



PHYTOCHEMICAL SCREENING & EVALUATION OF ANTIDIABETIC & ANTIHYPERLIPIDEMIC ACTIVITY OF ERYTHROXYLUM MONOGYNUM IN ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT

The objective of the present study was to evaluate the antidiabetic & antihyperlipidemic activity of *Erythroxylum monogynum* in alloxan induced diabetic rats. The preliminary phytochemical screening shows the presence of alkaloids, glycosides, carbohydrates, flavonoids, tannins, saponins, sterols & phenols. The antidiabetic & antihyperlipidemic effect of *Erythroxylum monogynum* was studied in alloxan (150mg/kg b.w., i.p.) induced diabetes in wistar rats for doses 100mg/kg b.w, 200 mg/kg b.w. and 400 mg/kg b.w. (p.o.) daily for 21 days, and the effect was compared with oral dose of 5mg/kg, b.w. glibenclamide. The effect of ethanolic bark extract of *Erythroxylum monogynum* on blood glucose, serum lipid profile [total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C) was measured in the diabetic rats. Diabetes mellitus which was induced by alloxan increase the blood glucose level and other biochemical parameter such as LDLc, VLDLc, TC, total triglycerides and cholesterol ratio and decrease the level of HDLc where as the stem bark ethonolic extract erythroxylum monogynum decreased the level of blood glucose level LDLc, VLDLc, TC, total triglycerides and cholesterol ratio and increased the level of HDLc since HDLc is a good cholesterol indicating erythroxylum monogynum to posses antidiabetic and antihyperlipidemic activity. Ethanolic extract of erythroxylum nonogynum produced it's effect at all the three doses i.e. 100mg/kg b.w, 200 mg/kg b.w. and 400 mg/kg b.w. (p.o.) daily for 21 days, and the effect was compared with oral dose of 5mg/kg, b.w. glibenclamide, it shown more potent effect at a dose of mg/kg b.w. by reducing blood glucose level by 56%, LDL by 53.82%, VLDL by 37.06%, TC by 31.69%. total triglycerides by 34.85%, cholesterol ratio by 57.81%, when compare to standard drug 58% BGL, LDL by 58.47%, VLDL By 39.64%, TC by 35.34%, TRG by 35.38% and cholesterol ratio by 60.34% respectively.

KEYWORDS: *Erythroxylum monogynum, Diabetes mellitus, Hyperlipidemia, maceration, Alloxan monohydrate, 5% Glucose, Ethanol, Glibenclamide*



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INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia, abnormal lipid and protein metabolism along with specific long-term complications affecting the retina, kidney and nervous system¹. Hyperglycemia is an important factor in the development and progression of the complications of diabetes mellitus². The pathogenesis of diabetes mellitus is managed by insulin and oral administration of hypoglycemic drugs such as sulfonylureas and biguanides³. Unfortunately, apart from having a number of side effects, none of the oral synthetic hypoglycemic agents has been successful in maintaining euglycaemia and controlling long-term microvascular and macrovascular complications⁴⁻⁵. The toxicity of oral antidiabetic agents differs widely in clinical manifestations, severity, and treatment⁶. The use of herbal medicines for the treatment of diabetes mellitus has gained importance throughout the world. The World Health Organization also recommended and encouraged this practice especially in countries where access to the conventional treatment of diabetes is not adequate⁷. There is an increased demand to use natural products with antidiabetic activity due to the side effects associated with the use of insulin and oral hypoglycemic agents⁸⁻⁹. The available literature showed that there are more than 400 plant species having hypoglycemic activity¹⁰⁻¹². Though some of these plants have great reputation in the indigenous system of medicine for their antidiabetic activities, many remain to be scientifically established. Hypercholesterolemia and hypertriglyceridemia are common complications of diabetes mellitus in addition to hyperglycemia¹³⁻¹⁵. The frequency of hyperlipidemia in diabetes is indeed very high, depending on the type of diabetes and its degree of control¹⁶. Despite the introduction of hypoglycemic agents from natural and synthetic sources, diabetes and its secondary complications continue to be a major medical problem. Many indigenous Indian medicinal plants have been found to be useful to successfully manage diabetes. One of the great advantages of medicinal plants is that these are readily available and have no side effects¹⁷. World Health Organization (WHO) has suggested the evaluation of the potential of plants as effective therapeutic agents, especially in area which we lack safe modern drugs¹⁸. Hyperlipidemia is a disorder of lipid metabolism manifested by elevation of plasma concentrations of the various lipid and lipoprotein fractions, which is the key risk factor for cardiovascular disorders (CVD)¹⁹ & has been reported as the most common cause of death in developed as well as developing nation²⁰⁻²¹. The current antihyperlipidemic therapy includes principally statins and fibrates; the former correct the altered blood lipid profile by inhibiting the biosynthesis of cholesterol and the latter acts by enhancing the clearance of triglyceride rich lipoproteins²². Since synthetic drugs have been shown to have side effects, clinical importance of the herbal drugs in treatment of hyperlipidemia has received considerable attention in recent years²³. Various medicinal products of herbal origin have been reported to have hypolipidemic and hypocholesteremic properties²⁴⁻²⁵. WHO has in fact recommended the use of indigenous plants as an alternative remedy especially

