International Standard Serial Number (ISSN): 2249-6807

International Journal of Institutional Pharmacy and Life Sciences 3(5): September-October 2013

INTERNATIONAL JOURNAL OF INSTITUTIONAL PHARMACY AND LIFE SCIENCES

Life Sciences **Research Article……!!!**

Received: 11-01-2013; Revised; Accepted: 25-10-2013

MEMBRANE BOUND ENZYMES AND LIPID LOWERING EFFECT OF *VITEX AGNUS-CASTUS* EXTRACT IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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Keywords:

Vitex agnus-castus, Diabetes Mellitus, Streptozotocin, Membrane bound ATPases, Lipid profile

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ABSTRACT

The aim of present study was to investigate the membrane bound ATPases and hypolipidemic activities of *Vitex agnus-castus* extract on normal and streptozotocin-diabetic rats. Experimental diabetes was induced by intraperitonal injection of streptozotocin (STZ) in a single dose of 50 mg/kg. Oral administration of methanolic extract of *Vitex agnus-castus* 200 mg/kg body wt was given orally for 45 days. Significant decreases in the activities of Na^+/K^+ ATPases, Mg^{2+}ATPases and Ca^{2+}ATPases were observed in the liver and kidney of STZ-induced diabetic rats. A significant increase in the levels of serum TC, TG, LDL and VLDL and decrease in the level of high density lipoproteins (HDL) were observed in STZ induced diabetic rats. Treatment with *Vitex agnus-castus* (200 mg/kg) in STZ-induced diabetic rats restored the enzyme activities and serum lipid profile to near normal levels.

INTRODUCTION

Diabetes mellitus, a common metabolic disorder, is characterized mainly by chronic hyperglycemia resulting from defects in insulin secretion and/or its action. This eventually leads to improper regulation of carbohydrate, protein and lipid metabolism that ultimately contributes to a key factor in the development and the progression of micro and macro vascular complications[1]. Both acute and late diabetic complications are commonly encountered. The long-term complications represented by cardiovascular and cerebro-vascular diseases, nephropathy, retinopathy and neuropathy are major causes of morbidity, disability and premature death in countries of the Eastern Mediterranean region[2]. The underlying causes of diabetic complications have been attributed to hyperglycemia, which results in oxidative stress, alterations in enzyme activities, protein glycosylation and several structural changes[3].

Medicinal plants continue to play an important role in the treatment of diabetes, particularly in developing countries where most people have limited resources and do not have an access to modern treatment[4]. The popularity of plant-based drug in the modern system of medicine is growing day by day as they are claimed to be safe, economical and yet efficacious[5,6]. A number of plants, including vegetables, are commonly consumed in India and other parts of the world and many of these are reported to possess antidiabetic potential[7]. More than 100 medicinal plants are mentioned in the Indian system of medicines including folk medicines for the management of diabetes, which are effective either alone or in combinations[8]. Presently, there is growing interest in herbal remedies due to the side effects associated with the oral hypoglycemic agents (therapeutic agent) for the treatment of diabetes mellitus. So the traditional herbal medicines are mainly used which are obtained from plants, it plays important role in the management of diabetes mellitus[9]. In recent years, herbal medicines have started to gain importance as a source of hypoglycemic agents. Herbal treatments are becoming increasing by popular as the herbal preparations have no least side effects[10]. Many traditional plant treatments for diabetes mellitus are used throughout the world[11]. The present study was done on streptozotocin induced diabetic rats to evaluate the role of *Vitex agnus-castus* in being an essential cause for the antidiabetic and antihyperlipidemic effects.

MATERIALS AND METHODS

Experimental Animals

Adult male albino rats of Wistar strain (160- 180 g) were procured from the Animal Experimental Laboratory of Tamil Nadu Veterinary and Animal Sciences, Chennai, India for the present the study. The animals were maintained in colony cages at 25 ± 2^{o}C, relative humidity of 45 ± 5% and maintained under 12 h light and 12 h dark cycles. The animals were fed with standard animals feed (Hindustan Lever Ltd.) and water *ad libitum*. All the animals were acclimatized for a week before use and they were maintained in hygienic environment in the animal house, J.J. College of Arts and Science, Pudukkottai. The study was conducted accordance with the rules and regulations of Institutional Animal Ethical Committee.

