CODEN (USA): IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

Available online at: http://www.iajps.com ***Research Article***

ANTIDIABETIC AND ANTIHYPERLIPIDEMIC ACTIVITY OF ETHANOLIC EXTRACT OF *ARTEMISIA NILAGIRICA* (clark) IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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Abstract:

Objective: *Diabetes mellitus is a serious health problem with continuously increasing rates of incidence and mortality. Diabetes mellitus is characterized by elevated plasma glucose concentrations resulting from insufficient insulin and insulin resistance, or both, leading to metabolic abnormalities in carbohydrates, lipids and proteins. The primary objective of this study was to determine the anti-diabetic activity of Artemisia nilagirica (clark) in streptozotocin induced diabetic rats.* ***Methods:*** *The anti diabetic effect of ethanol extract of leaf and flowering top of Artemisia nilagirica (clark) (200 and 400 mg/kg body weight) were administered orally in diabetic rats. After the oral administration of ethanol extract of Artemisia nilagirica (clark), blood glucose levels were monitored at specific intervals.* ***Results:*** *It was found that ethanolic leaf and flower extract were significant lowered blood glucose level. In the same study the action of the extract on diabetes induced hyperlipidemia was analyzed where the extract significantly lowered the elevated total cholesterol, triglycerides (TGL) and low density lipoprotein (LDL) level while increased the High density lipoprotein (HDL). Glibenclamide was used as a standard drug at a dose of 0.25 mg/kg.* ***Conclusion:*** *The experimental data revealed that the ethanolic extract has significant antidiabetic activity in Streptozotocin-induced rats compared to the standard drug.*

Keywords: *Artemisia nilagirica (clark), antidiabetic, glibenclamide, streptozotocin.*

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Please cite this article in press as Pradeep Pal et al , ***Antidiabetic and Antihyperlipidemic Activity of Ethanolic Extract of Artemisia Nilagirica (Clark) In Streptozotocin Induced Diabetic Rats****, Indo Am. J. Pharm. Sci, 2015;2(9).*

INTRODUCTION

Every year the number of diabetic patients is growing alarmingly all over the World. Diabetes is a chronic disease characterized by derangement in carbohydrate, fat, protein metabolism. Most of the hypoglycemic agents used in allopathic medicines are reported to have side effects in the long run. Therefore, there is a need to search for effective and safe drugs for diabetes [1]. 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 adequate. 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 agents [2, 3].

Artemisia Nilagirica (Clarke) (Hindi:Nagdona, Dauna, Tamil: Makkipu, Masipattiri, English:Indian Warmwood.) belongs to Asteraceae. It is the aromatic shrub found throughout the hilly districts of India. It grows at Mount Abu in Marwar and on the Western ghats and some parts of South India [4]. A tall aromatic perennial shrub, often gregarious, pubescent or villous throughout; lower leaves ovate in outline deeply pinnatisect with small stipule-like lobes at the base, pubescent above, white tomentose beneath, upper most smaller, 3-fid or entire, lanceolate; panicled racemer, outer flowers female, very slender, inner disk flowers fertile, bisexual, bracts ovate or oblong, margins scarious fruits oblong ellipsoid minute achene's. [5]. Plant contains sesquiterpene lactones, exiguaflavone A and B, macckianin and 2-(2, 4- dihydroxyphenyl) - 5, 6 methylenedioxybenzofuran [6]. The crude methanolic and ethanolic extracts of plant A. Nilagirica (Clarke) wile shows reasonably high potency against plasmodium falciparum. It is also said to be anthelmintic, antiseptic, expectorant, astringent, aromatic, antiinflammatory, appetizer, digestive and diuretic. It is also used in cough, asthma, leprosy, skin disease and as antiseptic [5]. Essential oils of Artemisia species have been widely used for a variety of medicinal purposes for many years.

This study was aimed at investigating the effects of ethanol extracts of the leaves and flowering top of *Artemisia nilagirica* (Clarke) on antidiabetic animal model**.**

MATERIAL AND METHODS

Plant Materials

The leaf and flowering tops of *Artemisia Nilagirica (Clarke)* was collected from forest area of Ooty district, Tamil Nadu, India in December 2011. The plant was identified by Dr. N. Selvaraj, professor and head Tamil Nadu Agriculture University Horticulture Research Station Ooty, Tamilnadu India. The plant specimens were deposited in the herbarium of Department of Pharmacognosy Mahakal Institute Of Pharmaceutical Studies,Ujjain, Madya Pradesh, Voucher no. MIPS/A/36/2011. The leaves and flowering tops were shade dried, reduced to coarse powder and stored in airtight container till further use.