in developing countries²⁶ *Erythroxylum monogynum* belonging to family Erythroxylaceae is a medicinal herb commonly known as Devadari & Red cedar. It is abundant in foothills scrub jungles up to 1000m in Peninsular India and Sri Lanka, Myanmar, Dry deciduous forests. In India it is seen in the states of Kerala, District/s: Kollam, Idukki, Pathanamthitta, Thiruvananthapuram, Palakkad, Karnataka: Chikmagalur, Coorg, Hassan, and Mysore. Found in foot hills, scrub jungles upto 1000 mts and deciduous forests. Leaves raw or cooked are eaten to a considerable extent in times of famine. They contain a bitter tonic principle which might serve to relieve the pangs of hunger. Biological activity of the plant is hypothermic & CNS active. Leaf is diaphoretic, stimulant, diuretic & stomachic. A decoction is used for malarial fever. Leaf juice given orally as a cooling beverage & also administered for treating jaundice. Leaves crushed with black pepper & the extract is given orally to kill the intestinal worms. Stem bark decoction is used to treat hiccups & given orally in morning time on empty stomach for 4 weeks to treat itches. Bark and wood are used as febrifuge. Infusion of the wood & bark is considered as stomachic, diaphoretic & diuretic. Oil from the seed is used to cure psoriasis, skin diseases. General stimulant other effects are related to the potentiation of responses of sympathetically innervated organs to catecholamines, and to sympathetic nerve stimulation causing tachycardia and mydriasis. It is used as an ophthalmic anaesthetic. Fruits are consumed to cure indigestion. Paste of roots in warm water is used to cure cough and skin diseases. A bark is made into a paste and applied in scabies and other skin diseases for quick healing. The treatment is to be continued twice daily (morning and evening) for 7 days, after which bath can be taken.²⁷ Methanolic extract of leaf showed hepatoprotective activity against paracetamol induced hepatotoxicity. Methanolic extracts of aerial parts of *E. monogynum* showed cytotoxic against brine shrimps & antitumour activity against the tumors induced by *Agrobacterium tumefaciens* using carrot disc anti-tumour bioassay²⁸. *E. monogynum* also possesses antioxidant activity²⁹. Phytochemical analysis & screening of antibacterial activity of the methanol and acetone leaf extracts of *C. guianensis* and *E. monogynum* was carried out against *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas putida* and *Staphylococcus aureus* & it was found to possess antibacterial activity³⁰. The objective of this research was to investigate the antidiabetic & antihyperlipidemic activity of ethanolic bark extract of *Erythroxylum monogynum* by estimation of blood glucose levels and plasma lipid profile such as total cholesterol, triglyceride, high density lipoprotein, very low density lipoprotein and low density lipoprotein in alloxan induced diabetic rats since the drug contains steroids and flavanoides.

