

Physiological Study on the Effect of *Acalypha wilkesiana* Leaves Extract on Streptozotocin-Induced Experimental Diabetes in Male Mice

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Abstract: Problem statement: Diabetes mellitus is a chronic disease that causes serious health complications and it is now one of the most common non-communicable diseases globally. It is the fourth or fifth leading cause of death in most high-income countries and there is substantial evidence that it is epidemic in many economically developing and newly industrialized nations. Recently, there has been a growing interest in antidiabetic agents from natural products especially those derived from plants. The antidiabetic agents have been focused on plants used in traditional medicine because that may be a better treatment than currently used synthetic drugs. **Approach:** The influence of *Acalypha wilkesiana* leaves extract on the levels of serum glucose, triglycerides, cholesterol, sodium (Na), potassium (K) and Calcium (Ca) was investigated using Streptozotocin (STZ)-induced diabetic mice. Diabetes was induced in male albino mice by single intraperitoneal injection of STZ (60 mg kg⁻¹ body weight). The experimental animals were divided into four groups. The first group was untreated and served as normal control. The mice of second group were used as diabetic control group. The diabetic mice of third group were treated orally with *A. wilkesiana* leaves extract for 30 days. The normal mice of fourth group were treated orally with *A. wilkesiana* leaves extract at the same dose given to third group. **Results:** The diabetic mice showed significant increases in the levels of serum glucose, triglycerides, cholesterol and Na after 10, 20 and 30 days. Also, the level of K was statistically increased after 20 and 30 days, while the level of Ca was significantly elevated after 30 days. The diabetic mice given *A. wilkesiana* leaves extract showed significant decreases in the levels of serum glucose, cholesterol and K. Insignificant alterations in the values of serum triglycerides, Na and Ca were noted in diabetic mice treated with leaves extract at all periods of experiment. **Conclusion/Recommendations:** These new data indicate that *A. wilkesiana* represents an effective antihyperglycemic, antihyperlipidemic adjunct and ameliorative role on electrolytes disturbances for the treatment of diabetes and a potential source of discovery of new therapeutic agent.

Key words: Streptozotocin, diabetes, *Acalypha wilkesiana*, serum parameters, mice

INTRODUCTION

Glucose is the major energy source of cells. A stable blood glucose is necessary since energy must be supplied to all cells at all times despite intermittent food intake and variable demands, such as the level of physical activity. The major regulatory hormone for intermediary metabolism is insulin, produced and secreted by the β -cells of the islets of Langerhans of the pancreas. Impaired control of blood glucose concentrations by insulin leads to diabetes mellitus. In patients with diabetes, an increased blood glucose concentration (hyperglycemia) causes an increased thirst, hunger and urine volume, but it is the chronic complications of diabetes that are the major health issues (Partanen *et al.*, 1995; Butler *et al.*, 1998; Marks and Raskin, 2000; Torffvit and Agardh, 2000; 2001;

Voutilainen-Kaunisto *et al.*, 2001). Diabetes is a major worldwide health problem predisposing to markedly increased cardiovascular mortality and serious morbidity and mortality related to the development of nephropathy, neuropathy and retinopathy. Diabetes mellitus is characterized by abnormal insulin secretion, derangement in carbohydrate and lipid metabolism and is diagnosed by presence of hyperglycemia (Zimmet *et al.*, 1997). For many patients, diabetes is only diagnosed and aggressively treated when one of characteristic diabetic complications develops. These include cataracts and retinopathy which lead to blindness, impaired kidney function leading to end-stage renal disease, diabetic neuropathy which may lead to tactile allodynia, ulcers or amputations, macrovascular disease such as atherosclerosis and impotence, or heart disease and stroke. In humans, diabetes is

associated with long-term cardiovascular damage (especially endothelial dysfunction, fibrosis and cardiomyopathy) with a much higher risk of coronary artery disease, heart failure, myocardial infarction and death (Tomlinson *et al.*, 1992). Diabetes can be induced by selective destruction of the insulin-producing β -cells of the pancreas with a single, rapid injection of Streptozotocin (STZ), a glucose moiety with a very reactive nitrosourea group from the mould *Streptomyces griseus*. This procedure, first introduced in 1963, has since been used in over 7600 PubMed citations, probably making this the second most used animal model of human disease after the Spontaneously Hypertensive Rat (SHR); cardiovascular changes following streptozotocin have been reviewed previously (Tomlinson *et al.*, 1992). STZ doses of 50-65 mg kg⁻¹ lead to hyperglycemia (20-30 mmol L⁻¹) but severe ketosis does not develop even if insulin is not administered. Higher doses (75 mg kg⁻¹ and above) result in spontaneous ketosis and death within days if insulin is not given.

