Research Article

Effects of *Cassia Alata* Treatment Towards Cardiovascular Oxidative Stress in Hyperglycemic Rats

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ABSTRACT

Hyperglycemia induced oxidative stress has been proposed as a cause of many complications in diabetes including cardiac dysfunction. The present study depicts the therapeutic effect of *Cassia alata* leaf aqueous extract on oxidative stress in aorta as well as heart of streptozotoc in hyperglycemic rats. Two days after diabetes induction, *Cassia alata* leaf aqueous extract was administered orally for 20 days (200mg/kg rat's weight). In the aorta and heart of hyperglycemic rats there was a significant increase in lipid peroxidation, decreased in total antioxidant activity (DPPH free radical scavenging activity) as well as decrease in antioxidant catalase activity. The administration of *Cassia alata* leaf aqueous extract to hyperglycemic rats has reduced lipid peroxidation (MDA levels), increased in total antioxidant activity (DPPH free radical scavenging activity) and antioxidant catalase activity as well as reduced in the blood glucose level. *Cassia alata* leaf aqueous extract provide a competent antioxidative mechanism to attack against the oxidative stress in the aorta and heart of hyperglycemic rats. This study suggests that *Cassia alata* may be a useful therapeutic alternative in the reversal of oxidative stress induced cardiac dysfunction in hyperglycemic condition as well as capable to act as antidiabetic agent.

Keywords: *Cassia alata,* hyperglycemic, lipid peroxidation (MDA levels), antioxidant activity, blood glucose level.

INTRODUCTION

Hyperglycemia is caused by a complex interaction of genetic, immunological and environmental factors. In general, hyperglycemia is due to impaired glucose utilization, abnormal insulin production, and increased glucose production. Metabolic dysregulation of glucose and insulin often leads to secondary multisystem pathology[1]. Oxidative stress is associated with the progression of diabetes which is stimulated by the free radicals such as reactive oxygen species (ROS), which includes superoxide ($O_2^{\cdot-}$), peroxyl, alkoxyl, hydroxyl and nitric oxide[2]. Patient with diabetes has rising levels of circulating markers of free radicals-induced damage and also reduced antioxidant defenses[3]. ROS is said to be increased when an organism is subjected to irradiation, chemicals or infection[4]. Production of a ROS load beyond the antioxidant capacity of the cell, will results in damage and oxidation of lipids, proteins, and nucleic acids, as well as of several other biomolecules. Presently, oxidative stress is demonstrated as mechanism underlying diabetes and diabetic complications[5]. The rise in oxidative stress and changes in antioxidant capacity has been observed in both clinical and experimental diabetes mellitus and are thought to be the etiology of diabetic complications[6]. Natural antioxidant is believed to prevent oxidative damage in diabetes with high oxidative stress[7]. Antioxidant such as vitamin C and E, enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidise (GSHPx) protects the cells against lipid peroxidation which is the initial step of many pathological processes[8].

The use of herbal medicines has gain rapid acknowledgements worldwide and its efficiency is associated with the content of the active compounds. *Cassia alata* has the properties to induce antioxidant effects[9]. The leaf of *C.alata* extract has been found to lower the blood sugar level[10]. The extraction from the leaves of *C.alata* showed a strong antioxidant activity[11].Hyperglycemia develops free radical condition which also impairs the endogenous antioxidant defense system in many ways during diabetes. This impairment leads to oxidative stress which contributes to the various diabetic complications. Hence, an effective treatment to balance the oxidative stress could help in the preventive measures. There has been increased importance in oxidative stress and its role in the development of diabetes complications. This current work describes the potential of treatment by the antioxidant properties in *C.alata* to overcome oxidative stress using hyperglycemic rat as a model.

MATERIALS AND METHODS

Sampling and extracting plant material

Fresh leaves of *C.alata* were collected from Dengkil, in the Sepang district of Selangor, and dried at room temperature (37°C) for about seven days. The leaves were pounded using mortar and pestle, and then grounded to fine powder. 200 gm of the powdered *C.alata* leaves was mixed with 2 L sterile distilled water. The mixture was boiled on a hot plate for one hour and left to cool. The mixture was then filtered through a cheese cloth. The liquid was filtered again with filter paper (Whatman No.1) in order to obtain refined extracts. The resulting filtrate was put in a rotary evaporator to produce a sterile aqueous extract stock of *C.alata* and stored at 4°C.

