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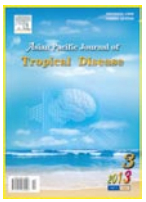

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Effect of *Evolvulus alsinoides* on lipid metabolism of streptozotocin induced diabetic rats

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PEER REVIEW

Peer reviewer

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Comments

This is a good study in which the authors evaluated the hypolipidimic activity in diabetes induced rats. The results are interesting and suggested that the ethanolic extract of *E. alsinoides* help to maintain the lipid levels in diabetic condition.
(Details on Page 187)

ABSTRACT

Objective: To determine the effect of ethanolic extract of *Evolvulus alsinoides* (*E. alsinoides*) on diabetes-induced changes in lipid metabolism. **Methods:** The ethanolic extract of *E. alsinoides* on serum and tissue lipid levels were examined in control and experimental group rats. **Results:** Oral administration of *E. alsinoides* extract to streptozotocin induced diabetic rats for 45 d significantly reduced the levels of triglycerids, phospholipids, cholesterol and free fatty acids in serum and tissues, it increases the high density lipoprotein in serum as that of control. **Conclusions:** The ethanolic extract of *E. alsinoides* supplementation is useful in hyperlipidemia prevention during diabetes mellitus.

KEYWORDS

Evolvulus alsinoides, Lipid metabolism, Streptozotocin, Convolvulaceae, Diabetes mellitus

1. Introduction

Diabetes mellitus is characterized by hyperglycaemia together with biochemical alterations of glucose and lipid metabolism. Liver is an insulin dependent tissue, which plays a essential role in glucose and lipid homeostasis and is severely affected during diabetes. Liver participates in the uptake, oxidation and metabolic conversion of free fatty acids, synthesis of cholesterol, phospholipids and triglycerides. During diabetes, profound alterations in the concentration and composition of lipids occur[1].

Evolvulus alsinoides L. (*E. alsinoides*) is an important medicinal plant (family: Convolvulaceae) employed for different ailments in India traditionally and grows in the open and grassy places almost throughout India and other subtropical countries. The entire plant is considered

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astringent and useful for treating hemorrhages, there are a variety of other medical applications, including as an adaptogenic, antiphlogistic, antipyretic, antiseptic, aphrodisiac, febrifuge, stomachic, tonic, vermifuge, against asthma, bronchitis, scrofula, syphilis, or in "controlling night emissions" and to promote wound healing[2].

The present study is aimed to find out the anti-lipidimic activity of *E. alsinoides* in serum and tissues of streptozotocin induced diabetic rats.

2. Materials and methods

2.1. Plant material

The whole plant of *E. alsinoides* used for this investigation was obtained from Coimbatore District, Tamilnadu, India. The plant was authenticated by Dr. P. Satyanarayana, Botanical Survey of India, TNAU Campus, Coimbatore (voucher number: BSI/SRC/5/23/2011-12/Tech.-514). Fresh plant material was washed under running tap water, air dried and powdered.

2.2. Sample extraction

A total of 100 g dried plant owder was extracted in 500 mL of ethanol in orbitory shaker for 72 h. Repeated extraction was done with the same solvent till clear colorless solvent was obtained. Obtained extract was evaporated and stored at 0-4 °C in air tight container.

2.3. Animals

Wistar albino rats weighing about 150-180 g were procured from Animal House, Karpagam University, Coimbatore, India. The animals were fed with rodent diet and water under standard conditions. The study was approved by Institutional Animal Ethical Committee.

2.4. Induction of experimental diabetes

Rats were rendered diabetic by a single intraperitoneal injection of freshly prepared streptozotocin (45 mg/kg body weight) in 0.1 mol/L citrate buffer (pH 4.5) in a volume of 1 mL/kg body weight[3]. Diabetes was identified in rats by moderate polydypsia and marked polyuria. After 48 h of streptozotocin administration, blood glucose levels were estimated and rats with a blood glucose ranging between 200-400 mg/dL were considered diabetic and used for the futher experiments.