Preparation of Plant Extract

The leaves and flowering tops part of dried powder material of *Artemisia Nilagirica* (Clarke) was extracted with 95% ethanol for 72 h, in a Soxhlet apparatus. The whole ethanol extract was collected in conical flask, filtered and the solvent was evaporated to dryness under reduced pressure. The w/w yield of the prepared extract was 12.25 %.

Preliminary Phytochemical Screening

The ethanol extract of *Artemisia Nilagirica* (Clarke) was subjected to various color reaction to identify the nature of the components by using standard phytochemical methods (7).

Experimental Animals

Male and female wistar albino rats having weight 180-230 gm are used. They were obtained from the College of veterinary science and animal husbandry, Mhow (M.P.) India. Animals were fed a standard pellet and water ad libitum and maintain ambient temperature at 24-28 ^{o}c, 60-70% relative humidity and 12 hr day and light cycle. The study was conducted after obtaining institutional animal ethical committee clearance**.**

Acute Oral Toxicity Study:

An acute oral toxicity study was performed as per OECD guidelines 420. By Acute toxic class method Swiss albino mice of female sex weighing 20-25gms were used for the study. Acute toxic class method is a stepwise procedure with use of three animals of a single sex per step. Depending on mortality or morbidity status of the animals. Average 2-4 steps may be necessary to allow judgment on the acute toxicity of the substance. Three animals were used for each step. The animal were placed individually and observed for any sign of toxicity, morbidity or mortality during the first 24hrs, with special given attention during the first 4 hours and daily thereafter for a total of 14 days [8, 9].

Induction of Diabetes

Diabetes mellitus was induced by single intraperitoneal injection of freshly prepared Streptozotocin (STZ 45 mg/kg body weight) in 0.1M citrate buffer (pH−4.5) in a volume of 1ml/kg body weight. The development of diabetes was confirmed after 48hrs of Streptozotocin injection. The animals having fasting blood glucose level more than 200mg/dl were considered as diabetic rats and used for the experimentation [10]

Experimental Design

Oral Glucose Tolerance Test (OGTT)

Fasted rats were divided into five groups of six rats in each. Group I served as normal control and received distilled water with Tween 80. Group II received standard drug Glibenclamide as an aqueous suspension at a dose of (0.25 mg/kg). Group III received ethanolic extract of *Artemisia Nilagirica* (200mg/kg) and Group IV recived ethanolic extract of Artemisia *Nilagirica* (400 mg/kg) as a fine Tween 80 suspension. After 30 min of extract administration, the rats of all groups were orally treated with 2 g/kg of glucose. Blood samples were collected from the rat tail vein just prior to glucose administration and at 0, 30 and 90 min after glucose loading. Blood glucose levels were measured immediately by using Glucometer [11].

Streptozotocin-induced Diabetic Model

The animals were segregated into five groups of six rats each. The ethanolic extract was administered for 28 days. Group I served as normal control rats administered drinking water daily for 28 days; Group II diabetic control rats administered drinking water with Tween 80 daily for 28 days; Group III diabetic rats administered standard drug glibenclamide (0.25 mg/kg); Group IV diabetic rats administered *Artemisia Nilagirica* (200 mg/kg); Group V diabetic rats administered *Artemisia Nilagirica* (400 mg/kg) for 28 days. The fasting glucose levels were determined on days 0, 7, 14 and 28 of extract administration. During the experimental period, the rats were weighed daily and the mean change in body weight was calculated [12].

Estimation of Biochemical Parameters:

The biochemical parameters were determined on day 28 after the animals were sacrificed by cervical dislocation. Total cholesterol, triglycerides (TGL), high-density lipoprotein (HDL) and low-density lipoprotein (LDL), were determined by the glucose oxidase method, using an auto-analyzer (13, 14).

Statistical Analysis: Results of estimation of biochemical and functional parameters have been reported as mean value ± SEM. The variation in a set of data has been estimated by performing one way analysis of variance (ANOVA). Individual comparisons of group mean values were done using Dunnet's test (Sigma stat 3.5). P values <0.05, were considered statistically significant.