Inducing diabetes in rats

Prior to inducing streptozotocin (STZ), the rats were abstained from food and water for 12 hours. The freshly prepared STZ solution was injected through the tail vein of the rats. The level of blood glucose or fasting blood sugar (FBS) was measured after 48 hour using glucometer. Rats with FBS level of above 200 mg/dl were taken for further investigation.

Preparing organ homogenate

Organ homogenate was prepared according to the method as previously described[12]. The organs, which are the heart and aorta were cut to 0.5 gm and diluted with 4 ml KC1 buffer. The organs were homogenized using tissue homogenizer and then centrifuged at 600G for 60 minutes. The supernatants were taken and stored in a freezer at temperature of -80°C.

Determining protein concentration

Protein assay was determined according to the method previously described[13]. The value of absorbance was measured against a blank at 540nm and each assay was repeated three times (triplicate). The concentration of protein was determined from a standard curve of bovine serum albumin at a range of 20 µg/ml to 100 µg/ml.

Lipid peroxidation activity

Lipid peroxidation activity was determined by the formation of malondialdehyde (MDA)-thiobarbituric acid reactive substrates (TBARS) according to the previous method[14]. The MDA production serves as an index of lipid peroxidation with maximum absorption at 535nm.

The first stock solution (4.0mM stock MDA) was prepared by hydrolyzing 9.6 µl of 1,1,3,3-tetramethoxypropane in 10 ml 0.1N HCl in 100°C for 15 minutes. The standard solution was prepared by taking 100 µl stock solutions and diluting it in 10 ml KCl buffer.

The second stock was prepared in five different concentrations (0 to 2.0 nmol/ml) by diluting it with KCl buffer. 0.1 ml organ supernatant was added to 0.4 ml distilled water and 2.5 ml TCA. The mixture was left at room temperature for 15 minutes. 1.5 ml of TBA was then added into the mixtures and placed in water bath at 100°C for 15 minutes until it turns pale pink.

After cooling, 4 ml of n-butanol was added and centrifuged for 10 minutes at 3000rpm. The values of absorbance were measured at 532nm in triplicate.

DPPH free radical scavenging activity

The total antioxidant assay (DPPH free radical scavenging activity) of organ homogenates was conducted according to previous method[15]. 1,1-diphenyl-2-picryl-hydrazyl (DPPH) plays a role in accepting an electron or hydrogen radical to become stable molecule. DPPH in absolute ethanol appears in deep violet color and shows a strong absorption band at 517nm. The value of absorbance was measured at 517nm in triplicate.

Estimating antioxidant catalase activity

The antioxidant catalase activity was assayed using the method previously described[16]. Dichromate in acetic acid was reduced to chromic acetate, when heated in the presence of hydrogen peroxide. After the mixture turned green, the value of absorbance was measured at 530 nm. A standard antioxidant catalase was prepared in the range of 0 to 400 µg/ml.

Statistical analysis

The data was analyzed using SPSS. Results are stated as mean±SEM. Statistical analysis was done using Independent t-test to determine the significant differences among the variables (three tests). $p<0.05$ is considered as significant.

RESULTS AND DISCUSSION

Comparison of the average parameters in this study was done between two groups of hyperglycemic rats treated with *C.alata* extracts and normal saline for 20 days.

Blood glucose level in hyperglycemic rats after 20 days of treatment

Figure 1 showed the percentage of reduction in blood glucose level of hyperglycemic rats. The group of hyperglycemic rats given normal saline (negative control) showed decreased in blood glucose level of 8.87%±4.24. The percentage of blood glucose level reduction in hyperglycemic rats treated with *C.alata* (41.15% ±2.89) was significantly different as compared to the negative control. The hyperglycemic rats treated with *C.alata* showed decreased in blood glucose level throughout the study.

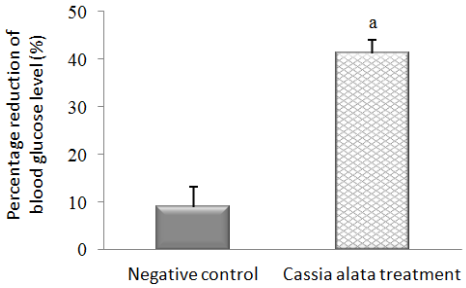

Figure 1: Percentage of blood glucose level reduction in hyperglycemic rats treated for 20 days. [a]Significantly different as compared to the negative control group.

