Study of lipid profile and lipid peroxide in diabetes mellitus treated with Vitex doniana ethanol leaf extract

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ABSTRACT

Several herbal preparations are used to treat diabetes, but their reported hypoglycemic effects are complex. In this study, the lipid profile and lipid peroxides in streptozotocin-induced diabetes in rats treated with the leaves of Vitex doniana ethanol extract on was evaluated. Twenty five male rats were used in the study by randomly allocating them into five groups, each of five rats. Diabetes was induced intraperitoneally using 50mg/kg streptozotocin. Normal rats received distilled water, while diabetic rats were treated with 100mg/kg ethanol extract and glibenclamide (2.5mg/kg) respectively for 28 days. Changes in levels thiobarbituric acid reactive substances (TBARS) in the liver as well as kidney, total cholesterol, low density lipoproteins (LDL), high density lipoproteins (HDL) and triglycerides (TG) were assayed. TBARS, LDL and TG levels were increased, while HDL level decreased significantly (P<0.05) owing to diabetic condition in control rats. Treatment with extract was able to revert these parameters to normal by increasing HDL and decreasing TBARS, LDL and TG levels. Hence, the extract was able to manage lipid peroxidation and hyperlipidemic conditions induced by streptozotocin, suggesting that it is a promising extract for the management of heart problems.

Key words: lipid profile, lipid peroxides, streptozotocin-induced diabetic, vitex doniana.

INTRODUCTION

Diabetes is a complex disease where the carbohydrate and fat metabolism are impaired [1]. Insulin affects many sites of mammalian lipid metabolism. It stimulates synthesis of fatty acid in liver adipose tissue and in the intestine. The insulin has also been reported to increase the cholesterol synthesis. The activity of lipoprotein lipase in white adipose is also increased. From this point of view the assessment of various lipid fractions and lipid peroxide in the cases of Diabetes Mellitus may be of some help in the prognosis of patients and in preventing the possibilities of complications or secondary disorders[2]. The occurrence of free radical induced lipid peroxidation causes considerable change in the cell membrane [3]. Peroxidation of lipid membrane has been related to the pathogenesis of many degenerative diseases, such as atherosclerosis, oxidative damage to DNA, aging, carcinogenesis, sickle cell disease and Diabetes Mellitus etc. [4]. Thus, the lipid peroxide in the blood provides useful information for the prognosis of diabetes in which secondary disorders are often fatal [5].

Vitex doniana sweet, (family Verbenaceae) is a perennial shrub widely distributed in tropical West Africa, and some East African countries including Uganda, Kenya and Tanzania; and high rainfall areas. It is found in the middle belt of Nigeria particularly Kogi, Benue, and parts of the savannah regions of Kaduna, Sokoto and Kano states [6]. It is
variously called *vitex* (English), *dinya* (Hausa), *dinchi* (Gbagyi), *ucha koro* (Igbo), *oriri* (Yoruba) *ejiji* (Igala) and *olih* (Etsako) [7]. *V. doniana* is employed in the treatment of a variety of diseases. Hot aqueous extracts of the leaves are used in the treatment of stomach and rheumatic pains, inflammatory disorders, diarrhoea dysentery and diabetes (Irvine, 1961; Etta, 1984) indicating that the plant’s leaves may possess antidiabetic properties among others. The roots and leaves are used for nausea, colic and epilepsy [8,9]. In North-Central and eastern parts of Nigeria, the young leaves are used as vegetables or sauces and porridge for meals, especially for diabetic patients.

**MATERIALS AND METHODS**

**Collection and Preparation of Plant Materials:**
Fresh leaves of *V. doniana* were collected from its natural habitat in Ankpa, Kogi State, and it was identified and authenticated by the Ethnobotanist in the Department of Medicinal Plant Research and Traditional Medicine of the National Institute for Pharmaceutical Research and Development (NIPRD) Abuja, Nigeria. A voucher specimen number NIPRD/H/6415 was deposited at the herbarium of the department. The plant material was dried in the laboratory at room temperature and pulverized using laboratory mortar and pestle.