In modern medicine, there is no satisfactory effective therapy to cure diabetes mellitus. The management of diabetes mellitus by insulin therapy, has several drawbacks like insulin resistance (Wild *et al.*, 2004) and in chronic treatment cause anorexia nervosa, brain atrophy and fatty liver (Tobias *et al.*, 2001). Ethnobotanical information indicates that more than 800 plant are used as traditional remedies for the treatment of diabetes (Pushparaj *et al.*, 2000), but many plants do not have a scientific scrutiny. Herbal medicines are used for primary health care, by about 80% of the world population particularly in the developing countries, because of better cultural acceptability, safety, efficacy, potent, inexpensive and lesser side effects (Pullaiah and Chandrasekhar Nadiu, 2003). The plant drugs are frequently considered to be less toxic when compared to synthetic drugs (Pari and Umamaheswari, 2000). More than 1123 plant species have been used to treat diabetes and more than 200 pure compounds have showed, lowering blood glucose activity (Grover *et al.*, 2002). The WHO Expert Committee recommended that the important to investigate the hypoglycemic agents from plant origin, which used in traditional medicine for the treatment of diabetes mellitus (Aларcon-Aguilera *et al.*, 1998). The antihyperglycemic agents have been focused on plants used in traditional medicine because that may be a better treatment than currently used synthetic drugs (Hu *et al.*, 2003). Additionally, many plant extracts and plant products have been shown to have significant antioxidant activity (Anjali and Manoj, 1995) which may be an important property of plant medicines

associated with the treatment of several ill fated diseases including diabetes. Thus, herbal plants are considered useful means to prevent and/or ameliorate certain disorders, such as diabetes, atherosclerosis and other complications (Scartezzini and Speroni, 2000).

Acalypha wilkesiana Muell Arg belongs to the family *Euphorbiaceae* (spurge family). Its other names include *A. amentacea* and *A. tricolor*, while its common names are copperleaf, Joseph's coat, fire dragon, match-me-if-you-can. It is native to Fiji and nearby islands in the South Pacificans is a popular outdoor plant that provides color throughout the year, although it is also grown indoors as a container plant. It is propagated by stem cuttings at any time of the year. Under ideal conditions, it grows as a spreading evergreen shrub with upright branches that tend to originate near the base and can get up to 3.1 m tall with a similar spread. It has leaves (12.7-20.3 cm long) that are alternate, elliptic to oval, serrate and multi-coloredans small inconspicuous flowers (10.2-20.3 cm) that hangs in catkin-like racemes beneath the foliage. *A. wilkesiana* has antimicrobial (Ogundaini, 2005; Akinyemi *et al.*, 2006; Oladunmoye, 2006) and antihypertension properties (Ikewuchi *et al.*, 2008). According to Ogundaini (2005), the expressed juice or boiled decoction is used for the treatment of gastrointestinal disorders and fungal skin infections such as *Pityriasis versicolor*, *Impetigo contagiosa*, *Candida intetrigo*, *Tinea versicolor*, *Tinea corporis* and *Tinea pedis*. The objective of the present investigation was to ascertain the scientific basis for *A. wilkesiana* leaves extract use in the treatment of diabetes mellitus. Therefore, this study was designed to investigate the protective effect of *A. wilkesiana* leaves on lowering the levels of serum glucose, triglycerides and cholesterol levels and its ameliorative influences on serum sodium (Na), potassium (K) and Calcium (Ca) disturbances in STZ-induced diabetic mice.

MATERIALS AND METHODS

Animals: The experiments were done using male albino mice of MF1 strain, weighing 27.2-33.8 g. The animals were maintained in controlled temperature (20±1°C), humidity (65%) and a 12 h dark-light cycle, with balanced food and free access to water. The protocol for these experiments was approved by the Committee on Experimental Animal Ethics of King Abdul Aziz University.