Induction of Diabetes mellitus

Diabetes mellitus was induced by single intraperitoneal injection of freshly prepared streptozotocin (STZ) 50 mg/kg body weight. STZ was dissolved in a freshly prepared 0.1 M cold citrate buffer pH 4.5. The control animals were administrated with only citrate buffer. Diabetes was developed and stabilized in the STZ treated rats over a period of 7 days. After 7 days of STZ administration, plasma glucose levels of each rat were calculated. Rats with fasting plasma glucose (FPG) range of 280 – 350 mg/dl were considered as diabetic and included in this study. Blood was collected by sin ocular puncture.

Preparation of the *Vitex agnus-castus* extracts (VACExt)

Fresh disease free leaves of *Vitex agnus-castus* was collected from in and around Tiruchirappalli District, Tamil Nadu, India and identified by Rev. Dr. John Britto, Botanist, St. Joseph's College, Tiruchirappalli. Voucher specimens were prepared in the form of herbaria and were deposited in Herbarium of St. Joseph's College, Tiruchirappalli. Shade dried and coarsely powdered leaves of *Vitex agnus-castus* (2 kg) were extracted with methanol by soxhelation at room temperature for 48 hour. The extract was filtered and concentrated under reduced pressure using rotary evaporator to get completely dried extract (VACExt). The yield of the crude methanol extract was about 120g was used for the present study.

Experimental protocol

The rats were divided into 5 groups of 6 rats each. VACExt was suspended in vehicle solution and administered orally using an intragastric tube for 45 days. Based on the tentative experiments, 200mg/kg b.w. VACExt was selected for the experiments.

Group 1 Normal rats + Vehicle alone
Group 2 Normal rats + 200mg/kg b.w. of VACExt
Group3 STZ induced diabetic rats + Vehicle alone
Group 4 STZ induced diabetic rats +200 mg/kg b.w. of VACExt
Group 5 STZ induced diabetic rats + Glibenclamide (0.6 mg/kg b.w.)

After 45 days of treatment, the 12 h fasted animals were anaesthetized between 7 am to 8 am, using ketamine (24 mg/kg b.w., intramuscular injection) and sacrified. Blood was collected in two different tubes (i.e.,) one with whole blood for serum separation and another with anticoagulant-potassium oxalate, sodium fluoride for plasma insulin assay.

Biochemical Analysis

The blood was collected and serum separated which was utilized for the analysis of lipid profile. Liver and kidney were dissected out, washed in ice-cold saline, and patted dry and weighed. 10% tissue homogenate was prepared from liver and kidney and used for the assay of membrane bound ATPases. The activity of Na^+/K^+-ATPase was assayed according to the procedure of Bonting[12]. The activity of Ca^{2+}-ATPase was assayed according to the method of Hjerken and Pan[13]. The activity of Mg^{2+}-ATPase was assayed by the method of Ohinishi *et al.*[14] .Total cholesterol in the plasma was estimated by the enzymatic method [15]. HDL-cholesterol was estimated using the diagnostic kit based on the enzymatic method of [16]. These were calculated using the formula[17]. Free fatty acids in the plasma and tissues were estimated by the method of[18]. Triglyceride in the plasma was estimated using the diagnostic kit based on the enzymatic method of [19].

RESULTS

In diabetic rats, the ATPase activity was decreased to 44.7% and 87.72% in the tissue liver and kidney respectively when compared with control rats. While oral administration of VACExt 200mg/kg significantly increased the ATPase to 1.62 and 1.28 1µmoles of pi liberated/h/mg protein in diabetic rats and it was near to the normal. The total ATPase level was increased in VACExt administrated rats than Glibenclamide treated rats. The administration VACExt to normal rats was not shown any significant effect ($P<0.05$) in the activity of ATPase (Fig. 1).

