Research Article

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Effects of *Cassia alata* leaf extract towards liver and renal microsomal oxidative stress in hyperglycemic rats

I. F. Abu, K. B. Manoharan, K. M. Ambia, R. M. Noah and R. Ishak*

Universiti Kuala Lumpur, Institute of Medical Science Technology, A1-1, Jln TKS 1, Taman Kajang Sentral, 43000 Kajang, Selangor, **Malaysia**.
***Corresponding author**

The effects of *Cassia alata* aqueous leaves extract, a traditional medicinal herb, was assessed in the liver and renal microsomal of streptozotocin (STZ) induced hyperglycemic rats. In diabetes, hyperglycemia increases the production of free radicals and simultaneously weakens the antioxidant defense mechanism. This leads to the damages on cellular organelles and enzymes, increasing lipid peroxidation, and develops insulin resistance in the body. *Cassia alata* extract was administered orally (200mg/kg) for 20 days to the hyperglycemic rats. The effects of *Cassia alata* in the liver and renal microsomal were measured and analyzed based on the levels of lipid peroxidation malondialdehyde (MDA), antioxidant enzymes catalase (CAT), and the total antioxidant of 2,2-diphenyl-1-picrylhydrazyl (DPPH). Treatments with *Cassia alata* showed a significant decrease of lipid peroxidation MDA levels in the liver and renal microsomal as compared to the control groups. The results also recorded an increase in the total antioxidant level of DPPH. Furthermore, the level of CAT enzyme in the group treated with *Cassia alata* has significantly increased in comparison to the control group. The results indicated that *Cassia alata* extracts have suppressed the oxidative stress in hyperglycemic induced rat and at the same time increased the enzymatic and non-enzymatic antioxidant.

Key words: *Cassia alata*, hyperglycemic, antioxidant, liver, renal, oxidative stress.

Diabetes mellitus is a group of metabolic disease characterized by hyperglycemia due to defects in insulin secretion, insulin action, or both (Kangralkar et al. 2010). Diabetes is not only a common chronic disease but it meets several criteria for public health disorders. The goal for the clinical management of all forms of diabetes is to control the metabolic abnormalities in order to prevent acute and long-term complications. In addition, the incidence of oxidative stress in diabetic complication has raised the production of free radicals and impaired the antioxidant defenses (Maritim et al. 2003). During diabetes, hyperglycemia causes increasing production of free radicals especially the reactive oxygen species (ROS) that attacked the tissues via glucose auto-oxidation and protein glycosylation mechanisms (Moussa, 2008). The glucose auto-oxidation and protein glycosylation mechanisms have the potential to cause damage as a result of free radical attack. Diabetes also affected the microsomes activity and the pattern of cytochrome P450 (Traverso et al. 1999). Alteration to the cytochrome P450 system may increase free radical production in the microsomal compartment. ROS increases the lipid peroxidation and its end product in the form of malondialdehye (MDA); a highly toxic carcinogen which leads to tissue damage.

In recent years, research on medicinal plant has gained a lot of attention in order to develop alternative remedy. Traditional medicine including medicinal plants is being used by over 80% of the world population (WHO, 2002). *Cassia alata* is a medicinal

plant that is traditionally used as laxative, and antifungal and antibacterial agents (Pharkphoom and Songsri, 2003). It has also been reported that *Cassia alata* has a strong antioxidant property. The leaves of *Cassia alata* contains polyphenol and flavonoid property that acts as protective shield against numerous free radical mediated diseases (Subramanian and Venugopal, 2001). *Cassia alata* extract has no effect on the glucose levels in normoglycemic animals, but it has reduced the blood sugar level in STZ induced hyperglycemic animals (Palanichamy et al. 1998). This work describes the antioxidant effects of *Cassia alata* aqueous extract in the liver and renal microsomal in hyperglycemic rat.