**Extraction**
The pulverized sample mixture was defatted with n-Hexane and extracted with ethanol using soxhlet extractor[10].

**Animal management**
Male albino rats (7-8 weeks old) were purchased from the animal house of the Department of Biosciences, Salem University, Lokoja, Nigeria. They were acclimatized for two weeks prior to commencement of experiment. They were kept at room temperature and maintained *ad libitum* on growers mash (feed) and weighed prior to experiment.

**Induction of diabetes**
Rats were fasted overnight and experimental diabetes was induced by intraperitoneal injection of streptozotocin (STZ) with a single dose of 50mg/kg body weight. STZ was dissolved in a freshly prepared 0.1M cold citrate buffer pH4.5 [11]. Control rats were similarly injected with citrate buffer. Because STZ is capable of inducing fatal hypoglycemia as a result of massive pancreatic insulin release, STZ treated rats were provided with 10% glucose solution after 6 hr for the next 24 hr to prevent severe hypoglycemia. After 3 days for development and aggravation of diabetes, rats with moderate diabetes (i.e. blood glucose concentration 250mg/dl) that exhibited hyperglycemia were selected for experiment [12].

**Experimental design**
In the experiment, the rats were divided into 5 groups of 5 rats each. Treatment was carried out orally for four weeks.

- Normal Control (N. control) Distilled water (5ml/kg)
- Diabetic Control (D. Control) Distilled water (5ml/kg)
- Diabetic Glibenclamide (D.STD) (2.5mg/kg)
- Diabetic Extract (D. Ethanol) ethanol extract (100mg/kg)
- Non diabetic Extract (N. Ethanol) ethanol extract (100mg/kg)

On the 28th day of post-treatment, the animals were fasted overnight, anesthetized with chloroform and sacrificed by humane decapitation. The blood was collected in test tubes and serum collected and stored in deep-freezer prior to analysis. Fasting blood glucose was and packed cell volume was monitored weekly. Liver and kidneys were surgically removed, immediately washed with ice-cold normal saline and stored in deep freezer.

**Tissue Preparation**
Weighed liver and kidney samples were homogenised separately in 10 parts (w/v) of ice-cold 50mM Tris-HCl, (pH 7.4) using a homogeniser (Janke and Kunkel, Germany). The homogenates were centrifuged at 3,000 rpm for 15 minutes and the supernatant collected. The supernatants were used for measurement of scavenging enzyme activities and lipid peroxides (TBARS).

**Determination of biochemical parameters**
**Thiobarbituric Acid Reactive Substances (TBARS)**
Hepatic Lipid peroxidation was determined as thiobarbituric acid reactive substances as described by [12]. Lipid peroxidation generates peroxide intermediates which upon cleavage release malondialdehyde, a product which react with thiobarbituric acid. The product of the reaction is a coloured complex which absorbs light at 535nm. The
extinction coefficient, 1.56x10^-5 M^-1 Cm^-1 was used in the calculation of TBARS and values were expressed as nmol/mg protein.

**Estimations of lipid parameters**
Serum was separated and analyzed for serum cholesterol [13], serum HDL [14], serum LDL [15].

**Statistical Analysis**
All the values estimations were expressed as mean ± standard deviation and analyzed for Duncan’s post-hoc ANOVA and student’s t-test using statistical package for social sciences (SPSS). Differences between groups were considered significant at $P < 0.05$ levels.

**RESULTS AND DISCUSSION**

**Thiobarbituric Acid Reactive Substances (TBARS) levels:**
TBARS level was significantly ($p<0.05$) elevated in the serum, liver and kidney of diabetic control rats, with the liver having the highest elevation when compared with the normal control rats (Figure 1, 2 and 3). This increase was reduced significantly ($p<0.05$) in the extract treated rats as well as in the rats treated with glibenclamide.