Preparation of leaves extract: Fresh young leaves of *A. wilkesiana* were directly collected from some botanical gardens in Cameron Highlands, Malaysia.

The leaves were thoroughly washed and dried at room temperature. The fine quality of dried leaves was kept in dry plastic container until use for extract preparation. The aqueous extract of leaves was prepared every ten days. The dried leaves (15 g) were put in 500 mL cold water and mixed in an electric blender for 10 min. The mixture was filtered and the resulting extract was stored in refrigerator for subsequent experiments.

Induction of diabetes: Experimental diabetes was induced in overnight fasted mice by intraperitoneal injection of STZ (Sigma-Aldrich Corp, St. Louis, MO, USA), 60 mg kg⁻¹ body weight, dissolved in 0.9% NaCl solution. After 5 days, mice with glycemia above 17 mmol L⁻¹ (at fasting state) were included in the study. Control mice were injected with saline solution.

Treatment: The treatment was started on the sixth day after STZ injection and this was considered as the first day of treatment. The treatment was continued for 30 days. The mice have been divided into four groups comprising 15 animals in each group as follows:

- Group 1:** Control mice given only saline solution
- Group 2:** Diabetic controls
- Group 3:** Diabetic mice treated orally with *A. wilkesiana* leaves extract (0.5 mL/mouse/daily)
- Group 4:** Normal mice given saline solution and treated orally with *A. wilkesiana* leaves extract at the same dose given to group 3

Serum analysis: After 10, 20 and 30 days of treatment, food was withdrawn from the mice and they were fasted overnight but had free access to water. The experimental animals were anaesthetized with diethyl ether. Whole blood samples were collected from orbital venous plexus and emptied into plain tubes and allowed to clot. The clotted blood samples were thereafter centrifuged to recover serum from clotted cells. Serum was carefully separated and stored frozen until used for glucose, triglycerides, cholesterol, Na, K and Ca determinations. Serum glucose, triglycerides and cholesterol were estimated using an automatic analyzer (Reflotron® Plus System, Roche, Germany). Serum Na, K and Ca were measured using Automated Clinical Chemistry Analysis System, Dimension® type RXL Max (Dade Behring Delaware, DE 19714, USA).

Statistical analysis: The data were expressed as mean± Standard Deviation (SD). Statistical comparisons were performed by one way Analysis Of Variance (ANOVA) followed by Duncans' Multiple Range Tests (DMRT). The results were considered statistically significant if the P-values were less than 0.05.

RESULTS

Significant increases in the levels of serum glucose, triglycerides, cholesterol and Na were observed in diabetic rats when compared to control mice and STZ plus leaves extract or leaves extract treated mice after 10, 20 and 30 days (Fig. 1). The level of serum K was statistically decreased in diabetic mice (group 2) after 20 and 30 days compared with other groups. The level of serum Ca was significantly increased in group 2 after 30 days. Serum glucose value was statistically increased in diabetic mice treated with leaves extract at all periods compared with control and leaves extract treated mice (Fig. 1). The level of cholesterol was increased in STZ plus leaves extract treated mice after 10 days compared with group 4 and after 20 days compared with control and leaves extract treated mice. In comparison with leaves extract treated mice, the level of serum K was statistically decreased in group 3 after 30 days. Insignificant changes in the values of serum triglycerides, Na and Ca were observed in STZ plus leaves extract treated mice at all periods of experiment. Moreover, the levels of all studied parameters were statistically unchanged in mice treated with only leaves extract (Fig. 1). Table 1 shows the percentage changes of serum parameters values of all treated groups (2, 3 and 4) compared with control mice (group 1). The maximum increases of serum glucose after 10 (345.8%), 20 (353.0%), 30 (445.2%) days were observed in STZ-diabetic mice, while the treatment of diabetic mice with leaves extract (group 3) reduced the elevations of percentage change after 10 (303.9%),

Table 1: Percentage changes of serum parameters values of STZ, STZ plus *A. wilkesiana* leaves extract and *A. wilkesiana* leaves extract treated mice after 10, 20 and 30 days compared with control values