MATERIALS AND METHODS

Collection and sampling of plant material

The leaves of *Cassia alata* was collected from Dengkil, Selangor, Malaysia. The leaves was cleaned with distilled water and dried at 40ºC for the period of 7 days. The leaves were pounded using mortar and pestle before it is grounded to fine powder using an electric blender. The powdered leaves are stored at 4ºC.

Preparation of *Cassia alata* aqueous extract

200g of the powdered *Cassia alata* leaves was mixed with 2 L distilled water. The mixture was boiled for 1.5 hours. Then, the mixture was cooled down to 40ºC and sieve through a cheese cloth. The liquid was filtered again using filter paper and evaporated until a concentrated extract of about 400 ml is obtained. The extract was stored in the refrigerator for future use.

Induction of diabetes on animals

30 male Wistar rats (180-200 gm) were used for this study. They were housed in standard cage in controlled environment with standard diet and water according to the guidelines provided by UniKL Animal Ethics Committee. The rats were fasted overnight for 12 hours before they were induced with streptozotocin (STZ). Prior to the injection, 50 mg/kg of STZ was dissolved in 4 ml of normal saline according to the method described by Sivaraj et al. (2009). STZ was injected at the tail vein within 10 minutes after preparation. After that, the rats were fasted for 24-48 hours in order to check their fasting blood glucose (FBS) using glucometer. The rats were deemed as diabetic when the level of FBS was above 10.5 mmol/dl (Hemalatha et al. 2010).

This experiment was performed on two groups of STZ induced diabetic rats. The first group of diabetic rats was treated with *Cassia alata* extracts. The second group of diabetic rats was given normal saline (control group). The extract was orally administered to the rats by single dosage of 200 mg per body weight for 20 days. After 20 days, the rats were humanely sacrificed and their liver and renal were harvested. The organs were homogenized and assessed using biochemical tests.

Preparation of liver microsomal

The liver was minced into small pieces and placed in small glass beaker at 4˚C. The small pieces of liver were dissolved in 4 ml/g of 0.25 M sucrose. Then, the liver was homogenated with homogenizer in cold condition. The preparation was based on the protocol and technique outlined previously by Lake, (1987).

Preparation of kidney homogenate

The kidney homogenate was prepared according to the method described previously by Noori et al. (2009). The kidney homogenate was prepared using a homogenizer in a control temperature of 4˚C. The homogenate (1:10 w/v) was prepared with 100 mmol 0.1 M Tris buffer, at pH 7.5. All homogenate was centrifuged at 600 G (for 60 minutes) at -80˚C and the supernatant was collected for the biochemical assays.

Malondialdehyde (MDA) lipid peroxidation assay

The lipid peroxidation was estimated according to the method previously described by Ledwozyw et al. (1986). MDA formed from the breakdown of polyunsaturated fatty acids serves as a convenient index to determine the extent of peroxidation reaction. MDA reacts towards thiobarbituric acid (TBA) to give a pink colored product with maximum absorbance of 535 nm.

2,2-diphenyl-1-picrylhydrazyl (DPPH) total antioxidant assay

The antioxidant activity of the homogenate was determined according to the method described by Bios, (1985). DPPH is a stable free radical by virtue of delocalization of the spare electron over the whole molecule. The

delocalization of spare electron is characterized by its maximum absorbance of 517 nm.

Estimation of catalase (CAT) enzyme

The catalase activity was measured using method described by Sinha, (1972). The dichromate in acetic acid was reduced to chromic acetate when heated in the presence of hydrogen peroxide and produced perchromic acid as an unstable intermediate. The green coloured product has a maximum absorbance of 530 nm.

Statistical analysis

The data was analyzed using SPSS. The results are expressed as mean ± SEM. Statistical analysis was done using Independent t-test to determine significant differences among the variables. $p<0.05$ is considered as statistically significant.

RESULTS AND DISCUSSION

Analysis of Weight Changes in Hyperglycemic Rats.

Figure 1 shows the percentage of weight changes in hyperglycemic rats. There is a marked difference in weight between the two groups. Hyperglycemic rats treated with *Cassia alata* (32.4%±7.7) have a reduced weight as compared to the control group (44.9%±10.9).

