Antihyperglycaemic activity of the flavonoid-rich fraction of the extract of Tamarindus indica L. on experimentally induced hyperglycaemic wistar rats

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ABSTRACT

Diabetes is the most common endocrine disease and its prevalence is reaching epidemic proportion worldwide. Tamarindus indica is a slow growing tree that is resistant to strong winds and perennial. The stem bark extract of the plant was investigated for its hypoglycemic action on experimentally induced hyperglycaemic Wistar rats. The oral LD₅₀ of the extract was found to be 1,265 mg/kg. The flavonoid-rich fraction lowered the Blood Glucose Level (BGL) in the three doses used (100, 200 and 400 mg/kg) there was a significant reduction with the 400 mg/kg dose at the 8th, 16th and 24th hour and the 200 mg/kg dose at 16 and 24 hours, and the 100 mg/kg dose at 24 hours. The flavonoid-rich portion of Tamarindus indica Linn significantly lowered elevated BGL in the experimental animal models.

INTRODUCTION

Diabetes mellitus is ranked seventh among the leading causes of death and is considered third when its fatal complications are taken into account (Trivedi et al., 2004). Diabetes mellitus is often linked with abnormal lipid metabolism and dyslipidemia and hyperlipidemia are recognized complications of diabetes mellitus characterized by increased levels of cholesterol, triglycerides and phospholipids and alterations in lipoprotein composition (Umesh et al., 2004; Sophia, and Manoharan 2007). Diabetes is the world’s largest endocrine disease with deranged carbohydrate, fat and protein metabolism (Shalam et al., 2006). The increasing prevalence of diabetes is reaching epidemic proportion worldwide. According to the World Health Organization (WHO) report, approximately 150 million people have diabetes worldwide, and this figure may double by the year 2025. As part of the pathogenesis of noninsulin-dependent diabetes mellitus (NIDDM), the skeletal muscle, liver and adipose tissue become resistant to the hormonal effects of insulin, which in turn leads to decrease insulin-mediated glucose disposal, hepatic glucose overproduction and a marked increase in lipolysis (Grover et al., 2001).

Fructose feeding leads to insulin resistance and a compensatory hyperinsulinemia responses (Reaven et al., 1988; Hwang et al., 1989). Nigeria is among the top five countries with the highest cost of diabetic care in Sub Saharan Africa (Opara, 2006).

Tamarindus indica Linn, belongs to the family Caesalpiniaceae, which is a sub-family in Leguminosae; a dicotyledonous. Caesalpiniaceae, is the third largest family of flowering plants (Lewis et al., 2005). The tamarind tree grows slowly and is resistant to strong winds and it is perennial. The stem bark of the plant is used locally in the management of diabetes mellitus but there is no scientific evidence to support this claim. This study aims to scientifically validate the hypoglycaemic activity of the plant.
METHODOLOGY

Plant collection
A sample of the plant (stem-bark of *Tamarindus indica* L.) was collected by scraping the trunk from Namaye in Bunkure Local Government Area of Kano state Nigeria. Botanical identification was done at the herbarium unit of the Department of Biological Sciences, Ahmadu Bello University Zaria. Mallam U. S. Gallah of the herbarium unit compared the sample with voucher specimen 00026.

The stem-bark was cleaned, and air dried under shade for 26 days. It was then pulverized using a pestle and mortar and then sieved to obtain the fine powder. The powder was weighed and kept in an air tight container.

Animals used in the Study
Male and female Wistar albino rats (weighing 150-200 g) obtained from the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria were used. The rats were housed in polypropylene cages at room temperature and maintained on standard laboratory animal feed obtained from the Department and water *ad libitum*, throughout the study. These studies were carried out in Ahmadu Bello University in accordance with the rules governing the use of laboratory animals as accepted internationally.

Flavonoids-rich fraction
The method described by Woo *et al.*, (1980) was followed. The method involves defatting initially with petroleum ether followed by extraction with 70% methanol solution. Polar compounds were further removed by dissolving the methanolic extract in diethyether solution with subsequent addition of water. N-Butanol was added to the water residue and the mixture shaken vigorously. The two distinct layers were then separated and 1% potassium hydroxide (KOH) solution was added to the n-butanol residue and gently shaken. The n-butanic portion contains the saponin-rich portion.

The KOH solution (alkaline fraction) was neutralized with diluted hydrochloric acid (HCl) then partitioned with n-butanol. The n-butanol fraction was removed, concentrated and tested for the presence of flavonoids. This fraction was subsequently referred to as flavonoid-rich fraction.

Acute toxicity studies
The oral median lethal dose (LD$_{50}$) of the extract in rats was conducted according to the method of Lorke (1983) with modifications. The method was divided into two phases. In the initial phase, 3 groups of three rats each were treated with the extract at doses of 10, 100 and 1000 mg/kg body weight orally and the rats were observed for clinical signs and symptoms of toxicity within 24 hours and death within 72 hours.

In the second phase, 4 groups each containing one fresh rat was administered with three more specific doses of the extract based on the result of the initial phase. The animals were also observed for clinical signs and symptoms of toxic effects and mortality for 14 days.

The LD$_{50}$ value was determined by calculating the geometric mean of the lowest dose that caused death and the highest dose for which the animal survived (0/1 and 1/1).

