

## Determination of the Hypoglycemic Effect of the Flower of *Tamarindus indica* on Alloxan Induced Diabetic Mice

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**Abstract:** The aim of the study was to examine whether the flower of the plant Tamarind (*Tamarindus indica*) can prevent alloxan induced diabetes mellitus. The mice were randomly divided into 3 groups of 3 mice each. Diabetes was induced in group 1 and 2 by Intraperitoneal (IP) injection of 180 mg alloxan/kg body weight. Group 1 was induced and treated with 10 mg/kg tamarind. Group 2 was induced (untreated) whereas Group 3 was used as a control (uninduced). Serum Glucose and Malondialdehyde (MDA) levels were determined. Tamarind flowers reduced fasting glucose level and MDA concentration significantly ( $p < 0.05$ ) in induced treated compared with induced untreated without regenerating the  $\beta$  cells. From the result obtained, it can be concluded that, tamarind flower has only hypoglycemic effect with no regeneration of  $\beta$ -cells on chemically induced diabetes.

**Keywords:** Diabetes mellitus, hypoglycaemic effect, oxidative stress, *Tamarindus indica*

### INTRODUCTION

The term diabetes mellitus describes a metabolic disorder of multiple aetiology, characterized by chronic hyperglycemia with disturbance of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both (Wild *et al.*, 2004). It is characterized by elevated blood glucose concentration caused by insulin deficiency, often combined with insulin resistance. Type-2 DM is more prevalent and account for about 90 to 95% of all diagnosed cases of diabetes (Wild *et al.*, 2004). The serious side effects of drugs currently available for treatment of diabetes mellitus warrant the need for alternative therapeutic interventions which are effective with fewer side effects. Ethnobotanical knowledge with its historical roles in diabetes has over 1,200 species of medicinal plants recognized throughout the world for their ability to treat diabetic indications have been currently giving attention to provide the alternative treatment (Frode and Medeiros, 2008).

*Tamarindus* is a monotypic genus and belongs to the subfamily Caesalpinioideae of the family Leguminosae (Fabaceae). *Tamarindus indica* L., commonly known as *Tamarindus* tree is one of the most important multipurpose tropical fruit tree species (Bhadoriya *et al.*, 2011). The fruit contains about 30% sticky edible pulp (Singh *et al.*, 2007)

It is used in treatment of many diseases such as fever, dysentery, jaundice, gonococci and gastrointestinal disorders (Khanzada *et al.*, 2008; Kobayashi *et al.*, 1996). The pharmacological effect *T. indica* fruit extracts as antibacterial (Al-Fatimi *et al.*,

2007) antifungal, hypoglycaemic (Maiti *et al.*, 2004), anti-inflammatory (Rimbau *et al.*, 1999) gastrointestinal (Coutino-Rodriguez *et al.*, 2001), hypolipomic (Mati *et al.*, 2005) and antioxidant activities (Al-Fatimi *et al.*, 2007) have been documented.

*T. indica* fruit is rich in phenolic compounds, cardiac glycosides (Rasu *et al.*, 1989), while the pulp contains pyrazines (trans-2-hexenal); and some thiazoles (2-ethylthiazole, 2-methylthiazole) (Hänsel *et al.*, 1992). Both the pulp and flower are rich in water soluble vitamins such as thiamine, riboflavin and ascorbic acid (Singh *et al.*, 2007). The flower is much richer in ascorbic acid than the pulp (Singh *et al.*, 2007). In Hausa community of Northern Nigeria, the flower of *T. indica* is used in the management of Diabetes mellitus. This work is an evaluation of flower of *T. indica* for its efficacy as antihyperglycaemic agent. Though the seeds of *T. indica* have been demonstrated to have antihyperglycaemic action (Maiti *et al.*, 2004, 2005), there is paucity of data about the potential of the flower currently used in Hausa land as anti diabetic agent. Thus the study attempted to provide scientific information on the effect of *T. indica* flower as antihyperglycaemic agent.

### MATERIALS AND METHODS

**Experimental animals:** The experiments were performed on male DW mice (approx. 30 g) obtained from school of veterinary medicine, Jos Nigeria. All aspect of animal care complied with the ethical guidelines and technical requirements approved by the institutional Animal Ethics Committee.

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Animals were housed in cages in an environmentally controlled animal facility with free access to a standard commercial diet and water *ad libitum*. The weight gain, food and water intake were determined daily in the morning. The experiment was conducted for a period of 5 weeks.

**Plant material:** The flower of tamarind was obtained from Bayero University Kano Old Campus, Kano Nigeria. The flower was plucked fresh from the tree and washed, pounded and dried under shade thereby avoiding exposure to sunlight. Dried flower was pounded again to powder. Ten gram of herb was use together with 490 g of fed making 500 g total to be fed to the diabetic induced mice. Thus, the concentration of the supplement fed to the diabetic test group was 2% w/w.

