EFFECTS OF *CASSIA ALATA* TREATMENT ON ERYTHROCYTE OXIDATIVE STRESS IN HYPERGLYCEMIC RATS

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ABSTRACT: Objective: *Hyperglycemia is known to cause oxidative stress that leads mainly to enhanced production of mitochondrial reactive oxygen species (ROS). ROS caused cellular damage through its oxidation ability and has been implicated in the pathogenesis of diabetes mellitus. In diabetes, persistent hyperglycemia increases ROS production through glucose autoxidation.* ***Materials & Methods:*** *This study investigates the effect of Cassia alata, a traditional medicinal herb on erythrocytes oxidative stress in streptozotocin (STZ) induced diabetic rats. The study was conducted in two groups; the first group consists of diabetic rats treated with Cassia alata extract, and the second group consists of diabetic rats fed with normal saline (control group). The Cassia alata extract was orally administered by single dosage of 200 mg per body weight daily for 4 weeks. Bloods from the rats were taken and undergoes biochemical parameter test.* ***Results:*** *The MDA level in the erythrocyte of hyperglycemic rats treated with Cassia alata extract was significantly different (p<0.05) compared to the control group. The level of antioxidant also showed a significant difference (p<0.05) with increased activity in the erythrocyte of hyperglycemic rats treated with Cassia alata extract as compared to the control group.* ***Conclusion:*** *This result indicates that Cassia alata is a plant possessing strong antioxidant properties which can be developed into a potential treatment for diabetic complication.*

Keywords: Oxidative stress, hyperglycemia, *Cassia alata*, erythrocyte

1. INTRODUCTION

Diabetes is characterized by the body's inability to metabolize glucose, and hyperglycemia is described as the condition with excessively high blood glucose levels (blood glucose > 15 mmol/l) [1]. Diabetes and hyperglycemia seems like a similar medical problem. People with diabetes have episodes of hyperglycemia from time to time, and hyperglycemia is one of the first symptoms to manifest before diabetes is diagnosed. During the development of diabetes, hyperglycemia causes the increase in free radicals production especially reactive oxygen species (ROS) which attacks the tissues through the glucose auto-oxidation and protein glycosylation mechanism [2]. The glucose auto-oxidation and protein glycosylation have the potential to cause a catastrophic damage as a result of the free radical attack, and complex antioxidant defence mechanisms have evolved to protect the body cells and tissues [3]. ROS together with increased lipid peroxidation and malondialdehye (MDA), a carcinogen of high toxicity, causes damage to the tissue [4]. The bad metabolic control of hyperglycemia would not prevent the alteration in peroxidation as well as vascular and secondary complication in diabetes [5]. Antioxidant based drugs formulated from different substances like glutathione peroxidase, flavonoids, and related polyphenols, have been discovered for prevention of oxidative stress and treatment of complex diseases [6]. Medicinal plant has gained a lot of attention in the pharmaceutical and drug discovery field in order to develop more efficient drugs for diabetes patient and to minimize diabetic complication. *Cassia alata* plants are commonly used as laxative and antifungal agents. It was reported that the leaves of *Cassia alata* have a strong antioxidant property, and contains polyphenol and flavonoid that acts as protective shield against free radical mediated disease [7-8]. Studies have shown that oxidative stress mediated by hyperglycemia-induced generation of free radicals contributes to the development of diabetic complication [9]. Thus, this study looked at the possible treatment of antioxidant components in *Cassia alata* leaves extracts in the erythrocytes of hyperglycemic rats.

2. MATERIALS AND METHODS

2.1. Plant Material and Extract Preparation

The leaves of *Cassia alata* was collected from Dengkil, Selangor. The plant was cleaned with distilled water and the leaves were dried at 40ºC for the period of 7 days [10]. The leaves were pounded using mortar and pestle before it is grounded to fine powder using an electric blender. 200 g of the powdered leaves were mixed with distilled water in two liters beaker. The mixture was boiled for 1 h 30 mins. The mixture was cooled down to 40°C and sieve through a cheese cloth. The liquid was filtered again using filter paper. The filtered solution was evaporated until the final volume is 400 ml. The extract was stored at 4°C in the refrigerator.