Alloxan-induced hyperglycaemia
Hyperglycaemia was induced by a single intraperitoneal injection of 150 mg/kg body weight of alloxan to 12 hours fasted rats (Dunn and Mc Letchie 1943; Goldner and Gomori 1944) Six hours after the alloxan administration, the rats were maintained on 5% glucose solution for the next 24 hours to prevent hypoglycaemia that may result from acute massive pancreatic release of insulin (Owu *et al.*, 2006). Seventy-two hours after drug administration, the rats were examined for hyperglycaemia by cutting the tail tip and using a one touch glucometer with compatible strips. Animals with fasting blood glucose of 180 mg/dL and less than 550 mg/dL were used in the study. Blood samples for blood glucose determination were collected from the tail at intervals of 0, 1, 4, 8, 16 and 24 hours. Determination of blood glucose level was done by the glucose-oxidase principle using the one touch Basic. (Rheney and Kirk 2000).

Group I: Received normal saline orally
Group II: Received 100 mg/kg body weight of flavonoids-rich fraction of methanol stem bark extract of *T. indica* orally
Group III: Received 200 mg/kg body weight of flavonoids-rich fraction of methanol stem bark extract of *T. indica* orally
Group IV: Received 400 mg/kg body weight of flavonoids-rich fraction of methanol stem bark extract of *T. indica* orally
Group V: Received metformin 250mg/kg body weight orally (Marta *et al.*, 2000; Solskov *et al.*, 2008).

Fructose-induced insulin resistance model
For this model the methods described by Dai *et al.*, 1995 and Vikrant *et al.*, 2001 were adopted. The animals were divided into six groups of five rats each.

Group I: Received 10%w/v Fructose solution *ad libitum* and 100 mg/kg body weight methanol stem bark extract of *T. indica* orally daily for 28 days
Group II: Administered 10%w/v Fructose solution *ad libitum* and 200 mg/kg body weight of methanol stem bark extract of *T. indica* orally daily for 28 days
Group III: Received 10%w/v Fructose solution *ad libitum* and 400 mg/kg body weight of methanol stem bark extract of *T. indica* orally daily for 28 days.
Group IV: Fructose – fed with 10%w/v fructose solution *ad libitum* in their drinker for 28 days only.
Group V : Received normal saline only
Group VI: Received 10%w/v fructose solution *ad libitum* and metformin 250mg/kg
All rats were fasted for half an hour prior to extract administration every day.
Data analysis

Results were expressed as mean ± SEM. Statistical analysis was performed by one-way analysis of variance (ANOVA). Student’s t-test at 95% level of significance was used to assess significant difference between the control and treated group. The results are presented in tables and charts.

RESULTS

The extract gave a yield of 8.4%, the oral LD50 in rats for the flavonoids-rich fraction was calculated to be 1,265 mg/kg body weight. The flavonoids-rich fraction lowered the BGL in the three doses used (100, 200 and 400 mg/kg) there was a significant reduction with the 400 mg/kg dose at the 8th, 16th and 24th hour and the 200 mg/kg dose at 16 and 24 hours, and the 100 mg/kg dose at 24 hours (figure 1b).

The three doses of the extract significantly lowered the BGL on the 10th and 20th days respectively (Table 1).

DISCUSSION

The study seeks to demonstrate the efficacy of flavonoids-rich portion of Tamarindus indica Linn in lowering an elevated blood glucose concentration as well as to investigate the
ability of the portion at preventing a rise in blood glucose level. The oral median lethal dose of the fraction was calculated to be 1,265 mg/kg. A scale proposed by Lorke, (1983) roughly classifies substance as; only slightly toxic (LD$_{50}$ up to 1000 mg/kg), LD$_{50}$ values greater than 1000 mg/kg are considered safe.

Alloxan is one of the various chemical methods of inducing experimental diabetes, alloxan diabetes has been commonly utilized as an animal model of insulin dependent diabetes mellitus (IDDM). In the present study, alloxan caused a significant increase in blood glucose concentration when compared to normal animals. It can be suggested that T. indica lowers the elevated glucose level by increasing peripheral glucose uptake. This is supported by the fact that metformin also lowered glucose level meaning that there is still some residual function of the β-cells.

High fructose intake over a long period has been shown to lead to rapid stimulation of lipogenesis and triglyceride accumulation; resulting in reduced insulin sensitivity and hepatic insulin resistance or glucose tolerance (Schwarz et al., 1993; Schwarz et al., 1994; Hellerstein et al., 1996).

Flavonoids are a group of polyphenolic compounds which are widely distributed in nature. They serve as growth inhibitors so that plant growth could be controlled by the balance between inhibiting and activating flavonoids (Furuya et al., 1962). They have been shown to be powerful antioxidants, stress modifiers, anti-allergic agents, antiviral compounds and anti-carcinogens (Harborne and Baxter 1993).

The flavonoid-rich fraction extracted from T. indica was also tested in both the alloxan and fructose induced hyperglycaemia and there was a reduction in elevated BGL with all the doses and at all the hours monitored. However, BGL was only significantly (p < 0.05) lowered after 8 hours for 400 mg/kg dose and after 16 hours for the 200 mg/kg dose. The 100 mg/kg dose only lowered the BGL after 24 hours. The antioxidant and free radical scavenging activities of flavonoids (Hillwel, 1994) could counteract the free radical generation responsible for alloxan-induced diabetes, and may contribute to the efficacy of the extract. All doses of the fraction significantly (p < 0.05) lowered the BGL after 10 days in the fructose-induced hyperglycaemic model.

Oral flavonoids have been reported to cause a significant blood glucose lowering effect in both normoglycaemic and diabetic rabbits (Ahmad et al., 2000). The flavonoids and polyphenols components of plants are well known antioxidants.

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