decline [24]. This is suspected to be the cause of decreased levels of F2-Isoprostan in this study did not significantly different with NC group because the dose given is too low.

The high levels of F2-Isoprostan in each treatment group, not significantly different from the NC group, indicated that lipid peroxidation persisted and was not fully suppressed by antioxidants. This can be compared with F2-Isoprostan levels in normal group was lower than the STZ-induced group. Changes in MDA levels significantly different from the NC group compared with F2-Isoprostan may also be caused by the inhibition of the cyclooxygenase enzyme (COX-2).

5. Conclusion

Administration of white tea, moringa and mixture of white tea+moringa may prevent or reduce MDA level in streptozotocin diabetic rats but not in F2-Isoprostan level. Mixture of white tea+moringa (WT+M) provides a better effect in lowering MDA levels than if administered singly. WT+M has no significantly different effect with green tea as control tea on MDA and F2-isoprostan changes.

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