2.2. Animals

30 adult male Wistar rats (180-200g) were used for this study. The animals were housed in a controlled environment with normal diet and water, in accordance with Universiti Kuala Lumpur, Institute of Medical Science Technology guidelines for care of laboratory animals as approved by the Animal Ethics Committee.

2.3. Induction of Diabetes on the Animals

This study was conducted in two groups of streptozotocin (STZ)-induced diabetic rats. The first group consists of 15 rats treated with *Cassia alata* extract, and the second group consists of 15 rats fed with normal saline (control group). The rats were fasted overnight for 12 h before inducing with STZ. STZ was given in a single injection. Prior to the injection, 50 mg/kg of STZ was dissolved in 0.9% normal saline as previously described [11]. STZ was injected at the tail vein within 10 mins after preparation. Then, the rats were fasted for 24 to 48 h. This is to evaluate the fasting blood glucose (FBS) using glucometer. When the FBS level is above 200 mg/dl, the animals are ready for further investigation [12]. The *Cassia alata* extract was orally administered with single dosage of 200 mg per body weight for 4 weeks for the first group. After 4 weeks, the rats were sacrificed and bloods from the rats were taken for biochemical tests.

2.4. Lipid Peroxidation Assay

The whole blood was washed using phosphate buffer saline (PBS) and was centrifuged for 5 mins at 1500 rpm. The samples were repeatedly washed (3 times) in order to obtain clean red cells. The lipid peroxidation assay was done according to the method described previously [13]. MDA formed from the breakdown of polyunsaturated fatty acids serves as a convenient index for the determination of the extent of peroxidation reaction. MDA is a product of lipid peroxidation which reacted s with thiobarbituric acid (TBA) to give a pink colored product. The absorbance was read using spectrophotometer at 535 nm and each assay was repeated 3 times.

2.5. Antioxidant Assay

The antioxidant activities of erythrocyte were determined according to the previous method [14]. This method uses the stable free 2,2-diphenyl-1-picrylhydrazyl (DPPH) to estimate the activity of antioxidant. DPPH is a stable free radical by virtue of delocalization of the spare electron over the molecule as a whole. The delocalization is characterized by an absorption band in solution centered at about 517 nm. The absorbance values of 517 nm were measured using spectrophotometer and each assay was repeated 3 times. The capability to scavenge DPPH radical was then calculated.

2.6. Statistical Analysis

Results were expressed as mean ± SD, and the statistical analysis was carried out using SPSS software package. The data was evaluated using Independent t-test and the variables are considered statistically significant when ($p < 0.05$).

3. RESULTS

3.1. Lipid Peroxidation Assay and MDA Level

Red blood cells (RBCs) are prone to lipid peroxidation by virtue of their function as oxygen carriers, and also because of their lipid composition. Figure 1 shows a significantly different MDA levels between hyperglycemic rats treated with *Cassia alata* extract (0.21±0.06), as compared to the control group (0.52±0.12). The MDA assay indicates that sucrose and phenol red uptake into RBCs has a higher correlation with incubation time under peroxidative stress. The phenol red uptake into RBCs with hydrogen peroxide (H_2O_2) signifies a direct linear proportional relationship. The assay also clearly shows that the uptake of sucrose or phenol red is specific for intact RBCs prior to hemolysis.

3.2. DPPH Scavenging Activity

Figure 2 shows the comparison of DPPH scavenging activity between *Cassia alata* treatment group and the control group. The group of hyperglycemic rats treated with *Cassia alata* extract has a significantly different DPPH scavenging activity (82.09±8.74), as compared to the value recorded by the control group (37.71±9.57). This result implies that the erythrocyte in hyperglycemic rats treated with *Cassia alata* extracts has a higher antioxidant activity.