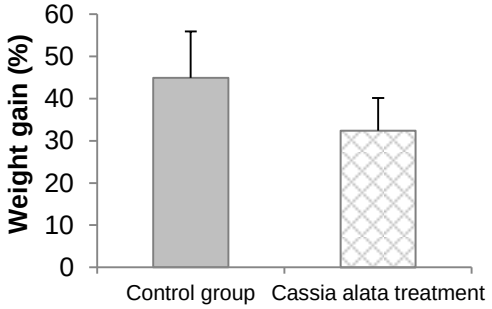

Figure 1: Percentage of weight gain in hyperglycemic rat.

Weight gain is one of the risk factor for diabetic patient. The causes of weight gain can be due to several factors including unhealthy lifestyle, high-calorie diet, sociocultural factors, and chronic medical illness. Treatment for diabetes involves weight management and medications to control blood-sugar levels. If left untreated, diabetes can elevate the risk of developing heart and blood-vessel diseases. Thus, reducing weight is important for prevention and treatment of diabetes.

Analysis of Lipid Peroxidation Assay (MDA)

Figure 2 shows the concentration of MDA in liver microsomal of hyperglycemic rats. The average concentration of MDA in liver microsomal after treatment with *Cassia alata* (0.32±0.1 nmol/mg protein) was significantly lower than the control group (2.07±0.93 nmol/mg protein).

Figure 3 shows the concentration of MDA in the renal microsomal. MDA level in the renal were significantly decreased with *Cassia alata* treatment (0.35±0.14 nmol/mg protein) as compared to the control group (2.87±1.98 nmol/mg protein).

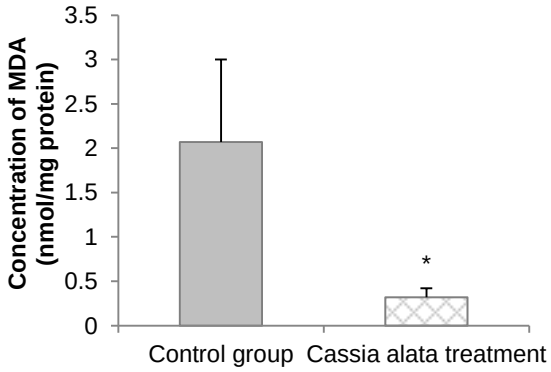

Figure 2: The concentration of MDA in liver microsomal of hyperglycemic rats.

*indicate significant difference compared to the control group (*p<0.05*).

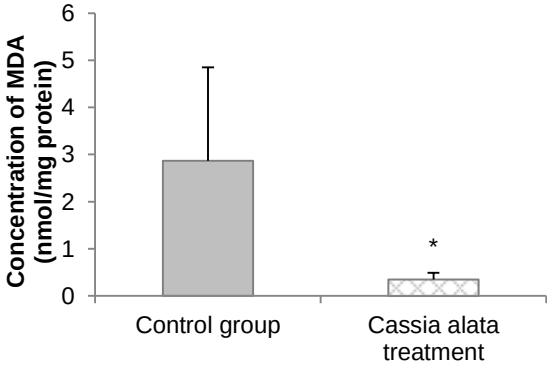

Figure 3: The concentration of MDA in renal of hyperglycemic rats.

*indicate significant difference compared to the control group (*p<0.05*).