RESULTS

Preliminary Phytochemical Screening

The phytochemical studies revealed the presence of flavonoids, steroids, terpenoids, saponins, tannins, proteins and essential oils.

Acute Oral Toxicity Study

The result of acute toxicity study of ethanol extracts of *Artemisia Nilagirica (Clarke)*, on laboratory animals showed that no lethality up to the dose of 2000 mg/kg body weight hence the animals were safe up to a maximum dose of 2000 mg/kg body weight.

Oral Glucose Tolerance Test (OGTT)

The effects of ethanolic extract of *Artemisia Nilagirica (Clarke)*, on the plasma glucose level are illustrated in table 1. Ethanolic extract showed significant reduction in plasma glucose level in rats at 30 min, and same was observed in standard drug. At 90 min, 200 mg/kg and 400 mg/kg body weight of both extracts treated rats produces significant reduction in plasma glucose level, while in glucose control rats, plasma glucose level was increased.

Effect of Ethanolic Extract on Streptozotocin-induced Diabetic Rats

The imitation of diabetes in the experimental rats was confirmed by the presence of a high fasting plasma glucose level. The effect of ethanol extract at different doses of *Artemisia Nilagirica (Clarke)*, on fasting plasma glucose level of normal and streptozotocin induced are depicted in table 2. The difference between the experimental and control rats in lowering the fasting plasma glucose levels were statistically significant in diabetic rats.

Effect of Ethanolic Extract on Biochemical Parameters in Streptozotocin-induced Diabetic Rats

The effect of different doses of the ethanolic extract on diabetes induced hyperlipidemia was also evaluated. It was observed that due to diabetes there was an increase in the total cholesterol levels as well as triglyceride levels. The HDL levels were reduced in the diabetic animals and the LDL levels were increased significantly (Table 3).

The ethanol extract showed a significant decrease in the total cholesterol levels and triglyceride levels. It also increased the HDL level and was successful in suppressing the LDL levels as compared to the standard drug by both extracts (Table 3).

Table 1: Effect of Ethanolic Extracts of *Artemisia Nilagirica* (Clarke) on Blood Glucose Level in Glucose Primed Rats.

Group	Blood Glucose level (mg/dl)		
	0 min.	**30 min.**	**90 min.**
Normal Control	**78.37±1.18**	**71.43± 1.15**	**72.67± 0.87**
Glucose Control	**85.03± 1.2**	**173.8 ±2.81**	**85.03 ±1.96**
Glucose + Glibenclamide	**92.1± 0.92**	**131.4± 1.73**	**94.73± 1.09**
***Ethanolic extract* (200 mg/kg)**	**96.2± 1.38**	**140.4± 0.96**	**98.9± 1.64**
***Ethanolic extract* (400 mg/kg)**	**93.8± 0.83**	**136± 2.02**	**96.67± 1.45**

*Values are expressed as mean ± SEM (Number of animals, n=6); significantly different at *P<0.05, when compared with glucose control group.*