4. DISCUSSION

Increasing evidence in the experimental and clinical studies suggests that oxidative stress plays a major role in the pathogenesis of both types of diabetes mellitus. Free radicals are formed disproportionately in diabetes by glucose oxidation, non-enzymatic glycation of proteins, and the subsequent oxidative degradation of glycated proteins.

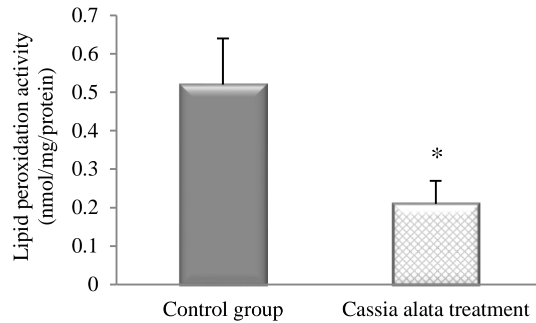

Figure 1: MDA level between hyperglycemic rats treated with *Cassia alata* extract and the control group. *Significantly different as compared to the control group.

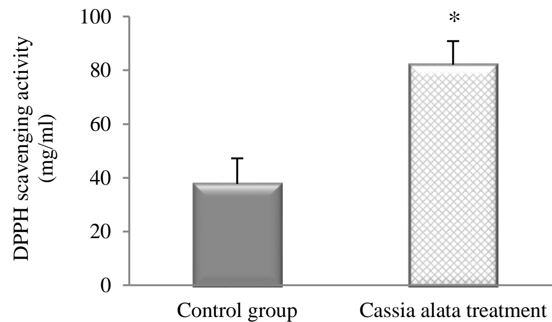

Figure 2: The DPPH scavenging activity between hyperglycemic rats treated with *Cassia alata* extract and the control group. *Significantly different as compared to the control group.

Abnormally high levels of free radicals and the simultaneous decline of antioxidant defense mechanisms can lead to damage of cellular organelles and enzymes, increased lipid peroxidation, and the development of insulin resistance. This study demonstrated significant differences in the erythrocyte's MDA level and DPPH scavenging activity between the hyperglycemic rats treated with *Cassia alata* extracts and the control group.

Lipid peroxidation is the process where free radicals "steal" electrons from the lipids in cell membranes and resulting in cell damage. It is believed that MDA arising from lipid peroxidation is an indicator for oxidative stress in the cells and tissue. The rise in MDA level in the erythrocyte of hyperglycemic rats indicates the increase in lipid peroxidation. The results of this present study strongly suggests that one of the main reasons for high MDA levels in the hyperglycemic rats could be due to the decreased activity of the defense system protecting the tissues from free radical damage. *Cassia alata* is rich with antioxidant components such as flavonoids, polyphenol, and tannins [15], and this may be one of the contributing factors that reduce the MDA level. Antioxidant works as free radical scavengers and thereby helps to reduce oxidative stress by preventing the free radicals from damaging the cells.

The TBARS levels were elevated and activities of antioxidant enzymes (SOD, glutathione peroxidase, and catalase) were significantly reduced in STZ-induced diabetic rats [16]. The results in this study suggests that the oxidative effects of DPPH scavenging activity in STZ-induced diabetic rats have significantly affected the DPPH levels and SOD activity after

the treatment with *Cassia alata* extract. This could be due to the phenolic compound found in *Cassia alata* plant. It is believed that the high antioxidant property in *Cassia alata* neutralizes free radical compounds which protect the cells from damage.

5. CONCLUSION

The treatment with *Cassia alata* extract has suppressed the oxidative stress in hyperglycemic rats. In addition, *Cassia alata* extract has also decreased the MDA level and increased the total antioxidant activity in erythrocyte. This effect could be ascribed to its flavonoid content and/or its antioxidant activity.

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