Progression of oxidation on peroxide leads to the production of several secondary products including MDA, a carcinogen of high toxicity. Hyperglycemic is widely known to cause the increased production of free radicals especially reactive oxygen species (ROS) which is generated during autoxidation of glucose. The mechanism by which increased oxidative stress in the liver and kidney microsomal in diabetic complication are not entirely known. Treatment with *Cassia alata* extract showed a good antioxidant property in order to reduce lipid peroxidation. *Cassia alata* is traditionally used to reduce lipid peroxidation and lowering blood glucose level (Hennebelle et al. 2009). The MDA level in the rat's liver has decreased when pre-treated with *Cassia alata* for 14 days. *Cassia alata* helps to regenerate tubular epithelium of the kidney tissues and possessed nephroprotectivity that prevent tissue damage caused by lipid peroxidation (Palanichamy et al. 1998). *Cassia alata* is also used in traditional medicine to cure cirrhosis and hepatitis. The leaves of *Cassia alata* contain hepatoprotective property and were used in the treatment of liver problems. The presence of active compounds such as anthocyanin and flavonoids may have helped in preventing liver and kidney damage through oxidative stress. The presence of *Cassia alata* antioxidant compounds is believed to be the key substances that protect the body from damage by free radical induced oxidative stress.

Analysis of Total Antioxidant Assay (DPPH).

Figure 4 shows the concentration of total antioxidant assay (DPPH) in the liver microsomal of hyperglycemic rats. The concentration of total antioxidant for hyperglycemic rats treated with *Cassia alata* (86.98±3.5 mg/ml) is higher and significantly different than the control group (69.23±8.4 mg/ml).

Figure 5 exhibits the concentration of total antioxidant in the renal of hyperglycemic rats. The average concentration of antioxidant for hyperglycemic rats treated with *Cassia alata* (86.98±4.5 mg/ml) differs significantly than the control group (66.95±6.1 mg/ml). The presence of low antioxidant concentrations has significantly delayed or prevents oxidation of the oxidant substrate.

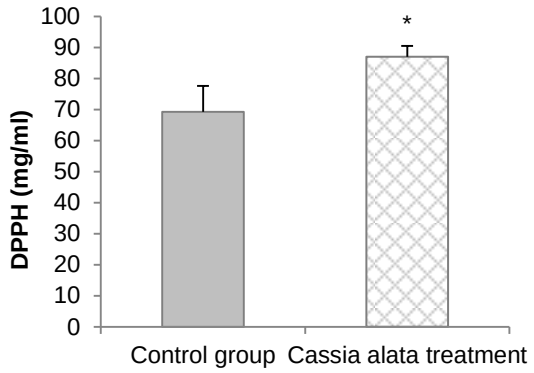

Figure 4: Total antioxidant assay (DPPH) in the liver microsomal of hyperglycemic rats.

*indicate significant difference compared to the control group (*p<0.05*).

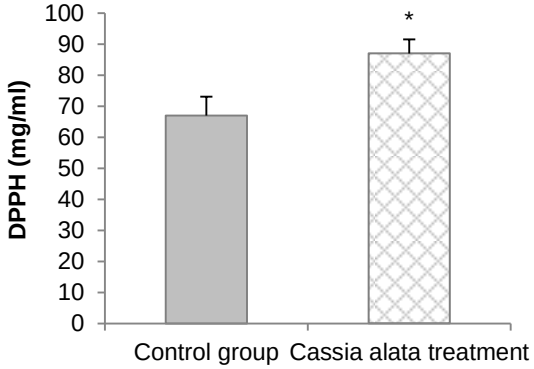

Figure 5: Total antioxidant assay (DPPH) in the renal of hyperglycemic rats.

*indicate significant difference compared to the control group (*p<0.05*).

The oxidation of substrates is prevented directly either by scavenging ROS or indirectly by inhibiting the production of ROS and promoting the antioxidant defence system. The rise of ROS level in diabetes could be due to the increased production and/or decreased destruction by non-enzymatic effect (Pisoschi and Negulescu, 2011). Exogenous sources of antioxidants from plants are deemed important to increase the endogenous antioxidant sources. *Cassia alata* extracts showed that the leaves contained a stronger antioxidant activity than the flower and pods. Phytochemical of *Cassia alata* proved that the plant is loaded with flavonoid, tannin, and polyphenol. The polyphenol group neutralizes the free radical, decomposing peroxides and triplet oxygen (Galato et al. 2001). *Cassia alata* also contains kaempferol

that helps to lower the risk of chronic diseases such diabetes and cerebrovascular disease (Pharkphoom and Songsri, 2003).