A significant decrease in the activities of Na^+/K^+ ATPase, Mg^{2+} ATPase and Ca^{2+} ATPase were observed in the liver and kidney of STZ-induced diabetic rats when compared to normal rats. The total ATPase level was decreased in diabetic rats. Oral administration of VACExt significantly increased Na^+/K^+ ATPase, Mg^{2+} ATPase and Ca^{2+} ATPase enzymes level to near normal (Table 1), than the standard drug Glibenclamide administrated rats. The Na^+, K^+-ATPase activity in tissues of liver and kidney were not shown any significant changes in VACExt treated normal rats. But in the diabetic rats the level of Na^+, K^+- ATPase (44.77% and 37.49%) were decreased in the tissues of liver and kidney respectively. Oral administration of VACExt 200mg/kg to the diabetic rats significantly increased (0.47 and 0.461µmoles of pi liberated/h/mg protein) the enzyme level in the tissue liver and kidney respectively to near normal. Similarly the diabetic rats were treated with glibenclamide for 45 days which significantly increased the enzyme level near to normal level (Table 1). In diabetic untreated rats, the enzyme Ca^{2+} - ATPase was significantly decreased to 41.17% and 36.95% in both the tissues liver and kidney respectively than the control rats. After administration with VACExt 200mg/kg to the diabetic rats for 45 days, the Ca^{2+} - ATPase enzyme was significantly increased level (50%) than control rats (Fig. 2).The diabetic rats have shown the decreased levels of Mg^{2+} - ATPase levels about 32.75% and 34.28% in both tissues liver and kidney respectively than the control rats. Oral administration of VACExt 200mg/kg and Glibenclamide 0.6mg/kg to the diabetic rats for 45 days significantly increased the enzyme level to near normal. The diabetic rats showed a decreased level of Mg^{2+} - ATPase levels (0.49 and 0.39461µmoles of pi liberated/h/mg protein) in tissues liver and kidney respectively. The Mg^{2+} - ATPase activity in tissues (liver and kidney) were not shown any significant changes in VACExt treated of normal rats (Table 1).

Fig. 1: ATPase levels in the tissues of Liver and Kidney and rats treated with the VACExt (200 mg/kg bw) and STZ.

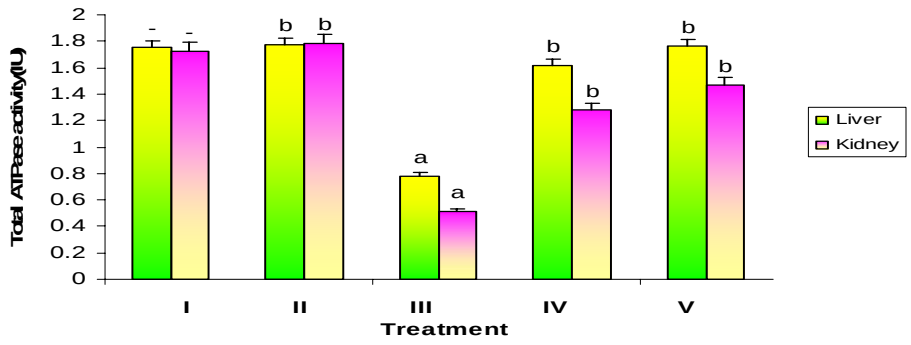


Table 1: Changes on the tissue total Na^+- K^+ ATPase and Mg^{2+} -ATPase levels in control and STZ-induced diabetic rats.

Groups	Na^+- K^+-ATPase (µmole of Pi liberated per hour/mg protein)		Mg^{2+} -ATPase (µmole of Pi liberated per hour/mg protein)	
	Liver	Liver	Kidney	Kidney
Group-I Control	0.67±0.06	0.58±0.06	0.35±0.03	0.56±0.05
Group-II VACExt 200 mg/kg	0.68±0.06 [b]	0.56±0.06[b]	0.33±0.03[b]	0.57±0.05 [b]
Group-III Diabetic control	0.30±0.03[a]	0.19±0.01[a]	0.12±0.02[a]	0.21±0.02[a]
Group-IV Diabetic + VACExt 200 mg/kg	0.47±0.03[b]	0.49±0.03[ab]	0.34±0.03[b]	0.46±0.04[b]
Group-V Diabetic + Glibenclamide 0.6mg/kg	0.50±0.05[b]	0.41±0.03[b]	0.32±0.02[b]	0.48±0.03[b]