![Fig. 1: TBARS levels in serum of normal and diabetic rats treated with V. doniana ethanol extract and glibenclamide](image)

**Fig. 1:** TBARS levels in serum of normal and diabetic rats treated with *V. doniana* ethanol extract and glibenclamide

*N. CONTROL = Normal Control; D. CONTROL = diabetic Control; D. STD = Diabetic Standard Drug (Glibenclamide); D. ETH = Diabetic Ethanol extract; N. ETH = Non Diabetic Ethanol extract*

![Fig. 2: TBARS levels in liver of normal and diabetic rats treated with *V. doniana* ethanol extract and glibenclamide](image)

**Fig. 2:** TBARS levels in liver of normal and diabetic rats treated with *V. doniana* ethanol extract and glibenclamide
Lipid profile levels

Figure 4, 5 and 6 shows the effects of the extract on total, LDL and HDL-cholesterol levels.

There was significant ($<0.05$) increase in the concentration of these parameters in the diabetic control groups compared to normal owing to diabetic condition. However, treatment with the extract was able to manage the condition by reverting these increase significantly ($<0.05$) to normal. Hence, a reverse observation was made in the HDL levels, reduced level of HDL was observed in the diabetic control group compared to normal (fig. 7). Treatment with the extract was able to raise the HDL level significantly compared with the normal group.
Fig. 5: Serum LDL-cholesterol concentration in normal and diabetic rats treated with *V. Doniana* ethanol extract and glibenclamide.

Fig. 6: Serum total cholesterol concentration in normal and diabetic rats treated with *V. Doniana* ethanol extract and glibenclamide.

Fig. 7: Serum total cholesterol concentration in normal and diabetic rats treated with *V. Doniana* ethanol extract and glibenclamide.
In the diabetes mellitus abnormal increased levels of lipid, lipoprotein and lipid peroxides in serum may be due to the abnormal lipid metabolism [16]. Maximum increase in lipid peroxide was found in group of diabetes mellitus with complication. Elevated levels of lipid peroxide in diabetes mellitus may be due to the alteration of function of erythrocytes membrane. This inhibits the activity of superoxide dismutase enzyme leading to accumulation of superoxide radicals which cause the maximum lipid peroxidation and tissue damage in diabetes [17].

Increase lipid peroxide may be due to the increased glycation of protein in diabetes mellitus. The glycated proteins might themselves act as a source of free radicals. There is a clear association between lipid peroxide and glucose concentration, which may be also thought to play a role in increased lipid peroxidation in diabetes mellitus. Higher levels of lipid peroxides were observed in diabetic animals with vascular complication. This increase in lipid peroxide may be due to the increased activity of the free radical formation. Free radicals interact in arachidonic acid metabolism, forming a toxic endoperoxidase. The lipid peroxide formed stimulates the cyclooxygenase, prostaglandin and thromboxane synthesis. This will cause increased platelets aggregation, leading to vascular complications [18]. We have found that serum total cholesterol is increased in diabetic group when compared with the controls. Some of the possible reason of higher concentration of serum cholesterol in diabetes may be attributed to the inhibition of cholesterol catabolism.

It has been suggested that the increase in triglyceride may be due to insulin deficiency which results in faulty glucose utilization, causes hyperglycemia and mobilization of fatty acids from adipose tissue. In diabetes blood glucose is not utilized by tissue resulting in hyperglycemia. The fatty acid from adipose tissue are mobilized for energy purpose and excess fatty acids are accumulated in the liver, Which are converted to triglyceride [19]. Chronic insulin deficiency might be associated with a diminished level of LDL receptor. This causes the increase in LDL particles and results in the increase in LDL-cholesterol value in diabetes mellitus.

High level of cholesterol, triglyceride, LDL-cholesterol and low HDL-cholesterol may also be due to the obesity [20,21].

In conclusion, *V. doniana* ethanol extract appears to be of benefit in dealing with diabetes and diabetes-induced complications. However, the estimation of lipid peroxide along with other lipid profile in the diabetes mellitus is very useful as it may serve as a useful monitor to judge the prognosis of diabetes. The detection of risk factor in the early stage of the disease will help to improve and reduce the morbidity rate.

**REFERENCES**