Estimation of Enzyme Catalase (CAT). Figure 6 represents the average enzyme CAT activity in the liver microsomal of hyperglycemic rats. The liver microsomal in *Cassia alata* treated rats has an average enzyme CAT (121.17±7.5 µg/ml) that is significantly higher than the control group (74.11±20.9 µg/ml).

Figure 7 shows the average enzyme CAT activity in the renal of hyperglycemic rats. The average enzyme CAT activity in the control group is recorded at 65.14±15.2 µg/ml, whereas, with *Cassia alata* treatment the enzyme CAT activity was at 115.57±7.0 µg/ml. The result was significantly different between the two groups (*p<0.05*).

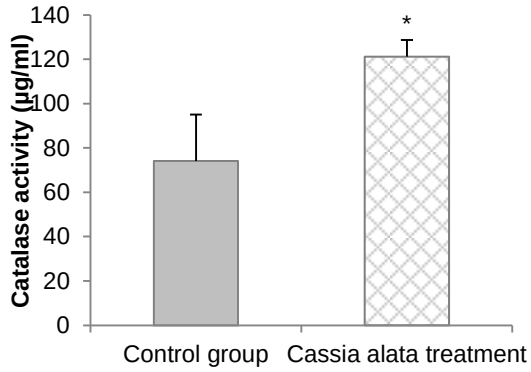

Figure 6: The average enzyme CAT activity in liver microsomal of hyperglycemic rats. *indicate significant difference compared to the control group (*p<0.05*).

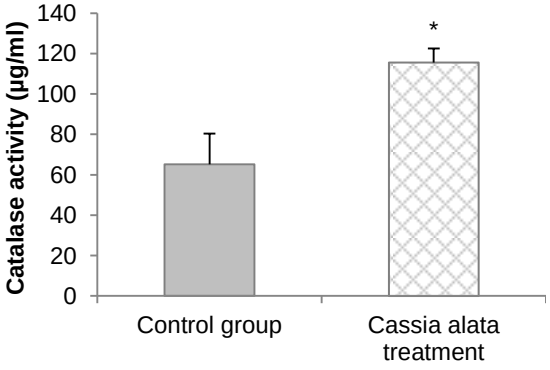

Figure 7: The average enzyme CAT activity in renal microsomal of hyperglycemic rats. *indicate significant difference compared to the control group (*p<0.05*).

Human body contains hydrogen peroxide (H_2O_2) which is a result of respiration and is made in all living cells. High content of H_2O_2 is harmful to body function and subsequently decreased the activity of catalase that catalyses H_2O_2. Catalase functions as a special peroxidation decomposition that breaks down H_2O_2 into oxygen and water. Treatment with *Cassia alata* has increased the enzyme CAT activity in hyperglycemic rats. The bioactive compound in *Cassia alata* extracts could directly eliminates free radical by increasing the enzyme activity level of catalase (Chung et al. 2004). Endogenous antioxidant is capable to regenerate each other and stabilizes the dynamic of the cells (Coleman et al. 2001). An overproduction of ROS especially in diabetes could not be properly balanced by antioxidant enzymes. Catalase in cells amounted to very little and very susceptible to the presence of peroxide. Due to this, only a small amount of this enzyme could act faster against H_2O_2 oxidant.

CONCLUSIONS

Cassia alata proved to be a good source for antioxidant, and has decreased the level of lipid peroxidation in hyperglycemic condition. The reduced level of MDA and the increased CAT activity in the liver and renal microsomal after treatment with *Cassia alata* pointed to the potential possessed by this plant. The plant has also suppressed the oxidative stress in hyperglycemic condition and able to imbalance free radical production. Thus, *Cassia alata* could be a useful therapeutic option to inhibit lipid peroxidation by acting as chain breaking agent, and increases antioxidant level in the body to help prevent tissue injury.

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