MATERIALS & METHODS

Plant material

The bark of *Erythroxylum monogynum* (Roxb) family Erythroxylaceae were obtained from Chittoor district during the months of February 2014. The species was identified & authenticated by Dr. Madhava Chetty, Assistant professor of Botany, Department of

Pharmacognosy, Sri Venkateshwara University, Tirupathi. With voucher number 1968

Preparation of extract³¹

The bark of *Erythroxylum monogynum* (E.M) was cleaned under tap water to remove dirt and dried under shade for about 10 days. After drying the bark of *Erythroxylum monogynum* was grounded using mixer grinder. Further the grounded powder of the bark was extracted using maceration technique using ethanol as solvent. In maceration (for fluid extract), whole or coarsely powdered plant-drug is kept in contact with the solvent in a stoppered container for a defined period until soluble matter is dissolved. This method is best suitable for use in case of the thermos labile drugs. Using this process the leaf powder was added in ethanol in the ratio 1:2 and vigorous shaking was carried out for 7 days continuously and was kept at room temperature. The mixture is then strained. The marc (damp solid material) is pressed & the combined liquids are clarified by filtration after standing. The extract was evaporated to dryness to get the residue. The residue thus obtained was ethanolic extract which was then dissolved in 0.9% normal saline and used in the experiment.

Phytochemical evaluation

Preliminary phytochemical screening was performed as per the standard methods³²

Chemicals

Alloxan monohydrate 150 mg/kg b.w, 5% glucose, Ethanol, Glibenclamide 5mg/kg. Alloxan was obtained from SISCO research laboratory pvt ltd, 5% glucose, Glibenclamide 5mg/kg has been obtained from imam medical and general stores and ethanol was obtained from scientific syndicate Nampally, hyd.

Animals

Healthy adult albino rats of wistar strain both male and female weighing 180-250gms each were used for the study. Animals were maintained under standard environmental conditions (22-28°C, 60-70% relative humidity, 12 hr L-D cycle) and fed with standard feed and water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethics Committee. All studies were performed in accordance with the guidelines for the care and use of laboratory animals, as adopted and promulgated by the Animal Care committee, CPCSEA, India. Animals were allowed to acclimatize to experimental conditions by housing them for 8-10 days period prior to experiments. CPCSEA registration no 1757/PO/RcBiBt/S/14/ CPCSEA.

Acute toxicity studies

Acute toxicity study was performed as per Organization for Economic Co-operation and Development OECD-425 guidelines. It was observed that there was no gross evidence of any abnormalities up to 4 hrs and no mortality was observed in animals up to the end of 48 hours at the maximum tested dose level of 2000mg/kg b.w. in rats. This was considered as Maximum Tolerated Dose (MTD) and thus, 1/10th of MTD i.e., 200mg/kg

b.w. was taken as test dose and double the test dose i.e., 400 mg/kg b.w. was also selected for the experimental studies.³³

Induction of diabetes in rats:³⁴⁻³⁵

Wistar rats (180-250gm) were made diabetic by a single i.p injection of Alloxan monohydrate at a dose of 150mg/kg using normal saline as vehicle which was freshly prepared immediately before injection. Alloxan also causes changes in serum lipid profile. The rats were maintained on 5 % glucose solution for next 24 hour to prevent hypoglycemia. The diabetic state was confirmed by the measurement of fasting blood glucose concentration, 3 days after the alloxan treatment using the blood samples drawn from tail vein. The rats with effective & permanent elevated blood glucose levels above 250mg/dl were considered to be diabetic & were used for the study.

Experimental design

The diabetic & hyperlipidemic rats were divided into 5 groups, each containing 6 animals.

Group I- Normal control rats received normal saline p.o.

Group II: Diabetic control rats received alloxan (150 mg/kg) b.w i.p+ normal saline p.o.

Group III: Alloxan induced diabetic rats + glibenclamide at a dose of 5mg/kg b.w in normal saline p.o.

Group IV- Alloxan induced diabetic rats + ethanolic bark extract of *Erythroxylum monogynum* (E.M) at a dose of 100mg/kg b.w in normal saline, p.o.

Group V- Alloxan induced diabetic rats + ethanolic bark extract of *Erythroxylum monogynum* (E.M) at a dose of 200mg/kg b.w in normal saline, p.o.

Group VI - -Alloxan induced diabetic rats + ethanolic bark extract of *Erythroxylum monogynum* (E.M) at a dose of 400mg/kg b.w in normal saline, p.o

The administrations of extract were continued for 21 days, once daily. Blood samples were collected through the tail vein on days 1, 7, 14 & 21 days after drug administration and the blood glucose levels were estimated using Accu-check glucometer.