2.5. Experimental protocol

The animals were divided into five groups of six animals each. Group I served as a control; group II consisted of streptozotocin-induced diabetic rats; group III consisted of streptozotocin-induced diabetic rats treated with glibenclamide (1.25 mg/kg body weight per day); groups IV consisted of streptozotocin-induced diabetic rats treated ethanolic extract of *E. alsinoides* (150 mg/kg body weight per day) and group V were normal rats treated with ethanolic extract of *E. alsinoides* (150 mg/kg body weight per day).

2.6. Biochemical studies

After 45 d of treatment, the animals were sacrificed under chloroform anesthesia. The blood was collected; serum was separated for biochemical estimations. Liver and kidney were quickly excised off, a portion of tissues washed with saline and homogenates was prepared, using 0.1 mol/L phosphate buffer, pH 7.4. The tissue homogenates were centrifuged and the supernatants were used for determing cholesterol, free fatty acid, triglyceride and phospholipid[4-7]. The serum samples were used for the determination of lipid profiles.

2.7. Statistical analysis

The values were expressed as mean±SD (n=6). The statistical analysis was carried out by one-way ANOVA

Table 1
Effect of ethanolic extract of *E. alsinoides* on lipid profiles of serum.

Particulars (mg/dL)	Control	Diabetic control	Diabetic+Glibenclamide	Diabetic+*E. alsinoides*	*E. alsinoides* alone
Phospholipids	163.28±4.72^{d}	223.09±8.33^{a}	169.93±3.38^{c}	179.74±7.90^{b}	162.65±7.82^{d}
Free fatty acid	5.23±0.13^{a}	8.86±0.18^{d}	7.83±0.23^{b}	8.50±0.29^{c}	5.29±0.19^{a}
Triglycerides	78.48±5.56^{a}	193.33±6.35^{b}	80.30±2.60^{a}	82.22±1.73^{a}	77.47±3.02^{a}
HDL	45.54±0.75^{c}	31.10±0.92^{a}	44.14±0.41^{b}	43.65±0.81^{b}	45.67±1.16^{c}
LDL	100.94±3.72^{a}	186.25±8.60^{d}	114.93±6.45^{bc}	119.99±4.29^{c}	112.42±4.32^{b}
VLDL	32.00±3.41^{a}	55.00±8.84^{d}	34.00±6.65^{bc}	38.00±4.46^{c}	35.00±4.46^{b}
Cholesterol	128.00±3.41^{a}	232.66±8.83^{c}	143.33±7.78^{b}	147.33±4.45^{b}	139.33±6.11^{b}

Values are expressed as mean±SD for six animals. Values not sharing common superscript letters (a-f) differ significantly at P<0.05 (DMRT).

using SPSS software (version 10). Statistical significance was considered at $P<0.05$.

3. Results

Table 1 shows the levels of phospholipids, free fatty acids, triglycerides, HDL, LDL, VLDL and cholesterol in serum of control and experimental groups. A significant elevation in serum lipids except HDL was observed in diabetic rats when compared with control rats. On oral administration of *E. alsinoides* extract to diabetic rats for 45 d, these values significantly restored near to normal. The comparison was done with standard drug glibenclamide treated group. The plant extract alone treated group did not show any significant change when compared to control rats.

In the present study, the phospholipids, triglycerides, free fatty acids and cholesterol levels were also estimated in tissues (liver and kidney), the results were represented in Tables 2 and 3. All the contents were increased in streptozotocin induced rats when compared to that of control. After treatment with plant extract and standard drug for 45 d, decreased levels of phospholipids, free fatty acid, cholesterol and triglycerides were showed. There was no significant difference found between control group and plant alone group.