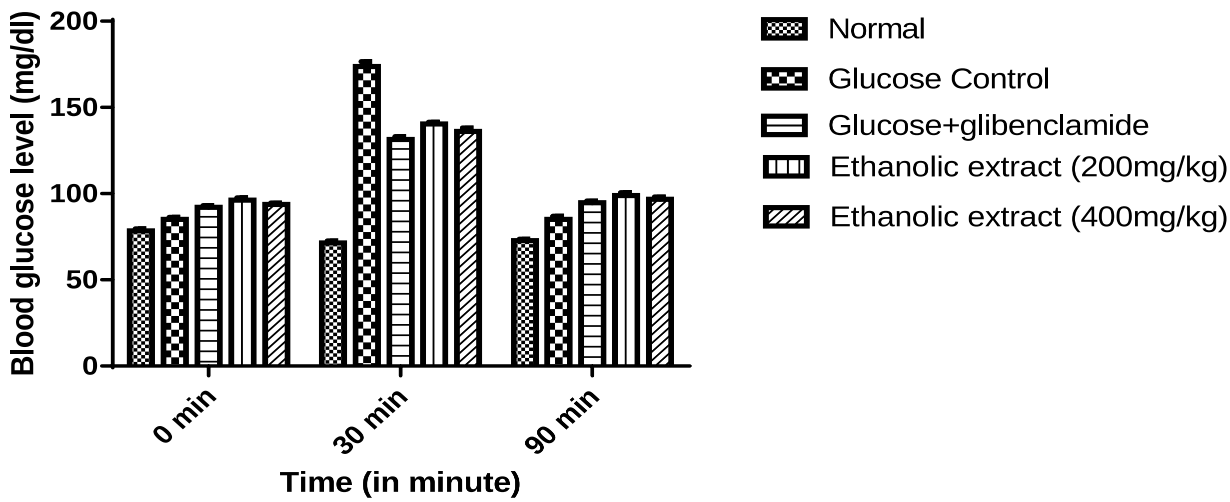

Fig 1: Effect of Ethanolic Extracts of *Artemisia Nilagirica* (Clarke) on Blood Glucose Level in Glucose Primed Rats

Table 2: Effect of Ethanolic Extract of *Artemisia Nilagirica* (Clarke) in Stz Induced Diabetes in Rats.

Group	Fasting plasma glucose concentration (mg/dl)			
	Day 0	**Day 7**	**Day 14**	**Day 28**
Normal Control	**87.8±0.94**	**93.48±1.74**	**108.3±3.80**	**84.83±0.84**
Glucose Control	**94.13±2.24**	**355±3.25**	**345.2±5.73**	**278.3±4.05**
Glucose + Glibenclamide	**96.57±2.01**	**107.2±2.44**	**95.9±0.85**	**88.2±0.87**
***Ethanolic extract* (200 mg/kg)**	**97.5±1.09**	**208.8±6.00**	**182.2±5.91**	**152.7±3.40**
***Ethanolic extract* (400 mg/kg)**	**102.2±1.84**	**185.3±0.70**	**162.9±3.34**	**113.8±4.45**

*Values are expressed as mean ± SEM (Number of animals, n=6); significantly different at *P<0.05, when compared with diabetic control group.*

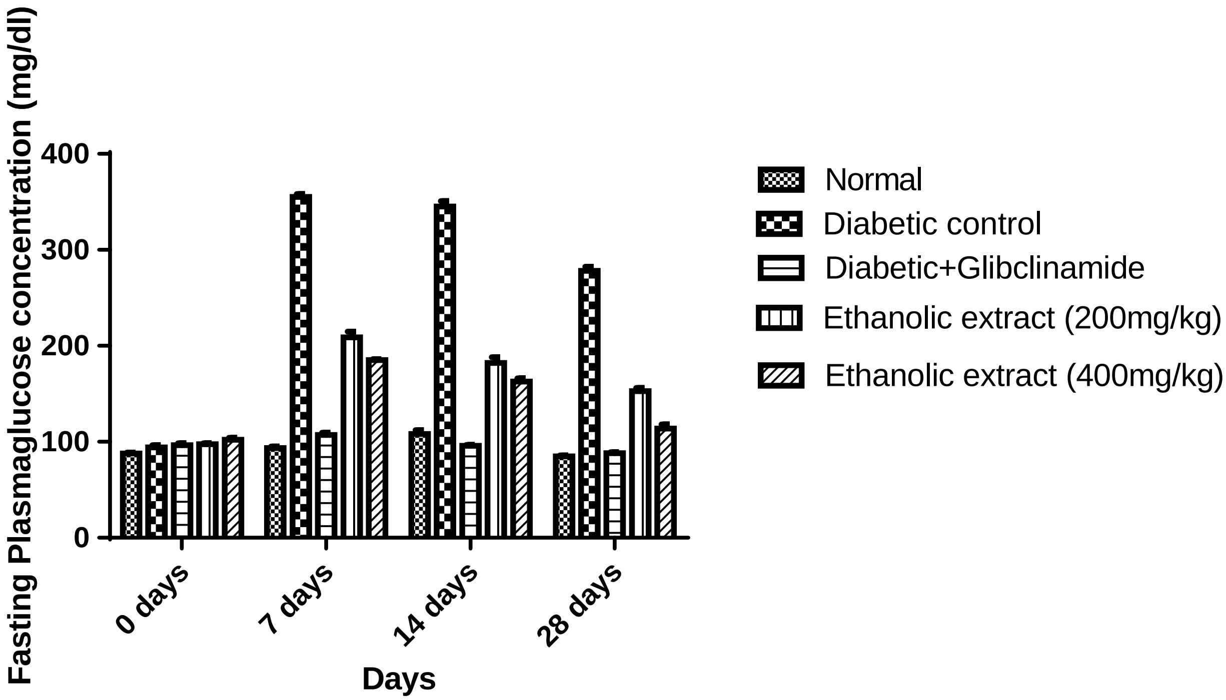

Fig 2: Effect of Ethanolic Extract of *Artemisia Nilagirica* (Clarke) In Stz Induced Diabetes in Rats

Table 3: Effect of Ethanolic Extract of *Artemisia Nilagirica (Clarke)* On Lipid Profile of Stz Induced Diabetes In Rats.