Estimation of blood cholesterol³⁶⁻³⁸

Serum lipid profile estimation at the end of 21 days, blood was collected from inferior vena cava, serum separated for determination of parameters like total cholesterol, HDL- cholesterol and triglycerides. VLDL cholesterol and LDL-cholesterol were calculated using the Friedewald's formula. $VLDL = \text{Triglycerides}/5$ $LDL = \text{Total cholesterol} - (\text{HDL} + \text{VLDL})$

STATISTICAL ANALYSIS

Results were expressed as Mean \pm SEM. Statistical analysis were performed with using graphpad prism version 5 one way analysis of variance (ANOVA) followed by Dunnet's t -test. P values less than * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ was considered to be statistically significant, when compared with control and standard group as applicable.

RESULTS

Table 1
Phytochemical analysis of ethanolic leaf extract of *Erythroxylum monogynum*.

Type of constituents	<i>Erythroxylum monogynum</i> ethanolic leaf extract
Alkaloids	+
Glycosides	+
Carbohydrates	+
Flavonoids	+
Tannins	+
Saponins	+
Sterols	+
Phenols	+
Proteins	-
Triterpenoids	-
Anthraquinones	-

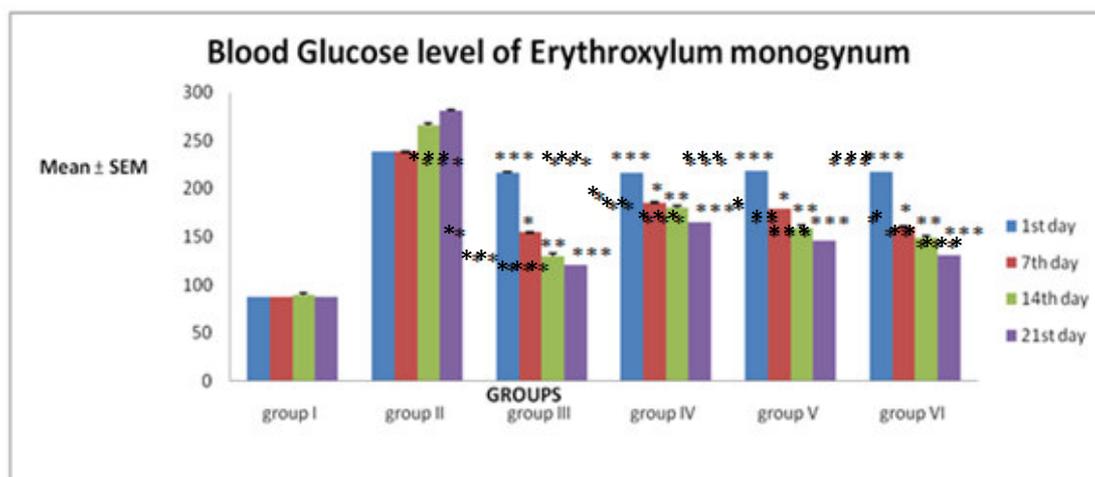
+ indicates presence & - indicates absence

Antidiabetic activity

The effect of *Erythroxylum monogynum* (EM) on serum glucose levels in diabetic rats depicted in (Graph 1). In animals treated with alloxan (150 mg/kg i.p) (Group II), a significant increase in serum glucose level was observed on 7th, 14th & 21st when compared with normal rats (Group I). Group III received glibenclamide (5 mg/kg

p.o.) showed decrease in serum glucose level when compared with diabetic control rats (Group II). After the oral administration of ethanolic leaf extract of *Erythroxylum monogynum* in diabetic control rats, a significant reduction in blood glucose level was observed on the 7th, 14th and 21st compared with diabetic control rats.