Parameters	Time (days)	Treatments		
		STZ	STZ + leaves extract	Leaves extract
Glucose	10	+345.8	+303.9	+0.23
	20	+353.0	+311.9	-3.30
	30	+445.2	+305.6	+0.70
Triglycerides	10	+27.5	+0.7	-0.73
	20	+33.1	+1.5	+0.73
	30	+40.3	-0.7	-4.00
Cholesterol	10	+29.6	+4.7	+0.47
	20	+34.4	13.0	+3.30
	30	+57.1	+8.1	-2.90
Na	10	+8.8	+2.0	+2.20
	20	+10.8	+1.0	+0.13
	30	+16.2	-0.3	-3.50
K	10	-4.4	+2.5	+4.70
	20	-9.2	+1.8	+3.10
	30	-11.9	-2.4	+3.90
Ca	10	-0.9	+3.0	-1.30
	20	+5.3	+3.1	+2.20
	30	+19.8	+4.9	+2.20

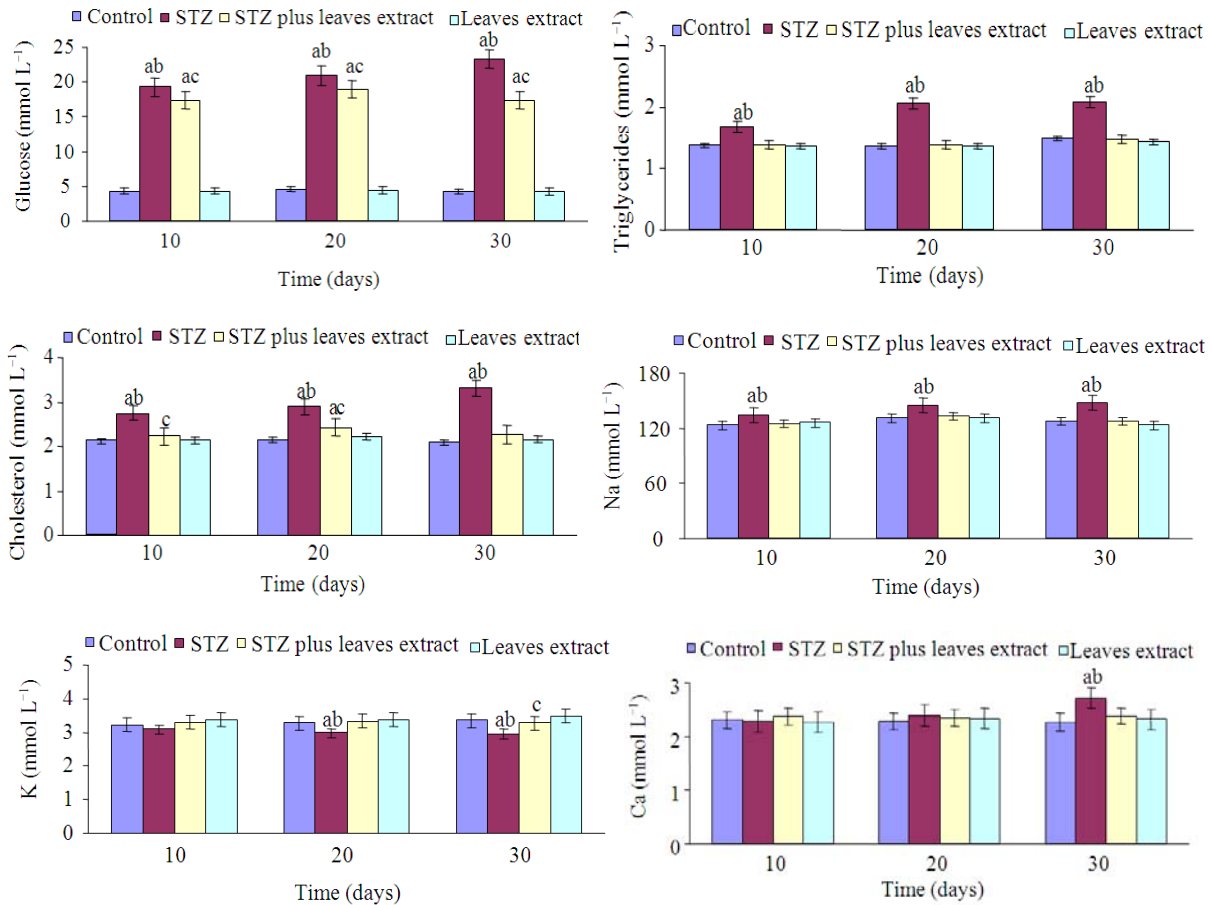


Fig. 1: Serum glucose, triglycerides, cholesterol, Na, K and Ca values of control (group 1), STZ (group 2), STZ plus *A. wilkesiana* leaves extract (group 3) and *A. wilkesiana* leaves extract (group 4) treated mice after 10, 20 and 30 days (n = 6); (a) Indicates a significant difference between control and treated groups; (b) Indicates a significant difference between STZ treated group and groups treated with STZ plus *A. wilkesiana* leaves extract and *A. wilkesiana* leaves extract; (c) Indicates significant difference between STZ plus *A. wilkesiana* leaves extract treated group and group treated with only *A. wilkesiana* leaves extract

20 (311.9%) and 30 (305.6%) days. The maximum increases of serum triglycerides (40.3%), cholesterol (57.1%), Na (16.2%) and Ca (19.8%) and the maximum decrease of K (11.9%) were noted in STZ-diabetic mice. From the present results, it is obviously that the percentage changes of serum parameters in group 2 were increased with the increases of experimental duration.

DISCUSSION

In the present study, the extract of *A. wilkesiana* leaves was investigated for antidiabetic activity on STZ-diabetic mice. The levels of serum glucose, triglycerides and cholesterol were significantly higher in diabetic mice