Group	Lipid Profile (mg/dl)			
	Triglyceride	Toal Cholestrol	HDL	LDL
Normal Control	87.07±1.75	95.5±3.1	52.77± 2.65	47.6± 1.15
Glucose Control	136.9±1.81	148.6±1.41	35.37± 2.50	118.4± 1.95
Glucose + Glibenclamide	85.63± 1.33	96.5±1.73	58.43±0.54	55.4±1.15
(200 mg/kg) 95±1.79	107.3±2.50	68.87±0.74	67±1.90	
Ethanolic extract (400 mg/kg)	89.8± 0.98	101.4±1.24	64±1.22	60.17±1.17

*Values are expressed as mean ± SEM (Number of animals, n=6); significantly different at *P<0.05, when compared with diabetic control group.*

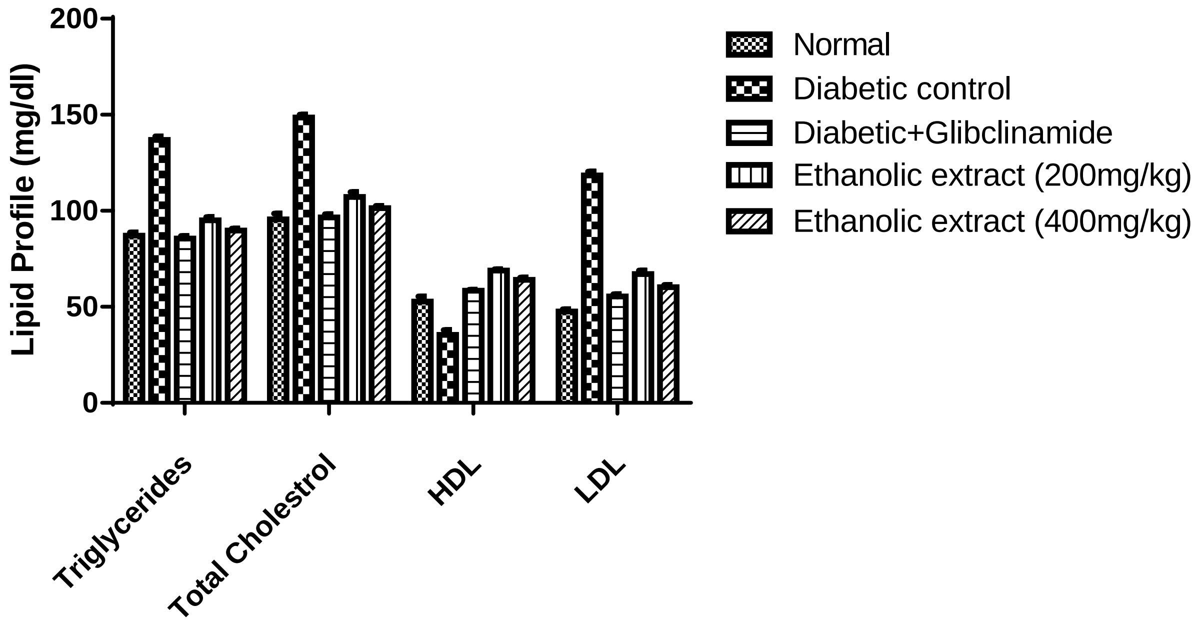

Fig 3: Effect of Ethanolic Extract of *Artemisia Nilagirica (Clarke)* On Lipid Profile of Stz Induced Diabetes in Rats.

DISCUSSION

The qualitative phytochemical analysis of the ethanolic extract of *Artemisia Nilagirica (Clarke)* revealed the presence of tannins, flavonoids, terpenes, saponins, steroids and alkaloids. These compounds have been reported to elicit a wide range of biological activities such as insulin-like effect, anti-hypercholesterol and hypotensive activity [15, 16]. For example, saponins are well-known to lower serum cholesterol by converting it to bile acids. The hypoglycemic and hypolipidemic properties of alkaloids, flavonoids and tannins have also been reported [17]. The presence of these compounds might contribute to the antidiabetic effect of this plant as observed in the present study.