Significant reduction on blood glucose level in hyperglycemic rats treated with *C.alata* could be due to the presence of the antidiabetic properties in the plant such as tannins, steroids, polyphenols, triterpenes and alkaloids[11]. The probable mechanism of actions is still unclear, but may control the blood glucose level by increasing glycolysis and decreasing gluconeogenesis with a lower demand of pancreatic insulin[17].

MDA levels in the heart of hyperglycemic rats

Figure 2 represents the MDA levels in the heart of hyperglycemic rats treated with *C.alata* and normal saline

(negative control). The average levels of MDA in hyperglycemic rats treated with *C.alata* (0.37±0.11) is significantly different as compared to the negative control (3.18±1.54). It is suggested that the oxidative pathways in hyperglycemia condition leads to the formation of free radicals and lipid peroxides[18]. The free radicals are found in normal human tissues, thus MDA which is one of lipid peroxidation products could be present in the normal tissues of rats[19].

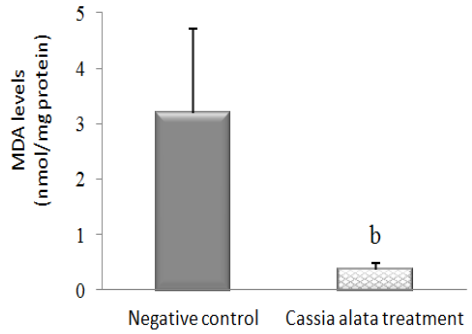

Figure 2: Comparison of MDA levels in the heart of hyperglycemic rats after 20 days of treatment. [b]Significantly different as compared to the negative control group.

MDA levels in aorta of hyperglycemic rats

Figure 3 illustrates the MDA levels in aorta of hyperglycemic rats treated with *C.alata* and normal saline (negative control). The average MDA levels in the rats treated with *C.alata* (0.31±0.10) is significantly lower than the negative control (3.55±1.74).

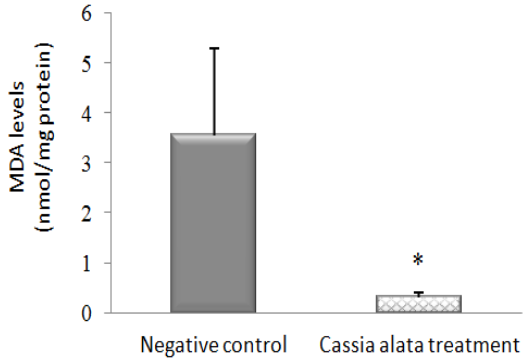

Figure 3: Comparison between the MDA levels in the aorta of hyperglycemic rats treated for 20 days. *Significantly different as compared to the negative control group.

Elevated levels of lipid peroxidation in diabetic rats are one of the characteristic of chronic diabetes[20]. In this study, higher level of MDA in negative control group indicates the tissues of heart and aorta were subjected to increase in oxidative stress. This situation can be ascribed to the different bioactive compounds present in the plant such as tannins, flavanoids and polyphenol[21]. Polyphenol have been reported to reduce lipid peroxidation, thus, administration of *C.alata* can act as free radical scavenger which is capable to reduce the MDA levels in short term period of time.

Total antioxidant activity in the heart of hyperglycemic rats

Figure 4 represents total antioxidant activity (DPPH free radical scavenging activity) in the heart of hyperglycemic rats treated with *C.alata* and normal saline (negative control). The average total antioxidant activity in rats treated with *C.alata* (84.92±9.82) is higher in comparisons with the negative control group (34.07±12.77).

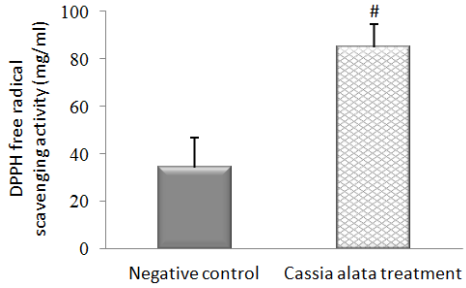

Figure 4: Comparison of the total antioxidant activity (DPPH free radical scavenging) in the heart of hyperglycemic rats after 20 days of treatment. [#]Significantly different as compared to the negative control group.

In this study, *C.alata* are shown to have the capability to act as free radical scavenger since the antioxidant activity in the heart were increased. This might be due to the stronger antioxidant activity possessed in the leaf of the plant[11]. Apart from that, there is evidence that phenolic compounds are a major contributor to the antioxidant activity of *C.alata*[22].