4. Discussion

Diabetes mellitus is a group of metabolic diseases characterized by abnormal metabolism of carbohydrate, proteins, fats resulting from defects in inadequate pancreatic insulin secretion with or without concurrent impairment of insulin action[8]. The disease is progressive and is associated with high risk of atherosclerosis, kidney and nerve damage as well as blindness. Abnormalities in the regulation of peroxide and transition metal metabolism are postulated to result in the development of the disease as well as its long-term complications[9].

A variety of derangements in metabolic and regulatory mechanisms, due to insulin deficiency, are responsible for the observed accumulation of lipids. It is well known that in uncontrolled diabetes mellitus, there will be a increase in total cholesterol, triglycerides and LDL cholesterol associated with decrease in HDL cholesterol which is often linked with hyperlipidaemia[10]. Hyperlipidemia certainly contributes to major risk factor for cardio vascular diseases[11]. During diabetic state, insulin deficiency contributes to derangements of various metabolic and regulatory mechanisms in body. At normal state insulin activates the lipolytic hormones action on the peripheral fat depots which hydrolyses triglycerides and prevents mobilization of free fatty acids. However, insulin deficiency inactivates the lipoprotein lipase which promotes liver

Table 2
Effect of ethanolic extract of *E. alsinoides* on free fatty acid, triglycerides and cholesterol of liver.

Particulars (mg/dL)	Control	Diabetic control	Diabetic+Glibenclamide	Diabetic+*E. alsinoides*	*E. alsinoides* alone
Phospholipids	30.44±0.29^{a}	49.36±0.46^{d}	28.78±0.33^{c}	26.19±0.43^{b}	30.46±0.25^{a}
Free fatty acid	9.52±0.18^{a}	16.64±0.47^{d}	10.54±0.25^{b}	11.25±0.30^{c}	9.59±0.36^{a}
Triglycerides	4.03±0.03^{a}	6.88±0.02^{d}	6.60±0.03^{c}	6.39±0.03^{b}	4.01±0.05^{a}
Cholesterol	9.23±0.07^{a}	11.91±0.05^{d}	10.32±0.04^{b}	10.70±0.03^{c}	9.28±0.09^{a}

Values are expressed as mean±SD for six animals in each group. Values not sharing common superscript letters (a-d) differ significantly at $P<0.05$ (DMRT).

Table 3
Effect of ethanolic extract of *E. alsinoides* on free fatty acid, triglycerides and cholesterol of kidney.

Particulars (mg/dL)	Control	Diabetic control	Diabetic+Glibenclamide	Diabetic+*E. alsinoides*	*E. alsinoides* alone
Phospholipids	21.84±0.29^{a}	34.87±0.41^{d}	20.46±0.34^{c}	18.18±0.19^{b}	21.83±0.29^{a}
Free fatty acid	8.74±0.11^{a}	13.30±0.17^{d}	10.75±0.43^{b}	11.14±0.17^{c}	8.74±0.31^{a}
Triglycerides	3.14±0.03^{a}	4.44±0.05^{d}	3.49±0.02^{c}	3.31±0.03^{b}	3.40±0.06^{c}
Cholesterol	8.02±0.13^{a}	10.40±0.49^{d}	9.05±0.05^{b}	9.59±0.05^{c}	7.78±0.07^{a}

Values are expressed as mean±SD for six animals in each group. Values not sharing common superscript letters (a-d) differ significantly at $P<0.05$ (DMRT).

conversion of free fatty acids into phospholipids and cholesterol and finally discharged into blood which resulted into elevated serum phospholipid level[12,13].

Our result showed significantly (P<0.05) fall in TC, TG, and LDL levels, as well as at the same time raised HDL level near to control on oral administration of *E. alsinoides* after 45 d repeatedly. This implies that plant may possess insulin-like activity which would be helpful to reduce the incidence of lipid born complications. The significant control on serum lipids may prevent from simultaneous coexistence of hypercholesterolemia and hypertriglyceridemia and also reduce the cardiovascular risk factors[14]. These findings are in agreement with previous studies carried out by Chakrabarti *et al*., who used aqueous and methanolic seed extract dose (250 mg/kg) of *Caesalpinia bonducellaon* in alloxan induced diabetic rat models[15]. Kaleem *et al*. also reported that the *A. squamosa* aqueous extract supplementation is useful in controlling the blood glucose level[16], improves the plasma insulin and lipid metabolism of streptozotocin induced diabetic rats.