Effect of ethanolic leaf extract of *Erythroxylum monogynum* on blood glucose levels (mg/dl) in alloxan induced diabetic rats on 1st, 7th, 14th & 21st day of the treatment



Graph 1
Blood glucose levels (mg/dl) on 1st, 7th, 14th & 21st day of

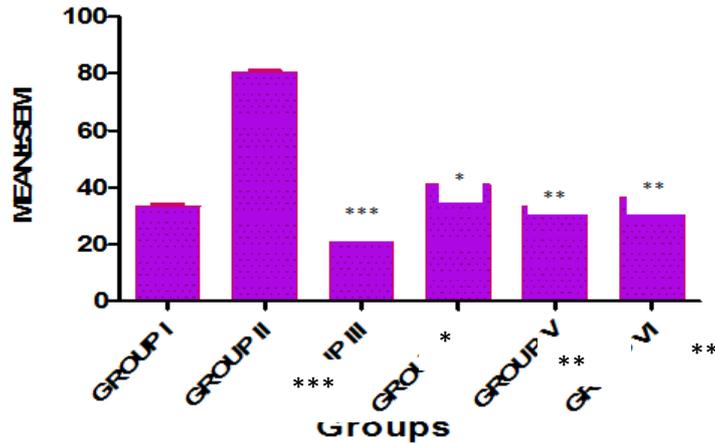
ANOVA followed by Dunnett's t-test *P<0.05, **P<0.01, ***P< 0.001 was considered significant comparing to diabetic control group. Here the administration of ethanolic extracts of plant stem bark for 21 days decreased the elevated blood glucose levels. The above result suggests that the combination of drug extract t 400mg/kg.b.w was more effective than 100 and 200mg/kg.b.w extracts and was almost comparable with the effect produced by standard drug glibenclamide treated group. The results are represented by means of graph 1 by using graph pad prism version 6.

Anti-hyperlipidemic activity

The lipid profiles in control and experimental rats are depicted in in alloxan induced diabetic rats. The diabetic control rats (Group II) showed significant increase in

serum triglycerides, total cholesterol, very low density lipoproteins (VLDL) and low density lipoproteins (LDL) while decrease in High density lipoproteins (HDL) when compared with normal (Group I). Standard glibenclamide (Group V) also reduced triglycerides, Total cholesterol, very low density lipoproteins (VLDL), low density lipoproteins (LDL) & increased High density lipoproteins (HDL) when compared with diabetic control rats (Group II). The ethanolic extract of *Erythroxylum monogynum* (EM) showed significant decrease in total cholesterol, LDL, VLDL, triglycerides and significant increase in HDL when compared with diabetic control group (Group II). All these effects were observed on day 21. The present experimental result indicated that ethanolic extract exhibited a potent serum lipid lowering properties in alloxan diabetic rats.

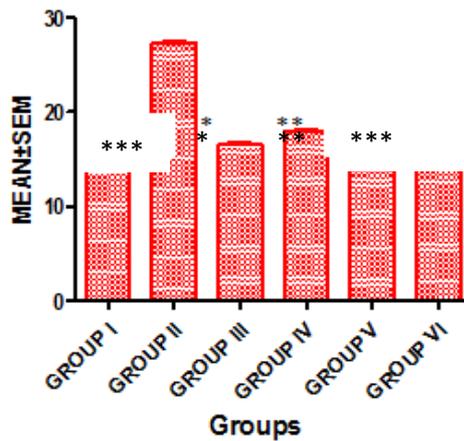
Effect of ethanolic leaf extract of *Erythroxylum monogynum* on serum lipid profile in alloxan (150mg/kg b.w) induced diabetic rats after 21 days of treatment



Values are expressed as Mean±SEM (n=6); *P<0.05, **P<0.01, ***P<0.001 was considered significant with respect to control group using ANOVA followed by Dunnett's t-test.

Graph 2

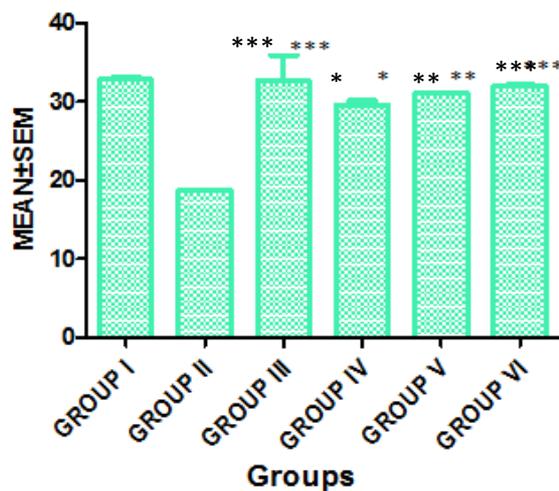
Effect of Erythroxylum monogynum on serum Very Low Density Lipoprotein in Cholesterol (VLDLc)



Values are expressed as Mean±SEM (n=6); *P<0.05, **P<0.01, ***P<0.001 was considered significant with respect to control group using ANOVA followed by Dunnett's t-test.

