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# The Combination of Black Soybean Tempeh and Purple Sweet Potato Affect Reactive Oxygen Species and Malondialdehyde Level in Streptozotocin-Induced Diabetic Rats

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## ABSTRACT

Hyperglycemia conditions increase free radicals in the body that cause oxidative stress. Oxidative stress increase lipid peroxidation activity and reactive oxygen species (ROS). An antioxidant can prevent a free radical movement. The materials that contain potent antioxidants are black soybeans tempeh (BST) and purple sweet potatoes (PSP). The antioxidants in the BST are isoflavones with their derivates, and PSP is anthocyanins. This study aimed to determine the effect of BST and PSP extract on reactive oxygen species (ROS) and malondialdehyde (MDA) levels. In this study, rats were given a high-fat diet, 10% sucrose drink, and injected with multiple low-dose streptozotocin to induce T2DM. The animal's experiment divided into six groups: healthy rats, DM rats, DM rats + glibenclamide, DM rats + combination of BST and PSP in 3:1, 1:1, and 1:3 respectively. ROS levels were determined using the ELISA method and MDA levels were determined using spectrophotometer according to Thiobarbituric Acid (TBA) method. Our result suggests that the combination of BST and PSP significantly reduces ROS and MDA levels.
**Keywords:** free radical; purple sweet potatoes; T2DM; tempeh

## INTRODUCTION

Diabetes mellitus is characterized by chronic hyperglycemic conditions (Baynest, 2015). There are two types of diabetes mellitus; type 1 diabetes caused a lack of insulin secretion by the pancreatic beta cells. Type two diabetes caused a decrease in the sensitivity of the target tissue of insulin (Ozougwu *et al.,* 2013). The prevalence of diabetes mellitus increases globally with population growth and an unhealthy lifestyle. 425 million people aged 20-79 had diabetes in 2017. This number is expected to increase to 625 in 2045 (Cho *et al.,* 2018).

Hyperglycemia is a marker of abnormalities in carbohydrate, lipid, and protein metabolism (Jung and Choi, 2017). Hyperglycemic can increase free radicals in cells and initiate Oxidative stress (Asmat *et al.,* 2016). Oxidative stress increases the oxidation of glucose, amino acids, and lipids, which affect the production of Malondialdehyde (MDA), and Reactive oxygen species (ROS) that trigger DNA damage (Sakuraba *et al.,* 2002). MDA is produced from oxidative degradation of polyunsaturated fatty acids (PUFA) and is an indicator of cell damage (Pasaoglu, *et al.,* 2004). ROS is reactive chemical that contain oxygen and work as free radical (Pasaoglu *et al.,* 2004). Antioxidant compounds can prevent a free radical (Lobo *et al.,* 2010). Materials that contain abundant antioxidants are black soybeans Tempe (BST) and purple sweet potatoes (PSP).

Black soybean Tempe is made from the fermented black soybean. Some antioxidants, such as 6-Tempe (6-OHD), 2,3-Dihydroxybenzoic acid (2,3-DHBA), 8hydroxydaidzein (8-OHD), and 8-hydroxygenistein (8-OHG) are derivatives of daidzein and genistein which derived from fermented soybeans (Rufer and Kulling, 2006). 3-hydroxyanthranilic acid (HAA) has an antioxidant ability higher than vitamin E at the same concentration (Esaki *et al.,* 1996).

Purple sweet potato abundant anthocyanin that one of the potent antioxidants (Gras *et al.,* 2017). Anthocyanin works by increasing the workings of insulin receptors, improving antioxidant status by suppressing MDA production, and enhancing Superoxide Dismutase (SOD) levels. Malondialdehyde (MDA) is a marker of oxidative (Castro-Acosta *et al.,* 2016).

Flavonoid compounds are reported to work in various systems such as the reproductive system (Gofur and Lestari, 2016), anticancer (Batra and Sharma, 2013). and reduce levels of AGE-RAGE which can trigger the formation of ROS (Gofur *et al.,* 2019).

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This study used a combination of an extract of black soybean Tempe and purple sweet potato. Tempe has proper absorption in the body (Astuti *et al.,* 2000). The combination of BST and PSP will improve antioxidant performance. Until now, there no research of the combination of black soybean and purple sweet potato extract in overcoming type 2 diabetes mellitus

## METHODOLOGY

### Subject

28 Wistar strain male rats with weights 185±15g. Rats were divided into six groups: healthy rats (N), DM rats (DM), DM rats + glibenclamide (DM +Glb), DM rats + combination of BST and PSP in 3:1, 1:1, and 1:3 (C1-C3) respectively.