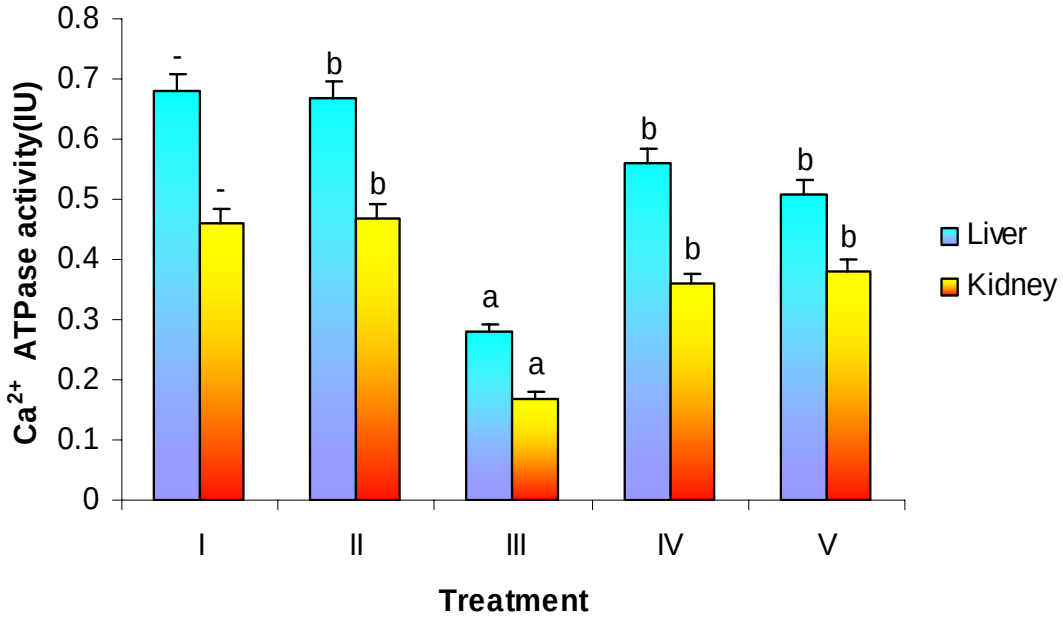
Each value is mean ± S.E.M for 6 rats in each group

a: $p<0.05$ by comparison with normal rats

b: $p< 0.05$ by comparison with streptozotocin induced diabetic rats

-: Not significant

Fig. 2: Effect of VACExt (200 mg/kg bw) on tissue total Ca^{2+} ATPase levels in control and STZ-induced diabetic rats.



In STZ induced diabetic rats, the serum cholesterol level was 203.9mg/dl. The cholesterol level was (172.43%) increased in diabetic rats when compared to normal rats. Oral administration of 200 mg/kg VACExt for 45 days decreased the cholesterol levels in diabetic rats. There was a significant decrease in serum cholesterol ($P<0.05$) in Glibenclamide (0.6mg/kg) treated rats, when compared to the vehicle-treated control rats (Table 2).

A marked increase in the frequency of triglycerides (155.23%) and free fatty acids (153.35%) were observed in diabetic control rats. Treatment with VACExt (200mg/kg) significantly reduced the lipid levels. The oral administration of Glibenclamide (0.6mg/kg) to STZ induced diabetic rats caused a significant decrease in the serum TG and free fatty acid ($P<0.01$) when compared to the control rats (Table 2).

The diabetic rats had elevated levels of LDL and VLDL and decreased level of HDL when compared with normal control rats. Oral administration with VACExt (200mg/kg) and Glibenclamide (0.6mg/kg) for 45 days significantly increased the HDL levels and decreased the LDL and VLDL levels towards near normal, respectively (Table 3).

Table 2: Changes on the concentration of serum cholesterol, triglyceride and free fatty acid in Control and STZ induced diabetic rats

Groups	Total Cholesterol (mg/dl)	Triglyceride (mg/dl)	Free fatty acids (mg/dl)
Group-I Control	118.25±6.5	52.34±1.31	62.86±2.47
Group-II VACExt 200 mg/kg	117.18±4.16[b]	52.79±1.16[b]	62.16±4.06[b]
Group-III Diabetic control	203.90±3.81[a]	81.25±6.28[a]	96.40±2.02[a]
Group-IV Diabetic + VACExt 200 mg/kg	124.47±5.91[b]	57.00±2.74[ab]	68.75±4.79[ab]
Group-V Diabetic + glibenclamide 0.6mg/kg	129.53±2.56[ab]	59.76±2.34[ab]	71.00±5.66[ab]

Table 3: Effect of VACExt treatment on serum HDL, LDL and VLDL levels in Control and STZ induced diabetic rats.