than in the control group. Similar observations were noted in different experimental diabetes investigations (Al-Attar and Zari, 2007; Geethan and Prince, 2008; Bang *et al.*, 2009; Eliza *et al.*, 2009; Roghani and Baluchnejadmojarad, 2010; Salahuddin *et al.*, 2010). STZ produces hyperglycemia in a concentration dependent model by selective β -cells cytotoxic effect (Saini *et al.*, 1996). The mechanism of action of STZ on rodent β -cells is related to uptake of STZ into β -cells and DNA strand breaks which then causes a lethal depletion of NAD, in β -cells (Swanston-Flatt *et al.*, 1989; Saini *et al.*, 1996). It has been shown that inhibition of free radical scavenger enzymes and enhancing production of the superoxide radical are the

mechanism of STZ on pancreatic β -cells (Swanston-Flatt *et al.*, 1989). From the present study, it is obviously that the administration of *A. wilkesiana* leaves extract to diabetic rats resulted in significant decline of serum glucose, cholesterol levels and protection of serum triglycerides level which statistically unchanged. The *A. wilkesiana* leaves extract might possess insulin like effect on peripheral tissues either by promoting glucose uptake and metabolism or inhibiting hepatic gluconeogenesis. A variety of derangements in metabolic and regulatory mechanisms, due to insulin deficiency, are responsible for the observed accumulation of lipids (Rajalingam *et al.*, 1993). The impairment of insulin secretion results in enhanced metabolism of lipids from the adipose tissue to the plasma. Further, it has been reported that diabetic animals treated with insulin shows normalized lipid levels (Pathak *et al.*, 1981). Thus, the results indicate that *A. wilkesiana* leaves shows insulin-like action by virtue of its lipid lowering levels.