**Induction and treatment:** All animals were fed on normal diet for 7 days of acclimatization. Diabetes was induced by an Intraperitoneal (IP) injection of 180 mg alloxan/kg b wt (Stanley and Venogopal, 2001).

Blood glucose levels were measured 48 h after alloxan administration. Development of diabetes mellitus was proven by sustained hyperglycemia (diabetic mice had glycaemia >11.11 mmol). This was based on the observation that only diabetic mice that had a fasting glucose greater than 200 mg/dL would be included in the study (Stanley and Venogopal, 2001).

**Experimental design:** The mice were randomly divided into 3 groups as follows:

**Group I:** Diabetic animals received the mixture of feed and plant

**Group II:** Induced diabetic animal receive normal diet

**Group III:** Uninduced animal received normal diet

**Glucose estimation:** Fasting blood glucose determination was done 1 week after alloxan induction of diabetes mellitus. The mice tail was nipped gently with sterilized blade and one drop of fresh venous were squeezed out and placed on the sample well of glucometer strip which was already inserted in the glucose monitoring meter. The glucometer was calibrated before use using standard Randox glucose kit. The mice were sacrificed after 5 weeks and fasting blood glucose level was determined by the glucose oxidase method according to manufacturer's procedure (Randox Laboratories Ltd. Ardmores, United Kingdom).

**Statistical analysis:** SPSS 14.0 statistical package was used in the analysis. All parameters were analyzed using descriptive and inferential statistics and the values were presented as mean±standard deviation for four mice in each group. The significance of difference in all the means of all parameters reported for the four groups of animals was determined using paired sample

Table 1: The weight of the mice before induction and also the weight gain after induction each week for 4 weeks

Weight gain of mice (g)			
Weeks	Group 1	Group 2	Group 3
1	22±5.41	29.3±1.15	24±1.73
2	20±2.98	28±1.00	25.3±1.52
3	22.2±2.50	25±1.00	28±1.52
4	23±2.00	23.3±2.08	27.6±2.51
5	25.2±3.40*	20.6±2.08	29.3±1.15*

Values are of mean±S.D.; n = 3; Group 1: Induced treated; Group 2: Induced untreated; Group 3: Un-induced untreated; \*: p<0.05

Table 2: The fasting blood glucose 1 week before induction and blood glucose level after induction

Blood glucose concentration (mmol/L)			
Weeks	Group I	Group II	Group III
1	22.6±2.55	23.6±1.61	5.2±0.65
2	21.7±4.94	22.7±0.62	4.7±0.47
3	16.1±4.39	23.8±1.35	5.1±0.26
4	12±2.43	24.7±2.31	5.0±0.25
5	9.7±1.61*#	26.7±1.34	4.9±0.37*

Values are mean±S.D.; n = 3; \*: p<0.05 compared with group II (diabetic control); #: p<0.05 compared with group III (normal control); Group I: Induced treated; Group II: Induced untreated; Group III: Un-induced untreated

Table 3: Serum malondialdehyde of mice for diabetic treated, diabetic untreated and non-diabetic untreated at the end of 5 weeks

Groups	Concentration of serum malondialdehyde (mmol/mL)
1	10.08±1.21
2	16.44±3.00*
3	10.61±0.39

Values are mean±S.D.; n = 3; \*: p<0.05 indicates statistical significance; Group 1: Induced treated; Group 2: Induced untreated; Group 3: Un-induced untreated

student t-test and a p-value of <0.05 (two tailed) was considered as significant.

## RESULTS

Table 1 shows the weekly body weight after induction of diabetes mellitus for 5 weeks. Groups I and III showed increasing weekly body weight, with a noticeable decrease in body weight in group II. The increase of body weight in diabetic supplemented (Group 1) and non-diabetic control on normal diet (Group 3) was significant (p<0.05) compared with diabetic control (group 2).

Table 2 shows the fasting blood glucose after 5 weeks of induction. Blood glucose level of group 1 (induced treated) was compared with group 2 (induced untreated) and also group 3 (unindicted untreated). The effect of tamarind on glucose level in the serum showed significant difference (p<0.05) compared to diabetic control, though it did not bring down blood glucose level effectively to normal level, but there was a change in blood glucose.

Table 3 shows the Malondialdehyde estimation at the end of 5 weeks, there was a significant difference compared with group 1 and 2, similarly there is a significant difference compared with group 2 and 3.