During diabetes, enhanced activity of the enzyme, increased lipolysis and releases more fatty acids into the circulation. The increased fatty acid concentration also increases the β-oxidation of fatty acids, producing more acetyl Co-A and cholesterol during diabetes. In normal condition, insulin increases receptor-mediator removal of LDL-cholesterol and decreased activity of insulin, during diabetes causes hypercholesterolemia. Hypercholesterolemia and hypertriglycedemia have been reported to occur in diabetic rats[17]. The increased concentration of free fatty acid may be due to lipid break-down and this may cause increased generation of NADPH-dependent microsomal lipid peroxidation.

Phospholipids are present in cell membrane and make up vast majority of the surface lipoprotein forming a lipid bilayer that acts as an interface with both polar plasma environment and non-polar lipoprotein of lipoprotein core[18]. Phospholipids levels increased in tissues of streptozotocin diabetic rats[18-21]. Administration of whole plant ethanol extract of *E. alsinoides* and glibenclamide decreased the levels of phospholipids in both liver and kidney. Based on our findings, administration of *E. alsinoides* helps to reduce the cholesterol, triglycerides, phospholipids, LDL, VLDL and also used to increase the HDL levels, this results was supported by other studies[22,23], showing that the administration of ethanolic and chloroform extracts of *E. alsinoides* inhibited the total cholesterol, triglycerides, low density lipoproteins level and significantly increased high density lipoprotein levels in experimentally induced hyperlipidemia rats.

From the present investigation, it can be concluded that ethanolic extract of *E. alsinoides* supplementation is quite beneficial in controlling the lipid levels during diabetes mellitus and also reduce the cardiovascular risk factors associated with diabetes.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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Comments

Background

Diabetes is the most common of the endocrine disorders and causes a serious challenge to healthcare worldwide. The prevalence of the different types of diabetes varies in different parts of the world. Recently, it has been reported that phytotherapy is considered to be less toxic and minimal or no side effects in comparison to modern allopathic medicines.

Research frontiers

Now a day diabetes mellitus has become more prevalent and considered to be a world-wide epidemic disorder. It causes rearrangement of lipid and protein metabolism. So this study is being performed in order to determine the antilipidimic activity of the whole plant ethanolic extract of *E. alsinoides* using streptozotocin induced rat model. The results showed a significant reduction in the lipid profiles thereby it prevents the atherosclerosis which is the main disorder may be caused by the over production of glucose in the body.

Related reports

There is no reports available for *in vivo* antidiabetic studies for this plant and this study was supported by Iyer and Patil, 2011 who studied the effect of *E. alsinoides* L. ethanolic extract and its fraction in experimentally induced hyperlipidemia in rats. They reported that this plant ethanolic extract and chloroform fractions caused inhibitory effects on total cholesterol and triglyceride level after olive oil administration and also increased the HDL level in rats.

Innovations & breakthroughs

Data represented in this study has showed good reduction in the cholesterol, phospholipids, triglycerides, low density

lipoprotein levels and also increased the high density lipoprotein levels as that of standard drug in streptozotocin induced rats.

Applications

The results of the present study suggest that this plant may act as a good hypoglycemic agent significant. Thus, it can be used in the prevention of atherosclerosis during diabetes mellitus.

Peer review

This is a good study in which the authors evaluated the hypolipidimic activity in diabetes induced rats. The results are interesting and suggested that the ethanolic extract of *E. alsinoides* helps to maintain the lipid levels in diabetic condition.

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