Diabetes is characterized by increased volume and metabolites excretions via the kidneys, usually in excess of normal thresholds. These usually give rise to derangements in homeostatic balance with respect to electrolytes. It is well known that alterations in mineral metabolism can induce disturbance in glucose metabolism (Kumar *et al.*, 1994; Rosolova *et al.*, 1997; Paula *et al.*, 1998) and glucose intolerance which also can interfere with mineral metabolism (de Lordes Lima *et al.*, 1998). There is evidence that STZ-induced diabetes in experimental animals alters trace mineral balance as a result of disturbances in pancreatic function. In the present study, the levels of serum Na and Ca were statistically elevated in diabetic mice, while the level of serum K was significantly declined. In contrast, Shaheen *et al.* (2007) showed that the levels of serum Na and Ca were significantly decreased in STZ-induced diabetic rats, whereas serum K levels were increased non-significantly as compared to control rats. Atangwho *et al.* (2009) reported that the serum concentrations of Na and K of STZ-diabetic rats were decreased compared to the non-diabetic control. Ikpi *et al.* (2009) demonstrated that the level of serum Na was significantly decreased in alloxan-induced diabetic rats, while the level of K was statistically unchanged compared with control rats. Akah *et al.* (2009) showed that the level of serum Na was significantly unchanged in alloxan-induced diabetic rats, while the level of K was statistically increased compared with control rats. Gayathri and Kannabiran (2008) showed that in STZ-diabetic rats, there was a significant increase in the serum Na, K

and Ca. Uchiyama and Yamaguchi (2003) and Yamaguchi *et al.* (2007) demonstrated that the levels of serum Ca were increased in STZ-diabetic rats. Yanardag *et al.* (2005) and Ozsoy-Sacan *et al.* (2006) reported that in the STZ-diabetic rats, the blood Na and K levels were statistically increased.

Disorders of sodium and water balance are very common. Sodium is the principal solute in the extracellular compartment and hence the plasma osmolality largely depends on the serum sodium concentration. A decrease or increase in the serum sodium level will have an effect on the plasma osmolality and this can have deleterious effects on the whole body-in particular, the central nervous system. Severe hypo-and hypernatraemia are associated with significantly high mortality and morbidity. Moreover, inappropriate treatment may result in treatment related complications such as osmotic demyelination syndrome. Hypernatraemia is defined as serum or plasma sodium higher concentration. Hypernatraemia represents a deficit of water in relation to the body's sodium stores. It can result from net water loss or hypertonic sodium gain. Sustained hypernatraemia can occur only when thirst or access to water is impaired (Genanri, 1998). K homeostasis is essential for normal myocardial function. K plays a central role in the maintenance of cellular polarization and is critical for the transmission of electrical impulses through the myocardium. Alterations in the normal balance between intracellular and extracellular K concentrations can lead to serious arrhythmias (Grumbach *et al.*, 1954; Ahmed *et al.*, 2007). The adverse association between hypokalemia and arrhythmias in animal models appears to be more significant in the presence of acute myocardial ischemia (Grumbach *et al.*, 1954; Yano *et al.*, 1991). The major causes of low serum K are (1) decreased K intake due to intravenous feedings which do not contain K, (2) increased loss of K in the urine due to accelerated tissue breakdown or renal lesions, (3) loss from the gastrointestinal tract due to diarrhea or fistulae and (4) shift between serum and cells due to metabolic causes, drugs or changes in pH. Serum Ca is usually measured to screen for monitor diseases of the bone or calcium regulation disorders due to hormonal disturbance (parathormone and calcitonin), vitamin D level and gastrointestinal absorption level of Ca and diseases of kidney. Yamaguchi *et al.* (2007) reported that the increase in serum Ca concentration in STZ-diabetic rats may result from the release of Ca from bone tissues; the femoral Ca content was found to decrease markedly in STZ-diabetic rats. Increased glucose oxidation in the presence of transition metals has been shown to produce membrane damage by

membrane lipid peroxidation and protein glycation (Hunt *et al.*, 1990). This could be the reason for the altered flux in electrolytes balance that resulted in the elevated extracellular concentration of Na and Ca in STZ-induced diabetic mice. Although the data obtained in this study do not allow any definite conclusions to be drawn on the mechanism of action of *A. wilkesiana* leaves extract on the levels of studied serum electrolytes in the experimental diabetic mice, it has been suggested that the active natural compounds of *A. wilkesiana* leaves might act by different pathways effect to cause the ameliorative activity.

CONCLUSION

Based on above mentioned observations, It can be concluded that this study shows for the first time that *A. wilkesiana* leaves extract supplementation is beneficial in lowering the level of blood glucose, improves the lipid and electrolytes profile in experimental diabetic mice. Further biochemical and pharmacological investigations will be required to study the influences of different concentrations and doses of *A. wilkesiana* leaves extract and its ingredients on STZ and other factors-induced diabetic animals.

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