### Preparation diabetic rat model

Rats were given a high-fat diet, 10% sucrose drink for 30 days, and injected with multiple low doses of Streptozotocin (STZ) (30mg / kg body weight in 0.1 citrates buffered saline, pH 4.5) intraperitoneally [16]. Blood glucose levels of rats were checked three days after injection. The rat was said to be diabetes if the blood glucose level exceeded 200 mg/dl.

### Preparation of extract

BST and fresh PSP were sliced, dried in a 45 °C oven, and then pulverized. 10 g of powder was extracted with 100 ml distilled water. The solution stirred for 30 minutes and then centrifuged 2500 rpm for 20 minutes. The supernatant was taken and stored in a dark bottle and cool place.

### Animal treatment

Diabetic rats were treated with the extract for 30 days. The extract was given orally (gavage) 1 ml volume per day. At the end of treatment, rats were sacrificed using anesthesia followed by cervical dislocation. Hepar was collected and wash three times using PBS. All procedures for maintaining and treating rats was approved from the Research Ethics Commission of Brawijaya University, Malang, with approval number 878-KEP-UB.

### Analysis of Malondialdehyde (MDA)

0.1 g of the liver was added with 1 ml TCA crushed in the mortal and centrifugated at 1000 rpm for 10 minutes. The supernatant was taken 400 µl, then added with TCA 10% and TBA 1% each 200 µl, HCL 1 M 0,2 ml, and equates 1 ml. The solution was vortexed and incubated in the waterbath at 95°C for 15 minutes, then incubated at 4° C for 15 minutes and centrifugated. The supernatant was taken, and absorbance measure tested using a UV-VIS spectrophotometer 532 nm in duplicate terms (1,1,3,3-tetra methoxy propane (TMP) as standard).

### Analysis of ROS activity

0.1 g of the liver was added with 1 ml PBS pH 7.2 crushed in the mortal and centrifuged at 5000 rpm for 5 minutes. Take the supernatant and move it into a microtube. Then ROS activity was - analyzed by the ELISA method.

### Data Analysis

Normality and homogeneity tests were performed, then the data tested with one-way ANOVA using data processing software. if significant ($p<0.05$) will be followed by Duncan Multiple Range Test (DMRT).

## RESULT AND DISCUSSION

The data collected in this study were the malondialdehyde level and ROS activity. Based on the one way ANOVA test, a $p <0.05$ indicates There is an effect of combination BST and PSP on ROS level in T2DM rats (Fig 1). Our result suggest that ROS level increase significantly in DM rats compared to normal rats (Figure 1). Treatment with combination of BST and PSP reduced ROS level significantly. The BST and PSP with ratio 1:1 showed the lowest ROS level compared to the other treatments. Our study indicates that the BST and PSP combination reduce the ROS level in the diabetic rats model.

Reactive oxygen species (ROS) are hydroxyl free radicals. Efforts to overcome free radicals in the body is consume foods that contain lots of antioxidants. BST and PSP are foods that contain high antioxidants. BST contains high antioxidants compared to soybeans, although tempeh itself is a product from soybeans, this is due to the activity of microorganisms during the fermentation process(Ruffer and Kulling, 2006). PSP contains antioxidants in the form of anthocyanin(Ji *et al.,* 2015) .

Soybeans are the main ingredient for making tempeh which contain daidzein and genistein, an antioxidant flavonoid that is highest compared to other processed products such as tofu. Flavonoid antioxidants contained in BST have a lot of functions such as anti-tumor or anti-cancer, antidiabetic, etc. Isoflavones belong to the flavonoid group, polyphenolic compounds that find in fruits, vegetables, and seeds (Kasote *et al.,* 2015).