The result of acute toxicity study of ethanol extracts of *Artemisia Nilagirica (Clarke)* on laboratory animals showed that the animals were safe up to a maximum dose of 2000 mg/kg body weight. There were no changes in normal behavior pattern and no signs and symptoms of toxicity and mortality were observed as per OECD guidelines ethanolic extract fall under class four values LD_{50} value being 2000 mg/kg. The pharmacological evaluations were therefore carried out at doses of 200 and 400-mg/kg body weight.

The fundamental mechanism underlying hyperglycemia in diabetes mellitus involves over-production and decreased utilization of glucose by the tissues. In our study, the difference observed between the initial and final fasting plasma glucose levels of different groups under investigation revealed a significant elevation in blood glucose in diabetic control group as compared with normal animals at the end of the 28-day experimental period. When ethanol extract of *Artemisia Nilagirica (Clarke)* were administered to glucose loaded normal rats fasted for 18 h, decrease in plasma glucose level was observed after 30 min. Ethnaolic extract at 200mg/kg and 400 mg/kg reduced plasma glucose level to normal at 90 min. During study it was found that both extracts control significantly the blood glucose level on streptozotocin induced diabetic rats. The ethanol extracts induced a significant reduction on blood glucose level in STZ-induced-diabetic rats as compared to the diabetic control group. But ethanol extract of *Artemisia Nilagirica (Clarke)* (400mg/kg) showed more significant antidiabetic activity as compared to 200mg/kg ethanol extract. The possible mechanism by which *Artemisia Nilagirica (Clarke)* brings about its hypoglycemic action in diabetic rat may be by potentiating the insulin effect of plasma by increasing either the pancreatic secretion of insulin from the existing beta cells or by its release from the bound form.

CONCLUSION

The marked increase in serum triglycerides and cholesterol observed in untreated diabetic rats. Under normal circumstances insulin activates enzyme lipoprotein lipase and hydrolyses triglycerides. Insulin deficiency results in failure to activate the enzymes thereby causing hypertriglyceridemia. The significant control of the levels of serum lipids in the both extracts of treated diabetic rats may be directly attributed to improvements in insulin levels upon *Artemisia Nilagirica (Clarke)* therapy. Elevation of plasma lipid concentration in diabetes is well documented. In insulin deficient diabetics, the plasma free fatty acid concentration is elevated as a result of increased free fatty acid outflow from fat depots, where the balance of the free fatty acid esterification–triglyceride lipolysis cycle is displaced in favour of lipolysis. Induction of diabetes with STZ is associated with the characteristic loss of body weight which is due to increased muscle wasting in diabetes. Diabetic rats treated with the extracts showed an increase in body weight as compared to the diabetic control which may be due to its protective effect in controlling muscle wasting i.e. reversal of gluconeogenesis.

Abnormalities in lipoproteins are very common in both NIDDM and IDDM. Although lipoprotein alterations appear to be an intrinsic part of these disorders, such alterations are also induced by diabetes associated complications such as obesity and renal disease. The total cholesterol, triglyceride levels, LDL and LDL were observed to be elevated in diabetics but reduced by both extracts as well as glibenclamide showing their beneficial effects. In the present study, HDL levels remained unchanged in diabetics compared to the other groups. These results suggest the beneficial effects of the natural extract in improving the imbalance in lipoprotein metabolism are also comparable to those of glibenclamide. The (400mg/kg) ethanol extract of leaves and flowering top of *Artemisia Nilagirica (Clarke)* produced more significant antidiabetic activity than ethanol extract of *Artemisia Nilagirica (Clarke)* (200 mg/kg), this indicates that the phytoconstituents responsible for antidiabetic activity. The present study has indicated the fact that the plant *Artemisia Nilagirica (Clarke)* has antidiabetic and antihyperlipidemic constituents and production of a safe antidiabetic drug is very much possible from leave and flowering tops.

ACKNOWLEDGEMENT

The authors are grateful to Dr. Vikas Jain and Dr. k.k. Shukla HOD in mahakal institute of phramaceutical studies. We are sincerely acknowledging Mr. Mausam Dubey for his technical support. The author gratefully acknowledge the Mr. Praveen vashishtha chairman of Mahakal institute of technology,Ujjain, Madya Pradesh, India for provided the abound facilities.

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