## DISCUSSION

Toxicity of alloxan in the destruction of  $\beta$  cells is elicited through its reduction by glutathione to dialuric acid, in which redox recycling process generated ROS that culminates in the damage the cells (Malaise, 1982). Furthermore, transition metals such as iron and copper, which are potentially involved in the generation of hydroxyl free radicals are also involved in the alloxan mediated killing of beta cells (Malaise, 1982). Tamarinda pericarp and flower are rich in flavanoids. The profile of polyphenolics in Tamarind pericarp and flower include proanthocyanidins in various forms, such as apigenin, catechin (procyanidin B2, epicatechineriodictyol, naringenin) (Bhadoriya *et al.*, 2011). The content of Tamarind seeds comprised only procyanidins, represented mainly by oligomericprocyanidin tetramer, procyanidinhexamer and procyanidinpentamer with lower amounts of procyanidin B2 epicatechin (Bhadoriya *et al.*, 2011). *T. indica* seed aqueous extract exhibited potent antidiabetogenic activity in Streptozotocin-induced diabetic male rats. The *T. indica* seed aqueous extract significantly reduced blood glucose levels (Maiti *et al.*, 2004). The hypolipidaemic effect was also demonstrated in hyperlipidaemic rats (Maiti *et al.*, 2005). The flower of *T. indica* has the highest content of vitamin C (Singh *et al.*, 2007), a water soluble vitamin with potent antioxidant property.

The administration of the tamarind flower lowered blood glucose levels in alloxan induced diabetic mice significantly. Though the leaves of *T. indica* had potent antidiabetogenic effect in rat, there was paucity of data to support the practice of the use of flower in the management of Diabetes mellitus in Hausaland. Photochemical analysis of the flower indicates the presence of flavonoids, proanthocyanidins and vitamin C (Bhadoriya *et al.*, 2011; Singh *et al.*, 2007). The  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibition of Tamarind leaves have profound effect in lowering post meal blood glucose level (Hamidreza *et al.*, 2010; Funke and Melzig, 2006). Similarly, proanthocyanidins of Persimmon inhibit digestive enzymes such as  $\alpha$  amylase and  $\alpha$  glucosidase enzymes in addition to prevention of the formation of advanced glycation products (Lee *et al.*, 2007). Hence, it is likely that the proanthocyanidins in the flower may have as well reduce the glycaemic index of the food consumed with flower of *T. indica* by acting on the carbohydrate digestive enzymes such as  $\alpha$  amylase and  $\alpha$  glucosidase.

Flavonoid and terpenes isolated from the other antidiabetic medicinal plants have been found to stimulate secretion of insulin (Marles and Farnsworth, 1995). Glucokinase (hexokinase IV) has a major role in the control of blood glucose homeostasis because it is the predominant hexokinase expressed in the liver, has very high control strength on hepatic glucose disposal and is the glucose sensor for insulin secretion in pancreatic  $\beta$ -cells (Chen *et al.*, 1998; Estrada *et al.*,

2005; Carrasco *et al.*, 2009). Thus, the flavonoids in the flower may have stimulating effect on insulin secretion from the remains of  $\beta$  cells not completely hitherto destroyed by alloxan. Flavonoids also inhibit glucose-6-phosphatase activity in the liver thereby suppressing gluconeogenesis and glycogenolysis and consequently reduces the hyperglycaemia (Chen *et al.*, 1998; Estrada *et al.*, 2005; Carrasco *et al.*, 2009). Serum molandialdehyde, was observed to have been significantly reduced in diabetic treated mice compared with diabetic control. Tamarind is rich in vitamin C, an antioxidant with potential of attenuating oxidative stress. Thus, the vitamin C content of tamarind may likely reduce oxidative damage associated with diabetes mellitus. Furthermore, the flavonoid content of the flower may as well reduce the oxidative stress. The free radical scavenging ability of many flavonoids-containing extracts has been postulated as the mechanism which affords relief in many distressful diseased conditions of the body, including diabetes mellitus (Tiwari, 2004). Though, from the experiment, it is difficult to suggest other mechanisms by which Taramind flower lowers blood glucose and increase body weight, however inhibition of amylase action may not be the sole mechanism. In animal studies, Tamarind has been found to lower serum cholesterol (D'Cruz, 2011). Due to lack of available clinical trials, there is insufficient evidence to recommend tamarind for the treatment of hypercholesterolemia (D'Cruz, 2011). Tamarind already has a history of traditional use as a diabetes aid. It has been noted that plants with hypoglycemic, or blood-sugar-lowering, potential may ultimately be better options than medicines used to manage diabetes because they are more harmonious with biological systems (Hamidreza *et al.*, 2010). The insulin therapy often used to treat diabetes has numerous drawbacks associated with long-term treatment (Hamidreza *et al.*, 2010). These include insulin resistance, fatty liver, anorexia nervosa and brain atrophy (Hamidreza *et al.*, 2010). Tamarind, on the other hand, may protect against oxidative damage associated with diabetes due to the plant's tannins, vitamin C which have antioxidant properties (Hamidreza *et al.*, 2010).

This study suggests that Tamarind flower has an effect on chemically induced diabetes mellitus. It is noted that the diabetic treated group has elevated fasting blood glucose than normal control. The amount given to the animal was extrapolated from what is currently used in hausaland for the management of diabetes. Thus, it is likely that higher dose would have more potent effect in lowering blood glucose.

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