Reactive Oxygen Species are reactive oxidants (Yuan *et al.,* 2008). The presence of high amounts of ROS in the body will cause oxidative stress. Each ROS that is formed can start a chain

Table I. Average of ROS level in each group

| Ros (U/mL) | Mean ± SD | P |
|---|---|---|
| N | 576.780[b] ± 28.890 | 0.03 |
| DM | 618.296[c] ± 30.446 | |
| DM + Glb | 547.036[ab] ± 24.503 | |
| DM + C1 | 557.406[ab] ± 20.195 | |
| DM + C2 | 522.520[a] ± 7.234 | |
| DM + C3 | 587.150[bc] ± 10.550 | |

Note: * Signifikan p < 0,05

Table I. ROS level after STZ injection (except N groups) with/without treatment for thirty days. N= healthy rats, DM (DM rats), DM + Glb (DM rats + glibenclamide), DM + C1-C3 (DM rats + the combination of BST and PSP extract with a ratio 3:1, 1:1, 1:3 respectively). The different letter indicate statistically differ compared to others group and vice versa (p<0.05) based on Duncan Multiple Range Test (DMRT) as a pos hoc test. Significance differences between groups are indicated by notation

Table II. Average of MDA level in each group

| MDA (ng/mL) | Mean ± SD | P |
|---|---|---|
| N | 452.833[b] ± 1.258 | 0.000 |
| DM | 492.166[c] ± 14.250 | |
| DM + Glb | 537.833[d] ± 11.250 | |
| DM + C1 | 444.000[a] ± 10.000 | |
| DM + C2 | 476.666[bc] ± 12.503 | |
| DM + C3 | 471.666[b] ± 2.516 | |

Note: * Signifikan p < 0,05

Table II. MDA level after STZ injection (except N groups) with/without treatment for thirty days. N= healthy rats, DM (DM rats), DM + Glb (DM rats + glibenclamide), DM + C1-C3 (DM rats + the combination of BST and PSP extract with a ratio 3:1, 1:1, 1:3 respectively). The different letter indicate statistically differ compared to others group and vice versa (p<0.05) based on Duncan Multiple Range Test (DMRT) as a pos hoc test.

reaction that continues until the ROS is removed with antioxidants (Ahmad *et al.,* 2017). Flavonoid compounds can act as a free radical scavenger (Halliwell, 2006). Antioxidants contained in a combination of treatment ingredients can synergistically reduce ROS levels (Procházková *et al.,* 2011).

Flavonoids contained in BST can immediately chelate free radicals and act as scavenger by donating hydrogen atom then flavonoid radical will react with the second free radical and form a stable quinone (Gofur *et al.,* 2018). Anthocyanins contained in PSP can reduce ROS activity by donating electrons to free radicals from the hydroxyl group (-OH), which are attached to the phenolic ring. These electrons will stabilize and inactivate free radicals. In this process, polyphenolic reducing agents turn into aroxyl radicals, which are relatively more stable because of the resonance of free radicals that have been reduced. The overall result is the cessation of damaging oxidative chain reactions (Nimse and Pal, 2015.

Our MDA result suggest that MDA level was increase in DM rats compared to normal groups (Figure 2). BST and PSP combination reduce MDA levels if compared with the DM group. HFD can mediate insulin resistance as a result of increased glucose. Adipose tissue in obese individuals releases more non-esterified fatty acids (NEFA), glycerol, and pro-inflammatory cytokines (Mukherjee *et al.,* 2013).

Antioxidants inhibit activity oxidants by donating one electron to an oxidant compound. Antioxidants prevent cell damage by inhibiting oxidation reactions by binding to free radicals and molecules that are very reactive so that cell damage can be prevented. Oxidation reactions with free radicals often occur in protein molecules, nucleic acids, lipids, and polysaccharides. Oxidation reactions are essential for life, but can also be damaging.

Plants and animals contain a variety of antioxidants (endogenous), such as glutathione, vitamin C, and vitamin E and enzymes such as catalase, superoxide dismutase, and peroxidase.

Low levels of antioxidants or inhibition of antioxidant enzymes causes oxidative stress can damage or kill cells (Valko *et al.,* 2007). Antioxidants (exogenous) from foods are isoflavones, flavonoids, and anthocyanins (Koulmanda *et al.,* 2003).

Antioxidant defense systems through several mechanisms, blocking the initial production of free radicals, changing oxidants into less toxic compounds, blocking the secondary production of toxic metabolites, and repairing damaged molecules. These defense mechanisms work together to protect the body from oxidative stress. Antioxidant defense systems consist of enzymatic and non-enzymatic antioxidants (Halliwell, 2006). Anthocyanins and isoflavones also increase insulin sensitivity decrease glucose Levels (Gofur *et al.,* 2019).

## CONCLUSION

Antioxidants contained in BST and PSP can immediately chelate free radicals so that cell damage can be prevented. Free radical damage if not dealt with quickly, will cause tissue damage. Black soybean Tempe and purple sweet potato extracts have a significant effect on reactive oxygen species (ROS) and MDA diabetes mellitus rat model.

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