Graph 3

Effect of Erythroxylum monogynum on serum High Density Lipoprotein Cholesterol (HDLc)

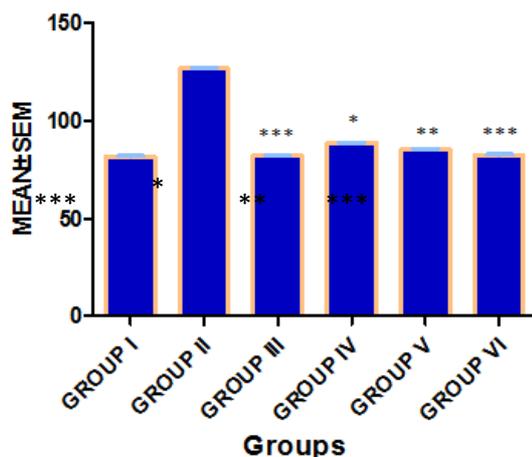


Values are expressed as Mean±SEM (n=6); *P<0.05, **P<0.01, ***P<0.001 was considered significant with respect to control group using ANOVA followed by Dunnett's t-test.

Graph 4

EFFECT OF ETHANOLIC LEAF EXTRACT OF *ERYTHROXYLUM MONOGYNUM* SERUM TOTAL CHOLESTEROL, TRIGLYCERIDES & CHOLESTEROL RATIO IN ALLOXAN (150mg/kg b.w) INDUCED DIABETIC RATS AFTER 21 DAYS OF TREATMENT

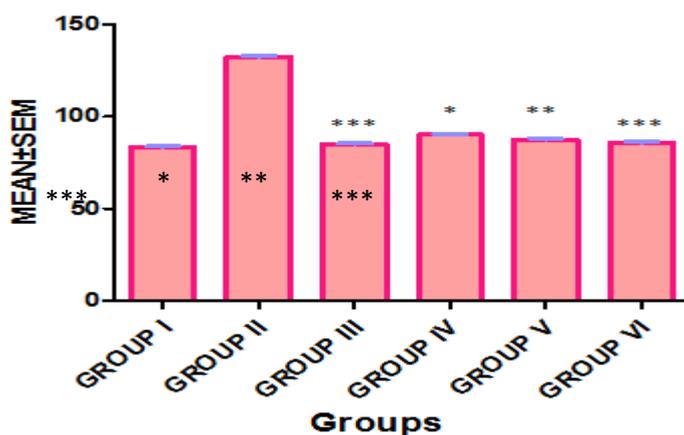
Effect of Erythroxyllum monogynum on serum Total Cholesterol (TC)



Values are expressed as Mean±SEM (n=6); *P<0.05, **P<0.01, ***P<0.001 was considered significant with respect to control group using ANOVA followed by Dunnett's t-test.

Graph 5

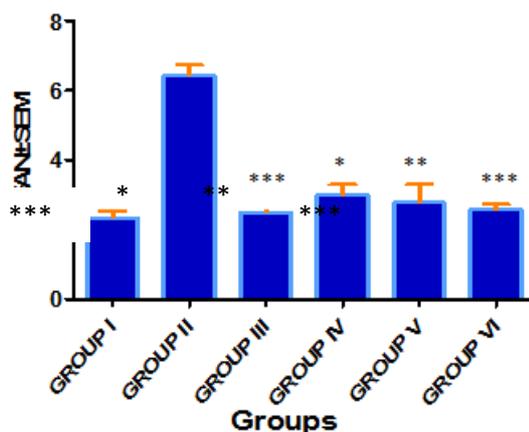
Effect of Erythroxyllum monogynum on Triglycerides (TG)



Values are expressed as Mean±SEM (n=6); *P<0.05, **P<0.01, ***P<0.001 was considered significant with respect to control group using ANOVA followed by Dunnett's t-test.