Groups	HDL-Cholesterol (mg/dl)	LDL-Cholesterol (mg/dl)	VLDL-Cholesterol (mg/dl)
Group-I Control	39.44±1.92	19.92±2.53	15.35±1.79
Group-II VACExt 200 mg/kg	40.93±3.32[b]	20.31±2.61[b]	16.61±2.29[-]
Group-III Diabetic control	22.09±1.73[a]	70.41±4.05[a]	38.82±2.27[a]
Group-IV Diabetic + VACExt 200 mg/kg	35.75±3.72[b]	28.92±3.59[b]	19.86±1.08[b]
Group-V Diabetic + glibenclamide 0.6mg/kg	33.69±1.38[b]	32.3±2.03[b]	22.18±2.54[b]

DISCUSSION

Total ATPases consists of Na^+ /K^+-ATPase, low affinity Ca^{2+}-ATPase and Mg^{2+}-ATPase. Insulin and catecholamines are the principal mediators of acute hormonal control of Na^+/K^+-ATPase[20]. In our study, diabetic rats exhibited decreased level of Na^+ /K^+-ATPase in the tissues, which coincide with the previous report of Kjeldsen *et al.* [21]. This might be associated with the deficiency of insulin, as insulin administration partially restored Na^+ /K^+-ATPase[22]. The oxidative damage of tissue lipids and proteins might have caused Na^+ /K^+-ATPase inactivation. Na^+ /K^+-ATPase is rich in thiol groups and oxidation of thiol groups has been reported to inhibit enzyme activity[23]. Na^+/K^+ -ATPase plays a central role in the regulation of intra and extracellular cation homeostasis. Alteration of this transport system was thought to be linked to several complications of diabetes [24]. Hyperglycemia can cause glycosylation of proteins and cellular lipid peroxidation, which, in turn, can cause inhibition/reduction in the activities of Na^+/K^+ and Ca^{2+} -ATPases. This result can, in turn, affect the intracellular concentrations of Na^+/K^+ and Ca^{2+} -ATPases, alter the signal transduction pathways, and affect contractility and excitability and cellular dysfunctions[25]. Increased glycoprotein components are related with increased glycation of membrane proteins and diabetic hyperlipidemia[26-28], which may also be responsible for the inhibition of the activities of ATPases. Glycation of ATPases is also possible during hyperglycemia.

Insulin directly regulates the membrane bound (Ca^{2+} /Mg^{2+}-) ATPase[29]. Low-affinity Ca^{2+} -ATPase is considered to be responsible for the shape and deformability of the

erythrocyte membranes[30]. In the present study, diabetic rats showed decreased activity of low affinity Ca^{2+} -ATPase and Mg^{2+}-ATPase. This could be due to insulin deficiency as insulin is the regulator of the enzyme. Diabetic rats had decreased activity of low affinity Ca^{2+} -ATPase as a consequence of interaction of glucose with these enzymes[30]. Increased lipid peroxidation can, in turn, diminish the activities of low affinity Ca^{2+}-ATPase and Mg^{2+}-ATPase in erythrocyte membrane when exposed to a higher glucose concentration- containing medium[25]. Oral administration of *Vitex agnus-castus* in STZ-induced diabetic rats significantly increased the activities of Na+/K+ ATPase, Mg^{2+}-ATPase and Ca^{2+}-ATPase in the liver and kidney which might be due to the protective effect of *Vitex agnus-castus* on the functional activity of membrane bound enzymes.

Excess of fatty acids in serum produced by the streptozotocin-induced diabetes promotes conversion of excess fatty acids into phospholipids and cholesterol in liver. These two substances along with excess triglycerides formed at the same time in liver may be discharged into blood in the form of lipoproteins[31]. The abnormal high concentration of serum lipids in the diabetic subject is due, mainly to increase in the mobilization of free fatty acids from the peripheral fat depots, since insulin inhibits the hormone sensitive lipase. Hypercholesterolemia and hypertriglyceridemia have been reported to occur in streptozotocin diabetic rats[32] and significant increase was observed in the present experiment also and it accordance with the above studies. The marked hyperlipidaemia that characterize the diabetic state may therefore be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat depots[33].

It is well known that in uncontrolled type 2 diabetes mellitus, shown an increase in the levels of TC, LDL and VLDL-cholesterol and triglyceride HDL level was declined by contributing to secondary complications[34]. High levels of total cholesterol and more importantly LDL-cholesterol in blood are major coronary risk factors. Insulin deficiency or insulin resistance may be responsible for dyslipidemia, because insulin has an inhibitory action on HMG-coA reductase, a key rate-limiting enzyme responsible for the metabolism of cholesterol rich LDL particles. Acute insulin deficiency initially causes an increase in free fatty acid mobilization from adipose tissue, this resulted an increase in the production of cholesterol rich LDL particle[35]. Oral administration of VACExt normalized these effects, possibly by controlling the hydrolysis of certain lipoproteins and their selective uptake and metabolism by different tissues.

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