Total antioxidant activity in the aorta of hyperglycemic rats

Figure 5 showed the total antioxidant activity (DPPH free radical scavenging activity) in the aorta of hyperglycemic rats treated with *C.alata* and normal saline (negative control). The average total antioxidant activity in rats treated with *C.alata* (84.53±7.06) is significantly high compared to the negative control group (33.28±9.10). *C.alata* extract helps to boost up and stimulate the endogenous antioxidant such as superoxide dismutase, gluthatione peroxidase[22]. Thus, the antioxidant property in *C.alata* is beneficial to build an antioxidant network inside the body to scavenge free radicals.

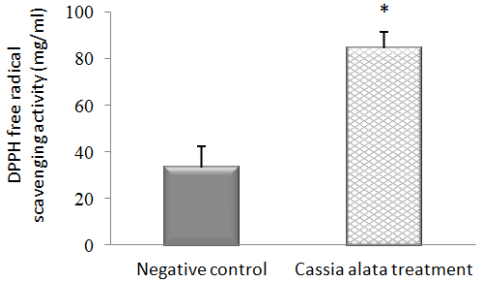

Figure 5: Comparison of the total antioxidant activity in the hyperglycemic rat's aorta (20 days treatment). *Significantly different as compared to the negative control group.

Activity of antioxidant catalase in the heart of hyperglycemic rats

Figure 6 showed the antioxidant catalase activity in the heart of hyperglycemic rats treated with *C.alata* and normal saline (negative control). The average antioxidant catalase activity in the group treated with *C.alata* (125.87±8.34) is significantly high as compared to the negative control group (69.04±16.39).

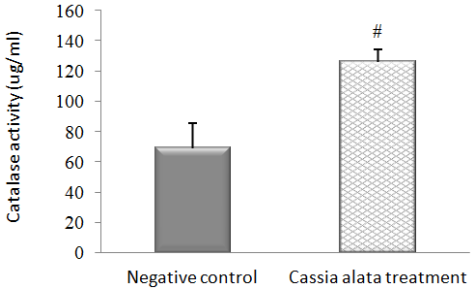

Figure 6: Comparison of antioxidant catalase activity in the hyperglycemic rat's heart (20 days treatment). #Significantly different as compared to the negative control group.

Besides increasing free radicals, the presence of oxidative stress also lowers the capacity of antioxidant defense mechanisms of the body[23]. In this study, the administration *C.alata* has perhaps increased the enzyme catalase in the body. The increase in catalase activity suggests a compensatory response to the oxidative stress due to an increase in endogenous H_2O_2 production[24]. The excessive amounts of peroxide are assumed to be present since catalase protects the cell against high peroxide levels.

Activity of antioxidant catalase in the aorta of hyperglycemic rats

Figure 7 represents the antioxidant catalase activity in the aorta of hyperglycemic rats treated with *C.alata* and normal saline (negative control). The average antioxidant catalase activity in the rats treated with *C.alata* (122.64 ±6.75) is significantly high as compared to the negative control group (65.79±14.64).

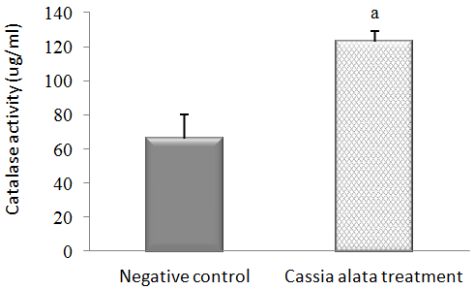

Figure 7: The comparison between antioxidant catalase activities in the aorta of hyperglycemic rats after 20 days of treatment. [a]Significantly different as compared to the negative control group.

The increase in catalase gene expression seems to be a natural response for the cells to cope with oxidative stress in streptozotocin induced diabetic rats[25]. The over expression of catalase with a cardiac specific transgene has increased the catalase activity to 60-fold which then provides significant protection from diabetes induced damage on cardiac[26].

CONCLUSION

The antioxidant enzymes in hyperglycemic condition required an additional mechanism in order to reduce the oxidative stress. *C.alata* is a plant which proved to possess an efficient anti-oxidative mechanism. This may perhaps improve the oxidative stress and expression of antioxidant enzymes. The phenolic contents were identified as having an antioxidant effect for the reduction of oxidative stress. Based on this study, *C.alata* has significantly reduced MDA levels and increased antioxidant activity, as well as lowering the blood glucose level. Therefore, *C.alata* could be a useful therapeutic option against oxidative stress induced cardiac dysfunction in hyperglycemia and as an antidiabetic agent.

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