Graph 6

Effect of Erythroxyllum monogynum on Cholesterol ratio (TC/HDL)



Values are expressed as Mean±SEM (n=6); *P<0.05, **P<0.01, ***P<0.001 was considered significant with respect to control group using ANOVA followed by Dunnett's t-test.

Graph 7

DISCUSSION

In the present study the stem bark of *Erythroxyllum monogynum* (Erythroxyllaceae) which was selected for the study is medicinally significant. Leaves of *erythroxyllum monogynum* were used in the treatment of jaundice. The bark is used as stomachic, diaphoretic, stimulant, diuretic, and also antibacterial activity. In the present study I investigated the antidiabetic and hypolipidemic potential of the plant which includes of *erythroxyllum monogynum* belonging to family *erythroxyllaceae* at doses of 100,200 and 400mg/kg.b.w and also other complications of diabetes in alloxan induced diabetic animal model. Diabetes is associated with accelerated atherosclerosis and microvascular complications which are the major cause of morbidity and mortality in those suffering from the disease(nishat Fatima, *phyllanthus embelica* endothelial dysfunction)³⁹. The increased risk of coronary artery disease in subjects with diabetes mellitus can be due to lipoprotein abnormalities. Hyperlipidemia is commonly associated with diabetes hence it is important to screen and treat these lipid abnormalities. *E.monogynum* also possesses antioxidant activity. Phytochemical analysis & screening of antibacterial activity of the methanol and acetone leaf extracts of *C.guianensis* and *E.monogynum* was carried out against *Escherchia coli*, *Klebsiela*, *Pseudomonas putida* and *Staphylococcus aureus* & it was found to possess antibacterial activity. Antidiabetic and antihyperlipidemic activity of *chonomorpha fragrans* and *erythroxyllum monogylum* combined ethanolic leaf extract in alloxan induced diabetic wistar rats at doses 200 mg/kg and 400 mg/kg b.w. was stated by kauser Fatima et.al⁴⁰. The effect of ethanolic bark extract of *Erythroxyllum monogynum* on blood glucose, serum lipid profile [total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C) was measured in the diabetic rats. Diabetes mellitus which was induced by alloxan increase the blood glucose level and other biochemical parameter such as LDLc, VLDLc, TC, total triglycerides and cholesterol ratio and decrease the level of HDLc where as the stem bark ethanolic extract *erythroxyllum monogynum* decreased the level of blood glucose level LDLc, VLDLc, TC, total triglycerides and cholesterol ratio and increased the level of HDLc since

HDLc is a good cholesterol indicating *erythroxyllum monogynum* to posses antidiabetic and antihyperlipidemic activity. Ethanolic extract of *erythroxyllum nonogynum* produced it's effect at all the three doses i.e. 100mg/kg b.w, 200 mg/kg b.w. and 400 mg/kg b.w. (p.o.) daily for 21 days, and the effect was compared with oral dose of 5mg/kg, b.w. glibenclamide, it shown more potent effect at a dose of mg/kg b.w. by reducing blood glucose level by 56%, LDL by 53.82%, VLDL by 37.06%, TC by 31.69%. total triglycerides by 34.85%, cholesterol ratio by 57.81%, when compare to standard drug 58% BGL, LDL by 58.47%, VLDL By 39.64%, TC by 35.34%, TRG by 35.38% and cholesterol ratio by 60.34% respectively.

CONCLUSION

In conclusion, this study revealed that oral administration of the ethanolic extract of both the plants exhibit antidiabetic and antihyperlipidemic activities via enhances insulin production in the alloxan- induced diabetic rats. Thus, oral use of this extract might positively affect the functional capacities of various rat tissues, particularly kidney and liver against toxic action of alloxan compound (dose of 150mg/kg BW). Hence, the therapeutic potential of this plant material should also be seen in combination with other medicinal agents and further biochemical and pharmacological investigations are needed to isolate and identify active ingredients in the extract using other models. The plant materials should be further investigated for use in humans to treat diabetes mellitus and the various complications of diabetes like damage to liver and kidney.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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